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STUDY OF WHEY PROTEIN ADSORPTION UNDER TURBULENT FLOW

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ABSTRACT

Reducing fouling in heat exchangers during treatment of milk products is one of the great challenges in the dairy industry. One approach to mitigate fouling is to alter the surface characteristics of the heat exchangers making them less prone to protein adsorption. On this background the European project MODSTEEL was established to study the use of new modified stainless steel surfaces to control fouling of milk components. This part of the project focuses on the adsorption of β-lactoglobulin (β-Lg) from a whey protein solution on unmodified and modified 316 2R stainless steel surfaces by *in situ* ellipsometry under well-defined flow conditions, in the turbulent regime. The effects of temperature, flow rate and surface modification, including SiF_3^+ and MoS_2 ion implantation, DLC (Diamond-Like Carbon) sputtering, and DLC-PlasmaCVD (Chemical Vapour Deposition), were investigated. The amount of protein adsorbed is discussed in relation to the thermal stability of β-Lg, surface properties and hydrodynamic conditions.

INTRODUCTION

Fouling of heat exchangers by dairy products is still an unsolved problem. The formation of a milk deposit can affect the product quality and decrease the process efficiency in terms of decreased heat transfer coefficients and increased pressure drops. Therefore the plant shutdown for cleaning, at least once a day, amounts to considerable costs. Fundamental understanding of the fouling mechanisms and development of practical and efficient methods to avoid or reduce fouling are therefore challenging topics of research. The present work is part of concerted European initiative, MODSTEEL, which was established to modify stainless steel surfaces to reduce fouling of milk components. The approach was to use and develop state-of-the-art material science methods to modify surfaces and in this work we will present the results for SiF_3^+ and MoS_2^{2+} ion implanted, DLC (Diamond-Like Carbon) sputtered, and DLC-PlasmaCVD (Chemical Vapour Deposition) modified surfaces.

β-Lactoglobulin (β–Lg) is the major whey protein and the main constituent in fouling deposits from dairy products. It is commonly present at concentrations of about 3 g L^{-1} in ordinary bovine milk (Walstra and Jenness, 1984). At 25 °C under the pH studied (pH 6.7) β–Lg is present as a dimer of two globular units, each with a molecular weight of 18,000 Da and a diameter of 3.6 nm. The protein has an isoelectric point of 5.2 and therefore carries a negative charge at milk pH (McKenzie, 1971).

Fluid temperature and shear rates are important factors that affect fouling rate. The influence of temperature on β–Lg adsorption onto stainless steel was studied by Kim and Lund (1998) by column adsorption chromatography. The increase in temperature slowed the adsorption rate and led to a higher adsorbed amount. Due to the low protein concentration used (20 μg mL^{-1}) they attributed the effect of temperature on the adsorption kinetics and on conformational changes of monomer and not due to the adsorption of β-Lg aggregates.

To study bulk and surface effects on whey protein fouling Belmar-Beiny et al. (1993) performed fouling experiments in a tubular heat exchanger at different inlet temperatures and Reynolds number in the turbulent region. The total amount of deposition increased with inlet temperature and decreased with the Reynolds number.

The influence of temperature, flow rate and surface modification on the adsorption behaviour of β-Lg was therefore investigated by *in situ* ellipsometry.

The surface characteristics of both unmodified and modified surfaces were determined by contact angle measurements, using the Wilhelmy plate technique, and roughness determination, using AFM, were performed on both. The topography of the stainless steel samples was imaged by AFM.

MATERIALS AND METHODS

Materials

Whey protein isolate (WPI, Lacprodan DI-9224) was provided by Arla Foods (Arla Foods, Ingredients amba, Denmark) and contains 88% (w/w) protein, from which 55% corresponds to β-Lactoglobulin, 0.1% lactose, 0.1% fat, 4% ash, 2.1% minerals, and 5.5% moisture. Alkaline detergent RBS35 (NFT 72151-72190) was obtained from Traitements Chimiques de Surfaces, Lille, France. All other chemicals were of analytical grade. The protein was dissolved in phosphate-buffered saline (PBS) (pH 6.7; 0.01 M phosphate; 0.15 M NaCl, ionic strength 0.17 M) to give a concentration of 5.4 g L^{-1}, corresponding to 3 g L^{-1} of β-Lactoglobulin. The water used was deonized and passed through a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA).

The unmodified surfaces tested were of type 316L with surface finish 2R (cold rolled, heated in a protective atmosphere and skin passed) received from a European manufacturer of stainless steel. The dimensions of the samples were 13 mm diameter and 0.6 mm thickness. The surface modifications consisted of SiF_3^+ and MoS_2^{2+} ion implantation, DLC sputtering and DLC-plasmaCVD, provided by the University of Stuttgart (Germany).

