Chapter 6

Complexation Between Casein Micelles and Whey Protein by Indirect UHT- Processing of Milk: Influence of Surface Hydrophobicity and Dye-Binding Characteristics of Micelles in Relationship with Their Physico-Functionality

Bärbel Lieske

Additional information is available at the end of the chapter

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1. Introduction

Milk is nearly always heat treated and the aim of heating was to make the milk safe for human health by killing microorganisms and inactivating the enzymes. Now there has been consistent interest in more novel and sophisticated strategies to improve the rheological properties and stability of acid gels by a more tailored complexation between casein and whey protein *via* SH/SS-exchange. The most frequented approach explored has been (ultra)high-pressure processing. Pressure treatment alone decreases the casein micelle size; however, this effect was less marked when heat and pressure treatments were combined and gels formed on acidification revealed a range of firmness, yield stresses and yield strains which were not to related to whey protein denaturation by oneself (1).Today, the understanding of the effects of high pressure seems quite advanced, e.g.; that the casein micelle properties were changed and that the denaturation of whey protein affected the gel assembly process in cheese and yoghurt (2). Investigations in the effect of high pressure (in the range of 50 to 350 MPa) on constituents of the colloidal phase of milk revealed that non-denatured β-lactoglobulin is left reaching maximum value of 62% in the range of 100 to 350 MPa (7). Other studies addressed the effects of pressure, pH, and temperature on the casein micelle dissociation in skim milk during high-pressure treatment with measurements of light scattering. A relationship between the barostability of casein micelles and pH was established, whereas no effect of temperature was ascertainable, and a mechanism for high pressure-induced disruption of micelle integrity is suggested in which the state of calcium

plays a crucial role in the micelle dissociation process (8). A study in high pressure effects on the structure of casein micelles in milk by cryo-transmission electron microscopy showed that in the range 150 to 300 MPa a large number of small micelles coexisted with a fraction of large micelles, which appeared perfectly spherical with smooth and well-defined surfaces (6).

The manufacture of acid gels from ultra-high pressure homogenization-treated milk in combination with a thermal treatment (90 °C/5min) resulted in a firmer gel quality to be used for improving the rheological properties and stability of yoghurt and, thus decreasing the need for additives (5). This expectation proved to be right as shown in two further experimental studies dealing with acid coagulation properties of set and stirred yoghurt (3, 4).

Thermo-sonification has proved to be an alternative technique to extent the shelf life of whole milk (10) and the combination of preheating (45°C) with thermosonification (24 kHz for 10 min) allowed the preparation of yoghurts with rheological properties superior (9) to those of control yoghurts produced from conventionally heated milk (90°C for 10min) (11). The effects of preheating milk (80°C for 10min) for preparing acid milk gels were studied with reference to the preheating pH by dynamic oscillation measurements resulting in firmer gel qualities at a higher pH (>6,65), probably due to greater involvement of S-S interactions (12). A short-time of preheating in terms of procedure is also an element of ultra-high temperature processing (UHT) of milk which is well-tried in the dairy practice today. Indirect UHT processing was used to prepare dairy desserts for studying the influence of varying concentrations of additives on the dessert rheology. As a result, a more extensive whey protein denaturation and subsequent complexation with casein micelles is believed to contribute to the improvement of rheological properties of the UHT desserts (13).

Own research into the effects on indirect UHT processing are in agreement with this and confirmed that perfect complexation may result only by the indirect procedure (14). Moreover, perfect complexation and inherent physico-functionality of the protein were to set not only by the technology alone but also by the current colloid-chemical status of the casein micelles in milk. In herd milk, this status may vary considerably in one period of lactation and inherent negative consequences are seen too late in the finished product.

This research was aimed at the investigation into the influence of a varying colloidal status of casein micelles on the resulting micellar complexation with whey protein on indirect UHT processing of skim milk. For this an own colloid-chemical approach is used to explain the structural changes of casein micelles with given examples of four herd milk samples and one dairy-based product for the purpose of confirmation

2. Materials and methods

2.1. Raw milk and thermal treatments

Bulked herd milk was collected from one farm in Schleswig-Holstein for a period of time of 8 month. Both separation of milk and thermal treatment were carried out using certified equipment. The skimmed milk was first regenerative (principle of heat recovery) preheated to 70-85 °C and then heated indirectly (126 °C for 4 s) and after that cooled down to 5 °C.

