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## Quantification of $\beta$ -Casein in Milk and Cheese Using an Optical Immunosensor

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$\beta$ -Casein was quantified in milk and cheese, using an optical immunosensor, based on surface plasmon resonance (SPR) measurement. The assay consists of a two-step sandwich strategy, with two anti- $\beta$ -casein antibodies directed against each extremity of the casein. This strategy permits only native  $\beta$ -casein to be quantified and not its degradation products. The calibration curve was obtained with a reference milk powder of known  $\beta$ -casein concentration. The analysis time per sample was less than 10 minutes. The antibody-coated surface could be used for more than 250 determinations. The detection limit was established at 85 ng  $\cdot$  mL<sup>-1</sup> and the intra- and inter-assay variation coefficients were 2.6 and 6.2% respectively. The method was applied to raw milk to quantify intact  $\beta$ -casein, with no pretreatment of the sample. A second application was realized with cheese, to follow the proteolysis of  $\beta$ -casein during ripening.

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**KEYWORDS:** Biosensor; milk;  $\beta$ -casein; proteolysis; cheese; quantification

### INTRODUCTION

In France, cows' milk is paid for according to its quality. Milk quality is assessed by measuring certain major constituents such as fat, total proteins, and somatic cells. Total milk proteins include whey proteins (WP) such as  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, serum albumin, or immunoglobulins, which make up 20% and caseins, 80%. In milk, hydrolysis of caseins by indigenous milk enzymes can occur, leading to the formation of degradation products of which the majority are soluble. However, among the different types of proteins present in milk, only native caseins will constitute the cheese matrix. WP and degradation products are eliminated mainly with the whey during draining. Therefore, to have an accurate estimation of the quality of milk used for cheesemaking, it seems to be more important to determine the concentration of native caseins in milk rather than its total protein content.

Four major caseins can be found in milk, namely  $\alpha$ S<sub>1</sub>-,  $\alpha$ S<sub>2</sub>-,  $\beta$ -, and  $\kappa$ -casein. The relative concentration of these four caseins in milk can vary according to different factors, such as the breed (1), the lactation stage of the cow (2), and the feeding (3). These variations can affect the coagulation properties of milk (4), the cheese yield (5), and the general quality of cheese. Therefore, the quality of milk is highly dependent on the relative proportion of these four caseins. Among the four caseins, the concentration of  $\beta$ -casein (35–45% of the casein content in milk) could have an influence on milk coagulation and curd syneresis (6, 7). This

casein is hydrolyzed in fresh raw milk by plasmin, the major indigenous enzyme in milk. During cheese ripening,  $\beta$ -casein proteolysis plays a major role in the formation of cheese texture and flavor. The majority of peptides found in cheese originate from  $\beta$ -casein (8).

Several biochemical methods have been developed to quantify the four major caseins in milk, such as gel electrophoresis (9, 10), fast protein liquid chromatography (FPLC) (11), high performance liquid chromatography (HPLC) (12, 13) and capillary electrophoresis (14, 15). However, none of these methods gave an optimum separation of all major milk proteins. In particular, separation of  $\alpha$ S<sub>1</sub>- and  $\alpha$ S<sub>2</sub>-casein by FPLC, or  $\alpha$ S<sub>2</sub>- and  $\kappa$ -casein by HPLC, was not always achieved (12, 16). Finally, a microparticle-enhanced nephelometric immunoassay has been developed to quantify  $\alpha$ S<sub>1</sub>-,  $\beta$ -, and  $\kappa$ -casein (17, 18) and applied to milk and dairy products (19). This technique uses polyclonal antibodies directed against the whole casein, rendering impossible the discrimination between native casein and their degradation products.