Before and after the adsorption experiments the surfaces were cleaned with the alkaline detergent RBS35. After the adsorption measurement the surfaces were kept in PBS buffer.

Experimental Rig

The rig used for the adsorption experiments is schematically shown in Fig. 1.

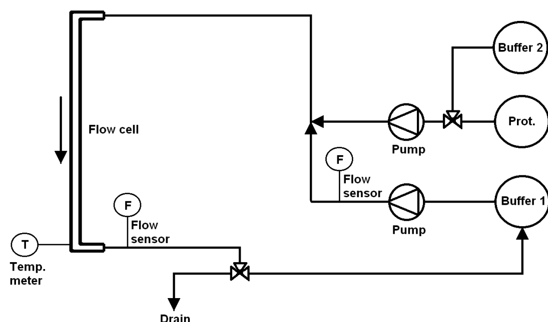

Fig. 1 Schematic diagram of the experimental set-up.

It consists of a flow cell, three solution reservoirs, two pumps, two flow sensors, two valves, a heater and a temperature meter. The set-up development of the flow cell is described by Karlsson, et al. (2001), where further details are given. The buffer 1 and protein reservoirs were made from jacketed glass vessels while the buffer 2 reservoir is a jacketed steel tank. A magnetic drive gear pump (MDG-M15T3-B, Iwaki, Japan) controlled by a frequency converter (SLm 750-1, Scandialogic AB, Sweden) was used for the circulation of the buffer 1 solution. The flow rate was monitored by two flow sensors with fiber optical output (0085 PHF and 0125 PBF, Equflow, The Netherlands) connected to a fiber converter (6700 D/D, Equflow, The Netherlands) and a digital indicator (6200 Flow, Equflow, The Netherlands). The buffer 1 and protein solution were pumped with a diaphragm pump. A three-way valve made of polyethylene was used to switch between the protein and buffer 2. The flow cell was made of stainless steel and has a trapezoidal cross-section. The cross-sectional area is 1.28 cm^2 and the hydraulic diameter 1 cm. The total length of the trapezoidal cross-section is 44 cm, of which 40 cm is upstream of the measuring position that allows the establishment of fully developed flow at the measuring position. Thin optical glass windows were mounted where the light enters and exits the flow cell. The surfaces were placed at the backside wall of the flow cell in a sample holder and were kept in place through a vacuum pump. A window made of Plexiglas in the front of the flow cell allowed inspection of the surface, in order to facilitate alignment and to make sure that no vapour bubbles were trapped during the experiment. A thermocouple (type K of jacket diameter 1.5 mm, Pentronic AB, Sweden) was placed at the outlet of the flow cell. The 3-way valve, made of polypropylene, allowed switching from recirculation of buffer in the beginning of each experiment, when the pure surface was characterised, to single pass of protein solution during the adsorption measurements. Viton tubing was used throughout, except in the outlet of the flow cell where PTFE was used. The inner diameter of the major part of the tubing was 12 mm. The flow cell, the tubing and the steel reservoir were insulated. Temperature control was achieved by circulating water from two heating baths through the reservoir jackets. The buffer 1 and protein reservoir had a volume of 3 L and 5 L, respectively. The buffer 2 reservoir had a capacity of 55 L.

Methods

Ellipsometry. The continuous monitoring of adsorption and desorption of β-Lg on the stainless steel plates was followed by in situ null ellipsometry. The method is based on the fact that polarized light changes its state of polarization when reflected at a surface. A detailed

description of the theory, instrumentation and applications of ellipsometry are given by Azzam and Bashara (1997).

A three-layer model was used to calculate the thickness and refractive index of the adsorbed film, where the three layers are the ambient medium, the protein film, and the stainless steel together with the chromium oxide and modified layer. Based on these parameters the amount adsorbed per unit area was calculated using the two-component formula (Cuypers et al., 1983) where the film is assumed to consist of a substance dispersed or dissolved in a medium. An ambient medium refractive index for PBS buffer of 1.332 and 1.321 was used at 25ºC and 85ºC, respectively. The values of the partial specific volume (ν) and the molar weight to molar refractivity ratio (M/A) used for β-Lg were 0.751 ml g^{-1} and 4.1 g ml^{-1}, respectively. A modified automated Rudolph thin film ellipsometer type 43 603-200E (Rudolph Research, Fairfield, N.J., USA) was used, with a polarized He-Ne laser beam of wavelength 632.8 nm incident at an angle of 68º in relation to a plane normal to the surface. The system allowed measurements every 4 sec. A detailed description of the apparatus is given in Nylander (1987).