2.2. Spectrophotometric analysis of ß-lactoglobulin (ß-Lg)

For quantification of β-Lg (15) from the pH 4,6 -filtrate a spectrophotometric method was applied using a molar absorption coefficient of 0.950 for 0, 1 % (w/w) β-Lg at 280 nm. All spectrophotometric results are means of triplicate determinations repeated twice.

2.3. Surface hydrophobicity of proteins in milk

The development of this method is dated back to 1990/91 and was refined further until 2004. The initial interest was to know the reason of interference of the non-ionic detergent Tween 80 in the Bio-Rad Protein Assay with a variety of proteins. The detergent binding turned out to be a measure of hydrophobic surface area of integral proteins and thus constituting the basis for an assay procedure to determine the hydrophobic potential on the surface of proteins. At first, the methodical research was turned to analyze only isolated proteins (16). A methodical description is given below.

2.3.1. Reagent and sample preparation

Detergent reagent: 0,25 % Tween ("commercial-grade") is prepared in destilled and/or deionized water, stored at room temperature for max. 3 days.

Dye reagent: The dye reagent is purchased as a five-fold concentrate, which must be diluted and filtered through a large-pored filter prior further use.

Sample preparation: Proteins are diluted to about 0,05-0,10 % using *aqua dest.* or 0,05M phosphate buffer. As a rule, the colour intensity of 50 µL diluted sample developed with 2,5 mL dye reagent should not exceed A 595nm, 1cm = 0,500 vs. *aqua dest*. or buffer.

Additional items required:

- Spectrophotometer: allowing measurements at 595 nm.
- Polystyrene cuvettes: 10 mm path length as semi-micro cuvettes.
- Test tubes: polystyrene test tubes (13 x 64 mm) each fitted with a mixing spatula (Boeringer, Mannheim, Germany)
- Dispenser and microliterpipets are necessary for precise dispensing the dye reagent resp. for adding the sample.
- Rack: Test tube rack to store the test tubes containing samples and blanks.

2.3.2. Assay procedure

The hydrophobicity of proteins and casein micelles is calculated from two different protein assays, which are developed at the same time. Triplicates for each single measurement are necessary.

1a) 50 µL protein (A_{sample}) is placed on the bottom of a dry and clean test tube whereas 50 µL *aqua dest*. or buffer are used as blank (A_{blank}).

1b) 50 µL 0,25 % Tween 80 is placed on the bottom of a dry and clean test tube and in addition to 50 µL protein is placed onto the droplet of Tween 80 (B_{sample}) whereas *aqua dest*. or buffer and Tween 80 are prepared as blank (B_{blank}).
2) Only the detergent-containing tubes (1b) are shaken for 10 min. (avoid foaming) to complete detergent binding at temperatures between 18-22 °C.
3) Add 2,5 mL diluted dye reagent to each test tube, insert mixing spatulas by use of a pincette to avoid any skin contact. Move the spatula several times up and down without foaming.
4) Allow standing for 12 min. to develop the colour. The colour intensity of each tube is measured at 595 nm vs. *aqua dest*. Avoid any warming of cuvettes by a prolonged standing inside the cuvette department of the photometer.
5) Calculation

Protein Hydrophobicity (PH) is defined as following (Nakai and Li-Chan) (17)

PH = (nonpolar residues)/ (nonpolar residues) – (polar residues)

Using this definition on detergent binding according the proposed method, PH is calculated as

$$\frac{SHP\ (\%) = \left(A_{sample} - A_{blank}\right) - \left(B_{sample} - B_{blank}\right) * 100}{\left(A_{sample} - A_{blank}\right)}$$

Analyzing the casein in its natural colloidal status (18) this method was modified to protect the micelle structure from an to early dissociation on dilution with water for a short time. Among some other things, the purity of water is one crucial point for the colloid-chemical analysis requiring a special conditioning procedure in some laboratories and need to do some research into the causes of this. The water quality plays also an important role in the dilution of the detergent for analysis; for this "protein-grade" Tween 80 is to be used. In original packing the detergent is diluted 10 % (v/v) in distilled, deionized and filtrated water and applicable for a limited period of time after opening. At present, all Tween-preparations on the market are plant-based and replaced the preferred high-grade detergent products of animal origin.

