Protein–calcium phosphate interactions in fouling of modified stainless-steel surfaces by simulated milk

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Abstract

The deposition behaviour of the main components of milk fouling (β-lactoglobulin and calcium phosphate) and its dependence on the surface energy properties of the fouling support were evaluated in this work. As deposition supports, several modified stainless-steel materials obtained by surface modification techniques were used. Four fouling systems were analysed: an aqueous solution that resembles the mineral composition of milk (simulated milk ultrafiltrate, SMUF), at 44 and 70 °C, and SMUF with addition of β-lactoglobulin at 50 and 85 °C. Both the final amount of deposits formed for each system on the different surfaces and the type of aggregates formed during the first stages of the process for each case were assessed. The number and size of the calcium phosphate aggregates initially formed can be used as a predictive approach to the final amount of deposit developed for each system since relationships between these parameters were established.
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1. Introduction

The minimization of the effects of fouling caused by milk during heat treatment has been one of the main challenges for researchers in the dairy field. Several techniques to modify the surface of stainless steels, the most common type of material used in process equipment in the food industry, have been developed recently with the goal of reducing the buildup of unwanted deposits in pasteurizers and sterilizers in dairy industry (Santos et al., 2004).

Milk fouling research has established clearly that calcium phosphate and whey protein are the main components of milk deposits (Jeurnink, Walstra, & de Kruif, 1996), the former predominating at lower temperatures. Literature on the building up of calcium phosphate layers from strictly mineral solution is abundant (House, 1999; Lu & Leng, 2005; Liu, Wu, Sethuraman, & Nancollas, 1997; Rosmaninho & Melo, 2006). Additionally, several studies have been carried out in recent times

concerning the deposition behaviour of whey proteins under different temperatures (Santos, Nylander, Paulsson, & Tragardh, 2006a; Santos, Nylander, Schillen, Paulsson, & Tragardh, 2006b), concentrations and denaturation ratios (Elofsson, Paulsson, Sellers, & Arnebrant, 1996). Also the effects of calcium (Christian, Changani, & Fryer, 2002) and phosphorous addition (Christian et al., 2002; Elofsson, Paulsson, & Arnebrant, 1997) and its relationship to the amount and rate of protein fouling has been studied. Some studies evaluated the balanced importance of surface and bulk liquid effects on protein deposition (Belmarbeiny, Gotham, Paterson, & Fryer, 1993). Morison and Tie (2002) added different protein concentrations to simulated milk ultrafiltrate (SMUF) and studied its effect on the amount of fouling. The effect of surface energy on the deposition behaviour of proteins or calcium phosphate has been studied as a possible guide for developing an anti-fouling strategy (Andritsos, Yiantsios, & Karabelas, 2002; Santos et al., 2006b) with no effective overall conclusions. However, no work has been reported yet on the combined effect of whey proteins and the nature of the deposition solid surfaces on the fouling behaviour of calcium

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phosphate under different temperatures. This situation occurs frequently when deposits are basically mineral and their growth and cleaning features are expected to be affected by the incorporation of whey protein aggregates in the fouling layer. Therefore, the present work looks at the interactions between whey protein and calcium phosphate deposition in cases where the fouling layer is of predominantly mineral nature. Special attention is also given to the role of the deposition surface (novel modified stainless-steel surfaces) since the latter is expected to affect the structure of the first layer attached and thereby to determine the overall development of the deposit.

2. Materials and methods

The experimental work was composed of three stages: (i) the characterization of the different stainless-steel surfaces, (ii) quantification of the amount of calcium phosphate deposit formed on the different surfaces at different temperatures and in the presence or absence of protein, and (iii) characterization of the initial stages of deposition and its connection to the surface properties and overall fouling behaviour.

2.1. Characterization of the stainless-steel surfaces

To evaluate the influence of the surface energy of the surfaces on the deposition process, different stainless-steel surfaces with similar roughness and a wide range of surface energy values were tested. The surfaces were all stainless-steel 316 2R (bright annealed) based which were subjected to several types of surface modification processes able to change their surface energy (Santos et al., 2004):

- *Turbulent ion implantation*: MoS_2 (molybdenum disulfide) ions were imbedded in the surface to a depth of approximately $0.2\,\mu m$ (produced at the University of Stuttgart, Germany).
- *Coating by a sol–gel technique*: stainless-steel surfaces were coated by a silica layer (produced at CPERI, Thessaloniki, Greece).
- *Coating by plasma chemical vapor deposition*: stainless-steel surfaces were coated by SiO_x and DLC–Si–O (DLC: diamond-like carbon) layers (produced at the University of Stuttgart, Germany).
- *Autocatalytic Ni–P–PTFE (nickel–phosphor–poly-tetra-fluorethylene) coating*: stainless-steel surfaces were coated by a Ni–P matrix where PTFE particles were fixed in a proportion of about 30% (manufactured by Novoplan, Aalen, Germany).

2.2. Contact angle measurements and surface energy determination

Contact angle values were measured by the sessile drop method in a contact angle meter (DataPhysics OCA15 Plus, DataPhysics Instruments GmbH, Filderstadt, Germany) using water, formamide and α-bromonaphthalene as reference liquids. The contact angles were measured automatically using an image-analysing system and were used to evaluate the surface characteristics of a solid in terms of its surface energy (γ, $mJ\,m^{-2}$) according to the approach of van Oss (1994). In this approach, the total surface energy of a solid or a liquid (γ^{TOT}) is obtained by the sum of an apolar Lifshitz-van der Waals component (γ^{LW}) and an acid–base polar component (γ^{AB})

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB}. \tag{1}$$

In turn, the γ^{AB} is divided into two non-additive parameters, the γ^- (electron donor component) and the γ^+ (the electron acceptor component) (van Oss, 1994)

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-}. \tag{2}$$

2.3. Calcium phosphate deposition with and without milk proteins

SMUF is an aqueous solution that simulates the mineral composition of milk and was used to evaluate calcium phosphate deposition. The solution was prepared according to Jenness and Koops (1962), with the pH value adjusted to 6.80 using diluted NaOH, and was kept overnight at $5\,°C$ before use. Another aqueous solution used was prepared by adding a commercial whey protein isolate (WPI, Lacprodan DI-9224, provided by Arla Foods Ingredients, Viby, Denmark) to the SMUF solution in order to obtain $3\,g\,L^{-1}$ of β-lactoglobulin (β-lg). This solution was named modified SMUF (MOD-SMUF) and its pH was also adjusted to 6.80. The deposition behaviour of these solutions was evaluated at two temperatures each, forming four systems: SMUF at $44\,°C$ and at $70\,°C$, MOD-SMUF at $50\,°C$ and at $85\,°C$. Each experiment was repeated three times, the amount of deposit formed is given as the average of the three experiments.

2.4. Rotating disk apparatus

The rotating disk apparatus is an apparatus composed of a hollow cone containing heated silicone oil that can freely rotate in the fouling solution contained in a cylindrical thermostatically (Rosmaninho & Melo, 2006). This apparatus was used to study the deposition of calcium phosphate onto stainless-steel surfaces (having a circular shape with $90\,mm$ diameter) under controlled hydrodynamics. The stainless-steel samples used as deposition surfaces were attached to the bottom of the heated cone by using a double-sided tape. The surface of the deposition sample was kept at a temperature approximately $1\,°C$ higher than the temperature of the SMUF and MOD-SMUF solutions.

2.5. Deposition experiments

The deposition experiments started by introducing the SMUF or the MOD-SMUF solution in the vessel of the