INFLUENCE OF WHEY PROTEIN DENATURATION ON ADHERENCE OF SOILING PARTICLES TO STAINLESS STEEL

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ABSTRACT

This work reports on the influence of β-lactoglobuline (β-LGB) and of its denaturation on the adherence of quartz particles, taken as a model of particulate soil, on stainless steel AISI 304 with mirror finish. The substrate was soiled with quartz suspensions in water or in β-LGB solutions as such or previously heated at 75°C, and dried at room temperature or in an oven at 75°C. Cleanability was evaluated after exposure to water in a radial flow chamber. Auxiliary characterizations were the surface tension and protein concentration of the solution, surface analysis of the substrate by X-ray photoelectron spectroscopy (XPS) and contact angle measurements.
The contact of stainless steel with β-LGB led to adsorption of the protein, which dominated the composition of the organic layer with respect to contaminants initially present, and was not markedly desorbed upon rinsing. The presence of β-LGB at the quartz particle/substrate interface slightly increased the adherence, which was further increased when the protein was denatured. On the other hand, denaturation of β-LGB enhanced its surfactant effect at the water/air interface. Comparison with systems investigated before suggests that the influence of protein via droplet spreading and soiling particles aggregation may be of minor importance compared to direct effects on the substrate/quartz interface. Stainless steel does not behave as a hydrophilic substrate owing to its surface contamination with organic compounds. It appears suitable to examine the influence of the initial surface state of stainless steel on its behavior regarding soiling and cleaning.

INTRODUCTION

Proteins are often used in food industry to improve fabricated foods qualities like texture and appearance. Whey proteins have high functional properties, which are valuable in numerous applications such as gelation, foaming and whipping, water retention, emulsification, and thermal stability. Whey is the liquid remaining after removal of casein from milk and contains mainly globular proteins (6 g/L in bovine milk). The main proteins are β-lactoglobulin (β-LGB), α-lactalbumin, bovine serum albumin (BSA), and immunoglobulins. β-LGB is the most abundant, representing more than 50% of the total whey protein (Fox and Mcsweeney, 1998).
In dairy, two kinds of fouling are identified as a result of heating: type A, soft and spongy deposits, containing more than 50% proteins, mainly β-LGB, and about 8% mineral compounds; type B, hard deposits, granular in structure, called mineral fouling, containing about 80% minerals, mainly calcium phosphate, and 15% proteins (Fickak et al., 2011; Changani et al., 1997; Visser and Jeurnink, 1997). A controversial question concerns whey protein implication in deposit layer formation and removal. Although the connection between the thermal stability and conformational changes of β-LGB is well established, the role of the denatured state in the build-up of the fouling deposit is still unclear. Trends reported in the literature are not conclusive and are focused on protein deposits or on the effect of mineral elements concentration on deposit formation (Blanpain-Avet et al., 2012; Bansal and Chen, 2006; 2009).

Fouling involving particle deposition and drying is of major concern for surfaces exposed to natural environments or surfaces of industrial equipments. Particulate soils may originate from splashing, or deposition in storage tanks, in the ducts or on the plates of heaters, coolers and other open surfaces. The problem is particularly severe in food and pharmaceutical processing, where fouling deposits may endanger microbial sterility and product purity (Stephan et al., 2004). The influence of macromolecules (Touré et al., to be submited; Touré et al., 2013; Touré et al., 2011) on the adherence of model particulate soils (quartz particles) was examined using substrates which differed according to hydrophobicity. The effect of dextran was weak, possibly owing to its low adsorption or easy desorption. The presence of BSA had little effect on the adherence to polystyrene but improved drastically the cleanability of glass. This was attributed to prevention of tight bonds between the particle soil and the substrate or to induction of a repulsion between the surfaces in contact. Since whey protein are often submitted to heating, the study of the influence of heating and protein denaturation on particle soil adherence will contribute to improve cleanability and better understand the mechanisms involved. To our knowledge, no data are available on this subject.

