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Membrane fouling during ultra- and microfiltration of whey and whey proteins at different environmental conditions: The role of aggregated whey proteins as fouling initiators

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ABSTRACT

For the concentration of whey proteins from whey by ultrafiltration (UF) or fractionation by microfiltration (MF), mass transfer through the membrane is limited by deposit formation which is variable and not well understood, yet. There is little knowledge on the predominant molecular mechanism responsible for membrane fouling during whey micro- and ultrafiltration. Some works indicate that whey protein aggregates induce or enhance membrane fouling. The impact of whey protein aggregates as well as the interaction of native whey proteins and whey protein aggregates during membrane fouling was studied in both dead-end lab scale and cross-flow pilot scale. By means of targeted heat-treatment, a defined amount of protein aggregates was formed in β-Lactoglobulin (β-Lg) model suspensions as well as in sweet whey. β-Lg aggregates were found to accelerate membrane fouling during MF and UF due to covalent thiol/disulfide reactions. For sweet whey cross-flow filtration, membrane fouling was accelerated by whey protein aggregates up to a certain degree of whey protein denaturation. Above a critical value of 30% protein denaturation, flux increased again. This effect is explained by an improved erosion of larger whey protein aggregates as well as a reduced reactivity of heat-aged whey protein aggregates.

1. Introduction

During filtration of whey, membrane fouling due to deposition of whey protein on the membrane surface is the major factor limiting concentration or fractionation efficiency. Especially at MF and UF of both sweet and acid whey long-term fouling and a progressive decrease in transmission is limiting the life time of the filtration plants [1]. Up to now, no investigations on causative and mechanistic reasons for the fouling process during MF and UF can be found. Investigations on the minor whey protein bovine serum albumin (BSA) conducted by Chandavarkar [2] and Kim et al. [3] have shown that protein aggregates are formed in shear flow during cross-flow filtration, which are deposited on the membrane surface afterwards. In contrast to BSA, the major membrane foulant during whey filtration β-Lg is not sensitive to shear forces [4]. Kelly et al. [5] as well as Kelly and Zydney [6,7] found that BSA-aggregates catalyze fouling when deposited on the membrane. The molecular mechanism involved in the fouling reaction was found to be based on the exposure of reactive thiol-groups in the deposit. This reactive initial deposit then serves as a nucleation site for further thiol oxidation and thiol-interchange reactions. Despite the fact that the proteins varied significantly in size, molecular structure and originated from various animal protein sources (Table 1), Kelly and Zyndey [8] observed that intensity of membrane fouling was correlated to the number of free thiols of the respective protein.

Like BSA, β-Lg contains one free sulfhydryl group (Table 1). Hence, membrane fouling based on thiol-interchange reactions as well as thiol oxidation probably also applies for β-Lg or other whey proteins. This reaction pathway via thiol/disulfide reactions is known for β-Lg aggregation during thermal processing [9]. Additionally, for the major whey protein β-Lg it was found that gel network formation is facilitated in the presence of calcium [10,11]. Based on this, Marshall et al. [12] assumed that calcium induced cross-linking was involved in β-Lg deposit formation.

It was shown that whey protein hydrophobicity increases as a consequence of protein unfolding, e.g. under heat-treatment [13]. Another reason for protein unfolding and an increase in hydrophobicity is surface denaturation, as it is reported for the adsorption of several proteins including β-Lg at hydrophobic surfaces.

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Table 1
Molecular characteristics of whey proteins.

Protein	Size[a] (kDa)	pI[a]	–SH[b]	–S–S–[b]	Concentration in whey[a] (g/L)	Mass portion[b] (%)
β-Lactoglobulin	18.3	5.13	1	2	3	60
α-Lactalbumin	14.2	4.2–4.5	0	4	1.2	20
Bovine serum albumin	66.4	4.7	1	17	0.4	3
Immunoglobulin G	161	–	–	–	0.6	10
Lactoferrin	76.1	9	0	17	0.02	< 0.1

[a] Farrel et al. [55].
[b] Edwards et al. [56].

Unfolding was found to increase when repulsive electrostatic interaction forces were reduced [14]. It can be assumed that hydrophobic whey protein aggregates can potentially serve as adsorption sites and induce protein unfolding during deposit layer formation on membranes.

Some works confirm this theory, since aggregates formed during whey heat-treatment enhance membrane fouling [12,15–18].

To suggest a molecular mechanistic description the role of whey protein aggregates on membrane fouling, the current study focuses on the fouling mechanism during whey filtration. Since β-Lg and its heat-induced aggregates are the major species made responsible for membrane fouling, UF as well as sequential MF experiments with suspensions of β-Lg in native ionic environment were carried out under variation of the protein's surface charge. To reduce the number of hydrodynamic forces acting during particle deposition, filtrations were performed in dead-end mode at first. Hence, an evaluation of the dead-end flux as a function of time at various environmental conditions can be used for the assessment of the molecular fouling mechanism for deposits formed by β-Lg. By an addition of a defined number of heat-induced β-Lg aggregates, their impact on membrane fouling was studied. In a final step, casein micelle-free milk serum (ideal whey) was heat-treated to achieve a defined whey protein denaturation. The impact of different degrees of whey protein aggregation was subsequently studied under cross-flow conditions.

