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Surface Hydrophobicity of Physicochemically and Enzymatically Treated Milk Proteins in Relation to Techno-functional Properties

BEATE HILLER AND PETER CHR. LORENZEN*

Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Location Kiel, Hermann-Weigmann-Strasse 1, D-24103 Kiel, Germany

Surface hydrophobicity (SH) of milk proteins treated physicochemically (by heating and Maillard reaction) or modified enzymatically (by transglutaminase, lactoperoxidase, laccase, and glucose oxidase) was assessed in relation to their techno-functional properties. Heat-treatment increased SH of whey protein isolate and decreased SH of sodium caseinate and bovine serum albumin. Maillard reaction of milk proteins caused time-depended decreases of SH. Only for total milk protein reacting with glucose and lactose elevated SH-values were detected. Protein modification with transglutaminase, laccase, and lactoperoxidase strongly increased the SH of whey protein isolate and total milk protein. Incubation with glucose oxidase elevated SH values of sodium caseinate, whey protein isolate, and total milk protein. When correlating SH with techno-functional properties, a positive correlation was observed between SH and foam formation, and a negative correlation was observed between SH and foam stability as well as emulsion stability. No clear correlation was detected between SH and emulsifying activity, surface tension, viscosity, and heat stability of enzymatically and physicochemically treated milk proteins.

KEYWORDS: Surface hydrophobicity; SDS binding method; techno-functional properties; milk protein

INTRODUCTION

Techno-functional properties of proteins such as water, fat and ligand binding (*1*), solubility (*2*), and surface properties result from a number of factors (*3*), especially protein hydrophobicity (*4*). The total hydrophobicity of proteins is given by their amino acid composition (*5*), but it disregards their secondary, tertiary, and quaternary structure (*6–8*). Thus, the effective hydrophobicity of exposed, nonpolar, cationic amino acid residues of proteins has been identified to mediate their interfacial adsorption, denaturation, and stabilization behavior (*6, 7, 9–11*).

Bigelow (*5*) calculated the total hydrophobicity of proteins from the amount of hydrophobic amino acid residues in their primary structure. Methods to assess surface hydrophobicity (SH) are based on the reaction of hydrophobic residues on the protein surface with *cis*-parinaric acid (CPA) (*9, 12*) 6-propionyl-2-(N,N-dimethylamino)-naphthalene (*1, 12*), 8-anilino-1-naphthalene sulfonate (ANS) (*6, 10, 12*), hydrophobic ligands, or emulsifier molecules (*7, 11, 13*). Keshavarz and Nakai (*7*) determined SH by hydrophobic ion exchange. However, the results of different methods are difficult to compare or characterized by a low degree of reproducibility. Moro et al. (*14*), for example, obtained a heat-induced rise in SH of whey proteins using the ANS method, whereas a diminution of SH was obtained using the CPA method.

Besides indicating techno-functional properties of proteins (*3*), SH studies were performed to characterize the influence of hydrostatic pressure (*15*), heat treatment (*12, 16, 17*), ultrafiltration (*18*), shearing (*19*), pH-value (*12, 20*), and ionic strength (*1, 10, 21, 22*). Only few studies have been undertaken to characterize the surface hydrophobicity of proteins treated physicochemically by Maillard reaction (*23–26*) or enzymatically by transglutaminase (*27–29*) lactoperoxidase (*30*), laccase, and glucose oxidase in relation to their techno-functional properties (*23, 31–33*).

For unmodified proteins, a positive correlation between SH and emulsifying properties was established, and a negative correlation was established between SH and interfacial tension (*7, 9*). No correlation was observed between overrun and SH (*34*), but a positive correlation between overrun and average hydrophobicity was found (*35*). According to Wierenga (*36*), a higher SH reduces the energy barrier for the adsorption of proteins to interfaces.

Correlations of SH and techno-functional properties were analyzed for heat-treated whey proteins (*13, 14, 37*); deamidated, reduced, and denatured soy proteins (*2*); and alkali- and acid-treated proteins (*10, 38*). Thus, a positive (*2, 10, 13, 14, 37*) or a negative correlation (*14*) between SH and interfacial properties, a positive relation between SH and solubility (*2*), as well as a

* To whom correspondence should be addressed. Telephone: +494316092264; fax: +494316092300; E-mail: peter-christian.lorenzen@bfel.de.

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positive relation between SH and gelling properties (*10, 38*), were described.

As only few studies have yet been performed to characterize the relation between SH and techno-functional properties of enzymatically and physicochemically treated proteins, the present paper gives an overview over SH values of milk proteins and their enzymatically (by transglutaminase, lactoperoxidase, laccase, and glucose oxidase) and physicochemically (by heating and Maillard reaction) derived modification products. Because no standardized method to assess SH exists, the SDS binding method was chosen to present this overview.