The present work investigates the effect of β-LGB and its denaturation on the adherence of particulate soils to stainless steel, the ubiquitous substrate in food industry, and on its cleanability. Quartz particles were taken as a simplified particulate soil model. β-LGB was chosen because it is the most abundant and the most heat-labile whey protein, and plays a key role in fouling (Robbins et al., 1999). The protein was involved in the soiling process by its introduction into the quartz suspension. A previous study with BSA showed that there is no significant difference in cleanability depending on whether the protein was brought by conditioning the substrate before soiling or by its presence in the soiling suspension, or both ways (Touré et al., to be submitted). The influence of denaturation was examined by a high temperature pretreatment of the soiling suspension or by drying the soiled substrate at high temperature. The soiling suspension was characterized at different stages regarding soluble protein concentration and surface tension. The substrate/solution interfaces were characterized by X-ray photoelectron spectroscopy (XPS) analysis of substrates conditioned with solutions in representative states and by contact angle measurements.

EXPERIMENTAL

Materials

Stainless steel (AISI 304-2R, 1mm thick) plates were provided by Arcelor (France). The face used for the study had a mirror finish and was protected with a plastic sheet which was removed before substrate preparation. The substrate was cut to the desired dimensions: 50 mm × 50 mm for fouling and cleanability assessment; 16 mm ×10 mm for surface characterization. The coupons were cleaned with ethanol, sonicated (ultrasonic cleaner Branson 3200, USA) in ethanol for 10 min, rinsed thoroughly with ethanol, dried with a gentle flow of nitrogen and wrapped in aluminium until use. Ground quartz particles (M400) were provided by Sibelco Benelux (Belgium). Quartz particles with a size about 10 to 30 µm were isolated from the initial batch (particle size distribution 1.1 to 60.3 µm) as described before (Touré et al., 2011) and used as a model of hard particulate soil. MilliQ water was produced by a MilliQ-50 system from Millipore (France). Absolute ethanol and β-lactoglobulin (β-LBG) from bovine milk (lyophilized powder; ≥85 %,) were purchased from Sigma-Aldrich (Wisconsin, USA).

Soiling procedure and cleanability assessment

Four kinds of quartz suspensions were prepared at a concentration of 150 g/L: (a) in water, (b) in a native β-LGB solution (3 g/L), (c) same as (b) and subsequent heating for 0.5 h at 75°C, (d) analogous to (c) but heated for 4 h. In addition, certain samples were soiled with suspension (a) or (b) and dried for 0.5 h in an oven at 75°C before the cleaning process. Further details on soil preparation and handling can be found in Touré et al. (2013, 2011). The choice of β-LGB

concentration in the suspension was based on β-LGB concentration in bovine milk (3 g/L) (Walstra and Jenness, 1984). The soiling procedure, cleanability assessment and data processing are detailed in previous studies (Detry et al., 2011; Touré et al., 2011; 2013). Briefly, suspension droplets were deposited by aspersion at room temperature. Cleanability assessment was performed at 20 °C in a radial-flow cell (RFC). Distilled water was flown during 5 min with a flow rate of 40 ml/min. Pictures taken before and after cleaning, were processed with a specific application of the Matlab software, which gave the radial position. The radial position at which the residual density became ≥50% was considered as the critical detachment radius (Goldstein and DiMilla, 1998; 1997). At least 10 repetitions of each experiment (soiling-cleaning) were made.

With the radial flow chamber used, the conversion of critical radius into critical wall shear stress is not reliable above flow rates of 20 ml/min (Detry et al., 2011). Therefore, the results of the present study are expressed in terms of critical radius, keeping in mind that for a defined flow rate, the higher the critical radius, the lower the adherence.