The experiments were performed using a single pass of protein solution in a flow cell connected to the ellipsometer. The WPI solution was not recirculated in order to be able to control the residence time of the protein solution at high temperature. The buffer 1 reservoir was filled with 54 L of PBS buffer, the buffer 2 with 2 or 3 L of PBS and the protein reservoir with 2.5 or 5 L of whey protein solution, depending on the adsorption time. The reservoirs were heated prior each experiment by setting the water bath to a determined temperature. For the high temperature experiments the protein solution and buffer 2 reservoirs were heated to 40°C and the buffer 1 to 94°C. The final temperature in the measuring position was 85°C. After aligning the laser beam and adjusting the polarizer and analyser to obtain minimum light intensity, measurements on the bare surface in buffer were started. This allows the determination of the refractive index of the substrate. The obtained refractive index is in reality a “pseudo refractive” index as it includes the presence of oxide layers as well as the modified surface layer on the steel plates. When the ellipsometric parameters from the bare surface were stable, the valve from the buffer 2 reservoir was opened and the three-way valve was turned from recirculation to one pass flow. When the temperature at the outlet of the flow cell was constant the adsorption was started by turning the valve to the protein reservoir, giving a final protein concentration of 0.3 mg ml^{-1}. After a certain adsorption time, the WPI solution was replaced with PBS by turning the valve again to the buffer 2 reservoir. The WPI solution was not recirculated to eliminate the possibility of fouling from material that had already passed through the systems.

Wilhelmy plate technique. Contact angle measurements were performed using the Wilhelmy plate technique. The stainless steel plates were suspended from the microbalance of a DST 9005 Dynamic Surface Tensiometer (Nima Technology, Coventry, UK) and the force, F(x), acting on the plate, with perimeter, P, versus depth of immersion, x, in pure water was recorded. The plate was moved at a constant speed of 2 mm min^{-1} and three immersion-retraction cycles were performed. The advancing and receding contact angle were calculated from the force F(x), using $F(x)/P = \gamma_L \cos \theta$, where γ_L is the water surface tension (72 mN m^{-1}) and θ is the water contact angle.

Atomic force microscopy. The roughness parameters, root mean square (RMS) and average roughness (Ra), of the different steel surfaces 316 2R were measured by atomic force microscopy in contact mode under air. At least 5 readings were taken for each surface tested. The Nanoscope® III AFM was equipped with an ultrasharp silicon cantilever (MikroMasch). The cantilever used for most of the surfaces has a length of 290 μm, a width of 40 μm, a resonant frequency of 15 kHz and a force constant of 0.12 N/m. The reflective side is coated with Aluminium. The tip has a radius of curvature less than 10 nm and a height between 15 and 20 μm. The force applied to the cantilever was kept constant at 50 nm.

RESULTS

Surface Characterization

Contact angle measurements. The surface modification techniques influenced the wettability of the stainless steel surfaces as apparent from Table 1. The MoS_2 implantation and DLC sputtering produced a more hydrophilic and hydrophobic surfaces, respectively, compared to the bare steel. The contact angle hysteresis, defined as the difference between the advancing and receding contact angle, is known to be influenced by the chemical (heterogeneity) and physical (roughness) nature of the solid surface. The modified surfaces tested, except the DLC sputtered, showed lower hysteresis values.

Roughness measurements. In general the surface roughness was not significantly affected by the modification methods. The ion implanted surfaces (SiF, and MoS_2), however exhibited similar and slightly lower RMS and Ra values compared to the unmodified surface (Table 2).

Topography. The unmodified surface 316 2R (Fig. 2 (a)) have a unidirectional surface topography with some surface defects. The modified surfaces MoS_2 and

Table 1. Water contact angle values, advancing and receding, and contact angle hysteresis (in degrees) for the unmodified and modified surfaces, measured by Wilhelmy plate method.

Samples	Adv.	Rec.	Hyst.
Unmodified	69 ± 1	14 ± 1	55
SiF Implanted	61 ± 10	22 ± 5	39
MoS_2 Implanted	49 ± 9	12 ± 5	37
DLC sputtered	84 ± 3	19 ± 5	65
DLC-plasmaCVD	65 ± 1	17 ± 1	48

Table 2- Roughness values for the unmodified and modified 316 2R steel surfaces, measured by atomic force microscopy.

Samples	RMS (nm)	Ra (nm)
Unmodified	38 ± 3	30 ± 2
SiF implanted	31 ± 3	24 ± 3
MoS_2 implanted	31 ± 3	25 ± 3
DLC sputtered	42 ± 2	30 ± 1
DLC-plasmaCVD	36 ± 6	28 ± 5

DLC-plasmaCVD 316 2R (Fig. 2 (c, e)) showed similar topographies compared to the unmodified one. Both SiF implanted and DLC sputtered exhibited a more heterogeneous topography featuring particles with a diameter larger than 100 nm. The surface profiles (Fig. 2) show similar patterns for the different surfaces.

Adsorption Behaviour

The results presented are the mean values of at least two experiments. All examples shown are from representative experiments.