MATERIALS AND METHODS

Proteins. Analytical grade α-lactalbumin, β-lactoglobulin, bovine serum albumin, α_S-casein, β-casein, and κ-casein were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). α-Lactalbumin and β-lactoglobulin (isolated according to ref *39*), as well as α_S-casein and β-casein (isolated according to ref *40*), were obtained by fractionating bovine milk (Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Kiel, Germany). Sodium caseinate was donated by Fonterra New Zealand's Dairy Company (Rellingen, Germany). Whey protein isolate was purchased from MILEI GmbH (Stuttgart, Germany). Low heat skim milk powder was obtained from Nordmilch (Bremen, Germany). Lactose hydrolyzed skim milk powder was produced by spray-drying skim milk (inlet/outlet temperatures of 180/80 °C) after incubation with β-Galactosidase [EC 3.2.1.23] (Lactozym 3000 L HPG; Novozymes Denmark) for 3 h at 40 °C in an enzyme–substrate-ratio of 1/100 (Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Kiel, Germany). Micellar casein was obtained from skim milk by microfiltration (cellulose acetate membrane, average pore size: 0.1 μm) and lyophilization (Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Kiel, Germany).

Enzymes/Cofactors. Transglutaminase [EC 2.3.2.13] from Streptoverticillium sp. (enzyme activity: 100 U/g) was donated by Ajinomoto (Tokio, Japan). Lactoperoxidase [EC 1.11.1.7] from bovine milk (enzyme activity: 950 U/mg) was donated by DMV International (Veghel, Netherlands). Laccase [EC 1.10.3.2] from Myceliophthera thermophila (enzyme activity: 10 500 PCU/ml) was from Novozymes A/S (Bagsværd, Denmark). Glucoseoxidase [EC 1.1.3.4] from *Aspergillus niger* (enzyme activity: 10 000 U/g) was supplied by Novozymes A/S (Bagsværd, Denmark). Hydrogen peroxide was from Merck (Darmstadt, Germany), and chlorogenic acid was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Sugars. D-(+)-Glucose monohydrate and D-(+)-lactose monohydrate were from Merck (Darmstadt, Germany). Citrus pectin (150 000 g/mol) and microbial dextran (40,000 g/mol) were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

Further Material. All further chemicals were analytical grade and were purchased from Merck (Darmstadt, Germany). Demineralized water was prepared with the Ultra Clear Water System (Barsbuettel, Germany). Dialysis membranes (cellulose acetate, molar mass cut of 12 000 g/mol) were from Medicell (London, United Kingdom).

Determination of Protein SH. The SH of milk proteins was determined using the SDS binding method (*41*) with slight modifications. Sodium dihydrogen phosphate dihydrate buffer (0.02 mol/L, pH 6.0) was prepared in demineralized water. Proteins were soluted in sodium dihydrogene phosphate buffer (w/v = 1.0 g/L). SDS reagent was prepared in sodium dihydrogen phosphate buffer (w/v = 40.37 mg/L). Methylene blue was dissolved in sodium dihydrogen phosphate buffer (w/v = 24.0 mg/L). Protein solution and SDS reagent were mixed (v/v = 1/2) and incubated for 30 min at 20 °C. Subsequently, the SDS−protein solution was dialyzed against sodium dihydrogen phosphate buffer (v/v = 1/25) for 24 h at 20 °C. Mixtures of 0.5 mL of dialysate, 2.5 mL of methylene blue, and 10.0 mL of chloroform were centrifuged at 2500*g* for 5 min (Labofuge M, rotor 2150; Heraeus Sepatech GmbH, Osterode, Germany). The extinction of the chloroform phase was assessed spectro-photometricly at a wavelength of $\lambda = 655$ nm (spectrophotometer Libra S12, Biochrom, Bremen, Germany).

Table 1. Conditions of the Incubation of Milk Proteins with Transglutaminase (TG), Lactoperoxidase (LPO), Laccase (PPO) and Glucose Oxidase (GOX)[a]

	reaction conditions				
	U/mg milk protein	pH	reaction temperature (°C)	reaction time (h)	cofactor (amount/mg milk protein)
SC + TG	0.050	6.7	6	16	
WPI + TG	0.050	7.0	6	16	
TMP + TG	0.050	7.0	40	4	
SC + LPO	47.500	6.8	40	16	1.80 μmol H_2O_2
WPI + LPO	9.500	7.2	50	1	18.00 μmol H_2O_2
TMP + LPO	9.500	7.0	50	1	1.80 μmol H_2O_2
SC + PPO	0.011	6.8	40	16	0.02 μmol CA
WPI + PPO	0.011	7.2	40	1	0.08 μmol CA
TMP + PPO	0.011	7.0	40	16	0.08 μmol CA
SC + GOX	0.500	6.8	40	16	
WPI + GOX	0.500	7.2	40	16	
TMP + GOX	0.010	7.0	20	16	

[a] SC, sodium caseinate; WPI, whey protein isolate; TMP, total milk protein in skim milk powder; CA, chlorogenic acid.

Chloroform served as solvent blank. A calibration curve was created for 0−10 μg SDS. The linear regression equation of the calibration curve was mathematically transformed and was used to calculate protein bound SDS according to the following equation: Surface hydrophobicity (μg SDS/500 μg protein) = 62.3052 × Extinction ($\lambda = 655$ nm) − 0.8349. Determinations were performed in triplicate. The coefficient of variation in this study differed between 1.2 and 5.4%.