Contact angle measurement and surface analysis

Static contact angles were measured using the sessile drop method with a goniometer (Krüss, France). The measurements involved at least 10 drops. The XPS analyses were performed on a SSX 100/206 photoelectron spectrometer from Surface Science Instruments (USA) equipped with a monochromatized micro focused Al X-ray source (powered at 20 mA and 10 kV). The analysis details were described by Toure et al. (to be submitted). The following sequence of spectra was recorded: survey spectrum, C 1s, Fe, Cr, O 1s, N 1s, S 2p and C 1s again to check the stability of sample charging and the absence of organic compounds degradation as a function of time. The data analysis was performed with the CasaXPS program (Casa Software, Teignmouth, UK). Molar concentration ratios were calculated from peak areas (linear background subtraction) normalized on the basis of the acquisition parameters and of sensitivity factors and transmission function provided by the manufacturer. The C 1s peak was decomposed by using a least square fitting procedure with a 85:15 Gaussian-Lorentzian product function.

Solution and supernatant characterization

β-LGB powder was dissolved in MiliQ water at concentration of 3g/L providing clear solution. Solutions were heated for 0.5 h or 4 h at 75°C and rapidly cooled down to room temperature. This resulted in white turbid liquids, indicating protein aggregation. After centrifugation using Beckman instrument (Coulter Inc., USA; 25696 x g for 30 min), the supernatants were collected for different uses. The UV-visible absorption spectra of β-LGB solution and supernatants collected after heating were recorded with a Shimadzu UV 2401PC spectrophotometer, using a quartz cell with a light path length of 10 mm and pure water (MilliQ) as reference. The soluble protein contents in the β-LGB solution and the supernatants were determined by the Kjeldahl method, multiplying the concentration by 6.32, specific of β-LGB. The surface tension of the liquids was measured at increasing dilution, with a Prolabo Tensiometer (Tensimat n°3) using the Wilhelmy plate method.

RESULTS AND DISCUSSION

Characterization of solutions

Figure 1 shows that the absorbance around 270-280 nm decreases from 2.34 for the native solution to 1.28 and 0.70 for the supernatant collected after heating at 75°C for 0.5 and 4 h, respectively. Note that preliminary tests of heating at different temperatures showed no important variation of absorbance at 60°C, a progressive variation at 75°C, and almost complete removal of proteins after 4 h at 90 or 100°C. Table 1 presents the protein concentration. Supernatants collected after 0.5 and 4 h heating show protein concentrations of

1.1 and 0.6 g/L, respectively, to be compared with2.5 g/L for the native solution. Heating the β-LGB solution at 75°C thus provoked aggregation of about 50 and 75% of proteins after 0.5 and 4 h, respectively (Table 1, Figure 1).

Figure 2 presents the evolution of the surface tension as a function of the concentration obtained by increasing dilution of the β-LGB solution and of supernatants collected after heating. The surface tension of the supernatant from 4 h heating is always lower compared to the supernatant from 0.5 h heating and to the native solution. The proteins remaining in the supernatant collected after 4 h are thus responsible for a lower surface tension, meaning a higher activity at the water/air interface. This in agreement with the observation (Schmitt et al., 2007) of a lowering of the surface tension of whey protein solution heated at 85°C in presence of NaCl, the decrease being higher as the NaCl concentration and protein aggregation increased. It was also found that heat aggregation of BSA provoked a decrease of the surface tension of the solution (McClellan and Franses, 2003). The lowering of protein solution surface tension as a result of heating reveals changes of conformation of the molecules still dissolved which are unfolded and expose hydrophobic residue

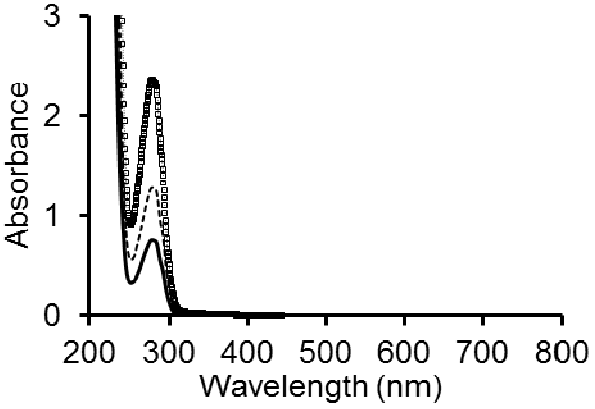

Figure 1. UV-visible absorption spectra: native β-LGB solution (▫▫▫); supernatant collected after heating 0.5h (---) and 4h (—) at 75°C.

