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# Cleaning beyond whey protein gels: Egg white

*Hui Li*[a,1], *Boris Koutzenko*[a,b,1], *Xiao Dong Chen*[a], *Romain Jeantet*[b], *Ruben Mercadé-Prieto*[a,*]

[a] *School of Chemical and Environmental Engineering, Soochow University, Suzhou, China*
[b] *Agrocampus Ouest, Rennes, France*

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ABSTRACT

There has been extensive research in the last decade trying to understand the fundamentals of dissolution of whey protein gels in order to lay the foundations of proteinaceous cleaning processes. To date, experimental data has been limited to whey proteins, raising questions of the applicability of the theoretical frameworks developed for whey proteins to other protein systems. In this work the dissolution of crude egg white gels is studied, which are rich in ovalbumin protein, and which have recently shown a different cleaning behavior to whey deposits. Egg white gels made in well-defined conditions were dissolved in a wide range of alkali and sodium chloride concentrations, and temperature. The dissolution rate was found to increase linearly with the alkali concentration up to 1 M, hence confirming the absence of an optimum cleaning concentration observed in whey protein cleaning studies. The apparent activation energy of dissolution for particulate egg white gels was 70–80 kJ $mol^{-1}$, suggesting that the dissolution of egg white gels is controlled by the β-elimination of disulfide bonds. Dissolution experiments with salts at different temperatures confirmed that swelling is very important in the dissolution of whey protein gels, but not in egg white gels.


## 1. Introduction

Fouling and cleaning are often considered to be inter-related: the nature of the fouling deposit will affect the cleaning performance (Wilson, 2005), and the cleaning step may affect the subsequent fouling formation by modifying the heat exchanger surfaces (Barish and Goddard, 2014). Hence, a holistic approach to the combined problem of fouling and cleaning, where the best cleaning protocol to apply is determined by the nature of the fouling layer, is a long term aim for the research community working in this area. In order to be able to fulfill this goal, it is essential to understand fouling, and the nature of the undesired material formed, as well as cleaning, how these deposits are removed by chemical and mechanical forces. The complexity of both processes, exacerbated by the use of chemically complex food products, has resulted in a slow buildup of solid fundamentals; a good example on dairy fouling is the recent work by Jimenez et al. (2013). The fundamentals of cleaning have attracted much less attention in the past, with the key exception of pure whey protein gels (Mercadé-Prieto et al., 2008; Saikhwan et al., 2010).

The dissolution of whey protein gels in alkali solutions involves the fast diffusion of alkali into the gel, followed by the swelling and subsequent break up of non-covalent interactions at pH above ~11.5. The dissolution rate increases pseudo-linearly at alkali concentrations higher than 0.01 M up to ~0.1 M. Dissolution is hypothesized to improve at higher alkali concentrations by a combination of faster and more extensive swelling, and due to faster cleavage kinetics (Mercadé-Prieto et al., 2008). However, if the alkali concentration is too high, typically >0.2 M, swelling becomes inhibited due to the large concentrations of ions in the cleaning solution

* Corresponding author. Tel.: +86 18501518178.
E-mail address: ruben@suda.edu.cn (R. Mercadé-Prieto).
[1] Both authors contributed equally.
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(Mercadé-Prieto et al., 2007b), resulting in the well-known optimum concentration for cleaning dairy deposits (Bird and Fryer, 1991). Nevertheless, many industrial plants operate at higher alkali concentrations (e.g. >2 wt%), where the lower dissolution rate at those high alkali concentrations is more than compensated by the high temperatures used (>70 °C) (Alvarez et al., 2010; Merin et al., 2002).

Very similar phenomenological observations have been consistently made on the cleaning of different kind of dairy fouling deposits, from milk, WPC, WPI or pure β-lactoglobulin (βLg) gels, the key protein causing dairy fouling. This strongly suggests that the general cleaning mechanisms discussed above for simpler models (particularly pure βLg) are relevant, if not directly applicable, to more complex systems such as milk fouling. However, it is impossible to claim to understand the cleaning or dissolution of proteinaceous deposits in general if only one kind of protein deposit is considered, as has been the case for whey proteins due to their relevance to dairy cleaning.

The recent work of Li et al. (2013a, 2013b) on the alkali cleaning of egg ovalbumin (OVA) fouling deposits and gels presents the first opportunity to test the universal applicability of the dissolution mechanisms found for whey protein gels. The preliminary results showed that egg white fouling deposits and gels, formed at similar conditions than those of whey proteins, present very different cleaning behavior. The most salient difference from those studies is the apparent lack of an optimum alkali concentration in cleaning (at least up to ~6 wt%, the maximum tested): cleaning proceeds faster at higher concentrations. The present study will start a more detailed characterization of the alkali dissolution of egg white heat induced gels using well established techniques, allowing a more reliable comparison with whey protein gels.