SH of Heat Treated Milk Protein. Solutions of sodium caseinate, micellar casein, whey protein isolate, and bovine serum albumin in sodium dihydrogen phosphate buffer (w/v = 0.1% protein) were heated from 20 to 80 °C, increasing the temperature by 1 °C/min. Every 10 min, aliquots were withdrawn, rapidly cooled to room temperature, and analyzed for SH as described above.

SH of Maillard Reaction Products of Milk Protein. Powder mixtures or lyophilized solutions of sodium caseinate or whey protein isolate with glucose, lactose, pectin, or dextran (w/w = 1/2) underwent Maillard reaction at 70 °C and 65% relative humidity for 0–240 h.

Under the same time/temperature/humidity conditions, Maillard reaction products of lactose-hydrolyzed skim milk powder, skim milk powder, skim milk powder/pectin mixtures (w/w = 1/2), and skim milk powder/dextran mixtures (w/w = 1/2) were produced. Samples were withdrawn after several heating times and were analyzed for SH as described above.

SH of Enzymatically Treated Milk Protein. Sodium caseinate, whey protein isolate, and total milk protein in skim milk powder (w/w = 5% protein) were incubated with transglutaminase, lactoperoxidase, laccase, or glucose oxidase in demineralized water under the reaction conditions given in **Table 1**. After enzymatic modification, protein samples were diluted with sodium dihydrogen phosphate buffer to a final protein concentration of w/w = 0.1% and then analyzed for SH as described above.

SH of Milk Protein in Relation to Techno-functional Properties. Techno-functional properties of milk proteins were plotted against their SH and regressed. Single techno-functional properties were determined as follows:

Heat stability was assessed as the time span needed for visibly denaturing milk proteins at 140 °C. Surface tension of aqueous protein solutions (w/w = 1% protein) was determined with a tensiometer (Krüss, Hamburg, Germany) by drop shape analysis. Emulsifying properties were assessed as described by Pearce and Kinsella (*42*). Emulsion activity was expressed as the Emulsion Activity Index (EAI) of freshly prepared emulsions. Emulsion stability was defined as the EAI of emulsions after storage (16 h and 20 °C) and centrifugation (10 min, 4000*g*, and 15 °C). Foaming properties were assessed by introducing air into aqueous protein solutions (w/w = 1% protein) by an Aero-Latte stirrer (5 min, 1000 turns/min, and 40 °C). Foam formation was expressed as overrun in percent and foam stability as the time span until half of the foam volume elapsed. Viscosity was

Table 2. Surface Hydrophobicity[a] (SH) of Milk Proteins

protein source	composition	SH (μg SDS/500μg protein) mean ± SD	Nötzold et al. (1991)	Kato et al. (1984)
α_S-casein (AG)	70% α_S-casein	2.78 ± 0.11		
β-casein (AG)	90% β-casein	2.16 ± 0.10	1.0 ± 0.3	
κ-casein (AG)	80% κ-casein	2.90 ± 0.12		2.9
α-lactalbumin (AG)	85% α-lactalbumin	3.50 ± 0.10		
β-lactoglobulin (AG)	90% β-lactoglobulin	4.74 ± 0.10	5.9 ± 0.3	6.00
bovine serum albumin (AG)	98% serum albumin	12.75 ± 0.15	13.5 ± 0.4	9.70
sodium caseinate (IP)	44% α_S-casein, 31% β-casein, 14% κ-casein	2.65 ± 0.12		
whey protein isolate (IP)	35% α-lactalbumin, 51% β-lactoglobulin	3.65 ± 0.10		
total milk protein (IP)	33% α_S-casein, 24% β-casein, 8% κ-casein, 7% α-lactalbumin 12% β-lactoglobulin,	1.60 ± 0.08		
α_S-casein (isolated according to LeMagnen and Maugas 1991) (PFEP)	45% α_S-casein, 34% β-casein	2.34 ± 0.07		
β-casein (isolated according to LeMagnen and Maugas 1991) (PFEP)	79% β-casein, 3% α_S-casein	2.16 ± 0.10		
α-lactalbumin (isolated according to Maubois et al. 2001) (PFEP)	24% α-lactalbumin, 54% β-lactoglobulin	5.71 ± 0.25		
β-lactoglobulin (isolated according to Maubois et al. 2001) (PFEP)	98% β-lactoglobulin, 2% α-lactalbumin	6.02 ± 0.20		

[a] Mean values ± standard deviations of at least triplicate determinations. AG, analytical grade; IP, industrial product; PFEP, protein fraction enriched product.

measured by shearing protein solutions (w/w = 5% protein) between 0 and 1000 turns/min at 20 °C with a rotation viscosimeter (UDS 200, Anton Paar GmbH, Ostfildern, Germany).

Statistical Analysis. SH values and techno-functional properties of milk proteins were determined at least in triplicate. Mean values and standard deviations are given.

The correlation between the SH of milk proteins and techno-functional properties was assessed by linear regression analysis. The linear regression equation $y = ax + b$ as well as the coefficient of determination R^2 were calculated and are given in the figure captions. SH was chosen as the independent variable x, single techno-functional properties were taken as the dependent variable y. Variable a as the slope of the linear regression equation was chosen to characterize the kind of correlation (negative correlation or positive correlation) as well as the strength of correlation between SH and techno-functional properties.

