

## An Analysis of Milk Fouling Formed During Heat Treatment on a Stainless Steel Surface with Different Degrees of Roughness

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### Abstract

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Results of research on the effect of stainless steel (SS) surface roughness on the amount and microscopic structure of milk impurities, formed under the influence of high-temperature milk processing are presented. Three types of plates of different roughness were used in the study:  $R_a = 0.028 \mu\text{m}$ ;  $R_a = 0.174 \mu\text{m}$ ;  $R_a = 0.445 \mu\text{m}$ . The plates were immersed in raw milk and heated at 85–90°C for 30 min, imitating pasteurisation conditions. As a result of this action, a milk sediment difficult to remove was created. The structure of impurities was determined by the Confocal Scanning Laser Microscope CSLM method. The analysis of the microscopic structure of formed milk impurities enabled their classification into three types depending on their structure and way of their bonding to the surface. The research results suggested that the roughness plays a prominent role in the level of fouling and probably in cleaning effectiveness.

**Keywords:** pollutions; surface area; roughness; milk thermal treatment

Fouling layers, formed during the thermal treatment of milk and milk products, are a severe problem for the dairy industry. Aggregated deposits negatively influence the heat exchange between flowing media, increase flow resistance and cause the corrosion of the equipment such as heat exchangers. The occurrence of milk fouling has also negatively influenced the stringent requirements for quality and hygiene in dairy processes (VISSE & JEURNINK 1997; AUGUSTIN *et al.* 2007). Depositions formed on heated surfaces depend on bulk and surface processes. The deposition is the result of a number of stages: denaturation and aggregation of proteins in the bulk, transport of the aggregated proteins to the surface, surface reactions resulting in the incorporation of protein into the deposit layer, and possible re-entrainment or removal of deposits (BANSAL & CHEN 2005). In general, milk fouling can be classified into two categories known as type A and type B. Type A is typical protein fouling which is formed at temperatures between 75°C and 110°C. The deposits are white, soft, and spongy, and their composition is 50–70% proteins, 30–40% minerals, and 4–8% fat. Type B is mineral fouling (known as milk

stone) and it takes place at temperatures above 110°C. Those deposits are hard, compact, granular in structure, and grey in colour, and their composition is 70–80% minerals, 15–20% proteins, and 4–8% fat (SANTOS *et al.* 2003; BANSAL & CHEN 2005; AUGUSTIN *et al.* 2007). The exact mechanisms and reactions between different milk components are not yet fully understood. Many authors established that the dominant protein in heat-induced fouling is  $\beta$ -lactoglobulin ( $\beta$ -Lg), which has globular nature and is heat sensitive. Upon heating of milk, the native protein  $\beta$ -Lg denatures and exposes the core containing reactive sulphhydryl groups. The denatured or unfolded protein molecules then react with similar or other types of protein molecules such as casein and  $\alpha$ -La and form aggregates (JEURNINK & KRUIF 1995; VISSE & JEURNINK 1997; BANSAL & CHEN 2005). The rate of fouling may be different for the denatured and aggregated proteins.

The fouling builds up fast and is usually difficult to remove with using standard procedures. Many researchers paid attention to various factors which influence the build-up of milk fouling. GRANT *et al.* (1996), GEORGIADIS and MACCHIETTO (2000), and

MAHDI *et al.* (2009) have shown that the thickness and the time of milk layer formation depend on: the concentration of proteins contained in the milk layer, heat treatment of milk, the concentration of dissolved calcium and phosphorus, as well as flow conditions, and the time of the milk contact with the hot surface. Hence, other researchers focus on factors which will minimize the build-up of milk fouling. It has showed that the amount of fouling is determined by the type of construction material used to manufacture the exchangers. BEUF *et al.* (2003) and ROSMANINHO *et al.* (2007) showed that the type of construction material, its finish, microstructure, charge and surface energy, as well as the presence of layers, significantly affect the rate of milk sediment accumulation. However, it has also been speculated that the limitation of the sediment layer formation can be affected by the modification of interphase interactions between the heat exchange surface and the sediment (AUGUSTIN *et al.* 2007). This can be done in two ways: by modifying the surface energy which is the decisive criterion for the suitability of the material for coating; and by modifying the geometrical properties of the heat surface.