The estimation of micellar SHP is carried out pH-depended between pH 6,0 and 7,5. For this, skimmed milk samples were held at 4-5 °C during pH adjustments at pH 6,0, 6,2, 6,4 and 6,6 ca. 8 gL^{-1} Glucono-delta-lacton (GDL) or NaOH (4 gL^{-1}) was used for adjustments at pH 6,8, 7,0, 7,2 and 7,4, respectively.

The eight samples were sealed and stored at 4-6 °C overnight. Before analysis the samples are equilibrated in a water bath; first, at 36 °C for 30 min while stirring and then 70 min rocking (120 rpm) at room temperature (19-21 °C). The procedure of equilibration is based on measurements of micellar turbidity. The SHP is analyzed from a watery dispersion (100-200 µL milk in 25 mL highly pure water) at 21 ± 1 °C. The measurements of samples were carried out in triplicate and six fold in case of the protein-free standards (blanks). The

experiments were repeated if the spread of readings do not agree with those permitted for the Bio-Rad- method.

3. Results and discussion

3.1. The influence of UHT-processing at 126 °C/ 4s on some major components in three different skim milk samples

Effects of indirect UHT-processing on some major components in the three skim milk samples are summarized in Tab.1.

No.	Milk Sample	pH	Protein %	β-Lg gL^{-1}	SHP %
1a 1b	Skim milk , indirect UHT (126°C/4s)	6,82 6,77	3,52 3,51	3,34 1,37	74,2 82,7
2a 2b	Skim milk, indirect UHT (126°C/4s)	6,77 6,77	3,51 3,49	3,34 1,35	65,4 66,3
3a 3b	Skim milk, indirect UHT (126°C/4s)	6,72 6,72	3,47 3,45	3,23 1,29	70,2 75,5

Table 1. Composition of three bulked raw milk samples before and on indirect UHT-processing at 126 °C for 4 s

The pH-value of samples No. 1 and 2 is between 6,77-6,82 pointing to an increased oxidative potential in both milk samples and thus to natural changes of the SH/SS- status of the proteins (19).

The high pH determined in sample No. 1a compared to No. 1b is explained by fact that this UHT- processing happened at the day of milk production but two days later to meet the demands of sample preparation in the colloid-chemical analysis (see 2.3). Within this period of time the pH of No. 1 increased in parallel with the decline of the inherent oxidative potential. In this course of events casein serves as the major radical scavenger in milk, whereas β-Lactoglobulin (β-Lg) and α-Lactalbumin (α-La) seem to be far less active (20). In agreement with it, the own results of β-Lg analysis in Tab.1 show a nearly constant rate of denaturation in all heat-processed samples and are probably more linked up with definite conditions on heat- processing.

From this point of view, samples No. 3 and No. 3b seem to be without criticism. In this instance the SHP gained about 5,3 % on UHT-processing. It is a definite indication that casein and whey protein became associated (reversible and/or irreversible) in this sample. Moreover, complexation is also verifiable for the other samples, the increase of SHP amounts to 8,5 % and 0,9 % for No. 1b and 2b, respectively. In both milk samples the participation of casein in complexation is varying. The underlying changes of the micelle status in milk are investigated and discussed below.

