Heat Exchanger Fouling and Cleaning XIV - 2022

Role of casein micelle on the whey protein fouling in a bench-scale fouling device

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Abstract

Fouling is an omnipresent issue in dairy production lines, however, the fouling mechanism is not fully understood and investigations arose mainly from experiments with model solutions that contained only whey proteins. The effect of casein on fouling has been rarely considered despite it is the major component of milk proteins. To partially fill this gap, in this work, the effect of casein on whey protein fouling behavior was investigated in a microchannel benchtop fouling device. This device provides a similar bulk fluid temperature profile to those performed in traditional plate heat exchangers (PHEs) but in a laminar regime. In unmodified pH conditions, such that the pH increases with elevated casein concentrations, the addition of casein significantly suppresses the deposition rate probably due to its calcium balancing capacity that reduces the ionic calcium level in the serum phase. While when the solution pHs were fixed at 6.6, casein has less calcium balancing capacity and therefore gradually loses its fouling mitigation effect, especially at high casein concentrations. A larger proportion of dissociated caseins was observed in unmodified pH conditions which might be responsible for fouling mitigation effect by expressing their chaperone-like functions to suppress the BLG denaturation.

Introduction

Fouling is a ubiquitous problem in dairy processing, the formation of the fouled layer reduces heat transfer efficiency therefore deteriorating the quality of the product. After decades of study, it has long been recognized that the thermal denaturation of the main component of the whey protein (*i.e.* β-lactoglobulin, BLG) is the key that drives fouling (Sadeghinezhad et al., 2015). However, the fouling mechanism is not fully understood and investigations arose mainly from experiments with model systems that contained only whey proteins. The role of casein on fouling has been rarely considered despite it being the major component of milk proteins. For example, the potential interactons between whey proteins and caseins are far from being well demystified and rarely studied as regards of fouling ability of processed protein solutions.

In our previous work, the addition of casein has been found to dramatically suppress whey protein fouling reaching a minimum deposition rate at casein/whey mass ratio of 0.2. Exceeding this critical value, fouling rate increased with elevated casein concentrations (Liu et al., 2021). In that study, the fouling experiments were performed in pilot plant plate heat exchangers (PHEs). Unfortunately, no analysis was performed to investigate the interactions between whey proteins and caseins occurred during thermal processing. Although being more meaningful in industrial aspects, the complexities of the fluid mechanics and configuration of

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PHEs make it hard to explain this contradictory effect of casein on whey protein fouling behavior. So in a second round, we have decided here to investigate the effect of casein on whey protein fouling behaviors in a benchtop fouling device. A rennenting-based technique was used to analyze the casein-whey interactions.

Experimental protocols

In order to rule out the complexities induced by turbulent flow in PHEs, a microchannel benchtop fouling device was designed, making it possible to achieve a similar bulk fluid temperature profile to those performed in PHEs but in a laminar regime (Figure 1). The microchannel provides a rectangular cross-section of 2 × 3.5 × 158 mm^3 (width × height × length. Fouling solutions were reconstituted from a whey protein isolate powder (WPI) and a casein powder supplied by Ingredia, France. It was decided to fix the whey protein concentration at 0.5 wt% to simulate the whey protein content in raw milk (Farrell Jr et al., 2004), with varying casein concentrations to yield different Casein/WPI mass ratios up to four (*i.e*. the ratio in milk). Notice that previous research has revealed a necessity of introducing Ca^{2+} to obtain appropriate fouling in a pilot-scale PHE (Liu et al., 2021), therefore, it was decided to add 42 ppm Ca^{2+} in all fouling solutions. The solution pH was either uncontrolled such that it increased with elevated Casein/WPI ratios or fixed at pH 6.6 by adding concentrated HCl, if needed. Fouling experiments were performed in the custom-built laboratory-scale fouling device at a fixed temperature profile by setting a flow rate at 2 $mL \cdot min^{-1}$ and a constant surface temperature of the plate heater at 90 °C. This temperature profile mimics a high-temperature-short-time (HTST) pasteurization process (*i.e*. 60 to 83 °C in the u-shape tunnel).