RESULTS AND DISCUSSION

Determination of Protein SH. The SH of milk proteins was determined according to Kato et al. (*41*) with slight modifications. Anionic SDS molecules were bound to solvent exposed, hydrophobic amino acid residues and quantified spectrophotometricly. The resulting SH values of proteins are listed in **Table 2** in comparison to values Nötzold et al. (*43*) and Kato et al. (*41*) obtained with the same method. In agreement with Nötzold et al. (*43*) and Kato et al. (*41*), low SH values were observed for caseins and total milk protein, whereas comparably higher values were obtained for whey proteins (**Table 2**).

Regarding whey proteins, the highest SH values are displayed for bovine serum albumin (**Table 2**), in agreement with Konieczny and Uchmann (*4*), Alizadeh-Pasdar and Li-Chan (*12*), and Cardamone and Puri (*6*). Moro et al. (*14*) and Cardamone and Puri (*6*) reported that high SH values of serum albumin as well as β-lactoglobulin correspond to their biological function of transporting hydrophobic molecules bound to their surface. Minor SH values were obtained for α-lactalbumin (**Table 2**) and reflect its compact molecular structure (*44*). Wagner and Guéguen (*8*) also characterized globular, tightly packed protein molecules by a low SH.

Low SH values of caseins obviously conflict with the random coil, open-chain structure of casein molecules and their hydrophobic behavior (*44, 45*). Low SH values possibly reflect strongly hydrophobic interactions of casein monomers (*8, 43*), thus hiding hydrophobic amino acid residues from being assessed by the SDS binding method.

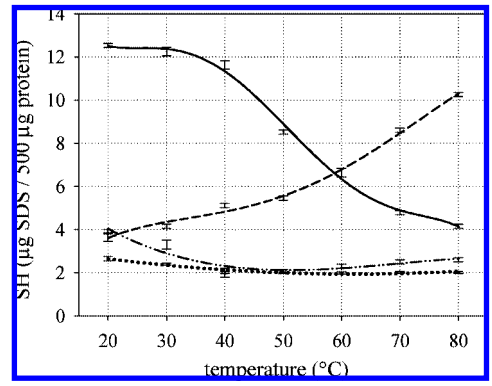

Figure 1. Surface hydrophobicity (SH, mean values ± standard deviations of at least triplicate determinations) of bovine serum albumin (—), sodium caseinate (· · · ·), micellar casein (— · · — · ·) and whey protein isolate (— — —) as affected by heat treatment (heating protein (w/w = 0.1% milk protein in sodium dihydrogene phosphate buffer) from 20 to 80 °C by +1 °C/min).

The SH values of milk protein preparations consisting of more than one protein monomer fraction correspond to their composition of different monomers (**Table 2**). This is to be seen for sodium caseinate, whey protein isolate, skim milk protein, and protein enriched fractions isolated according to Maubois et al. (*39*) or LeMagnen and Maugas (*40*) (**Table 2**). For instance, lower SH was obtained for analytical grade α-lactalbumin than for an α-lactalbumin enriched product (isolated according to ref *39*). Although analytical grade α-lactalbumin consisted of 85% α-lactalbumin, the α-lactalbumin enriched product (isolated according to ref *39*) consisted of 24% α-lactalbumin and of 54% of the more hydrophobic β-lactoglobulin (**Table 2**).

SH of Heat Treated Milk Protein. Whey protein isolate, bovine serum albumin, sodium caseinate, and micellar casein were analyzed for heat-induced changes in SH as diagramed in **Figure 1**.

Figure 1 shows that the SH of whey protein isolate increased by a factor of 3 during heating in the temperature range from 20 to 80 °C. Euston et al. (*46*), Sava et al. (*47*), Kim et al. (*37*), Bonomi and Iametti (*17*), and Belloque and Smith (*19*) explained a rise in SH with a heat-induced unfolding/denaturation of the tertiary and secondary structure of whey proteins, especially their α-helical regions (*46*). Thus, hydrophobic amino acid residues are exposed. When heating whey proteins, Moro et al. (*14*) and Kim et al. (*37*) observed a rise in SH, surface activity, and molecular flexibility, as well as a diminution of the interfacial area each protein molecule covered. According to Konrad and Lieske (*48*), heated and more surface-hydrophobic whey proteins form highly stable foam lamellae and surface films as compared to untreated whey proteins. Associated with increased SH (**Figure 1**), Konrad and Lieske (*48*) and Lorenzen (*3*) reported improved techno-functional properties of whey protein heated under predenaturing conditions.

Figure 1 shows a higher increase in SH of whey proteins in the temperature range from 55 to 80 °C than in the range from 20 to 55 °C. According to Belloque and Smith (*19*), these different denaturation kinetics are attributable to a reversible denaturation of whey protein molecules at temperatures below 55 °C and, according to Moro et al. (*14*) and Konrad and Lieske (*48*), to an irreversible denaturation of whey proteins at temperatures above 60 °C.

