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Determination of a molecular fouling model for the micro- and ultrafiltration of whey: A recombination study from single whey proteins to complex mixtures

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ABSTRACT

Since there has been a lack of knowledge concerning the molecular understanding of the specific fouling mechanism during deposit layer structure formation, in this study membrane fouling during the filtration of single whey protein fractions was investigated at various pH and ionic strength. During the filtration of both individual whey proteins and whey protein mixtures, membrane fouling was found to be strongly correlated to repulsive inter-particle forces and pH-dependent protein cross-linking reactions. A disproportionate decrease of the specific fouling resistance from pH 6.4 to pH 7.5 was observed when proteins with a free thiol group were present. At pH 3, α-lactalbumin exists in apo-form, which may cause an increased fouling resistance at acidic pH. Based on the insights gained from this study, membrane fouling during the filtration of whey protein mixtures comprising different native whey proteins in either acid or rennet whey can be explained.

1. Introduction

In dairies producing rennet cheese or acid curd cheese, sweet and acid whey, respectively, are produced as the aqueous phase from the protein aggregation procedure. Sweet and acid whey are often blended prior to whey protein concentration by means of membrane filtration. This yields a fundamentally different filtration behaviour in comparison with separate processing of the two whey types. To understand protein fouling on membrane surfaces on a molecular level, fundamental knowledge of the molecular mechanism responsible for fouling is required. Thus far, there is a lack of knowledge on the cause and mechanistic explanations regarding the fouling process and the resulting structure of the deposited protein layer, occurring during the filtration of sweet and acid whey.

Some authors identified the molecular fouling mechanism in single component systems of certain whey proteins with sulphydryl groups. It was concluded that fouling was facilitated when electrostatic repulsion was weak and that thiol–disulphide reactions were involved in membrane fouling (Kelly, Senyo Opong, & Zydney, 1993; Kelly & Zydney, 1995, 1997; Steinhauer, Marx, Bogendörfer, & Kulozik, 2015b). However, these studies did not take into account interactions of different whey proteins in mixtures, as they occur during whey ultrafiltration (UF). For bovine serum albumin (BSA) it was found that the flux decreased as pH was shifted towards the isoelectric point (pI) (Casa, de la Guadix, Ibáñez, Camacho, & Guadix, 2008; Chan & Chen, 2001; Fane, Fell, & Suki, 1983; Ghosh & Cui, 1998). A similar observation was made by Palecek and Zydney (1994) who carried out UF with a variety of model proteins including the whey proteins bovine serum albumin (BSA) and immunoglobulins (Ig). For β-lactoglobulin (β-Lg), Ibáñez, Almécija, Guadix, and Guadix (2007) and Marshall, Munro, and Trägårdh (2003) determined an increasing fouling resistance at higher ionic strengths. Marshall et al. (2003) postulated the possibility of calcium crosslinking followed by a subsequent gelation of the protein on the membrane surface. In contrast, Ibáñez et al. (2007) concluded that membrane fouling at increased salt concentrations (in this case NaCl) was also facilitated, due to the decreased effective range of electrostatic repulsion. Studies on the thermal gelation of β-Lg (Barbut & Foegeding, 1993; Xiong, Dawson, & Wan, 1993) showed that the gelation of whey proteins was enhanced in presence of calcium ions even at ambient temperature. In conclusion, it can be assumed that calcium bridging and reduced repulsive forces due to an increased ionic strength enhance the deposit layer formation.

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Besides single component systems, there are studies dealing with the interaction of proteins during deposit formation of complex systems. Van den Berg and Smolders (1989) carried out filtration experiments at pH 7.4 with mixtures of BSA (pI 4.7) and lysozyme (pI 11.8) or α-lactalbumin (α-La, pI 4.3). At this filtration pH, BSA as well as α-La are negatively charged, while lysozyme has a positive net charge. For filtrations with mixtures of BSA and α-La, a higher flux level was observed as compared with filtrations with BSA and lysozyme. The lower flux level for proteins with opposite net charge was attributed to electrostatic attraction and, therefore, a higher deposition probability. Only a few studies were published dealing with the interaction of colloids during deposit formation in complex dairy systems like milk. These studies investigated membrane fouling due to casein micelle deposition as it occurs during the filtration of milk (Bouzid et al., 2008; Kühnl et al., 2010; Rabiller-Baudry, Gesan-Guiziou, Roldan-Calbo, Beaulieu, & Michel, 2005; Steinhauer, Kühnl, & Kulozik, 2011; Steinhauer, Kulozik, & Gebhardt, 2014). They reported that different types of interactions occur which can be related to electrostatic interactions and van-der-Waals forces, but additional forces have to be taken into account if electrostatic interactions are weak. Kühnl et al. (2010) postulated that this is the case for milk due to its high content of monovalent and divalent ions, which shield the charges at the protein surfaces. They explained charge effects on flux by solvation forces (hydrophilic repulsion and hydrophobic attraction).