Effect of temperature. The adsorption behaviour of β–Lg at stainless steel surface at 25 and 85 °C was investigated and the results are shown in Fig. 3. The adsorbed amount versus time 25°C reaches a plateau before rinsing with PBS. The adsorbed amount is much higher (by a factor of 7) at 85°C and increases with time without reaching a plateau value. At room temperature 14% of protein was desorbed from the surface during rinsing with protein free buffer solution, whereas at 85°C no desorption was observed (Fig. 3).

Effect of flow rate. The protein solution was pumped through the flow cell at flow rates of 1, 3 and 4.5 L min^{-1}, corresponding to Reynolds number of 3 800, 11 300 and 17 100, respectively. The adsorption rates were calculated by fitting a straight line to the adsorption curve for the first 30 seconds (initial rate) and between 3 and 7 min from the start (final rate). For the early stages of adsorption the rate at which the protein adsorbed at the surface increased with the Reynolds number. This increase was more pronounced in the turbulent regime (Table 3). An increase in the Reynolds number in the turbulent regime did not influence the final adsorption rate or the amount adsorbed at 7 min. When the flow changed from transition to turbulent regime increased of both, the final adsorption rate and adsorbed amount, was observed (Fig. 4, Table 3).

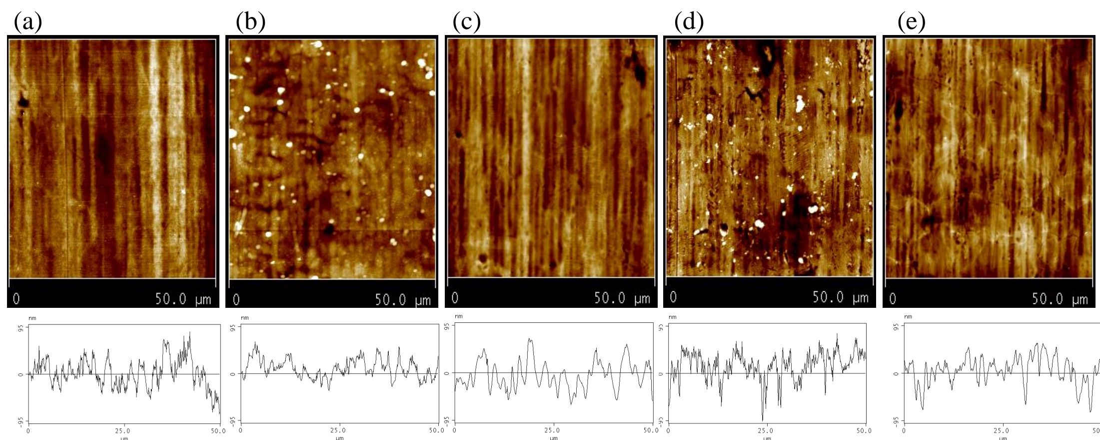

Fig. 2 AFM height images and profiles of the surfaces 316 2R: (a) Unmodified; (b) SiF ion implantation; (c) MoS_2 ion implantation; (d) DLC sputtered; and (e) DLC-plasmaCVD.

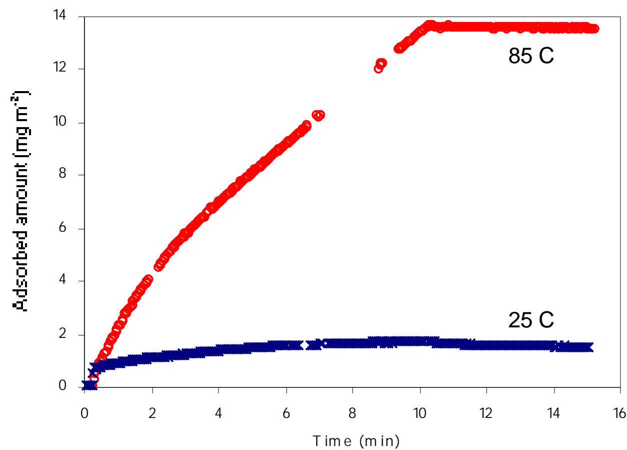

Fig. 3 Adsorption curves for β–Lg on 316 2R steel surfaces at different temperatures. β–Lg was adsorbed from a 0.3 g L^{-1} solution in PBS at pH 6.7. The adsorption was recorded for 10 min, with a Reynolds number of 11 300 (85°C) or 4400 (25°C), followed by rinsing with PBS for 5 min.