3.2. Research into demands on the colloid-chemical status of natural casein micelles relative to their suitability as a physico-functional food ingredient

3.2.1. The influence of oxidative stress on the colloidal-chemical status of casein and on results of thermal complexation by indirect UHT

In Figs. 1-3, the colloid-chemical profiles of three skimmed bulk milk samples (Figs.1a-3a) are compared with their profiles resulting from UHT-processing at 126 °C/ 4s (Figs.1b-3b). In the profiles two colloid-chemical criteria are compared *vs.* the pH-value, i.e., *(i)* the overall surface hydrophobicity of micelles SHP) and *(ii)* the corresponding absorbance of the protein-dye complex (O.D. at 525nm) developed with the Bio-Rad protein assay. Data of absorbance are of use for a better understanding of the natural pH-depended structural changes of micelles in milk.

Here the profiles of SHP and O.D. *vs.* pH are applied to visualize the influence of the colloidal micellar status in raw milk on the complex formation between casein and whey protein by indirect UHT-processing.

First, the profile of skimmed raw milk depicted in Fig.1a will be viewed as this milk is *(i)* is suitable to visualize a nearly perfect thermal modification (Fig.1b) and thus *(ii)* suited to explain the interpretation of an ordinary colloid-chemical profile of milk, very briefly.

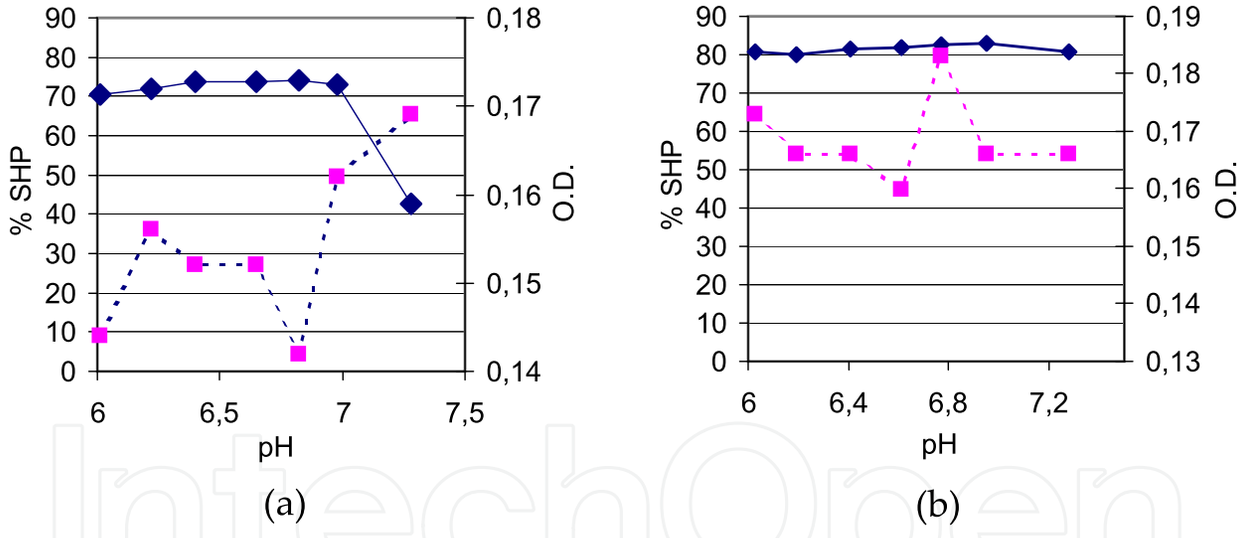

Figure 1. a: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of skimmed raw milk. Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,82. N = 3.
b: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of milk treated by indirect UHT-processing (126 °C/4s). Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,77. N = 3.

At natural pH of milk, casein micelles are very voluminous and exhibit both, a great surface area (O.D.) and a high hydrophobic potential (SHP) of the protein, too. A very hydrophilic surface layer, developed by the C-terminal glycosylated residues of κ-casein, is protecting the hydrophobic core of micelles to get collapsed in contact with other micelles. On lowering the pH, the colloidal calcium phosphate becomes increasingly dissolved. This is paralleled to

a gradual decrease in micellar surface area and thus to a more dense protein structure. In the profile of milk (Fig.1a) two minima of dye binding (O.D.) are detectable, one at natural pH and the other at pH 6,0 and both are pointing to a denser micelle structure. At pH 6,0 the total dissolution of colloidal calcium is completed whereas the dense micelle structure at the natural pH stems from a partial oxidation of micellar κ-casein at the surface *via* SH/ SS-exchange; a result of immunological reactions in the bovine mammary gland (see 3.1). At pH ≥ 6,82 the SHP decreases considerably due to the beginning of micellar disintegration with a stronger binding of calcium to individual caseins. It is paralleled with the dissociation of whole κ-casein from the micellar surface at pH 6,9. With advanced disintegration (pH > 6,9) the dye binding increases further showing intersection with the graph of SHP (Fig. 1a) This intersection is a common feature of the colloidal profiles of raw milk.