However, there is still a lack of information on colloidal interactions between single whey protein fractions and whey protein mixtures. Especially for the deposit formation during the filtration of whey and whey proteins, no systematic study has been conducted thus far with regard to particle–particle and particle–deposit interactions. Therefore, the focus of this study was on the fouling behaviour of single whey protein fractions at various environmental conditions, i.e., pH and ionic strength. Based on this, membrane fouling of binary and complex mixtures like whey was investigated to also understand the interactions of various protein components during deposit formation as a function of the aqueous phase composition.

2. Materials and methods

2.1. Preparation of whey protein stock suspensions for dead-end filtration

Highly purified major whey proteins β-Lg (>98%) and α-La (>92%) were isolated in native state based on the method according to Toro-Sierra, Tolkach, and Kulozik (2013) from whey protein isolate (WPI 895, Fonterra, Auckland, New Zealand). The fractions of β-Lg and α-La were either used individually or β-Lg was mixed gravimetrically with α-La and/or native BSA powder (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in the ratios given in Table 1. Additionally, the above mentioned whey protein isolate (WPI) was used for filtration experiments as a reference for native sweet whey.

Table 1
Molar mixing ratio of whey proteins used for filtration experiments.[a]

Protein	Suspension number (No.)							
	1	2	3	4	5	6	7	8
β-Lactoglobulin	100		80	50	95	50	78	33.4
α-Lactalbumin		100	20	50			18	33.3
Bovine serum albumin					5	50	4	33.3

[a] Values are given as percentages.

All protein powders were dissolved in either ultra-pure water (Milli-Q, Millipore, Eschborn, Germany) or protein-free milk serum (PFMS, see Table 2), using 10 g of powder and 90 g of solvent. PFMS was produced by UF of skim milk obtained from a local dairy using a spiral wound UF membrane (cut off 10 kDa, GR81PE 6338/30, DSS Silkeborg AS, Silkeborg, Denmark, Material: PES) at a processing temperature of 10 °C to avoid calcium phosphate precipitation (Hanemaaijer, Robbertsen, van den Boomgaard, & Gunnink, 1989). Suspensions were prepared with a protein content of about 10% one week prior to the experiment to ensure equilibration. A prefiltration (0.2 μm, Merck KGaA, Darmstadt, Germany) for the removal of insoluble particles was carried out. The completeness of the separation process was checked by particle size analysis (see Section 2.5). All samples were preserved from bacterial spoilage by addition of 0.02% (w/w) sodium azide (Sigma–Aldrich Chemie GmbH) and stored at 4 °C.

2.2. Quantitative analysis of single whey protein fractions and preparation of feed suspensions

Concentrations of native whey protein stock solutions containing β-Lg, α-La or BSA were quantified by reversed phase high-performance-liquid-chromatography (RP-HPLC) (Voswinkel & Kulozik, 2011). For the filtration experiments, the stock solutions were diluted by the addition of a certain volume of Milli-Q water or PFMS to a protein concentration of 2.2%.

2.3. Sweet whey and acid whey for cross-flow filtration

For crossflow filtration, sweet whey from Edam cheese production (pH 6.4) and acid whey (pH 4.5) from cottage cheese production were obtained from local dairies. The composition of the applied whey types and the solvent PFMS (pH 6.8) are given in Table 2. The methods used are presented above (Section 2.2) and in Steinhauer, Schwing, Krauß, and Kulozik (2015c).

2.4. Filtration rigs and filtration procedure

For lab-scale investigations on the interaction of whey proteins during deposition, an unstirred dead-end UF rig (AMICON 8050, Merck-Millipore, Billerica, MA, USA) was used. It was equipped with fully retentive UF membranes (Pall Omega PES, cut off 30 kDa, Pall Corporation, Port Washington, USA) and pressurised with nitrogen gas by a pressure control unit (AL-PRESS, Bronkhorst, Ruurlo, The Netherlands). Transmembrane pressure (TMP) and temperature were kept constant during filtration (2×10^5 Pa; 20 ± 0.5 °C).

Cross-flow filtrations were carried out using a lab-scale filtration rig (Spiralab, TAMI Deutschland GmbH, Hermsdorf, Germany), operated using a frequency-controlled gear pump (VGS 200, Verder Deutschland GmbH, Haan, Germany). For filtration, ceramic INSIDE®DisRAM microfiltration membranes (nominal pore size:

Table 2
Composition of different whey types and protein-free milk serum.[a]

Component	Sweet whey	Acid whey	PFMS
Protein (%, w/w)	0.54 ± 0.02	0.59 ± 0.01	0.00 ± 0.01
Lactose (%, w/w)	3.40 ± 0.09	4.00 ± 0.08	4.19 ± 0.07
Lactate (%, w/w)	0.038 ± 0.004	0.9 ± 0.005	0.0036 ± 0.002
Calcium (%, w/w)	0.032 ± 0.001	0.1 ± 0.09	0.037 ± 0.01
pH	6.4 ± 0.2	4.5 ± 0.1	6.8 ± 0.1
Conductivity (mS cm^{-1})	5.28 ± 0.12	8.50 ± 0.43	5.56 ± 0.21

[a] Values are means from duplicate experiments ± standard deviation; data for sweet and acid wheys are as originally presented by Steinhauer et al. (2015c).