Among the different parameters characterising a surface, one of the most important roles in maintaining hygiene is played by surface topography. The topography of the surface is given by its roughness, porosity and any irregularities. HOLAH and THORPE (1990) indicated that the surface must be smooth, continuous and free of cracks, crevices, and cavities, since these are critical for the maintenance of hygiene. The microstructure of construction materials not only has an impact on the effective removal of post-production impurities, but also initiates the process of their formation. General recommendations for construction materials used in contact with food can be found in European Regulations (EC) No. 852/2004 and No. 1935/2004. Recommendations for the surface finish and its roughness are included in European Hygienic Engineering & Design Group (EHEDG 2004, 2007). The authors propose the use of an optimal surface roughness, not exceeding  $R_a = 0.8 \mu\text{m}$ . They suggest that this is a sufficiently smooth surface that does not cause any accumulation of impurities and can be easily cleaned and maintained (HOLAH 2000; LELIEVELD *et al.* 2003; TAMIME 2008). Many authors studied the influence of the surface type on soil contamination and cleanability (SANTOS *et al.* 2003; ROSMANINHO *et al.* 2007; GORDON *et al.* 2014). A comprehensive overview of previous research on this subject was presented by DETRY *et al.* (2010). The

tests were aimed at the influence of surface energy, chemical composition, and topography such as surface roughness on the build-up of milk fouling. KARLSSON *et al.* (1998) studied the fouling and cleaning behaviour of both 304 and 316 stainless surfaces of  $R_a < 0.1 \mu\text{m}$  toward  $\beta$ -lactoglobulin. No differences were reported in terms of fouling and cleaning between polished and unpolished surfaces. JULLIEN *et al.* (2002) studied stainless steels with different surface finishes (2B and 2R corresponding to  $R_a$ : 0.1–0.5 and 0.005–0.1  $\mu\text{m}$ ) soiled with *Bacillus cereus* and they did not observe any differences in bacterial contamination or cleanliness between the tested surfaces. However, another research suggests that the surface roughness does indeed influence soil adsorption and hence cleanliness. LECLERC-PERLAT and LALANDE (1994) observed a difference in cleanability toward yogurt contaminated with spores of *Bacillus stearothermophilus* for stainless steel surfaces where the  $R_a$  varied between 0.11 and 0.3  $\mu\text{m}$ . The result of their research was that the cleanability increased with decreasing  $R_a$ .

This study attempts to determine the influence of different roughness of acid-proof stainless steel AISI 316 surfaces on the microstructure and amount of milk sediments forming at high temperatures. The aim of this work was to find a relationship between the problems of milk fouling and the roughness of the material, in order to find the optimal stainless steel surface to be used in the construction of heat exchanger equipment for the dairy industry.

## MATERIAL AND METHODS

**Surfaces and their characterisation.** The stainless steel surfaces tested in this study were AISI 316 with a diameter of 0.1 m and area of 0.079 m<sup>2</sup>, sourced from a manufacturer of installations and equipment for the dairy industry. The plates, which are designated A, B and C, differed in their surface microstructure. Their roughness and topography were determined by laser scanning using the Confocal Scanning Laser Microscopy (CSLM) method. A single sampling area depended on the size of deposits created on plates and ranged from 250 mm to 2500 mm. Contour images for subsequent sections of the surface sample were obtained from these measurements. The results of the measurements used for the studied stainless steel surface are shown in Figure 1. The surface structure is most often analysed by means of its so-called profile. This is a sectional view of the surface measured in 2D