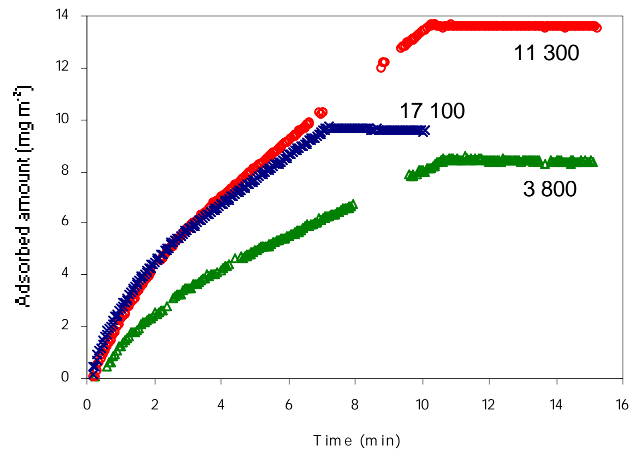

Fig. 4 Adsorption curves for β–Lg on 316 2R steel surfaces at different flow rates. β–Lg was adsorbed from a 0.3 g L^{-1} solution in PBS at pH 6.7 and at a temperature of 85°C. The adsorption was recorded for 10 min, or 7 min for the higher Reynolds number, followed by rinsing with PBS for 5 min.

Table 3- Adsorption rates for the initial and final stages of adsorption and adsorbed amounts at 7 min on the 316 2R surface, for different Reynolds number.

Reynolds number	Adsorption rate ($mg\ m^{-2}\ min^{-1}$) Initial[a]	Final[b]	Adsorbed amount ($mg\ m^{-2}$)
3 800	2.21	0.70	6.06 ± 0.06
11 300	2.92	1.02	9.87 ± 0.29
17 100	4.28	0.99	10.03 ± 0.51

[a] Deviation from the mean less that 0.6 mg m^{-2} min^{-1}
[b] Deviation from the mean less that 0.04 mg m^{-2} min^{-1}

For the DLC sputtered samples the adsorption experiment was also performed for turbulent flow with the two Reynolds number. The differing flow conditions in this case did influence the adsorption (Fig. 5), where a similar behavior was observed for the DLC and unmodified surfaces at a Reynolds number of 17 100. Since the adsorption on the DLC surface at a Reynolds number of 17 100 was performed with an interval of more than one month from the adsorption experiment at a Reynolds number of 11 300, the properties of the DLC surface might have changed during that period of time. In order to compare the adsorption behavior for the new and fouled surface, the experiment was repeated for the later surface with the Reynolds number of 11 300. The adsorption curves for the new and fouled

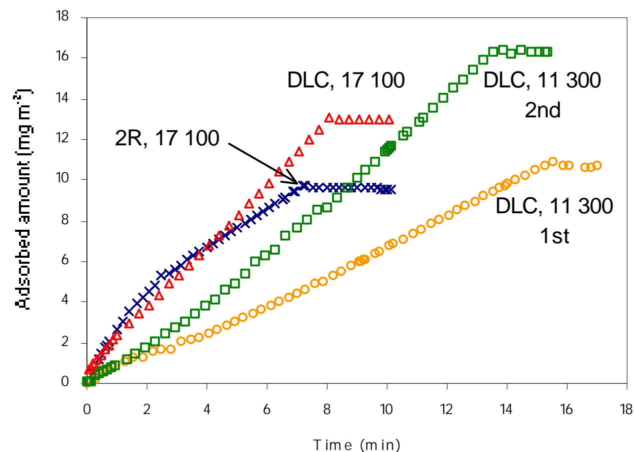

Fig. 5 Adsorption curves for β–Lg on 316 2R and DLC sputtered surfaces at different flow rates. β–Lg was adsorbed from a 0.3 g L^{-1} solution in PBS at pH 6.7 and at a temperature of 85°C. After adsorption was recorded during different times, rinsing with PBS was started.

surface had a similar behavior for the first minutes but differed for the remaining time, with twice the adsorption rate and adsorbed amount (Fig. 5).

Effect of surface properties. The effect of surface modification on protein adsorption was studied at a flow rate of 3 L min^{-1} and a temperature of 85°C. From the adsorption curves in Fig. 6 it can be concluded that the surface modification indeed affects the amount of proteins adsorbed. The adsorption kinetics were calculated for the first 30 seconds (initial) and between 3 and 15 min (final) and the results are presented in Table 4, where the adsorbed amount recorded after 15 min adsorption is also included. During the first minutes the adsorption was faster to the MoS_2 surface and slower to both DLC sputtered and DLC-plasmaCVD compared with the adsorption to the unmodified steel. The adsorbed amount after 15 minutes was also higher at the MoS_2 surface and lower for the other surfaces compared to the unmodified stainless steel surface. The final adsorption rate was not significantly influenced by the different surfaces. The SiF surface exhibited a slightly lower initial adsorption rate and adsorbed amount after 15 minutes (Table 4).