To get a better view of the potential interactions between whey protein and caseins, a renneting-based technique was used to analyze the fouling solutions as shown in Figure 2. This technique allows to reveal different molecular status of caseins between dissociated form and micellar structures as well as repartition of BLG associated between micellar and serum phase. To summarize, this procedure aimed at separating four different classes of protein particles:

i) *Fouling solutions before heating*: determines total amount of both whey and casein proteins;
ii) *Curd*: consists of micellar caseins and whey protein-bound casein micelles;
iii) *Precipitation*: includes protein aggregates in the serum phase (*e.g*. BLG-BLG, BLG-αLa, BLG-κ-casein aggregates);
iv) *Supernatant*: contains only native whey proteins and dissociated caseins that are released from micelle.

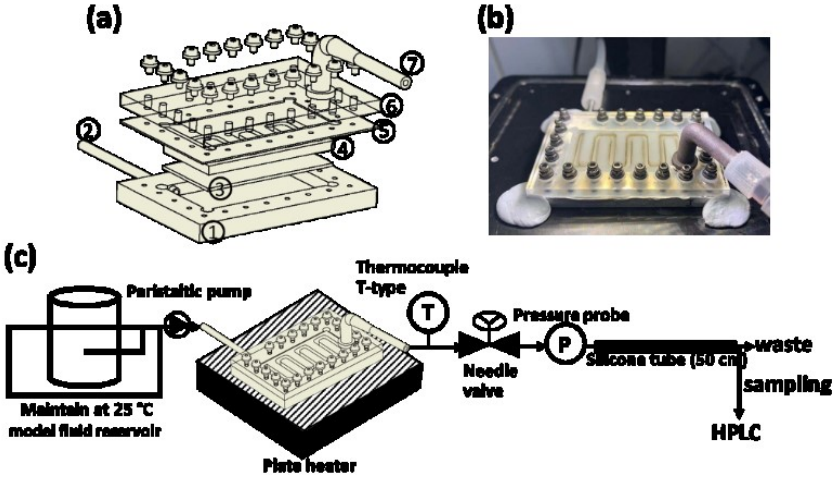

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Figure 1. (a) 3D schematic of the dismantled benchtop fouling rig. The device is assembled with (from bottom to up) aluminum alloy substrate, 68 × 44 × 6 mm^3 (1), 304 stainless steel tube (i.d. 2 mm) (2), 316 stainless steel plate, 48 × 24 × 1 mm^3 (3), silicone tunnel wall (4), silicone rubber (5), PMMA cover (6) and 3D-printed nylon bend tube (7). (b) The picture of the assembled state of the fouling rig mounted upon a plate heater. (c) experimental set-up for fouling runs.

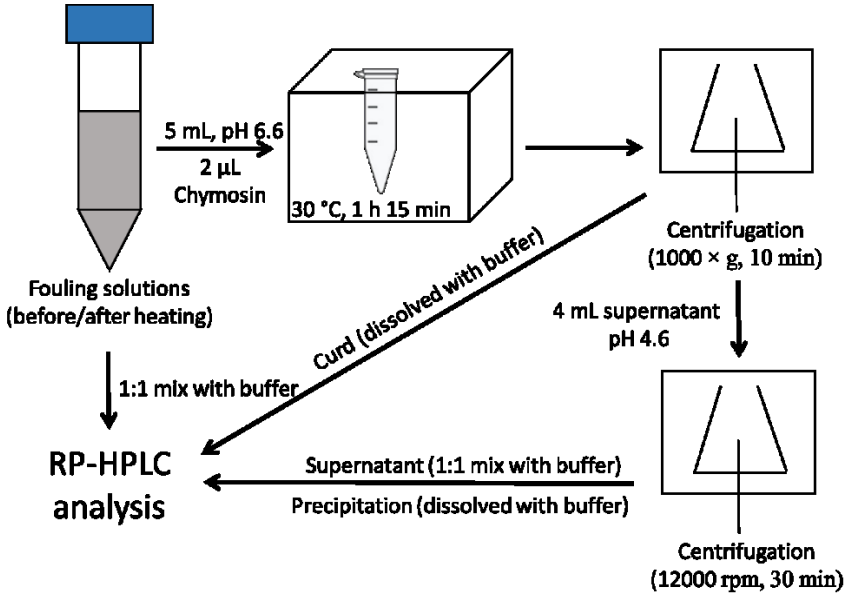

Figure 2. Schematic diagram of milk protein fractionation with renneting-based methodology.