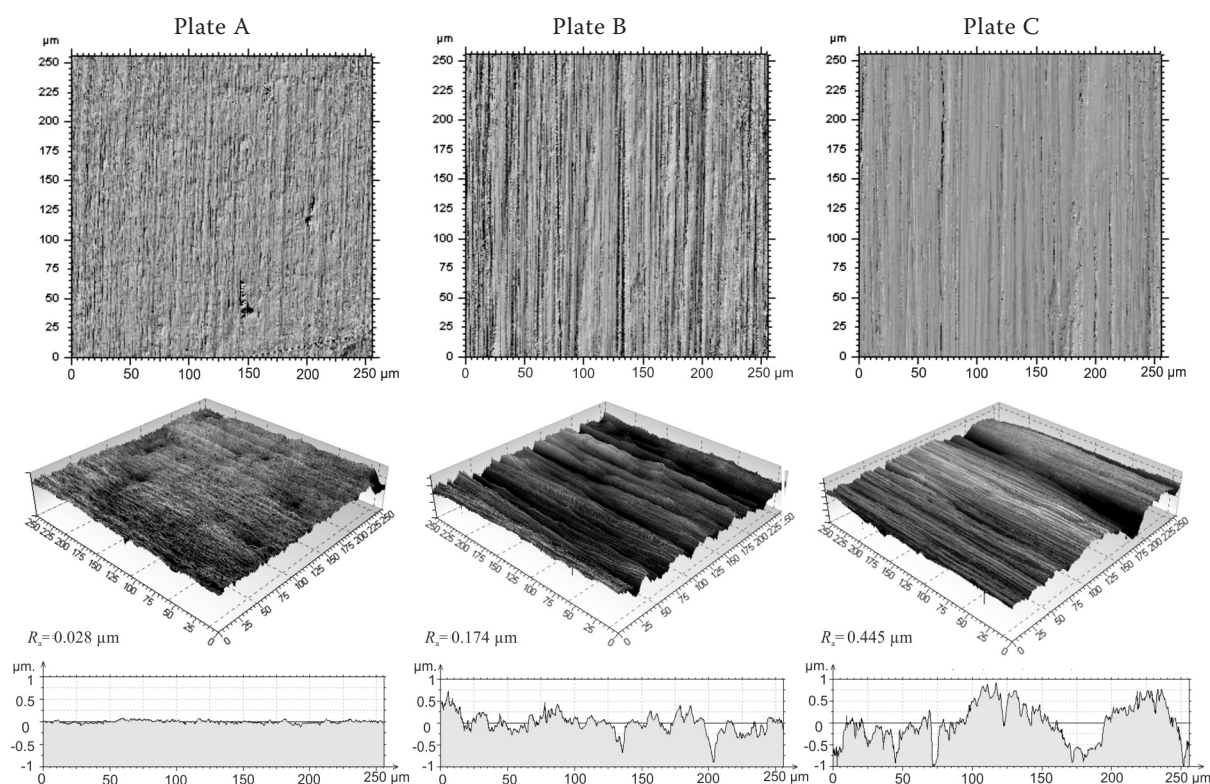


Figure 1. Microstructure of the studied surface with different roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )

described in accordance with ISO 4287:1997 – Geometrical products specification (GPS) – Surface texture: Profile method – Terms, definition and surface texture parameters. and ISO 25178:2013 – Geometrical product specifications (GPS) - Surface texture: Areal – Part 604: Nominal characteristics of non-contact (coherence scanning interferometry) instruments norms. Inequalities in profiles are described by the  $R_a$  symbol, which is a parameter to assess surface roughness. The plates used in the research clearly differed in their surface finish and their  $R_a$  values fit EHEDG recommendations (2004).

**Preparation of milk deposits.** The plates were placed on the bottom of a stainless steel vessel filled with standardised untreated whole milk at a temperature of  $T = 90^\circ\text{C}$ . In order to maintain the required temperature the bottom of the vessel was heated. The test plates were subjected to treatment under these conditions for 30 minutes, which resembled typical milk pasteurisation. The mass of created deposits on the plates was evaluated in 5, 10, 20 and 30 min of heating. Due to the mechanism of milk sediment formation on hot surfaces, as described in previously published studies (JEURNINK & KRUIF 1995; BANSAL & CHEN 2005), standardised and unpasteurised milk was used in the research (Table 1). This is the most

suitable medium for the formation of milk sediments resembling industrial sediments. Every five minutes, the mass of fouling formed during heat treatment on the plates was monitored by measuring the plate weight. On this basis, the rapidity of deposit formation and the relationship between roughness ( $R_a$ ) and the amount of fouling layers was determined. After soiling, any milk sediment layer unrelated to the surface was rinsed by the immersion of the plate in a dish with clean water. The topography of deposits (thickness and structure) was evaluated using the CSLM method. Each of the tests was done in three replications.

**Morphological evaluation of fouling deposit via CSLM.** After soiling, samples were washed by im-

Table 1. Milk composition used in the soiling of stainless steel plates

Details of milk composition	Value
Protein content (%)	3.29
Casein (%)	2.51
Fat (%)	3.7
Density ( $\text{g}/\text{cm}^3$ )	1.029
pH (–)	6.78
Acidity ( $^\circ\text{SH}$ )	7.8