## 2. Materials and methods

Crude egg white powder was obtained from Sigma (A5253), with a nominal ovalbumin (OVA) composition of 62–88%. The moisture content of the powder was determined at 7.6 wt%. Solutions of crude egg white powder yielded a precipitate when trying to obtain transparent (stranded) gels, such as when dialyzing to decrease the salt content. In order to avoid the presence of a precipitate before forming gels, a simple purification method was developed as follows. About 20 wt% egg white powder solutions were centrifuged for 2 min at 13,000 rpm (Neofuge 18R, Heal Force) in 50 mL tubes. About 4 wt% of the solution was separated, with a resulting protein concentration after centrifugation of ~16.6 wt% at pH ~6.4. Whey protein isolate (WPI) was used for comparison purposes, and was obtained from Davisco (USA).

Some egg white solutions where further purified by dialysis using 6–8 kDa MWCO membranes (Spectra Laboratories) first with 100 mM Tris buffer at pH ~8.1 for 150 min, followed by a second dialysis for 150 min using a 10 mM buffer solution. The pH of the egg white solution was finally increased to 10 using 1 M NaOH, resulting in a final OVA concentration of 10.3 wt% after the dilution during dialysis.

Heat induced egg white gels were made inside cylindrical plastic capsules (9.1 mm i.d., 40 mm high) held inside a water bath for 1 h, at 80 °C for pH 10 gels, and at 80 or 90 °C for pH 6.4 gels. WPI gels were made at 15 wt%, pH 6.8 at 80 °C for 1 h for comparison. Gels were kept at 4 °C overnight before use, with the top 2 mm removed with a spatula to ensure an even surface. Dissolution experiments were performed as described previously (Mercadé-Prieto and Chen, 2006), by submerging the capsule with the gels in ~280 mL alkali solutions of known concentration. A small part of the solution was recirculated using a peristaltic pump through a UV spectrophotometer (Mapada UV-1600), using a flow cell cuvette. Absorbance measurements at 280 nm were taken every 2 s; the protein concentration was calculated from previously determined calibration curves at different alkali concentrations, for both WPI and egg white. Gels were dissolved under limited stirring for at least 2 h. For experiments at high dissolution temperatures, the Erlenmeyer with the alkali solution was immersed in a water bath at a controlled temperature.

The swelling equilibrium of gels was performed gravimetrically as explained previously (Mercadé-Prieto et al., 2007b), where the equilibrium swelling ratio is calculated from the initial gel weigh $m_0$ and the final swollen weight $m_{sw}$ as $SR = m_{sw}/m_0 - 1$. The effect of different NaOH concentrations on the swelling of different gels was studied. About 0.35 M NaCl was added to the solutions; otherwise gels would swell extensively, becoming too weak to handle or dissolving too quickly.

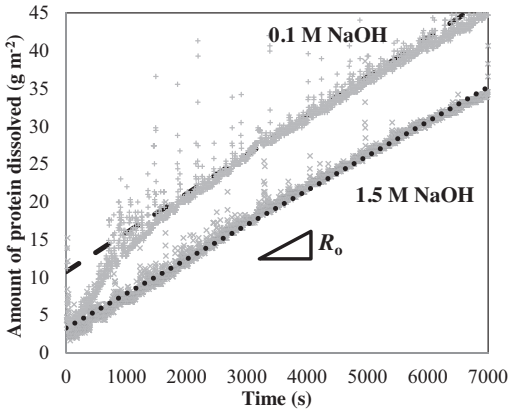


**Fig. 1 – Dissolution of a 20 wt% crude egg white gel without centrifugation in 0.1 and 1.5 M NaOH at 21 °C. Raw experimental points show artifacts like bubbles in the flow cuvette during the continuous UV measurements. The constant dissolution rate $R_o$ at 0.1 M was 0.0052 g m$^{-2}$ s$^{-1}$ and at 1.5 M was 0.0044 g m$^{-2}$ s$^{-1}$.**

## 3. Results

### 3.1. Dissolution of egg white gels in alkali

Following the initial work by Li et al. (2013b) on egg white fouling, heat induced gels were initially made of 20 wt% crude egg white powder. The resultant gels were very hard to the touch, and were very difficult to dissolve at ambient conditions regardless of the NaOH concentration used. Fig. 1 shows examples at 0.1 and 1.5 M NaOH, where a constant dissolution rate $R_o$ is evident for the whole dissolution time. The values of $R_o$ were at least a magnitude lower than previously observed for whey protein gels, with typical values for the later of 0.02–0.1 g m$^{-2}$ s$^{-1}$ at 0.1 M NaOH and room temperature (Mercadé-Prieto et al., 2006), depending on the gelation conditions. For comparison, 15 wt% WPI gels were also dissolved at room temperatures, resulting in the same alkali dependence reported previously (Mercadé-Prieto and Chen, 2006), with a $R_o$ value in 0.1 M NaOH of 0.092–0.1 g m$^{-2}$ s$^{-1}$.

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