Figure 1 also displays that the SH of bovine serum albumin decreased by a factor of 3 upon heating from 20 to 80 °C. Kato et al. (*41*) found out that the SH of serum albumin particularly decreased at temperatures of 40−70 °C. This decline was explained by a heat-induced loss of *cis*-parinaric acid binding sites in the heat-sensitive serum albumin structure (*14*). Laligant et al. (*49*) discussed that heat-induced protein unfolding caused protein aggregation through hydrophobic interactions, thus decreasing SH.

Figure 1 demonstrates that the SH of casein was less affected by heat treatment than that observed for whey proteins. The SH of sodium caseinate slightly decreased upon heating from 20 to 80 °C (**Figure 1**). The findings correspond to the random coil, open-chain structure of casein molecules undergoing no major modification in this temperature range. Fundamental dephosphorylation, fragmentation, elimination, and aggregation reactions of caseins were reported to set in at heating temperatures above 120 °C (*44*).

The SH of micellar casein declined upon heating from 20 to 50 °C and subsequently increased upon heating from 50 to 80 °C (**Figure 1**). A rise in SH due to heating micellar casein from 50 to 80 °C corresponds to a release of protein monomers from casein micelles, a less dense casein micelle structure, and a dissociation of κ-casein molecules (*50*). In contrast, Yuksel and Erdem (*16*) observed a reorganization of casein micelles with increasing temperature, forming more compact aggregates.

SH of Maillard Reaction Products of Milk Protein. Sodium caseinate, whey protein isolate, and total milk protein were modified with glucose, lactose, pectin, and dextran through Maillard reaction and were analyzed for changes in SH (**Table 3**). **Table 3** shows that the SH of sodium caseinate decreased upon heating in the presence of sugars with increasing reaction time. This decrease in SH relates to the Maillard reaction between hydrophilic sugar molecules and hydrophobic amino acid residues mainly on the protein surface (*51, 52*) and on the formation of heterogeneous protein/saccharide- and protein/protein-cross-linked polymers and aggregates (*26, 53*).

A decrease in SH upon Maillard reaction with pectin and dextran was also detected for whey protein isolate and total milk protein (**Table 3**). Concerning a Maillard reaction with glucose and lactose, a rise in SH was detected for total milk protein, and the SH values of whey protein isolate first decreased and again increased with incubation time (**Table 3**). Rises in SH resulted from temperature-induced protein unfolding as well as protein denaturation/unfolding upon conjugation with sugar molecules as described by Wooster and Augustin (*54*) for whey protein/dextran conjugates. Van Teeffelen et al. (*55*) observed a diminution of SH of β-lactoglobulin through Maillard reaction with glucose, and Handa and Kuroda (*33*) observed that of egg white protein with glucose. In relation to unmodified proteins,

Table 3. Surface Hydrophobicity[a] (SH) of Milk Proteins and Their Maillard Reaction Products[b]

Maillard reaction with	reaction time (h)	SH (μg SDS/500 μg protein). SC	WPI	TMP
		2.65 ± 0.05	3.65 ± 0.20	1.60 ± 0.06
glucose	0	2.72 ± 0.18	6.22 ± 0.16	3.04 ± 0.20
	1	2.81 ± 0.07	3.84 ± 0.25	3.62 ± 0.17
	4	1.86 ± 0.13	4.40 ± 0.21	4.01 ± 0.16
lactose	0	4.85 ± 0.38	6.61 ± 0.06	1.60 ± 0.11
	1			3.25 ± 0.22
	4			5.55 ± 0.35
	24	2.53 ± 0.25	4.45 ± 0.25	
	96	1.70 ± 0.09	5.86 ± 0.24	
pectin	0	6.34 ± 0.25	8.21 ± 0.35	7.65 ± 0.29
	1	2.23 ± 0.11		
	4	1.00 ± 0.04	5.02 ± 0.05	2.03 ± 0.07
	24		2.90 ± 0.11	0.83 ± 0.06
dextran	0	3.87 ± 0.11	5.28 ± 0.23	4.89 ± 0.25
	5			2.55 ± 0.19
	24	1.92 ± 0.09		0.80 ± 0.07
	120	1.84 ± 0.13		
	240		5.18 ± 0.15	

[a] Mean values ± standard deviations of at least triplicate determinations. [b] Maillard reaction of protein-carbohydrate-powder-mixtures (protein−carbohydrate-ratio: w/w = 1/2) at 70 °C, 65% r. h., 0–240 h. SC, sodium caseinate; WPI, whey protein isolate; TMP, total milk protein.

Table 4. Surface hydrophobicity[a] (SH) of enzymatically treated milk proteins[b]

enzymatic treatment	SH (μg SDS/500 μg protein) SC	WPI	TMP
	2.65 ± 0.05	3.65 ± 0.20	1.60 ± 0.11
transglutaminase	2.71 ± 0.21[c]	6.59 ± 0.18[d]	1.91 ± 0.11[e]
lactoperoxidase	2.61 ± 0.15[f]	4.77 ± 0.14[g]	2.75 ± 0.20[h]
laccase	2.55 ± 0.13[i]	6.27 ± 0.05[j]	4.20 ± 0.12[k]
glucose oxidase	4.21 ± 0.27[l]	6.41 ± 0.26[m]	4.01 ± 0.21[n]