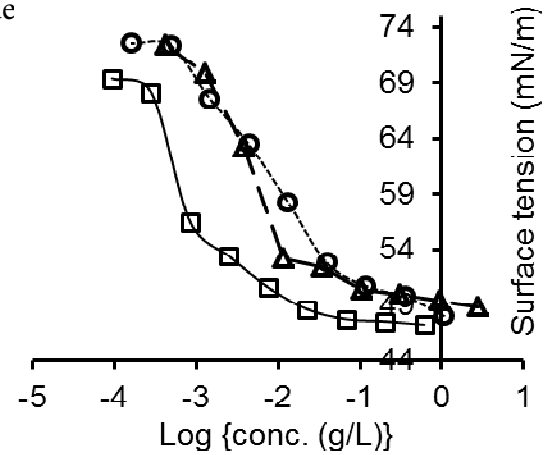

Figure 2. Variation of the surface tension as a function of the protein concentration resulting from increasing dilution of: native β-LGB solution (Δ); supernatant collected after heating 0.5h (○) and 4h (□) at 75°C.

Table 1. Protein concentration of native β-LGB solution and supernatants collected after heating at 75°C (standard deviation, 3 replications)

	Native	Supernatant heating 0.5 h	Supernatant heating 4 h
Concentration (g/L)	2.5 ± 0.2	1.1 ± 0.2	0.6 ± 0.1

Surface chemical composition

Figure 3 shows representative O 1s and C 1s XPS peaks recorded on stainless steel substrates (SS) just cleaned, or rinsed twice after immersion in the solution of β-LGB or the supernatants collected after heating. The carbon peak of cleaned stainless steel is due to contaminants remaining after cleaning or adsorbed from the surroundings, either the ambient atmosphere or the spectrometer vacuum chamber. The C 1s peak was decomposed (Rouxhet and Genet, 2011; Rouxhet et al., 2008), allowing the possibility of 4 components. The component attributed to carbon only bound to carbon and hydrogen [**C**-(C,H)] was set at 284.8 eV. Other components were found at 286.3 ± 0.2 eV, assigned to carbon making a single bond with oxygen or nitrogen [**C**-(O,N)], and 288.0 ± 0.2 eV, assigned to carbon typical of amide function in proteins [N-**C**=O]. For certain samples, a very weak component was found near 289.3 eV, which may be due to ester or carboxyl.

The O 1s peak component near 529.7 eV is attributed to metal oxides of stainless steel. The peak showed a maximum at about 531.0 eV for conditioned substrates, which may comprise oxygen of amide of β-LGB [N-C=**O**] and of metal hydroxides of the substrate. The contribution near 533.0 eV is attributed to C–OH or C–O–C. The position and shape of the O 1s peak varied according to the respective contributions. Its decomposition was not performed, owing to the overlap of contributions of the substrate, protein and organic contaminants.

The XPS spectra provide the concentrations of elements and of specific forms of carbon, given in mole fractions with respect to the sum of all elements except hydrogen. Owing to the complexity of the adsorbed layer, the adsorbed amount cannot be expressed in mass per unit area or thickness. However, comparisons of adsorbed amounts can be based on the sum of concentrations of elements due to the adsorbed layer, Σadlayer, and due to the stainless steel, Σsubstrate, which may be evaluated as follows.