Next, the colloid-chemical profile of the heat-processed milk in Fig.1b will be viewed. On this treatment the micellar SHP is determined on very high level (≥80 %) irrespective of the pH-value. The course of O.D. indicates a maximum at natural pH whereas next to the natural pH there is a slight increase towards pH 6,0 and constant readings towards the neutral point. This means that the micellar colloidal status became static and thus without the known dynamic equilibriums between micelles in the colloidal milieu of milk, such as the equilibrium between dissolved and colloidal calcium and phosphate. It looks very much as if most of the colloidal calcium remained associated with the heat-modified micelles and this might explain the distinct white colour of this milk. The colloidal changes have positive effects on the rheological properties in both directions, in sour milk products as well in dietetic foods giving priority to a more neutral pH-value.

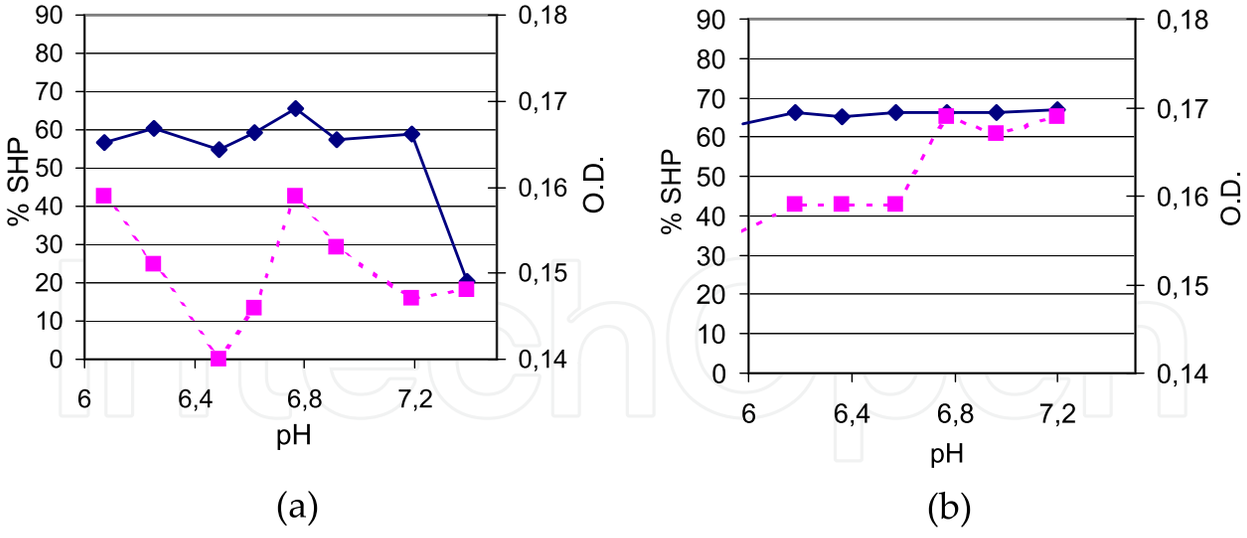

Figure 2. a: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of skimmed raw milk representing elevated oxidative stress resulting from increased incidence of herd mastitis. Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,77. N = 3.
b: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of milk treated by indirect UHT-processing (126 °C/4s) representing elevated oxidative stress resulting from increased incidence of herd mastitis. Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,77. N = 3.