Biosensor systems, using the principle of surface plasmon resonance (SPR) (20), are a recent addition to research and a new analytical tool. Among the optical biosensors commercially available, the Biacore system (Biacore International SA, Uppsala, Sweden) is one of the most commonly used. Indeed, it has already allowed the detection of staphylococcal enterotoxins (21, 22), drug residues (23–26) or vitamins (27, 28), in milk and dairy products. Several analysis kits are now commercialized to quantify contaminants or vitamins in food products. In this context, the development, using Biacore, of an immunoassay

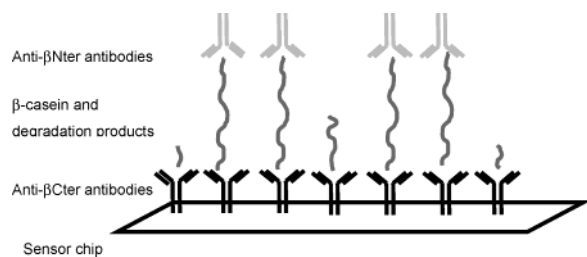
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Muller-Renaud et al.



**Figure 1.** Strategy used to quantify  $\beta$ -casein.

allowing the simultaneous quantification of the four caseins would constitute a progress in the characterization of milk quality.

To determine the feasibility of the approach, this paper describes the development of a first immunoassay for quantification of  $\beta$ -casein in milk using Biacore. The method needs to be able to quantify specifically native  $\beta$ -casein only and not its degradation products. The strategy used is a sandwich immunoassay using two monoclonal antibodies, one specifically directed against the C-terminal extremity and the other directed against the N-terminal part of the protein. The antibody directed against the C-terminal extremity was immobilized on the sensor chip surface and used to capture the protein and degradation products including this extremity. The second antibody directed against the N-terminal extremity, injected after the milk sample, was able to recognize native  $\beta$ -casein only (Figure 1). This method was developed and validated with milk. To demonstrate its versatility, the technique was also applied to the monitoring of  $\beta$ -casein proteolysis during cheese ripening.

## MATERIALS AND METHODS

**Reagents.**  $\beta$ -casein was purified as described previously (29).

$\alpha$ S1-,  $\alpha$ S2-, and  $\kappa$ -casein,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, immunoglobuline G and bovine serum albumin were obtained from Sigma-Aldrich (Sigma-Aldrich, St Quentin Fallavier, France).

**Milk and Cheese Samples.** *Milk Powder.* Skimmed low heat milk powder (SRTAL-INRA, Poligny, France) was used for biosensor calibration. Milk was reconstituted in  $\text{CaCl}_2$  10 mM to a final concentration of 12% (w/v). The concentration of each casein in milk powder was determined by reverse phase HPLC (RP-HPLC) (Nutrinov, Vezin-le-Coquet, France).

*Milk Samples.* Six herds (three Montbeliarde and three Holstein), each comprising 10 cows, were milked four times over a three month period, for a total number of 48 milk samples. Samples were aliquoted and stored at  $-20^\circ\text{C}$ . Before analysis, the top fat layer was removed by centrifugation (1800g,  $4^\circ\text{C}$ , 15 min).

*Cheese Samples.* Comté is a French Swiss-type cheese made from raw milk. Four different Comté cheeses were ripened according to the following process: pre-ripening,  $13^\circ\text{C}$ , 49 days; warm room,  $17^\circ\text{C}$ , 35 days; cold room,  $6^\circ\text{C}$ , 63 days. Cheeses were sampled after 7, 49, 85, 122, and 183 days of ripening. One gram was finely grated and dispersed in 40-mL buffer (0.4 M trisodium citrate, 0.15 M NaCl, 75 mM EDTA, 10 mM DTT, 8 M urea) for 1 h at  $40^\circ\text{C}$  and 45 min at room temperature. The solid fat layer was discarded by centrifugation (1800g,  $4^\circ\text{C}$ , 15 min) and the solubilized cheeses were aliquoted and stored at  $-20^\circ\text{C}$ .