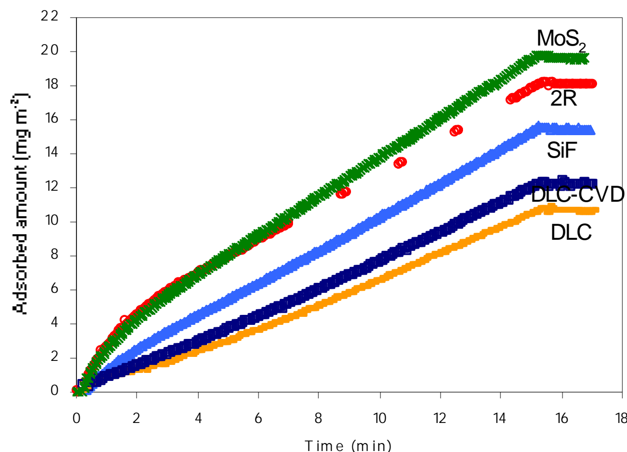

Fig. 6 Adsorption curves for β–Lg on unmodified and modified 316 2R surfaces at a Reynolds number of 11 300. β–Lg was adsorbed from a 0.3 g L^{-1} solution in PBS at pH 6.7 and at a temperature of 85°C. The adsorption was recorded for 15 min, followed by rinsing with PBS for 2 min.

Table 4- Adsorption rates for the initial and final stages of adsorption and final adsorbed amounts, for the surfaces tested.

Samples	Adsorption rate ($mg\ m^{-2}\ min^{-1}$)		Adsorbed amount ($mg\ m^{-2}$)
	Initial[a]	Final[b]	
316 2R	2.92	1.02	18.28 ± 0.37
SiF implanted	2.25	0.94	14.57 ± 0.95
MoS_2 implanted	4.42	1.12	19.42 ± 0.38
DLC sputtered	1.24	0.76	11.01 ± 0.24
DLC-plasmaCVD	0.81[c]	0.78	13.34 ± 1.07

[a] Deviation from the mean less that 0.4 mg m^{-2} min^{-1}
[b] Deviation from the mean less that 0.04 mg m^{-2} min^{-1}
[c] Deviation from the mean of 0.32 mg m^{-2} min^{-1}

DISCUSSION

No correlation was found between the contact angles and the surface roughness. This can be due to the low roughness values measured on these surfaces. The same was observed by Busscher et al. (1984) who found no systematic dependence of contact angles on surface roughness for polymer surfaces with Ra < 0.1 µm. The lower contact angle hysteresis determined on the modified surfaces (except for the DLC surface) is probably due to the chemical nature of the solid since the surface roughness was not significantly affected by the modification techniques.

Although the surface free energy of the solid is known to be one factor that influences protein adsorption, no simple relation between surface free energy and protein adsorption is apparent. Some researchers found higher adsorption rates and amounts of protein adsorbed at low energy surfaces (Yoon and Lund, 1994; Arnebrant and Nylander, 1986; Krisdhasima et al., 1992; Santos et al., 2001), while others reported similar rate and extent of protein adsorption at surfaces differing in surface energy (Adesso and Lund, 1997). In this work a higher surface energy (lower water contact angles) resulted in higher adsorbed amounts of protein (Fig. 7). For the adsorption rates this trend was not consistent.

The protein adsorbs at the bare surface in a conformation that depends on the temperature. During the first minutes of adsorption the native protein, present at room temperature in the form of monomers or dimers, or the denatured or/and aggregated protein, formed at the higher temperature, will adsorb at the surface. The slightly slower initial adsorption rate at 85°C can be due to competition between aggregation in bulk and adsorption at the surface.

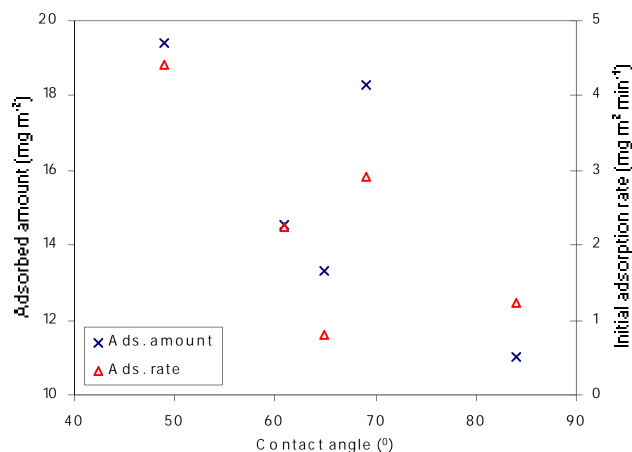

Fig. 7 Water contact angle versus adsorbed amount of β–Lg after 15 min adsorption and initial adsorption rate on the surfaces tested. The Reynolds number was 11 300 and the temperature 85°C.