$\Sigma adlayer = C_{tot} + N + O_{org}$, where the third term is an evaluation of oxygen due to β-LGB and organic contaminants;

$\Sigma substrate = Fe + Cr + O_{inorg}$, which accounts for metal elements and oxygen bound to them.

Based on the amounts of different amino-acids in β-LGB (Walstra and Jenness, 1984), the elemental composition and the expected contributions to the C 1s peak components were computed as performed before for BSA and validated by correlations between independent spectral data (Touré et al., to be submitted). This provides the formula $C_{3.97}O_{1.20}N_1S_{0.04}H_{6.38}$ and the molar mass of 18263 Dalton, with molar ratios O/N = 1.20 and $C_{ox}/N = (C_{tot} - C_{284.8})/N = 2.05$. There is no significant difference between variants A and B of β-LGB.

The contribution of β-LGB to the organic adlayer should be $C_{ox\beta\text{-}LGB} = 2.05*N$ and $O_{\beta\text{-}LGB} = 1.20*N$. The contributions of O due to organic contaminants should be $O_{cont} = C_{oxcont} = (C_{ox} - 2.01*N)$ if oxidized carbon is present in the form of alcohol, aldehyde, ketone or ester functions, as expected. The concentration of organic oxygen can then be deduced:

$$O_{org} = 1.20*N + (C_{ox} - 2.05*N) = C_{ox} - 0.85*N \quad (1)$$

where 1.20 *N and (C_{ox} - 2.0*5N) are oxygen contribution due β-LGB and organic contamination, respectively.

The concentration of oxygen due to stainless steel substrate may then be evaluated as:

$$O_{inorg} = O_{tot} - O_{org}. \quad (2)$$

The XPS analysis was performed on coupons prepared in different ways: just cleaned, or previously immersed in the protein solution either at room temperature or at 75°C for 0.5 or 4 h, or in the supernatant collected after heating the β-LGB solution at 75°C for 0.5 or 4 h. The analysis was performed after rinsing or not with water (immersion twice for 5 min), and flushing with nitrogen for removing the liquid film.

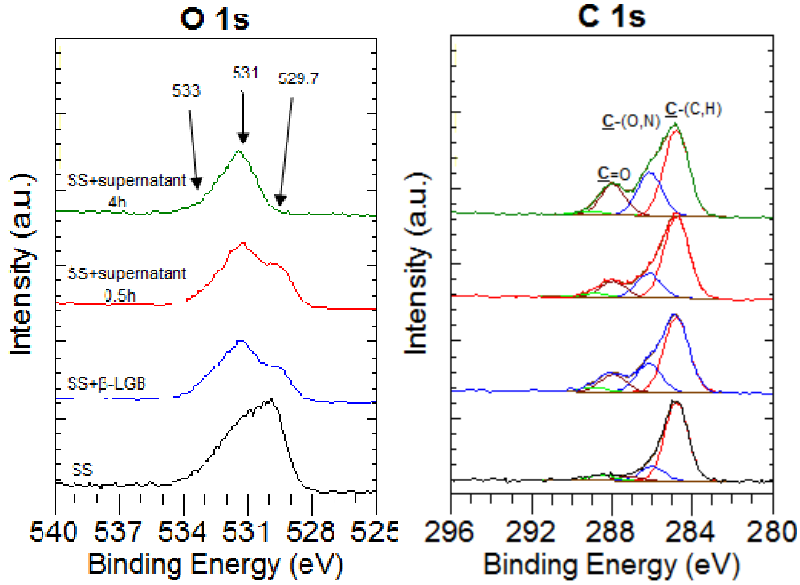

Figure 3. Representative O 1s and C 1s peaks recorded on stainless steel, as such (SS), or immersed in β-LGB solution or in supernatants collected after heating 0.5 or 4 h at 75°C and rinsed. Illustration of C 1s peak decomposition.