**Antibodies.** *Monoclonal Antibodies.* Two monoclonal antibodies (Mabs) were used, one directed against the N-terminal extremity of  $\beta$ -casein (called anti- $\beta$ Nter), and the other directed against the C-terminal extremity (anti- $\beta$ Cter). These antibodies were previously raised and characterized in our laboratory (29). Ascites were obtained according to the procedure of Jones et al. (30).

Anti- $\beta$ Cter Mabs were purified from ascitic fluid by affinity chromatography using a HiTrap NHS-activated HP column (Amersham Pharmacia Biotech, Uppsala, Sweden), according to the manufacturers recommendations. Briefly, 10 mg of  $\beta$ -casein in 1 mL of coupling buffer (0.2 M  $\text{NaHCO}_3$ , 0.5 M) were covalently immobilized on the column.

Any excess active groups were deactivated by washing with 0.5 M ethanolamine, 0.5 M NaCl, pH 8.3, and the nonspecifically bound ligands were washed out with 0.1 M acetate, 0.5 M NaCl pH 4. Ascitic fluid (1 mL) diluted in 4 mL of running buffer (75 mM Tris-HCl pH 8) was then injected. The column was washed with running buffer. Anti- $\beta$ Cter antibodies were eluted with elution buffer (100 mM glycine-HCl, 0.5 M NaCl pH 2.7), and the eluted fraction was neutralized by addition of 1/4 volume of 1 M Tris-HCl pH 9. The purified antibodies were aliquoted and stored at  $-20^\circ\text{C}$ .

**Polyclonal Antibodies (Pabs).** Polyclonal antibodies directed against  $\alpha$ S1-,  $\alpha$ S2-, and  $\kappa$ -casein were produced according to the procedure described by Senocq et al. (31).

**Biosensor Assay.** *Apparatus.* The optical biosensor used to perform the assay was a Biacore 3000 (Biacore).

*Detection Principle.* The BIA-technology allows real-time measurement of molecular interactions using surface plasmon resonance (SPR) detection (20). One of the reactants is immobilized on an extended carboxymethyl dextran matrix layered on a gold surface, and the other is introduced in a continuous flow over the sensor surface. The SPR detects and measures changes in refractive index due to the binding and dissociation of interacting molecules in proximity to the gold surface. The change in refractive index is proportional to the quantity of analyte interacting with the ligand. The interaction causes a shift in the angle of index at which the SPR phenomenon occurs. These shifts, monitored continuously over time, are shown as sensorgrams and expressed in resonance units (RU). The integration of SPR detection, a microfluidic system and operator designed sensor surfaces into one automated analytical system provides quantitative analysis of biospecific interactions.

*Preparation of the Sensor Surface.* Anti- $\beta$ Cter Mabs were immobilized covalently on a carboxymethyl 5 sensor chip, CM5 (Biacore) by amine coupling, as described previously (20). Briefly, a continuous flow of 10 mM HEPES pH 7.4 containing 150 mM NaCl, 3.4 mM EDTA, and 0.005% Surfactant P20 (HBS-EP, Biacore) over the sensor surface at  $5\ \mu\text{L} \cdot \text{min}^{-1}$  was maintained. The CM5 carboxymethylated dextran matrix was activated by injection of  $35\ \mu\text{L}$  of a solution containing 0.05 M N-hydroxysuccinimide (NHS), 0.2 M N-ethyl-N'-(3-di-ethylaminopropyl)carbodiimide (EDC) (Biacore). Next,  $35\ \mu\text{L}$  of Mabs at a concentration of  $0.2\ \text{mg} \cdot \text{mL}^{-1}$  in sodium acetate 10 mM, pH 4.5 (Biacore) were injected, followed by  $35\ \mu\text{L}$  of 1 M ethanolamine (Biacore) to block remaining NHS-ester groups. The immobilization level was 10000 RU, corresponding to  $10\ \text{ng}/\text{mm}^2$  of Anti- $\beta$ Cter Mabs. The Mabs were immobilized on flow channel Fc 2, with Fc1 being used as reference cell.