Results and discussions

A clear view of how casein affects the whey fouling behavior can be seen in Figure 3. When the pH of the fouling solutions was unmodified such that it increased with elevated Casein/WPI ratios (from pH 6.6 without additional casein to pH 7.1 at Casein/WPI of 4), the total fouling was dramatically suppressed. The minimum value locates at Casein/WPI of 0.1, where ~90% of fouling was mitigated. This trend is in accordance with those results performed in the pilot plant PHEs, where the minimum value was found at Casein/WPI of 0.2 (Liu et al., 2021). When additional Ca^{2+} was absent as in the case of Casein/WPI at 0.8 (Fig. 3(a)), no deposit was visualized after two hours of fouling run, even though it contained a similar ionic calcium level (introduced by casein powder) to that without casein but with additional Ca^{2+} (data not shown). This behavior is in line with those obtained in the pilot-scale experiments, and it was proposed that the casein micelle shifts the ionic calcium in the serum phase towards the formation of micellar calcium phosphate during heating, resulting in low calcium content in the serum phase, and low BLG denaturation level, therefore a low level of fouling (Fig. 3(b)) (Liu et al., 2021).

Nevertheless, when the solution pHs were fixed at 6.6, casein gradually lose its fouling mitigation effect with elevated Casein/WPI ratios, resulting in an even higher deposition rate at Casein/WPI of 4. These different fouling behaviors showed a strong correlation to the corresponding BLG denaturation level as shown in Figure 3(b): the BLG denaturation level decreased with the addition of casein and was kept almost constant at a relatively low value (around 30%) for unmodified pH conditions. However, when pH was fixed at 6.6, the BLG denaturation level remained at a high level regardless of casein concentrations. This denaturation of BLG changes can be explained by a lower capacity of casein controlling the level of calcium content in the serum phase, especially at low pH conditions; the free calcium

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concentrations obtained when pH was fixed at pH 6.6 are significantly higher than those obtained with unmodified pH (data not shown). This higher calcium content should be responsible for the high BLG denaturation level as well as the fouling behavior.

It is important to bear in mind that caseins, especially at such low concentrations, might not be in their micellar structure. This can be crucial as the functionality of micellar casein differs dramatically to that in the dissociated form. For instance, when caseins are in micellar structure, κ-casein is the most likely one to interact with denatured whey proteins under thermal stress due to their superior location in the micellar surface. However, for α_s/β-caseins that are naturally buried inside the interior of the micelle are less responsible for casein-whey interactions, unless they are dissociated. In almost all researches that used casein micelle powders, the casein solutions were reconstituted to reach the level that is similar to milk (*i.e.* 2.5%) or even higher (*e.g.* 5%, 10%) (Chandrapala et al., 2014). Therefore, for our fouling solutions, it is hypothesized that a large proportion of caseins are in their dissociated form, especially at low casein concentrations. One should notice that the term "dissociated casein" does not refer to individual casein proteins (in monomer form). More likely, they tend to become κ-casein-depleted casein submicelles that have an increasingly loose structure and are much larger, and stronger hydrated compared to the native micellar structure probably due to the cleavage of the linkage between caseins and colloidal calcium phosphate as described in (Aoki et al., 1990).

Our results suggest that more than 50% of the caseins at low Casein/WPI ratios (<1) are in the dissociated form (Figure 4). Whilst this proportion reduces significantly at Casein/WPI ratios ranging from 1 to 4. At the ratio that is close to milk (*i.e.* 4), more than 90% of caseins are in their micellar form. Fixing solution pH at 6.6 generally decreases the proportion of dissociated caseins (Fig. 4(b)). It was finally proposed that a larger proportion of dissociated caseins in unmodified pH conditions could express their chaperone-like functions to suppress the BLG denaturation as well as subsequent fouling behaviors.