mersion in pure water and subsequently air-dried and mounted onto an Olympus LEXT OLS4000 Confocal Scanning Laser Microscope (Olympus, Tokyo, Japan). The apparatus enabled the generation of an image based on the reflection of light from the focal plane. Light, creating an image with different depths of focus, was eliminated using a confocal double circular aperture. The light source was a 120 mW laser diode, emitting a wavelength of  $\lambda = 405$  nm (violet colour). The system was equipped with a set of five microscopic lenses for various magnifications (5 $\times$ , 10 $\times$ , 20 $\times$ , 50 $\times$ , 100 $\times$ ). Spatial and high quality 3D images were obtained through precise scanning of an object in the  $x$ – $y$  axes by using a miniature electro-mechanical element. Digital image creation, 3D rendering and image analysis were performed using the TalyMap Platinum 4.0 software (DygitSurf, Besançon, France). Images were taken from each sample with fouling deposits resulting in 30 series of images in each replication, which gave 90 samples in total. The substitute diameter (average length of the symmetry  $x$ - and  $y$ -axis) and height were determined for each deposit. This gave information about the heterogeneity of fouling layer. On this basis, the relationship between roughness ( $R_a$ ) and the size of fouling layers was determined.

**Statistical analysis.** To assess the repeatability of the tests of deposits (mass and size) three experiments were performed for each surface after the built-up of milk fouling on them. The test results were averaged and the regression function was described as a relationship between roughness ( $R_a$ ) and the amount and size of milk fouling.

## RESULTS AND DISCUSSION

**Analysis of the amount of fouling deposit.** Visual assessment and weight measurement of the plates clearly indicates that with decreasing ratios of the surface finish  $R_a$  the amount of impurities forming on the plates also decreased (Figure 2).

On plate A ( $R_a = 0.028$   $\mu\text{m}$ ) fine, dot-like protein deposits are visible, while on plate C ( $R_a = 0.445$   $\mu\text{m}$ ) they are larger and more numerous. They occur in clusters, thereby forming a thick, porous layer easily absorbing more layers of impurities. The fouling trends observed at each plate (Figure 3) were quite similar to each other. We can divide this process into two periods, i.e. induction which is usually required for the formation of protein aggregates or insoluble mineral complexes before a noticeable amount of deposits is formed and fouling period in which the milk fouling grows. This is consistent with results presented by many authors (VISSE & JEURNINK 1997; SANTOS *et al.* 2003; AUGUSTIN *et al.* 2007). The only difference between presented trends in Figure 3 is that plate C has a greater amount of deposits than the other analysed plates. Finally, after heating for 30 min, on average about 70 g of milk fouling per  $\text{m}^2$  of the analysed area was formed on plate C while on plate A it was about 40 g/m (Figure 3).

Based on results the trend of the dependence of an amount of milk layer (change of layer weight) formed on the stainless steel surface on its roughness was determined. The results show that this dependence can be described linearly (Figure 4). The coefficient of correlation  $R$  calculated for a confidence level of  $\alpha = 0.05$  was  $R = 0.999$ . This means there is a perfect positive linear relationship between the mass of milk deposits formed on the analysed plate surface and its roughness  $R_a$ .

**Morphological analysis of fouling deposit via CSLM.** Figure 5 shows the CSLM results of individually adsorbed milk sediments. Based on the analysis, it was found that the deposits are characterised by a conical shape, fixed to the SS surface, upwardly tapered. This shape is repeated on each of the studied plates. This result is consistent with previous reports on the formation of milk sediments on surfaces (CHANGANI *et al.* 1997; VISSE & JEURNINK 1997). These studies describe the basic layer of protein molecules adsorbed to the SS, on top

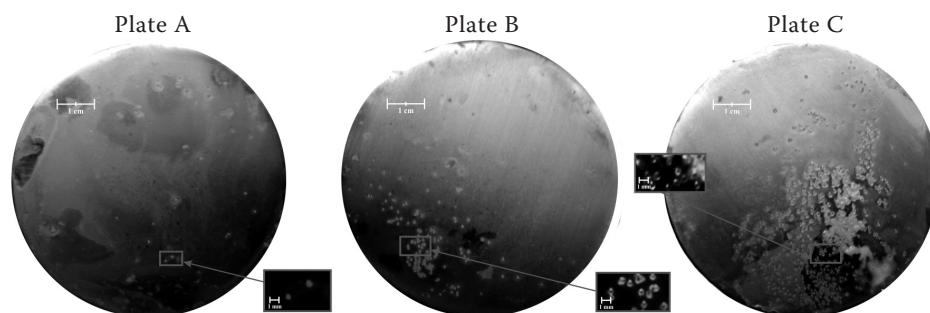


Figure 2. Stainless steel surface roughness (Plate A:  $R_a = 0.028$   $\mu\text{m}$ ; Plate B:  $R_a = 0.174$   $\mu\text{m}$ ; Plate C:  $R_a = 0.445$   $\mu\text{m}$ ) soiled with hot milk