[a] Mean values ± standard deviations of at least triplicate determinations. [b] SC, sodium caseinate; WPI, whey protein isolate; TMP, total milk protein in skim milk powder. [c] 0.05 U of transglutaminase/mg milk protein, 6 °C, 16 h, pH 6.7. [d] 0.05 U of transglutaminase/mg milk protein, 6 °C, 16 h, pH 7.0. [e] 0.05 U of transglutaminase/mg milk protein, 40 °C, 4 h, pH 7.0. [f] 47.5 U of lactoperoxidase/mg milk protein and 1.8 μmol H_2O_2, 40 °C, 16 h, pH 6.8. [g] 9.5 U of lactoperoxidase/mg milk protein and 18.0 μmol H_2O_2, 40 °C, 1 h, pH 7.2. [h] 9.5 U of lactoperoxidase/mg milk protein and 1.8 μmol H_2O_2, 40 °C, 1 h, pH 7.0. [i] 0.011 U of laccase/mg milk protein and 0.02 μmol chlorogenic acid, 40 °C, 16 h, pH 6.8. [j] 0.011 U of laccase/mg milk protein and 0.08 μmol chlorogenic acid, 40 °C, 1 h, pH 7.2. [k] 0.011 U of laccase/mg milk protein and 0.08 μmol chlorogenic acid, 40 °C, 16 h, pH 7.0. [l] 0.5 U of glucose oxidase/mg milk protein, 40 °C, 16 h, pH 6.8. [m] 0.5 U of glucose oxidase/mg milk protein, 40 °C, 16 h, pH 7.2. [n] 0.01 U of glucose oxidase/mg milk protein, 40 °C, 16 h, pH 7.0.

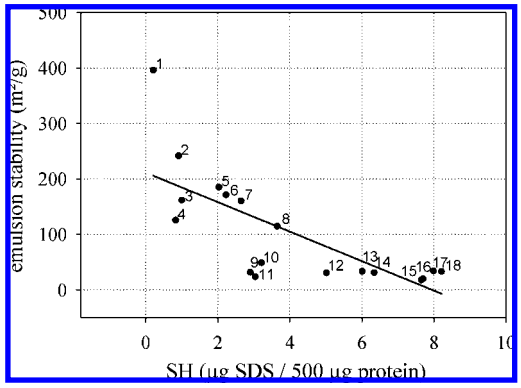

Figure 2. Correlation of surface hydrophobicity (SH) and emulsion stability as assessed for sodium caseinate, whey protein isolate, and total milk protein and their Maillard reaction products with pectin (protein/carbohydrate-ratio: w/w = 1/2); linear regression equation: $y = -26.6x + 211.4$; $R^2 = 0.75$. (1) lyophilized sodium caseinate/pectin-solution (70 °C, 65% relative humidity, 1 h), (2) lyophilized total milk protein/pectin-solution (70 °C, 65% relative humidity, 5 h), (3) sodium caseinate/pectin-powder-mixture (70 °C, 65% relative humidity, 4 h), (4) total milk protein/pectin-powder-mixture (70 °C, 65% relative humidity, 24 h), (5) total milk protein/pectin-powder-mixture (70 °C, 65% relative humidity, 5 h), (6) sodium caseinate/pectin-powder-mixture (70 °C, 65% relative humidity, 1 h) (7) sodium caseinate (70 °C, 65% relative humidity, 0 h), (8) whey protein (70 °C, 65% relative humidity, 0 h), (9) whey protein/pectin-powder-mixture (70 °C, 65% relative humidity, 24 h), (10) lyophilized whey protein/pectin-solution (70 °C, 65% relative humidity, 5 h), (11) total milk protein (70 °C, 65% relative humidity, 0 h), (12) whey protein/pectin-powder-mixture (70 °C, 65% relative humidity, 5 h), (13) lyophilized sodium caseinate/pectin-solution (70 °C, 65% relative humidity, 0 h), (14) sodium caseinate/pectin-powder-mixture (70 °C, 65% relative humidity, 0 h), (15) total milk protein/pectin-powder-mixture (70 °C, 65% relative humidity, 0 h), (16) lyophilized total milk protein/pectin-solution (70 °C, 65% relative humidity, 0 h), (17) lyophilized whey protein/pectin-solution (70 °C, 65% relative humidity, 0 h), (18) whey protein/pectin-powder-mixture (70 °C, 65% relative humidity, 0 h).

Bouhallab et al. (*23*) characterized β-lactoglobulin/lactose-complexes by a high SH, and Pan et al. (*24*) characterized casein/dextran-complexes by a high total hydrophobicity.

SH of Enzymatically Treated Milk Protein. Sodium caseinate, whey protein isolate, and total milk protein were incubated with transglutaminase, lactoperoxidase, laccase, and glucose oxidase and were analyzed for changes in SH (**Table 4**). **Table 4** shows that the SH of sodium caseinate remained unaffected by incubation with transglutaminase, lactoperoxidase, and laccase, whereas glucose oxidase activity increased the SH of sodium caseinate by a factor of 1.6. Regarding whey protein isolate and total milk protein, **Table 4** elucidates that the SH of these proteins was increased by any enzymatic treatment by a factor of up to 2.6.