Table 2 gives the proportion of elements belonging to the organic adlayer (∑adlayer) and to the substrate (∑substrate), respectively, and the molar ratios O_{org}/C_{tot}, N/C_{tot} and O_{org}/N determined on the different samples and expected for β-LGB. In XPS, the first layer at the surface with a thickness equal to the electron inelastic mean free path (order of 3 nm) and the next layer of the same thickness are responsible for 63 and 23% of the signal, respectively. It appears that the organic adlayer is thick enough to strongly screen the contribution of the substrate to the XPS spectrum. Comparisons between the molar ratios O_{org}/C_{tot}, N/C_{tot} and O_{org}/N of β-LGB conditioned samples, the values expected for pure β-LGB and the values measured on native substrate; indicate that the organic layer was dominated by the protein for most conditioned samples. In particular, it shows that rinsing provokes only a moderate desorption, as also observed for BSA and other substrates. This means that β-LGB was not quickly desorbed during the cleaning test. A detailed comparison between different samples according to their history (heating in the β-LGB solution, treating with supernatant, heating time) may not be made owing to the lack of replicates allowing the significance of differences to be ascertained.

Table 2. Surface chemical composition measured by XPS on stainless steel samples with different treatments as indicated.

Conditioning liquid	Heating	Rinsing	Proportion (%) of elements due to adlayer	Proportion (%) of elements due to substrate	Molar ratios in organic adlayer O_{org}/C_{tot}	Molar ratios in organic adlayer N/C_{tot}	Molar ratios in organic adlayer O_{org}/N
none	none		55.0	45.0	0.22	0.02	12.1
	4 h		57.5	42.5	0.18	0.02	7.8
β-LGB	none	none	95.6	4.4	0.29	0.20	1.5
		twice	80.6	19.4	0.29	0.15	1.9
	0.5 h	none	95.8	4.2	0.28	0.16	1.7
		twice	78.0	22.0	0.25	0.12	2.1
	4 h	none	100.6	-0.6	0.31	0.20	1.5
		twice	103.2	-3.2	0.31	0.19	1.6
supernatant 0.5 h	none	none	98.1	1.9	0.32	0.22	1.5
		twice	94.1	5.9	0.30	0.19	1.6
supernatant 4 h	none	none	82.3	17.7	0.28	0.15	1.8
		twice	76.9	23.1	0.23	0.13	1.9
computed for β-LGB			100.0	0.0	0.30	0.25	1.2

Contact angles and surface cleanability

The water contact angle of the non-conditioned stainless steel (Figure 4B) is higher than expected for chromium and iron oxides constituting the surface, which is attributed to the presence of organic contaminants, as revealed by XPS (Table 2). The slightly higher contact angle measured with the supernatant containing a low concentration of β-LGB in denatured form is difficult to interpret as it characterizes the contact between 4 phases: naked substrate, substrate with adsorbed protein, liquid, and air.

Figure 3A presents the critical detachment radius measured on stainless steel pretreated in different ways: dried at room temperature after soiling with a quartz particles suspension in water and in β-LGB solution, or with a quartz suspension in β-LGB solution heated previously at 75°C for 0.5 or 4 h; dried at 75°C after soiling with a suspension in water or β-LGB solution. Remember that the higher the critical detachment radius the lower the particle adherence. The presence of β-LGB in the quartz suspension decreased the critical detachment radius (b compared to a). This was further decreased when the soiling suspension containing β-LGB was previously preheated 4 h (d) or when the sample soiled with a suspension in β-LGB solution was dried at 75°C (f). Note that protein aggregates were not identified under the microscope. In absence of β-LGB, the enhanced adherence of the quartz particles observed after drying at high temperature compared to room temperature (e compared to a) may be due to a more complete removal of the water, as observed for soiling with starch particles (Detry et al., 2011).