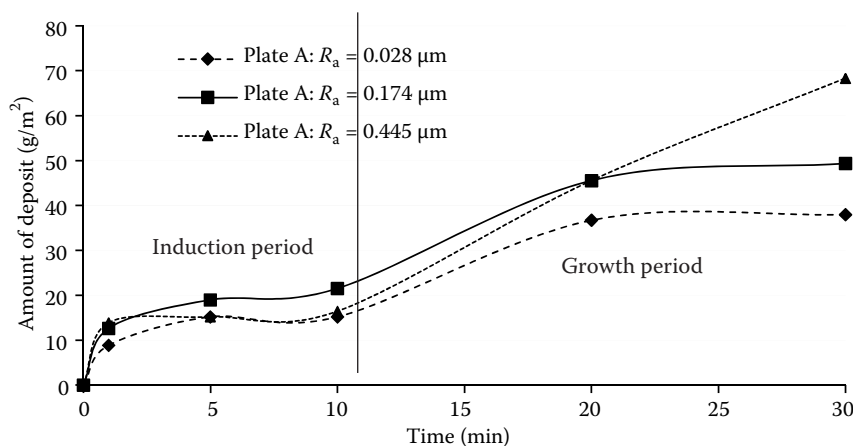


Figure 3. The effect of roughness on mass fouling created on plates during milk heat treatment

Plate	Mass fouling created on plates after different time of milk heat treatment (g/m <sup>2</sup> )					
	0	1 min	5 min	10 min	20 min	30 min
A	0	26.58 ± 0.84	45.57 ± 0.84	45.57 ± 0.85	110.13 ± 6.75	113.92 ± 4.22
B	0	37.97 ± 1.69	56.96 ± 0.84	64.56 ± 0.84	136.71 ± 3.38	148.10 ± 1.69
C	0	41.77 ± 2.53	45.57 ± 2.53	49.37 ± 2.53	136.71 ± 6.75	205.06 ± 5.06

of which protein aggregates are deposited (formed after exposure of the hydrophobic core and the reactive free SH groups during the thermal denaturation of  $\beta$ -lactoglobulin). It was also observed that some of the particular deposits could be rounded at the top and others could be broken and look like craters. The appearance of the adsorbed deposits, with empty spaces or visible craters in the structure (Figure 5), is similar to that reported by other studies (HINTON *et al.* 2002; CHEN *et al.* 2004; BOYCE *et al.* 2010). The craters are a result of breaking air bubbles saturated with water vapour and forming on the hot surface which comes into contact with the milk. As a result of elevated temperature, the

pressure inside the bubble increases due to which it breaks in the next phase. The bubbles and craters formed on the SS surface absorb more compounds ( $\beta$ -lactoglobulins, casein micelles and agglomerates of denatured proteins), which are responsible for the formation of milk sediments (WALSTRA *et al.* 2006). Images of the microstructure of formed milk sediments in the studied areas are shown in Figure 5.

The number, height, and diameter of individual deposits differed on each of the analysed surfaces (Figure 6). This was already observable in the visual assessment. For the smooth plate (plate A) with a roughness of  $R_a = 0.028 \mu\text{m}$ , a lot of fine, usually unbroken bubbles were formed with an average diameter of  $252.8 \mu\text{m}$ . While on the other plates, resulting sediments of larger diameters were mainly distributed in clusters and resembled craters ( $575.0 \mu\text{m}$  – plate B and  $1002.6 \mu\text{m}$  – plate C). The average substitute diameter deposits formed on the individual plates are shown in relation to the surface roughness (Figure 7). The coefficient of correlation  $R$  calculated for a confidence level of  $\alpha = 0.05$  was  $R = 0.991$ . On this basis it can be stated that there is a perfect positive linear relationship between the size of milk deposits forming on the analysed plate surface and its roughness  $R_a$ .