Former investigations revealed that protein oligomers and polymers formed upon incubation of milk proteins with transglutaminase, lactoperoxidase, laccase, or glucose oxidase (results not shown). Thus, the degree of cross-linking as well as the tertiary structure of protein polymers and oligomers determines their SH. The SH of transglutaminase modified products results from the formation of ϵ-(γ-glutamyl-)-lysine cross-linked protein polymers (*56*) characterized by large, compact structures with a high degree of cross-linking (*57*). Upon incubation with transglutaminase, a diminution of SH was reported for sodium caseinate (*27*), soy, and wheat protein (*28, 29*), and an increase was observed for soy protein films (*57, 58*).

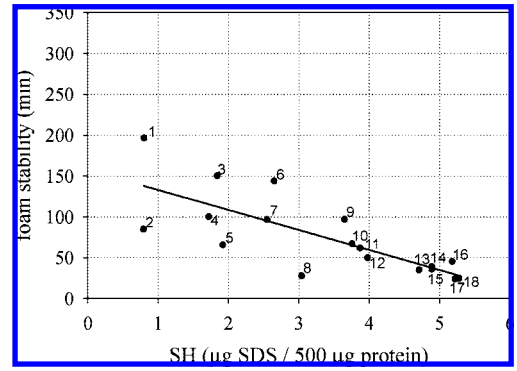

Figure 3. Correlation of surface hydrophobicity (SH) and foam stability as assessed for sodium caseinate, whey protein isolate, and total milk protein and their Maillard reaction products with dextran (protein/carbohydrate-ratio: w/w = 1/2); linear regression equation: $y = -24.5x + 157.5$; $R^2 = 0.71$. (1) total milk protein/dextran-powder-mixture (70 °C, 65% relative humidity, 24 h), (2) lyophilized total milk protein/dextran-solution (70 °C, 65% relative humidity, 5 h), (3) sodium caseinate/dextran-powder-mixture (70 °C, 65% relative humidity, 120 h), (4) lyophilized sodium caseinate/dextran-solution (70 °C, 65% relative humidity, 24 h), (5) sodium caseinate/dextran-powder-mixture (70 °C, 65% relative humidity, 24 h), (6) sodium caseinate (70 °C, 65% relative humidity, 0 h), (7) total milk protein/dextran-powder-mixture (70 °C, 65% relative humidity, 5 h), (8) total milk protein (70 °C, 65% relative humidity, 0 h), (9) whey protein (70 °C, 65% relative humidity, 0 h), (10) lyophilized sodium caseinate/dextran-solution (70 °C, 65% relative humidity, 0 h), (11) sodium caseinate/dextran-powder-mixture (70 °C, 65% relative humidity, 0 h), (12) lyophilized whey protein/dextran-solution (70 °C, 65% relative humidity, 48 h), (13) lyophilized total milk protein/dextran-solution (70 °C, 65% relative humidity, 0 h), (14) total milk protein/dextran-powder-mixture (70 °C, 65% relative humidity, 1 h), (15) total milk protein/dextran-powder-mixture (70 °C, 65% relative humidity, 0 h), (16) whey protein/dextran-powder-mixture (70 °C, 65% relative humidity, 240 h), (17) lyophilized whey protein/dextran-solution (70 °C, 65% relative humidity, 0 h), and (18) whey protein/dextran-powder-mixture (70 °C, 65% relative humidity, 0 h).

Oxidases have been described to cross-link protein by oxidation of aromatic and sulfur-containing amino acid residues and spontaneous isopeptide bonding of oxidized protein groups (*59, 60*) The reaction products were characterized as heterogeneous oligomers and polymers of extended structure (*59*). According to Davies and Delsignore (*61*), protein oxidation of bovine serum albumin causes an unfolding of the secondary and tertiary protein structure and a rise in hydrophobicity. Hydrophobic amino acid residues originally oriented toward the interior of the protein molecule are exposed through oxidation. Hirano et al. (*30*) discussed a lactoperoxidase-induced rise in protein hydrophobicity as the cause for the formation of softer yogurt gels made from lactoperoxidase treated milk.

Correlation of SH with Techno-functional Properties. The SH of physicochemically and enzymatically treated milk proteins was plotted against their techno-functional properties. Linear regression analysis was applied to characterize the kind of correlation (negative correlation or positive correlation) as well as the strength of correlation between SH and techno-functional properties by the slope of the linear regression equation and by the coefficient of determination R^2.

No correlation was observed between SH and heat stability, viscosity, emulsifying activity, and surface tension of the analyzed proteins (results not shown). A negative correlation was observed between the SH of Maillard products and emulsion stability (**Figure 2**) as well as foam stability (**Figure 3**). As diagramed by the linear regressions plots, linear regression