*Assay Principle.* This assay consists of a sandwich test with two monoclonal antibodies, one specifically directed against the N-terminal extremity and the other directed against the C-terminal part of the protein (Figure 1).

Anti- $\beta$ Cter antibodies were immobilized on the sensor surface. A continuous flow was maintained at  $30\ \mu\text{L} \cdot \text{min}^{-1}$ . Sample ( $30\ \mu\text{L}$ ) was injected, followed by the injection of  $30\ \mu\text{L}$  of anti- $\beta$ Nter monoclonal antibodies (diluted 1/1000 in HBS-EP buffer). The regeneration of the sensor surface was realized by two  $15\ \mu\text{L}$  injections of 5 mM NaOH.

*Calibration Curve.* To study the matrix effect, 3 calibration curves were realized with three different media. The first calibration was realized with different concentrations of purified  $\beta$ -casein ( $0-8\ \mu\text{g} \cdot \text{mL}^{-1}$ ) in HBS-EP buffer. The other calibrations were realized with serial dilutions of milk powder and milk of known  $\beta$ -casein content. The dilutions of milk powder and milk were established in correspondence with the concentrations of purified  $\beta$ -casein. The calibration curve was established in response to the corresponding binding of anti- $\beta$ Nter antibodies on the native  $\beta$ -casein (Figure 2).

*Assay Specificity.* The specificity was assessed by replacing Mabs anti- $\beta$ Nter with Pabs directed against  $\alpha$ S1-,  $\alpha$ S2-, and  $\kappa$ -casein as the detector.

*Detection Limit.* The detection limit was calculated from the mean of measurement observed from a representative blank sample ( $n = 20$ ) plus three times the standard deviation ( $s$ ) of the mean ( $\text{mean} + 3s$ ).

*Assay Precision.* The repeatability was defined by determining intra-assay (within run) and inter-assay (between runs) variation. Intra-assay

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Quantification of  $\beta$ -Casein in Milk and Cheese

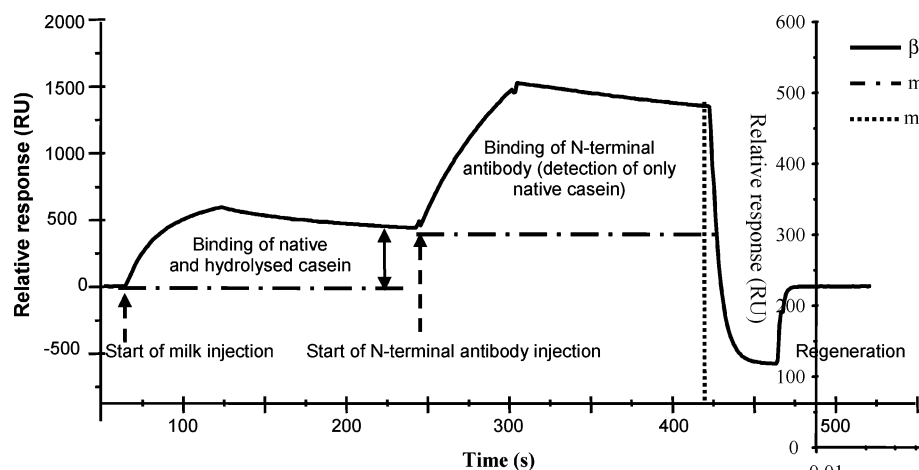


Figure 2. Sensorgram obtained with a milk sample.