It was also observed that the bubbles on plate A are not so easily broken and are higher than the bubbles formed on the other plates, while on plates B and C, there were small bubbles which were not broken and their dimensions were comparable with each other. The

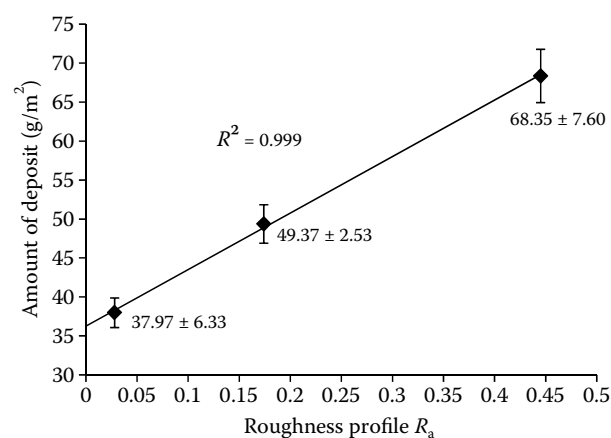


Figure 4. The relationship between the of amount deposits and stainless steel surface roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )

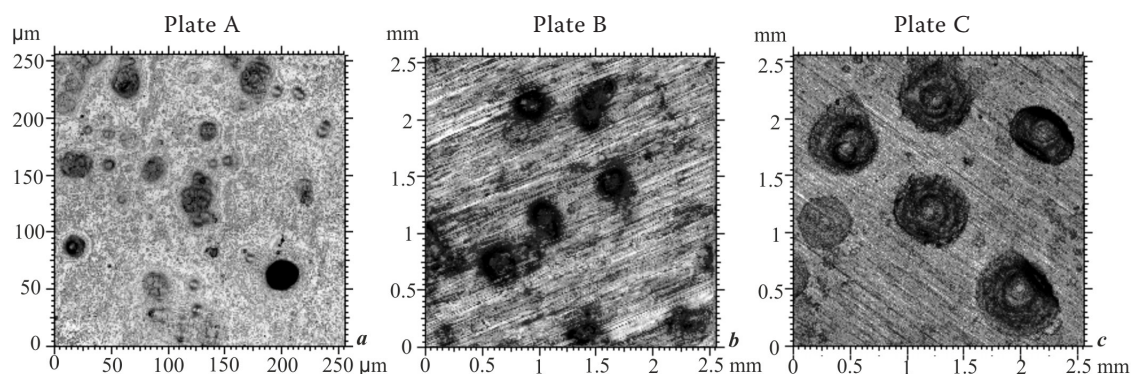


Figure 5. Clusters of individual protein deposits formed on individual plates with different surface roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )

CSLM analysis indicates that the amount of sediments on plate A, in the form of unbroken bubbles, ranged from  $130 \mu\text{m}$  to  $300 \mu\text{m}$ , and the remaining amount on the plates ranged from  $30 \mu\text{m}$  to  $180 \mu\text{m}$ . This suggests that the bubbles on plates with greater surface roughness break much faster. They form a layer that easily absorbs other impurities and are conducive to the accumulation of successive layers of sediments. As a result, large agglomerates of subsequent deposits are formed in the vicinity (Figure 8).

The formed clusters are irregular. They have many empty spaces and their shape and structure resemble craters. This description is consistent with that of the previously published literature (HINTON *et al.* 2002; CHEN *et al.* 2004). On plate A, the majority of deposits occurred individually in the form of unbroken bubbles. The clusters consisted of numerous broken bubbles, closely adjacent. The thickness of impurity deposits formed on plate A (diffused bubbles) ranged from  $40 \mu\text{m}$  to  $140 \mu\text{m}$ , while on plates B and C it was comparable and it was in the range of  $120$ – $250 \mu\text{m}$ . The obtained results are not consistent with the results published by BOYCE *et al.* (2010). The authors show that in fact the thickness of the deposit of the formed sediments on the SS surface was in the range

of  $340$ – $710 \mu\text{m}$ , with the majority of the deposit in the range of  $460$ – $510 \mu\text{m}$ . This incompatibility may result from the soiling method. They also prolonged the duration of keeping the plates in the hot milk to 90 minutes. They also determined that the formed sediments consist primarily of proteins, lipids and polysaccharides. On the basis of this comparison, it can be observed how the duration of the high-temperature heat treatment of milk impacts on the thickness of the formed sediments.

Further studies on the microstructure of the formed milk sediments indicated the presence of an additional, near-surface layer entirely covering the researched surfaces. The results obtained by the LSCM method showed that, with increasing surface roughness, the thickness of the deposit increases (Figure 9). On plate A, its thickness ranged from 3 to  $7 \mu\text{m}$ , while on plates B and C it was in the range of  $8$ – $16 \mu\text{m}$ . The formed layer was very strongly bound to the surface and difficult to remove by rubbing. It can initiate the formation of further layers of sediments, including individual deposits.