The faster adsorption rate at 85°C for the later stages of adsorption is caused by the continuous build up of β–Lg aggregates at the surface, leading also to a higher adsorbed amount. This temperature dependence of the adsorption curve has also been observed in previous studies. (Kim and Lund, 1998; Elofsson et al., 1996). An increase in temperature not only causes conformational changes of the protein but also decreases the solid surface energy (McGuire and Sprout, 1990). Since higher adsorbed amounts were found at the higher temperature and lower surface free energy (Fig.7), the effect of temperature on the thermal instability of the protein is the dominating factor, rather than the effect on the surface free energy. The adsorbed amount reached after 10 min at 25°C was 1.7 mg m^{-2}, corresponding to less than monolayer coverage of monomers or dimers of β–Lg adsorbed side-on, which amounts to 2.7 mg m^{-2}. However, taking into account the parking limit (i.e., the fraction of an area that can be occupied by objects of one size randomly placed on a surface) a value of about 1.4 mg m^{-2} would be a more realistic value for a monolayer of β–Lg monomers (Nylander, 1998). In this case the value 1.7 mg m^{-2} would correspond to a monolayer of β–Lg adsorbed side-on. At 85°C a high adsorbed amount was obtained (13.1 mg m^{-2}) corresponding to the formation of multilayers of protein. At this temperature no desorption was observed during rinsing suggesting that the adsorption occurred in an irreversible way. Due to the larger size of the aggregates they posses a higher number of binding sites compared to the monomer, making them difficult to remove from the surface. The tendency of the protein to desorb from the surface seems to be also dependent on the contact time, where longer adsorption times can cause conformational or orientational changes of the protein leading to little or no desorption. Such conformational changes can lead to intermolecular interactions and bonds, e.g. disulphide bridges, which are bond to be strong within the adsorbed layer. In a previous study where the adsorption was carried out for 30 minutes no desorption was observed either at 25°C or 85°C (Karlsson et al., 1999).

The Reynolds number of 3 800, 11 300 and 17 100 corresponds to the residence times of 37, 12 and 8 seconds, respectively. A lower flow rate corresponds to a longer residence time. It has been shown that β–Lg conformational changes are time dependent, these changes are therefore expected to be more extensive at a lower flow rate. This would in turn lead to a more surface active protein with a higher tendency to adsorb. The decrease of the initial adsorption rate with the flow rate suggests that other factors are involved (Table 3). Lower flow rates correspond to a lower mass transfer coefficient and less amount of protein is flowing pass the surface. For the lowest flow rate the subsequent protein layers are therefore expected to build up at lower rate. At higher flow rates the probability of collisions between the denatured protein molecules, leading to aggregate formation in the bulk, and between these aggregates and the surface are higher, increasing the rate and extent of adsorption. On the other hand, the shorter protein residence time will give fewer unfolded protein molecules and consequently leads to a lower amount of aggregates at the surface. In addition the higher shear stress at the deposit/fluid interface can also remove the already adsorbed aggregates. The similar rate and extent of adsorption for both turbulent flow rates, suggests that these effects compensate each other. For the DLC sputtered surfaces the higher rate and extent of adsorption obtained at the higher Reynolds number (Fig. 5), reflects the role of the influence of the surface properties. The difference between the new and reused samples can be due to the cleaning procedure, which either is not efficient enough to remove the foulant from the surface or changes the surface property of the material. Protein adsorption has previously been reported to be different on new compared to reused stainless steel surfaces by Karlsson et al. (1999). In previous studies a decrease in the total amount of whey protein fouling with an increase in the Reynolds number in the turbulent region was observed by Belmar-Beiny et al. (1993). The influence on the adsorption behaviour of the flow rate in the turbulent region, was also found to be small, whereas the initial adsorption was considerably slower in the laminar region (Karlsson, et al., 2001). They concluded that in the laminar region the initial adsorption rate was controlled by a

combination of mass transfer limitations and adsorption kinetics.

The final adsorption rate was similar for the modified surfaces (Table 4), suggesting that the deposit growth depends mainly on interactions between the protein in the bulk and the deposits building up on the surfaces. The slightly lower adsorption rate observed for the DLC sputtered and DLC-plasmaCVD can be due to a different conformation of the protein in the first layers making the subsequent deposition less favourable.

In the present study we try to mimic the relevant conditions in a typical commercial dairy plant as far as possible. As our experimental cell works under atmospheric pressure it is however not possible to reach the UHT treatment temperatures (typically 140°C). We have therefore chosen 85°C, which above the unfolding temperature of the whey proteins that is bound to be the most important factor. At normal pasteurisation of milk (72-75°C), protein deposit is not such large problem. The wall of the equipment and the surface to be tested were not heated directly although they were heated during buffer recirculation. However in modern heat exchangers the temperature difference between the wall and the solution is minimized, amounting to a degree or less. Regarding the residence time used in the present study, 8-37 s, they are in the same range as used during pasteurisation (15-20 s) and slightly longer than the residence time during UHT treatment (4 s).