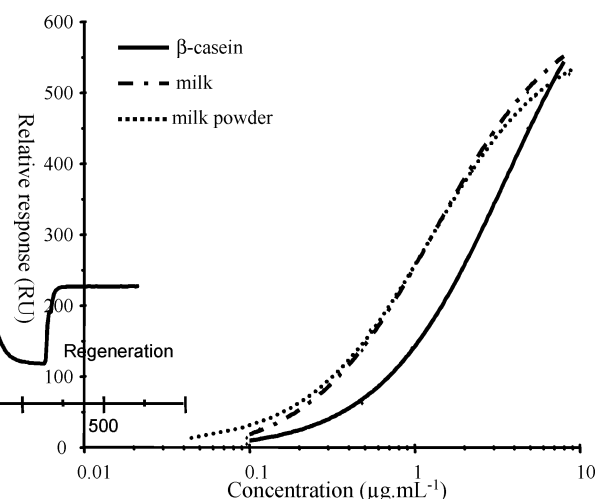


Figure 3. Illustration of matrix effects. Calibration curve realized with milk, milk powder, or purified  $\beta$ -casein.

variation was determined by 10 successive analyses of the same sample at three different dilutions (1/20 000, 1/15 000, and 1/10 000). Inter-assay variation was evaluated by analysis of the same sample in 10 different runs.

To determine the accuracy, the recovery after addition of exogenous  $\beta$ -casein was calculated. Four different concentrations of reconstituted milk powder corresponding to 2.3, 5.8, 8.2, and 11.6  $\text{mg} \cdot \text{mL}^{-1}$  of  $\beta$ -casein were added to a sample of raw milk. Each sample analyzed was diluted to 1/10 000.

**Quantification in Milk and Cheese Samples.** Milk samples were diluted 1/10 000 in HBS-EP buffer. Milk cheese samples were diluted to 1/5000 and to 1/3000 in HBS-EP buffer. To determine  $\beta$ -casein concentration, each sample was analyzed in duplicate.

RESULTS AND DISCUSSION

**Characteristics of the Assay. Specificity of the Antibodies.** The specificity of both the anti- $\beta$ Nter and anti- $\beta$ Cter antibodies has been previously tested in our laboratory, against different fragments of  $\beta$ -casein. Anti- $\beta$ Nter and anti- $\beta$ Cter antibodies were specific for the N-terminal (f1–25) and C-terminal (f184–209) fragments, respectively (29). The specificity of the anti- $\beta$ Cter antibodies immobilized on the chip was tested against the principal milk proteins ( $\alpha$ S<sub>1</sub>-,  $\alpha$ S<sub>2</sub>-,  $\kappa$ -casein,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, immunoglobulin G, and bovine serum albumin). The different proteins were injected at concentration of 10  $\mu\text{g} \cdot \text{mL}^{-1}$  onto an anti- $\beta$ Cter coated sensor chip. No cross-reactions were observed.

**Assay Specificity.** In milk, 95% caseins are organized as micelles in association with calcium and phosphate. To know if the response after milk injection was due to binding of a casein complex and not only  $\beta$ -CN, the secondary antibodies anti- $\beta$ Nter were replaced by Pabs, specific from  $\alpha$ S<sub>1</sub>-,  $\alpha$ S<sub>2</sub>-, or  $\kappa$ -casein. No binding of these probes on the immobilized antigen was observed.

**Time of Analysis.** The analysis for one sample, including the regeneration, is approximately 10 min.

**Stability.** The regeneration conditions were optimal with addition of 5 mM NaOH. A sensor surface could be used for more than 250 cycles without any significant decrease in baseline.

**Matrix Effect.** Figure 3 shows the calibration curves obtained with purified  $\beta$ -casein, a reference milk powder, and raw milk. Standard curves obtained with milk powder and raw milk were shown to be identical. Significant differences were observed with purified  $\beta$ -casein, for a same  $\beta$ -casein concentration the response was higher with milk powder. Therefore, to avoid a