Based on the results discussed above, subsequent milk sediment layers formed on the stainless steel surfaces due to the heat treatment of milk have been

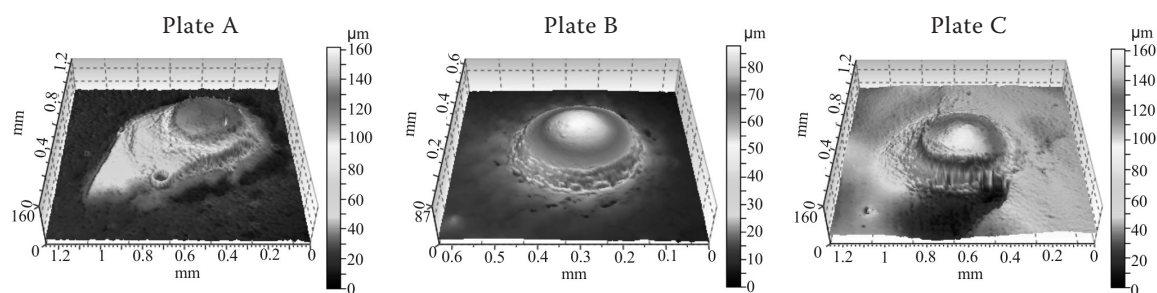


Figure 6. Single, unbroken protein milk deposits formed on individual plates with different surface roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )



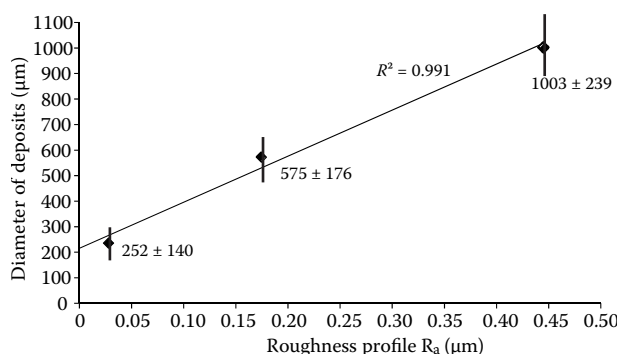


Figure 7. Relationship between the size of milk deposits and Stainless steel surface roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )

distinguished (Figure 10). The first outer layer, which is not bound with the surface, is formed by the pure milk layer entirely covering the researched areas. In the above-mentioned studies, this layer was rinsed by immersing individual plates into water in order to carry out a further analysis using CSLM. Another layer is formed as bubbles occurring in single and in large agglomerates. This fouling is insoluble aggregates of protein that are formed by denaturation in the bulk solution. These aggregates adhere to the

deposit layer formed in the induction period, causing the deposit to grow (GRANT *et al.* 1996). They are further embedded in a third layer of sediments, bonding them together. This layer was called the near-surface, because it was located closest to the surface of the researched plates and it covered them evenly. GRANT *et al.* (1996) indicated that this layer is a compact sublayer and is composed of calcium phosphate and proteins. This is also in agreement with BOYCE *et al.* (2010), who reported two fouling layers. The first layer closest to the SS was described as fouling material composed predominantly of non-hydrophobic protein in contrast to the remainder of the deposit which also contained hydrophobic protein and lipids. Similar results were reported by CHANGANI *et al.* (1997) and VISSER and JEURINK (1997).

## CONCLUSIONS

Based on the survey of measurements, it can be stated that the surface roughness has a significant impact both on the amount and thickness of milk sediments formed during the heat treatment of milk and on their topography. It has been observed that the