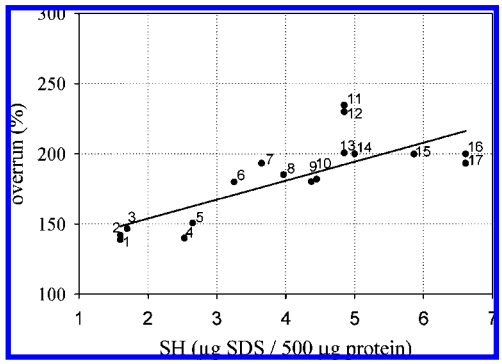

Figure 4. Correlation of surface hydrophobicity (SH) and overrun as assessed for sodium caseinate, whey protein isolate, and total milk protein and their Maillard reaction products with lactose (protein/carbohydrate-ratio: w/w = 1/2); linear regression equation: $y = 13.5x + 126.8$; $R^2 = 0.85$, (1) total milk protein in skim milk powder (70 °C, 65% relative humidity, 4 h), (2) total milk protein in skim milk powder (70 °C, 65% relative humidity, 0 h), (3) sodium caseinate/lactose-powder-mixture (70 °C, 65% relative humidity, 96 h), (4) sodium caseinate/lactose-powder-mixture (70 °C, 65% relative humidity, 24 h), (5) sodium caseinate (70 °C, 65% relative humidity, 0 h), (6) total milk protein in skim milk powder (70 °C, 65% relative humidity, 1 h), (7) whey protein (70 °C, 65% relative humidity, 0 h), (8) lyophilized whey protein/lactose-solution (70 °C, 65% relative humidity, 1 h), (9) lyophilized sodium caseinate/lactose-solution (70 °C, 65% relative humidity, 1 h), (10) whey protein/lactose-powder-mixture (70 °C, 65% relative humidity, 96 h), (11) sodium caseinate/lactose-powder-mixture (70 °C, 65% relative humidity, 0 h), (12) lyophilized sodium caseinate/lactose-solution (70 °C, 65% relative humidity, 0 h), (13) lyophilized whey protein/lactose-solution (70 °C, 65% relative humidity, 4 h), (14) sodium caseinate/lactose-powder-mixture (70 °C, 65% relative humidity, 4 h), (15) whey protein/lactose-powder-mixture (70 °C, 65% relative humidity, 24 h), (16) whey protein/lactose-powder-mixture (70 °C, 65% relative humidity, 0 h), (17) lyophilized whey protein/lactose-solution (70 °C, 65% relative humidity, 0 h).

equations reveal that a Maillard reaction-induced decrease in SH by 1 μg SDS/500 μg milk protein causes a rise in emulsion stability by 26.6 m^2/g (**Figure 2**) and in foam stability by 24.5 min (**Figure 3**). Enhanced emulsion and foam stability correspond to the more hydrophilic, water-binding behavior of Maillard reaction products due to a modification of hydrophobic amino acid residues on the protein surface with hydrophilic carbohydrate molecules (*51, 52*). **Figure 4** displays a positive correlation between SH and overrun of protein/lactose-Maillard-products as an increase of SH by 1 μg SDS/500 μg milk protein effected overrun by +13.5%. This result agrees with the observation of Wierenga (*36*) that hydrophobic amino acid residues on the molecule surface accelerate the interfacial adsorption behavior of proteins.

Regarding literature, only a few studies have been undertaken to characterize the correlation between SH and techno-functional properties of enzymatically and Maillard reaction-treated proteins. Whereas for unmodified proteins (such as bovine serum albumin, β-lactoglobulin, and κ-casein) a positive correlation of SH and emulsifying properties and a negative correlation between SH and interfacial tension was established (*7, 9*), the results obtained in this study relate to changes in protein structure as induced by physicochemical modification.

Bouhallab et al. (*23*) and Morgan et al. (*31*) observed a positive relationship between SH and protein solubility and hydration properties of β-lactoglobulin after Maillard reaction with lactose. Concerning casein macropeptide and lactose as Maillard reaction substrates, Moreno et al. (*32*) observed a negative association of SH and solubility and a positive connection between SH and emulsifying properties. According to Handa et al. (*26*), less hydrophobic egg white protein performed better gelation properties after Maillard reaction with sucrose. Whey proteins heated without the addition of saccharides were described by a positive (*13, 14, 37*) or a negative correlation (*14*) between SH and interfacial properties.

To conclude, the paper presents an overview over SH values of physicochemically or enzymatically treated milk proteins in relation to their techno-functional properties. Heat-treatment increased SH of whey protein isolate and decreased SH of sodium caseinate and bovine serum albumin. Maillard reaction of milk proteins essentially caused time-dependent decreases of SH, and increased SH values were detected after enzymatic cross-linking of milk proteins. When correlating SH with techno-functional properties, a positive correlation was observed between SH and foam formation, and a negative correlation was observed between SH and foam stability as well as emulsion stability. No correlation was detected between SH and emulsifying activity, surface tension, viscosity, and heat stability of enzymatically and physicochemically treated milk proteins. Discrepancies between obtained results (**Figures 2−4**) and reported data result from the fact that only a few studies have yet been performed to characterize the relation between SH and techno-functional properties of enzymatically and physicochemically treated proteins using different analytical methods. Regarding the analyzed, heterogeneous protein products in this study, it has to be taken into account that protein aggregates and polymers of high molar mass were produced, which, for example, were described to perform lower emulsifying activity but higher emulsion stability (*56*).

The results demonstrate a correspondence of SH and protein structure, interactions, and certain techno-functional properties which may be of interest for creating novel food ingredients by physicochemical and enzymatic protein modification.

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