Table 1. Determination of Recovery after Exogenous Addition of Different Concentrations of  $\beta$ -casein in a Raw Milk Sample<sup>a</sup>

	theoretical $\beta$ -casein concn (mg/mL)	$\beta$ -casein concn obtained (mg/mL)	recovery (%)
milk		13	
milk + 2.3 $\text{mg} \cdot \text{mL}^{-1}$	15.3	15.2	99.3
milk + 5.8 $\text{mg} \cdot \text{mL}^{-1}$	18.8	18.0	95.9
milk + 8.2 $\text{mg} \cdot \text{mL}^{-1}$	21.2	20.9	98.6
milk + 11.6 $\text{mg} \cdot \text{mL}^{-1}$	24.6	24.1	98

<sup>a</sup> Four different quantities of reconstituted milk powder corresponding to 2.3, 5.8, 8.2, and 11.6  $\text{mg} \cdot \text{mL}^{-1}$  of  $\beta$ -casein were added to a raw milk sample. Each sample analyzed was diluted 1/10 000. Responses were the mean of three determinations.

matrix effect, the standard curve was established with reference milk powder.

**Standard Curve.** The curve was established from the response of N-terminal antibodies to  $\beta$ -casein concentrations in reconstituted milk ranging from 0 to 9.32  $\mu\text{g} \cdot \text{mL}^{-1}$  (Figure 3). A new calibration may be necessary after 100 cycles.

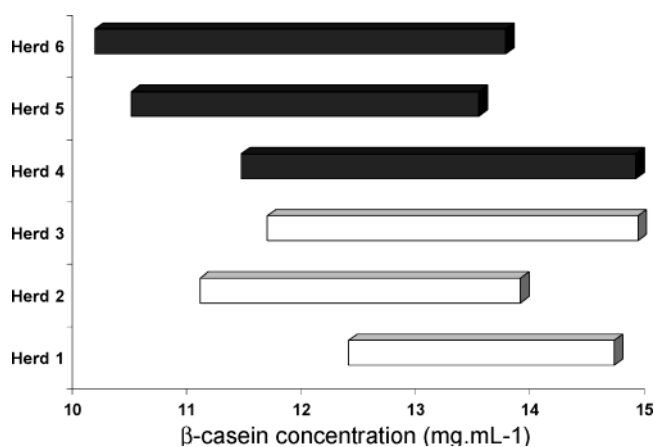
**Detection Limit.** The detection limit, determined from the mean measurement of a representative blank sample ( $n = 20$ ; mean + 3s), was established at 85  $\text{ng} \cdot \text{mL}^{-1}$ .

**Assay Precision.** The repeatability determined with different dilutions of a raw milk sample, established for intra- and inter-assay was 2.6 and 6.2%, respectively. The accuracy of the assay, estimated by exogenous additions of known  $\beta$ -casein concentrations in a raw milk sample and expressed as recovery (%), was between 95.9 and 99.3% (Table 1).

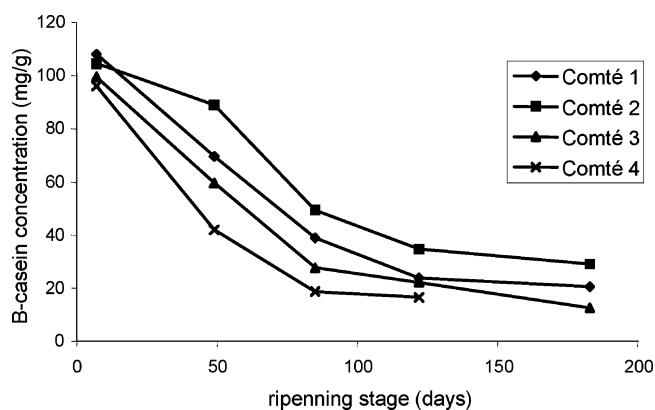
**Analysis of Milk Samples.** Milk samples ( $n = 48$ ), from six cow herds (3 Holstein, Herd 1, 2, 3; and 3 Montbeliarde, Herd 4, 5, 6) collected at eight different milking times, were analyzed. The concentrations of intact  $\beta$ -casein detected in the milk samples ranged from 10.1 to 14.8  $\text{mg} \cdot \text{mL}^{-1}$ . The range of  $\beta$ -casein concentration obtained for each herd is represented in Figure 4.