CONCLUSIONS

In this study the influence of the wettability, surface roughness and surface modification of stainless steel samples on the adsorption behaviour of β–Lg was evaluated. The main conclusions can be summarised as follows.

1. The modification techniques did not affect the roughness or the topography of the bare steel but did alter their surface energy.
2. Both surface and bulk conditions affected the adsorption behavior of β–Lg and strong effect of temperature was observed.
3. The DLC sputtered surface showed to be most promising in terms of reducing protein adsorption, however their surface properties were altered after cleaning.

NOMENCLATURE

β–Lg β–Lactoglobulin
WPI Whey Protein Isolate
DLC Diamond Like Carbon
CVD Chemical Vapour Deposition
PBS Phosphate Buffered Saline

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REFERENCES

Azzam, R.M.A., and Bashara, N.M., 1977, *Ellipsometry and Polarized Light*, North Holland, Amsterdam.

Arnebrant, T., and Nylander, T., 1986, Sequential and Competitive Adsorption of β–Lactoglobulin and κ–Casein on Metal Surfaces, *J. Colloid Interface Sci*., Vol. 111, No. 2, pp. 529-533.

Addesso, A., and Lund, D.B., 1997, Influence of Solid Surface Energy on Protein Adsorption, *J. Food Proc. and Preserv*., Vol. 21, pp. 319-333.

Belmar-Beiny, M. T., Gotham, S. M., Paterson, W. R., and Fryer, P. J., 1993, The effect of Reynolds number and fluid temperature in whey protein fouling, *J. Food Engineering*, Vol. 19, pp. 119-139.

Busscher, H. J., Van Pelt, A. W. J., De Boer, P., De Jong, H. P., Arends, J., 1984, The effect of surface roughening of polymers on measured contact angles of liquids, *Colloids and Surfaces*, Vol. 9, pp. 319-331.

Cuypers, P.A., Corsel, J.W.W., Jansen, M.P., Kop, J.M.M., Hermens, W.Th., and Hemker, H.C., 1983, The Adsorption of Prothrombin to Phosphstidylserine Multilayers Quantitated by Ellipsometry, *J. Biol. Chem*., Vol. 258, No. 4, pp. 2426-2431.

Elofsson, U.M., Paulsson, M.A., Sellers, P., and Arnebrant, T., 1996, Adsorption during heat treatment related to the thermal unfolding/aggregation of β–Lactoglobulins A and B, *J. Colloid Interface Sci.*, Vol. 183, pp. 408-415.

Karlsson, C. A.-C, Wahlgren, M. C., and Trägårdh, A. C., 2001, Non-invasive monitoring of protein adsorption and removal in a turbulent flow cell, *Colloids and Surfaces B: Biointerfaces*, Vol. 20, pp. 9-25.

Karlsson, C. A.-C, Wahlgren, M. C., and Trägårdh, A. C., 1999, Some surface related aspects of the cleaning of new and reused stainless steel surfaces fouled by protein, *Int. Dairy J.*, Vol. 8, pp. 925-933.

Krisdhasima, V., McGuire, J., and Sproull, R., 1992, Surface Hydrophobic Influences on β–Lactoglobulin Adsorption Kinetics, *J. Colloid Interface Sci*., Vol. 154, No. 2, pp. 337-350.

Kim, J.C., and Lund, D.B., 1998, Milk Protein/Stainless Steel Interaction Relevant to the Initial Stage of Fouling in

Thermal Processing, *J. Food Process Eng.*, Vol. 21, pp. 369-386.

McKenzie, H.A., 1971, *Milk Protein - Chemistry and Molecular Biology*, Academic Press, New York, pp. 257.

Nylander, T., 1987, Proteins at the Metal / Water Interface- Adsorption and Solution Behaviour, Ph. D. Dissertation, Dept. of Food Technology, Lund University, Lund, Sweden.

Nylander, T., 1998, Protein Adsorption in Relation to Solution Association and Aggregation, *in* Biopolymers at Interfaces, Malmsten, M., Marcel Dekker, New York, pp. 409-452.

Santos, O., Nylander, T., Paulsson, M., and Trägårdh, C., 2001, Adsorption behaviour of β–Lactoglobulin on different types of stainless steel surfaces, *Proc. of the Heat exchanger fouling, Fundamental Approaches & Technical Solutions Conference 2000*, Davos, Switzerland, 8-13 July.

Walstra, P., and Jenness, R., 1984, *Dairy Chemistry and Physics*, Wiley, New York.

Yoon, J., and Lund, D.B., 1994, Magnetic Treatment of Milk and Surface Treatment of Plate Heat Exchangers: Effects on Milk Fouling, *J. Food Science*, Vol. 59, No. 5, pp.964-980.