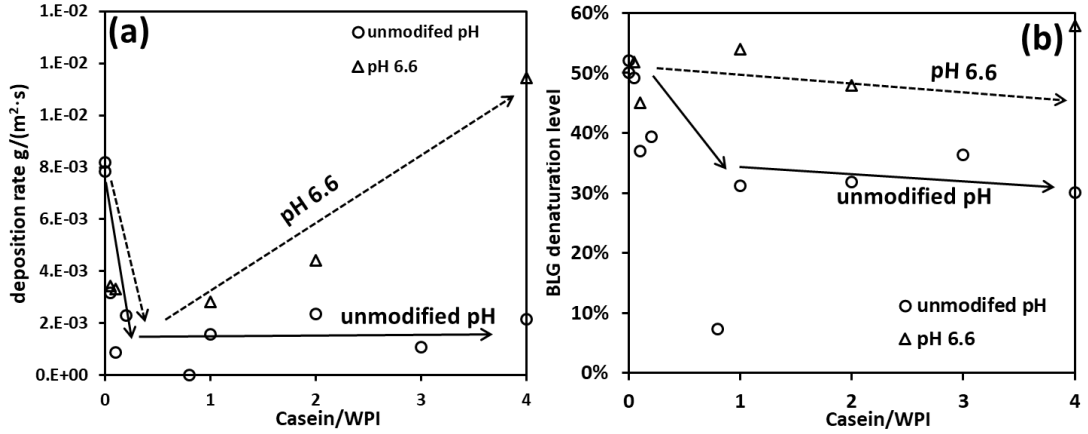

Figure 3. (a) Effect of Casein/WPI mass ratio on the total fouling deposition rate in the custom-built laboratory-scale fouling device. (b) Denaturation level of BLG after thermal treatment in the benchtop fouling device at various Casein/WPI ratios. The pH of fouling fluids was either unmodified such that it increased with elevated Casein/WPI ratios or kept constant at pH 6.6 in the micro fouling rig. Note the value of zero at Casein/WPI of 0.8 in (a) was obtained without additional ionic calcium. Arrows are used to guide the eyes.

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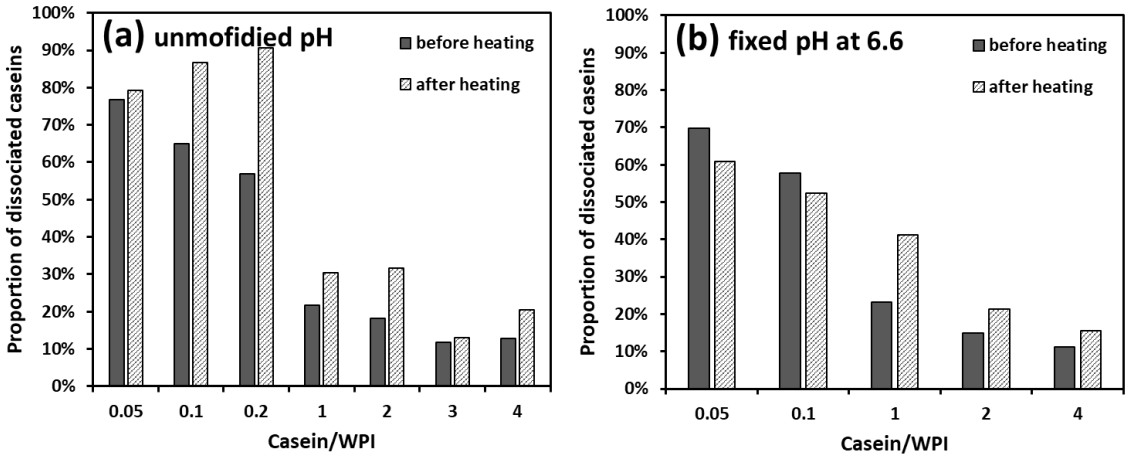

Figure 4. The proportion of caseins in their dissociated form in the fouling solutions at various Casein/WPI ratios under unmodified pH conditions (a) or fixed pH at 6.6 (b). The proportions were calculated using the sum of all casein concentrations (*i.e.* sum of α_s/β/κ-casein) detected in the supernatants divided by the values obtained from the solutions before fractionation.

Conclusions

In this work, the effect of casein on whey protein fouling was investigated in a custom-built benchtop fouling device. Despite different flow schemes, caseins were confirmed to have a similar fouling mitigation effect to those reported in PHEs, probably due to their calcium balancing capacity that reduces the ionic calcium levels in the serum phase. However, if the solution pHs were fixed at 6.6, casein gradually loses its mitigation effect, resulting in even higher fouling rates at high casein concentrations. The corresponding BLG denaturation levels were found to correlate well with the fouling behaviors. A larger proportion of caseins was found to be in the dissociated form, especially at unmodified pH conditions that might be able to express their chaperone-like functions to suppress the BLG denaturation as well as subsequent fouling. These findings reveal a potential application of casein on fouling mitigation.

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