In the following the profiles of samples No. 2a and 2b will be viewed. The herd milk was sampled at the time of increased udder infections. The somatic cell count (SCC) and colony forming units (CFU) are determined at $5{,}81 \cdot 10^5$ per mL and $5{,}1 \cdot 10^3$ per mL, respectively, and inherent effects on the bulked raw milk are seen in the graph of SHP in Fig. 2a. The natural pH was determined at pH 6,77. At this point a partial dissociation of κ-casein is seen, however, complete dissociation of κ-casein from the micellar surface as well as intersection of the two graphs occurred at pH 7,4 pointing to an exceptionally high oxidative potential in this milk sample. Here, the dissociation of κ-casein in two stages is put down to the fact that the herd milk was collected in one milk tank comprising both, first-grade raw milk and milk taken from infected mammary glands. In practice, all casein micelles are contributed in reducing the oxidative potential in milk on udder infections. This oxidation is the reason for poor/non renneting milk with insufficient gel matrix formation (19). In agreement with this, the micellar defects of the milk sample in Fig. 2a led to a partial dissipation of the gel matrix of Mozzarella Cheese on prolonged storage. The interpretation of micellar self-oxidation in the profiles SHP/O.D. vs. pH was very difficult (21) compared to the confirmative proof of mykotoxins or antibiotics (next chapter). Here, the presence of mykotoxins in milk was tested by a rapid immunoaffinity-based method for determination by fluorometry and antibiotics by use of the qualitative BR Test "AS".

Usually, the course of micellar dissociation is associated with a significant increase in protein-dye binding due to the occurrence of a vast number of submicellar particles. In the profile of raw milk, in Fig, 2a, the value of O.D. at pH 7,4 is even below that determined at pH 6,0. From this it can be drawn that the dissociation of casein micelles results in significant larger protein particles than in high-quality milk. The inherent change of the SH/SS-status blockades the free sulfhydryl groups in some places at the surface and within the individual micelles and thus reducing their chance to be fully involved in complexation with whey proteins by UHT *via* SH/SS-exchange as seen in Fig.2b. On UHT- processing at 126 °C/4s a constant SHP is determined between pH 6,0 and pH 7,2 amounting to ca. 66 %. It is paralleled with a continuous increase of O.D. indicating to a less stable complexation due to the scavenger function of free SH-groups of casein discussed before.

3.2.2. Influence of protein-bound antibiotics on the colloid-chemical status of casein and on inherent thermal complexation of whole milk protein

The udder health problems on herd level mentioned above were regulated by a medical treatment with antibiotics (AB). The milk samples used for the colloid-chemical analysis were collected some days after the legitimated waiting period. At that time the "PR" Test reacted still positively on AB's. The results obtained are summarized in Figs.3a and 3b.

The profile of the raw milk (Fig. 3a) shows maximum hydrophobicity at natural pH (pH 6,72) and thereafter the SHP decreases steadily coming up to total dissociation of micelles at pH 7,4. The overall graph of O.D. is on low level indicating to a denser micelle structure. The peak of dye binding at pH 7,2 has been turned out to be a distinguishing feature of protein-bound AB's in raw milk and appeared never in the profiles of high-grade milk samples.

Another group of foreign substances in milk (e.g. toxins of chemical and microbial origin) are occurring protein-bounded as well and are recognizable by their typical signs in the colloidal