**Analysis of Cheese Samples.** The method for determination of intact  $\beta$ -casein developed here was applied to cheese to follow the evolution of  $\beta$ -casein concentration during ripening. Four Comté cheeses were analyzed at five stages during ripening (Figure 5). The four Comté cheeses showed similar profiles for the decrease in native  $\beta$ -casein concentration. The concentra-

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**Figure 4.**  $\beta$ -casein concentration calculated in 48 samples of raw milk. Six herds, three Holstein (Herds 1, 2, and 3) and three Montbeliarde (Herds 4, 5, 6) each constituted of 10 cows, were milked eight times over a three month period. Responses were the mean of two determinations.



**Figure 5.** Percentage of intact  $\beta$ -casein in four Comté cheeses during ripening. The four Comté cheeses were produced by the same ripening process (pre-ripening, 13 °C, 49 days, Warm room, 17 °C, 35 days, Cold room, 6 °C, 63 days).

tion of intact  $\beta$ -casein at the beginning of ripening was between 96 and 108 mg  $\cdot$  g<sup>-1</sup>. For cheese 4, the concentration of intact  $\beta$ -casein at the end of ripening was not determined because the signal response obtained was below the detection limit. The majority of  $\beta$ -casein was hydrolyzed during preripening and ripening in the warm room periods, with a concentration of intact  $\beta$ -casein between 18 and 38 mg  $\cdot$  g<sup>-1</sup> at the end of these periods. The quantity of intact  $\beta$ -casein remaining at the end of the ripening process ranged from 29 to 12.6 mg  $\cdot$  g<sup>-1</sup> for cheeses 1–3.

The development of an immunoassay for the quantification of  $\beta$ -casein in milk and cheese using Biacore is presented in this study. The strategy used is a sandwich assay with two monoclonal antibodies directed against each extremity of the protein. Thus, only intact  $\beta$ -casein can be quantified and not its degradation products.

To quantify  $\beta$ -casein in milk, a calibration curve was established with purified  $\beta$ -casein. However, a matrix effect was observed in comparison with the calibration curve obtained with a milk sample. The use of milk powder for calibration eliminated the matrix effects. For a same  $\beta$ -casein concentration, the response obtained with milk or milk powder was higher. To discover if the higher response observed with milk or milk powder was due to the binding of a casein complex, and not only  $\beta$ -casein, the secondary antibodies anti $\beta$ -Nter were replaced

Muller-Renaud et al.

by Pabs, specific for  $\alpha$ S<sub>1</sub>-,  $\alpha$ S<sub>2</sub>-, or  $\kappa$ -casein. No binding of these probes on the immobilized antigen was observed, demonstrating that only  $\beta$ -casein had been captured by the anti- $\beta$ Cter. Two hypotheses could explain the variation between purified  $\beta$ -casein and  $\beta$ -casein in milk. First, the variation could be due to a difference in the structure of  $\beta$ -casein in milk and in purified solution. The epitope recognized by Mab anti $\beta$ -Nter seems to be more accessible in milk than in purified  $\beta$ -casein. It is quite possible that the purification process of  $\beta$ -casein led to changes in the conformation of the protein, rendering difficult the accessibility of the N-terminal extremity by the anti- $\beta$ Nter. The second explanation of the higher response in milk and milk powder could be due to an unspecific binding of a minor constituent present in milk. In this case, the unspecific constituent should have two distinct epitopes that are recognized by both the anti- $\beta$ Cter and anti- $\beta$ Nter antibodies.

This technique was applied to quantify  $\beta$ -casein in 48 milk samples. The  $\beta$ -casein concentrations determined within each herd throughout the experiment varied significantly. This variability could be attributed to the limited size of the herds (10 cows each).