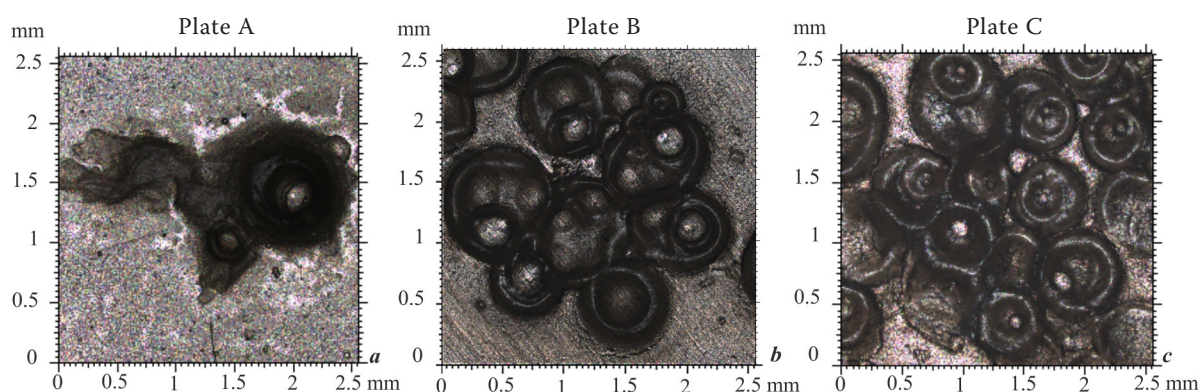


Figure 8. Clusters of deposits formed on the individual plates with different surface roughness (Plate A:  $R_a = 0.028 \mu\text{m}$ ; Plate B:  $R_a = 0.174 \mu\text{m}$ ; Plate C:  $R_a = 0.445 \mu\text{m}$ )

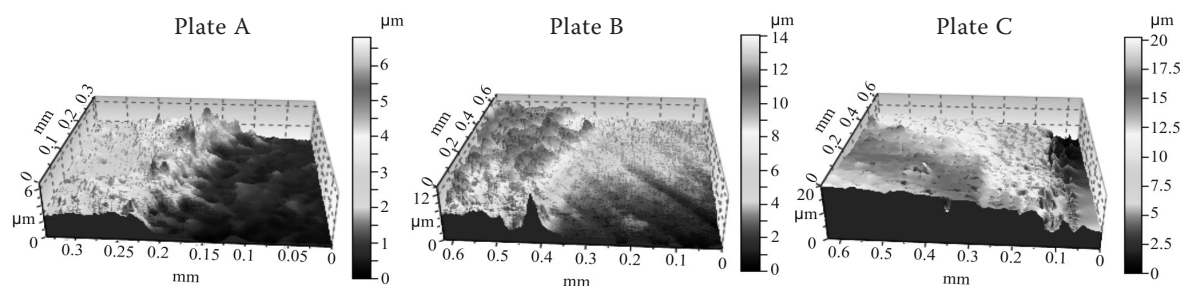


Figure 9. The thickness of milk sediment layer near the surface

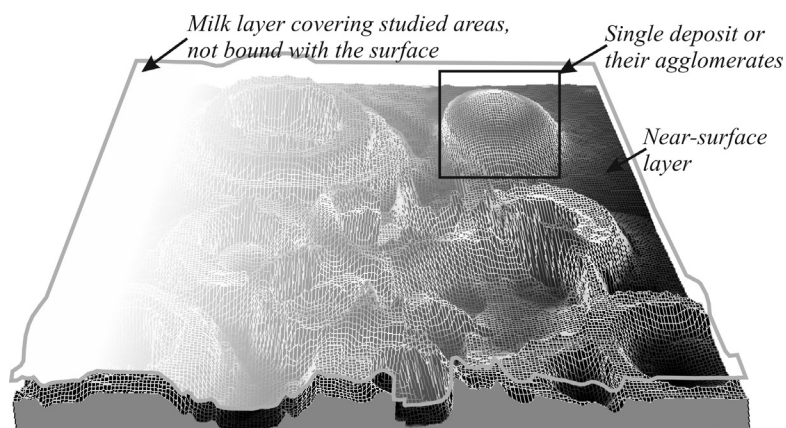


Figure 10. Layers of forming milk sediments on hot surfaces

lower the surface roughness, the less milk deposits, the longer the time of impurity deposition and the greater the probability of an easier cleaning process. Due to food safety and hygienisation of devices, it has been reported that it is preferable to use surfaces which are as smooth as possible. In this study, the best surface for this purpose was a polished surface (A), of the parameter  $R_a = 0.028 \mu\text{m}$ . The least milk sediments were formed on such a surface. This finish is very expensive – food and production equipment producers cannot often afford it. It has also been reported that surfaces with higher roughness profiles (plates B and C) favour the formation of milk impurities. The degree of roughness is directly proportional to the mass of fouling and the size of formed aggregates.

The analysis of microscopic measurement results also enabled the selection of individual layers in the milk sediments. This division is different from that presented previously in this particular area (BANSAL *et al.* 2003; AUGUSTIN *et al.* 2007). Specifying the three layers of sediments which are formed may be important in the process of cleaning the surface on which they are formed. Their composition and adhesive forces binding with the surface can help in the selection of active substances contained in the cleaning agents and the development of cleaning regimes and programs.

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