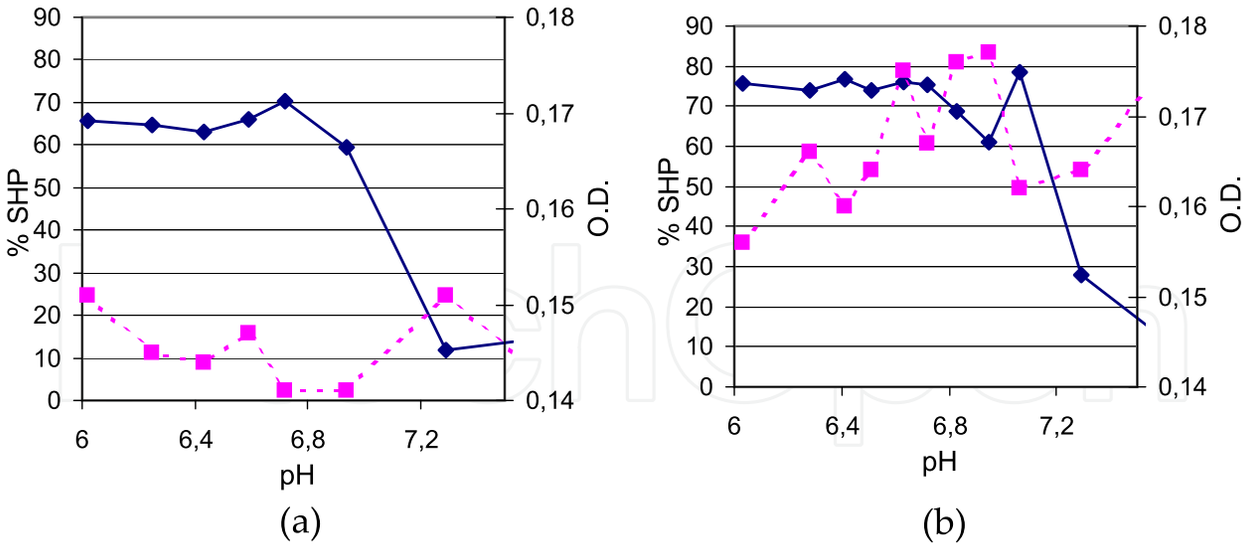

Figure 3. a: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of skimmed raw milk containing protein-bound antibiotics. Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,72. N = 3.
b: Effect pH on micellar surface hydrophobicity and dye-binding characteristics of milk treated by indirect UHT-processing (126 °C/4s) containing protein-bound antibiotics. Micellar surface hydrophobicity (SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5; orig. pH 6,77. N = 3, repeated twice

profiles, except for mykotoxins. These were taken up by the micellar surface and thus evident at each pH of analysis. Mykotoxins are competing increasingly with the non-ionic detergent for the places at the casein micelle surface to dock with on gentle warming, e.g.; on a prolonged standing inside the cuvette department of the photometer. In this case the readings of absorbance must happen right after the cuvette is placed into the photometer.

Next, the graphs of SHP and O.D. resulting from UHT-processing will be viewed (Fig.3b). Between pH 6,0 and pH 6,8 a high SHP is determined and a further peak of SHP occurred at pH 6,95 paralleled with a somewhat lower value of O.D. Generally, the graph of O.D shows a great variation *vs.* pH-value. The high readings of O.D. are indicating to a labile structure of the complexed protein on account of protein-bound AB's embedded. For this reason the complexed protein begins to dissociate at an early stage at pH 6,8 and inherent dissociation of the protein-bound AB's happens in the peak of SHP at pH 6,95. The bounding of AB`s to casein in Fig. 3b is noticeable increased compared to the raw milk (Fig. 3a).It might explain that protein-bound AB's in heated milk samples are underestimated owing to their lacking analytical availability. This event gives rise to a common misinterpretation believing that AB's in milk are degradable on heating (22).

Own investigations of evidencing protein-bound AB's immediately resulted in a qualitative rapid method derived from HPLC working with the Source 15 PHE PE 4,6/100 for separating the individual casein fractions by Hydrophobic Interaction Chromatography (HIC)(21). Whenever a casein sample with protein-bound AB's is dissolved and prepared for injection onto the column a very stable turbidity is perceptible. This turbidity has been used to indicate protein-bound AB's in milk sample. A brief description of the methodical principle is given now: an

approximate protein concentration of 7 mg/mL is dissolved in 4 M guanidine thiocyanate (GdmSCN) prepared in 0,1 M phosphate buffer, pH 7,2 (PBS) and then homogenized using the Ultra-Turrax TP 18/10 (Janke& Kunkel KG, IKA Werk Staufen; FRG). The completely dispersed protein sample is diluted to 10 mL with PBS and vortex-mixed. The turbidity is measured at 320-330 nm. The readings are compared with AB-free milk analyzed in parallel.