The  $\beta$ -casein concentration calculated with the primary response after milk injection was compared to the concentration determined with the secondary response after the binding of the N-ter antibodies (data not shown). For all samples,  $\beta$ -casein concentrations determined with the primary response were higher than those determined with the secondary response. The primary response corresponds to the fixation of  $\beta$ -casein and C-terminal fragments of  $\beta$ -casein on the chip, whereas the secondary response corresponds to the detection of the entire protein by the specific antibodies from the N-extremity of  $\beta$ -casein. These results confirm that the strategy developed here allowed quantification of native  $\beta$ -casein only and not its proteolysis products. The difference in concentration between the two responses was not always the same, which tends to demonstrate the fact that the level of proteolysis was different according to the milk samples. It is known that  $\beta$ -casein is rapidly cleaved by plasmin in fresh raw milk and that this enzymatic activity can vary from one milk to another (typically 10–20% of  $\beta$ -casein, in weight) (32–34). The other endogenous milk enzymes, cathepsin-D could be responsible for part of the  $\beta$ -casein proteolysis (35).

The immunosensor was able to quantify native  $\beta$ -casein in milk, and the technique was applied to cheese to follow the proteolysis of  $\beta$ -casein during ripening. The major part of the  $\beta$ -casein hydrolysis occurred during preripening and ripening in the warm room periods. This intense phase of proteolysis was observed by Bican and Spahn (36), who have shown that all degradation products could be seen after 30 days of maturation. In Swiss-type cheeses, such as Comté, the cooking of the curd extensively inactivates the coagulant and simultaneously enhances plasmin activity, which becomes predominant in the proteolysis of caseins (37). Plasmin could be responsible for the proteolysis during preripening and ripening in the warm room. Recently, Senocq et al (38) have demonstrated, by the measurement of  $\beta$ -casein cleavage by plasmin at the Lys28-Lys29 site, that during ripening in the warm room, the activity of plasmin was intense. The cheese sample 4 presented a more important degree of proteolysis than the other three cheeses. Plasmin activity was quantified in all cheese samples at the beginning of ripening and a higher plasmin activity was observed in cheese 4, which could explain a more intense proteolysis. At the end of ripening, the quantity of native  $\beta$ -casein in cheeses 1 to 3 ranged from 29 to 12.6 mg  $\cdot$  g<sup>-1</sup>

Quantification of  $\beta$ -Casein in Milk and Cheese

(i.e., from 25 to 12% of the quantity of native  $\beta$ -casein at the beginning of ripening). In Cheddar cheese, 60–70% of  $\beta$ -casein is hydrolyzed during maturation (39), and it has been demonstrated that in Swiss-type cheeses, proteolysis of  $\beta$ -casein was higher than that in Cheddar (40, 41). Grappin et al. (42) compared proteolysis in Swiss-type cheeses and reported that Comté cheese showed an average proportion of 10.5% of residual native  $\beta$ -casein at the end of maturation.

The immunoassay developed here is able to quantify native  $\beta$ -casein in milk and cheese. It could therefore be of great interest in routine analysis. However, the Biacore device, used to detect the antigen–antibody interactions, is a tool for research applications that remains expensive. An alternative would be the development of a Biacore kit dedicated to the specific quantification of  $\beta$ -casein in dairy products.

## ABBREVIATIONS USED

WP, whey protein; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography; SPR, surface plasmon resonance; CM5, carboxy methyl dextran; HBS-EP buffer, HEPES buffer saline; EDTA, ethylenediaminetetraacetic acid; NHS, *N*-hydroxysuccinimide; EDC, *N*-ethyl-*N'*-(3-diethylaminopropyl) carbodiimide; DTT, dithiothreitol; Mab, monoclonal antibody; Pab, polyclonal antibody; RU, response unit; Fc, flow channel.

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## LITERATURE CITED

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