2. Materials and methods

2.1. Preparation of native and heat-denatured β-lactoglobulin solutions

Native β-Lg was isolated from native whey protein isolate (WPI 895, Fonterra, New Zealand, Lot. No. CT 08) following a procedure published by Toro-Sierra et al. [19] and finally sprays dried. The protein content of the dry powder was 99.73% as determined using a LECO protein analyzer (Model: FP-528, Leco, Mönchengladbach, Germany). The yield of the native whey protein β-Lg was quantified by reversed phase high-performance-liquid-chromatography (RP-HPLC) based on an established method [19]. A yield of > 98% native β-Lg was found in the powder.

Native β-Lg solutions (2.2%-w/w) were prepared by dissolving β-Lg isolate in protein free milk serum. Protein-free milk serum was manufactured from skim milk obtained from a local dairy using a spiral wound UF membrane (MWCO 10 kDa, GR81PE 6338/30, DSS Silkeborg AS, Silkeborg, Denmark, Material: PES) at a processing temperature of 10 °C to avoid calcium phosphate precipitation [20]. For thermodynamic equilibration all solutions were prepared one week prior to the experiment, pre-filtered (0.2 μm, Merck KGaA, Darmstadt, Germany), preserved from bacterial spoilage by the addition of 0.02% (w/w) sodium azide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and stored at a temperature of 4 °C.

Aliquots of the pre-filtered solution were heated at native pH 6.8 for 20 min, 40 min and 4 h at 65 °C. Thus, a defined amount of β-Lg aggregates was formed. The relative aggregate concentration for the filtration experiments was adjusted by adding 5% of heat-treated β-Lg suspension to the native one (total protein yield constant). The aggregate containing solution was thermodynamically stable and did not show sedimentation or size enlargement of the aggregates during storage.

For studying the impact of thiol-interchange reactions and thiol oxidation on membrane fouling, the reactive sulfhydryl group of β-Lg was inactivated stoichiometrically. To achieve this, solutions were incubated with NEM (N-Ethylmaleimide, Sigma-Aldrich Chemie GmbH; Steinheim, Germany) at pH 7. NEM is commonly used for this purpose in studies on the targeted heat-induced (whey) protein aggregation [21].

2.2. Preparation of whey (casein free milk serum)

Whey was produced as casein free milk serum. This kind of whey, sometimes referred to as 'ideal whey' was prepared from skim milk obtained from a local dairy by MF–UF diafiltration at a filtration temperature of 10 °C. It contains all low molecular weight constituents including the whey proteins. The native whey protein content in this study was found to vary around 0.66 ± 0.01% w/w. The applied MF rig (7P19-40GL, APV, Silkeborg, Denmark) was equipped with seven ceramic 0.1 μm-membrane elements (Type EP1940, PALL, Germany) and operated in uniform transmembrane pressure mode at a mean transmembrane pressure (TMP) of $0.2 \cdot 10^5$ Pa. For UF, the above mentioned rig was used (Section 2.1). A comparable setup was described by Kulozik and Kersten [22].

As can be seen in Fig. 1, MF permeate containing a portion of the milk whey protein content (mean sieving coefficient ≈ 0.5) was transmitted to the feed vessel of the UF rig. During UF, whey proteins were fully retained and protein-milk serum (UF permeate) was returned to MF feed vessel. UF permeate volume throughput was adjusted to the one of MF. During diafiltration total protein content as well as individual protein fractions were analyzed in permeate and retentate samples of UF and MF by RP-HPLC to track the progress of the fractionation procedure with regard to whey protein yield. Once the required whey protein level was reached diafiltration was terminated.

2.3. Dead-end filtration rig

For studying membrane fouling with isolated native and heat-denatured β-Lg, a dead-end microfiltration cell (AMICON 8050, Merck-Millipore, Billerica, USA) was used. It was equipped with a MF membrane (Pall Supor PES, nominal pore size: 0.1 μm, Pall Corporation, Port Washington, USA) or a UF membrane (Pall

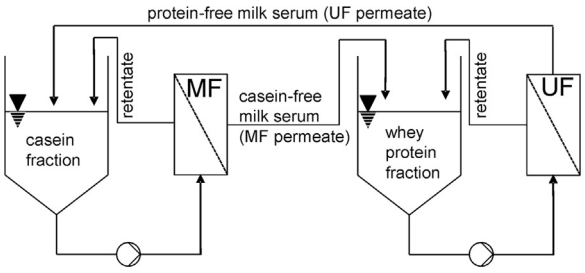

Fig. 1. Processing scheme for the production of ideal whey