In theory, the raw milk sample in Fig. 3a meets important requirements of a thermal complexation of whole milk protein; in particular, there is no oxidative potential owing to the immune system in the mammary gland is turned off as long as AB's are left (23).

The increased SHP occurring between pH 6,0 and pH 6,8 (Fig. 3b) bears out this assumptions, however, the structural stability indicated left to the original pH of milk is wearing off near to the neutral point owing to protein-bound AB's in this sample.

This will be substantiated with another profile shown in Fig. 4 assigned to a liquid foodstuff (pH 7,0) produced from concentrated skimmed milk. The packaged foodstuff showed emulsion-problems on short storage and was recalled. It should be added that the qualitative BR Test "AS" turned out negatively whereas the qualitative "Turbidity"-test reacted positively on AB's.

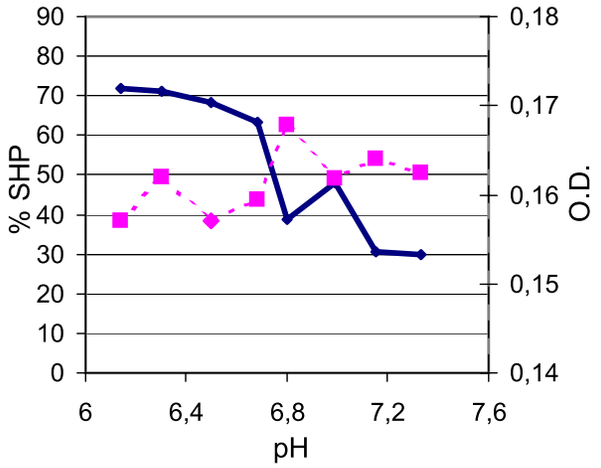

Figure 4. Effect pH on micellar surface hydrophobicity and dye-binding characteristics of milk protein as dietetic constituent in "ready-to-meal" product representing elevated emulsifying problems due to protein-bound antibiotics. Micellar surface hydrophobicity ((SHP; ♦—♦) and dye-binding (O.D.; ■---■) were determined from pH 6 to 7.5. N = 6.

4. Conclusions

Milk protein of high physico-functionality resulting from indirect heat processing at 126 °C/4s makes definite demands on the colloid-chemical status of casein micelles in raw milk. The ideal complexation between casein and whey protein in milk required that all superficial sulfhydryl-groups of the micelles were free and available to take part in SH/SS-exchange between the milk proteins. In practice, a less demanding standard is sufficient for normal commercial use. The analysis of the colloid-chemical status of casein micelles in milk can give an answer whether a thermal modification is to make good economic sense or not.

With the colloid-chemical research two reasons for an inadequate complex formation on indirect UHT-processing were turned out and, both were to trace back to bacterial infections of

the mammary gland; i.e., *(i)* the self-oxidation of micellar sulfhydryl groups in milk whereby the casein is acting as the main scavenger in reducing the natural oxidative potential in milk and *(ii)* the presence of protein-bound antibiotics used to control the pathogens of mastitis.

Effects attributable to oxidation *(i)* are negligible as far as it remained on low level. On higher oxidative stress it requires a thermal processing very soon after the milk production is finished or alternatively, the fresh raw milk is bulked separately according to quality for avoiding a further spreading of the oxidative activities in whole bulked milk. The prospects for improving the physico-functionality of casein *via* complexation are steadily decreasing on advancing oxidation. The effects of micellar self-oxidation are paralleled with an increased pH of milk what is just right to be used as criterion to make a distinction between varying raw milk qualities.

The presence of protein-bound antibiotics in milk *(ii)* is a serious problem in various respects. Here the complexation of raw milk containing protein-bound antibiotics is considered. Under these circumstances the immune reactions of the mammary gland is inactive and thus all micellar sulfhydryl-groups were available to get involved in intermolecular complexation and in addition to also the pH of milk agrees with the theory (24). The fact is however that the molecular-structural organization inside the micelles is messed up and thus only a labile complexation between casein micelles and the whey protein can be formed on indirect UHT-processing.

Author details

Bärbel Lieske
Germany

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