The influence of BSA on contact angle and cleanability of glass (Touré et al., to be submitted) led to emphasize a direct action of the protein at the interface. This did not act by influencing droplet spreading and soiling particles aggregation. It might prevent the formation of tight bonds between glass and silica upon drying or prevent the formation of junctions made of hydrophobic organic contaminants, or which may act as a detergent. While the presence of BSA increased drastically the cleanability of glass, it decreased slightly the cleanability of polystyrene. The presence and the denaturation of β-LGB also tend to decrease slightly the cleanability of stainless steel. An important remark is that stainless steel does not behave as a hydrophilic substrate owing to its surface contamination with organic compounds. Therefore, it appears suitable to examine the influence of the initial surface state of stainless steel on its behavior regarding soiling and cleaning.

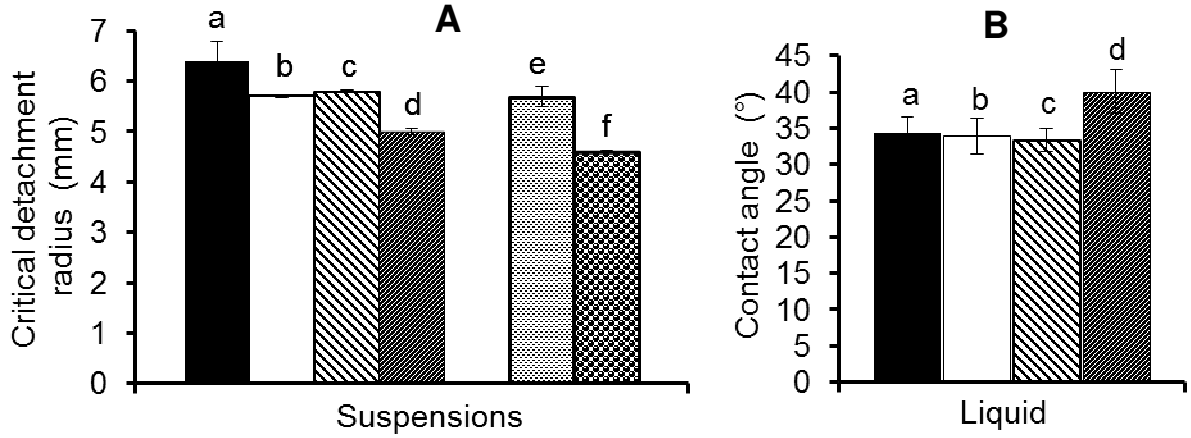

Figure 4. A. Critical detachment radius measured on stainless steel: samples dried at room temperature after soiling with a quartz particles suspension in water (a), in β-LGB solution (b), or with a quartz suspension in β-LGB solution previously heated at 75°C for 0.5 h (c) or 4 h (d); (e) and (f), same as (a) and (b), respectively, except that soiled samples were dried at 75°C. B. Contact angle measured on stainless steel with water (a), native β-LGB solution (b), supernatant collected after heating at 75°C for 0.5 h (c) and 4 h (d).

CONCLUSIONS

The contact of stainless steel with β-LGB present in the soiling quartz particles suspension led to adsorption of the protein, which dominated the composition of the organic layer with respect to contaminants initially present, and was not markedly desorbed upon rinsing.
The presence of β-LGB at the quartz particle/substrate interface slightly increased the adherence, which was further increased when the protein was denatured. Comparison with systems investigated before suggests that the influence of protein via droplet spreading and soiling particles aggregation is of minor importance compared to direct effects on the substrate/quartz interface. It is important to realize that stainless steel does not behave as a hydrophilic substrate owing to its surface contamination with organic compounds. A broader study, including the effect of substrate hydrophobicity, is under way to further elucidate the effect of soluble protein and their denaturation on the particulate soils adhesion.

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REFERENCES

Bansal, B. and Chen X.D. (2009) Fouling of heat exchangers by dairy fluids – a review, *The Berkeley Electronic Press*, **RP2**, 149–57.

Bansal, B. and Chen X.D. (2006) A critical review of milk fouling in heat exchangers, *Science,* **5**(2), 27–33.

Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T. and Delaplace, G. (2012) Analysis by raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger, *J. Food Eng.,* **110**(1), 86–94.

Changani, S.D, Belmar-Beiny, M.T and Fryer, P.J. (1997) Engineering and chemical factors associated with fouling and cleaning in milk processing, *Exp. Them. Fluid Sci.,* **14**(4), 392–406.

Detry, J.G., Sindic, M., Servais M.J., Adriaensen, Y., Derclaye, S., Deroanne, C. and Rouxhet P.G. (2011) Physico-chemical mechanisms governing the adherence of starch granules on materials with different hydrophobicities, *J. Colloid Interface Sci.,* **355**(1), 210–21.

Fickak, A., Al-Raisi, A. and Chen, X.D. (2011) Effect of whey protein concentration on the fouling and cleaning of a heat transfer surface, *J. Food Eng.* **104**(3), 323–31.

Fox, P.F. and Mcsweeney, P.L.H. (1998) Milk proteins, *Dairy Chem. Biochem.,* **1**, 146–264.

Goldstein, A.S. and Dimilla, P.A. (1997) Application of fluid mechanic and kinetic models to characterize mammalian cell detachment in a radial-flow chamber, *Biotechnol. Bioeng.,* **55**(4), 616–29.

Goldstein, A.S. and DiMilla P.A.. (1998) Comparison of converging and diverging radial flow for measuring cell adhesion, *AIChE J.,* **44**(2), 465–73.

McClellan, S.J. and Franses E.I. (2003) Effect of concentration and denaturation on adsorption and surface tension of bovine serum albumin, *Colloids Surf. B. Biointerfaces,* **28**(1), 63–75.

Robbins, P.T., Elliott, B.L., Fryer, P.J., Belmar, M.T. and Hasting, A.P.M. (1999) A comparison of milk and whey fouling in a pilot scale plate heat exchanger: implications for modelling and mechanistic studies, *Food Bioprod. Process,* **77** (C2)(2), 97–106.

Rouxhet, P.G., Misselyn-Bauduin, A.M., Ahimou, F., Genet, M.J., Adriaensen, Y., Desille, T., Bodson P. and Deroanne, C. (2008) XPS analysis of food products: toward chemical functions and molecular compounds, *Surf. Interface Anal.,* **40**(3-4), 718–24.

Rouxhet, P.G. and Genet M.J. (2011) XPS analysis of bio-organic systems, *Surf. Interface Anal.,* **43**(12), 1453–70.

Schmitt, C., Bovay, C. Rouvet, M., Shojaei-Rami, S. and Kolodziejczyk E. (2007) Whey protein soluble aggregates from heating with nacl: physicochemical, interfacial, and foaming properties, *Langmuir Acs J. Surfaces Colloids,* **23**(8), 4155–66.

Stephan, O., Weisz, N., Vieths, S., Weiser, T., Rabe, B. and Vatterott, W. (2004) Protein quantification, sandwich elisa, and real-time pcr used to monitor industrial cleaning procedures for contamination with peanut and celery allergens, *J. AOAC Int.,* **87**(6), 1448–57.

Touré, Y., Rouxhet, P.G. and Sindic, M. (2013) Influence of soluble proteins on the adherence of particulate soils, *Proc. Int. Conf. Heat Exch. Fouling Clean.,* **X**, 285–290.

Touré, Y., Rouxhet, P.G., Dupont-Gillain, C.C. and Sindic, M. (2011) Influence of soluble polysaccharide on the adherence of particulate soils, *Proc. Int. Conf. Heat Exch. Fouling Clean.,* **IX**, 219–26.

Visser, J. and Jeurnink, T.J.M. (1997) Fouling of heat exchangers in the dairy industry, *Exp. Therm. Fluid Sci.,* **14**(4), 407–24.

Walstra, P. and Jenness, R. (1984) Dairy Chemistry and Physics, *John Wiley Sons, New York*, 467 pp.