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## Assessment of changes in crystallization properties of pressurized milk fat<sup>1</sup>

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### ABSTRACT

The aim of the study was to demonstrate the use of fractal image analysis as a possible tool to monitor the effect of pressurization on the crystallization pattern of anhydrous milk fat. This approach can be useful when developing new products based on milk fat. The samples were subjected to different hydrostatic pressure (100, 200, 300, and 400 MPa) and temperature (10 and 40°C) treatments. The crystallization microphotographs were taken with a scanning electron microscope. The image analysis of scanning electron microscope photographs was done to determine a fractal dimension. Milk-fat pressurization under the applied parameters resulted in slight, but statistically significant, changes in the course of crystallization curves, related to the triacylglycerol fraction crystallizing in the lowest temperature (I exothermic effect). These changes were dependent on the value of pressure but not dependent on the temperatures applied during the process of pressurization (at either 10 or 40°C). In turn, significant differences were observed in crystallization images of milk-fat samples subjected to this process compared with the control sample. The results of additional fractal analysis additionally demonstrated the highest degree of irregularity of the surface of the crystalline form for the nonpressurized sample and the samples pressurized at 200 and 300 MPa at 10°C. The lowest value of fractal dimension—indicative of the least irregularity—was achieved for the fat samples pressurized at 400 MPa, 10°C and at 100 MPa, 40°C. The possibilities of wider application of the fractal analysis for the evaluation of effects of parameters of various technological processes on crystallization properties of milk fat require further extensive investigations.

**Key words:** milk fat, triacylglycerol composition, pressurization, physical and crystallization properties, fractal analysis

### INTRODUCTION

Milk fat remains the subject of interest to the consumers and many research groups (Breitschuh and Windhab, 1998; Datta and Deeth, 1999; Frede and Buchheim, 2000; Herrera and Hartel, 2000; Lopez et al., 2001, 2006; Shi et al., 2001; Roginski et al., 2003; Fox and McSweeney, 2006; Verret et al., 2009; Rønholt et al., 2012). Use of milk fat as a food component is affected by seasonal and regional variability of its physical properties. Many studies showed that the physical properties of milk fat depend largely on the composition of its main constituent, triacylglycerols (**TAG**), given the type and position of esterified FA (Shi et al., 2001; Wright and Marangoni, 2006). Consequently, the range of milk-fat melting temperatures is very broad and usually is between  $-40$  and  $+40^{\circ}\text{C}$ . Various polymorphic forms of crystallizing TAG ( $\gamma$ ,  $\alpha$ ,  $\beta'$ ,  $\beta$ ) might also affect the physical properties of milk fat (Breitschuh and Windhab, 1998; Wright and Marangoni, 2006; Lopez and Ollivon, 2009). The knowledge of crystallization kinetics is, therefore, essential for monitoring the technological parameters of production of many foods based on milk fat, especially those influencing their functional properties, in particular texture (Herrera and Hartel, 2000; Roginski et al., 2003; Wright and Marangoni, 2006). This may be particularly significant to dairy products with the highest fat content; that is, butter and anhydrous milk fat, which is more often applied in various branches of the food industry. Apart from milk-fat composition (including the content of incomplete acylglycerols), the key parameter that affects rheological properties of such products is the ratio of solid and liquid phases of milk fat at given temperature (Shi et al., 2001; Foubert et al., 2004). On the micro scale, these characteristics may be determined by the crystallization image that covers not only size of the crystals but also their quality, as indicated by their shape and even acylglycerol composition.

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Many possibilities for milk-fat modification are offered by existing technologies. A significant range of modifications of the physicochemical properties of milk fat may be ensured by such processes as fractionation (Van Aken et al., 1999; Lopez et al., 2006), hydrogenation (Augustin and Verteeg, 2006), and esterification (Tynek and Ledóchowska, 2003; Wright and Marangoni, 2006). The composition and physical properties of milk fat may as well be modified by the nutritional methods; that is, cow feed supplementation with so-called protected lipids, which prevent biohydrogenation of unsaturated FA in the rumen. Pressurization seems to be an interesting physical method applied to modify milk-fat properties (Buchheim et al., 1996; Datta and Deeth, 1999; Huppertz et al., 2002; Trujillo et al., 2002).

Initial studies focused on the size of fat globules and crystallization processes occurring after pressurization of milk or cream (Buchheim et al., 1996; Frede and Buchheim, 2000; Gervilla et al., 2001; Fox and McSweeney, 2006; Verret et al., 2009). It was shown that the application of pressure in the range of 100 to 500 MPa at 25 and 50°C to sheep milk resulted in an increased content of fat globules with diameters of 1 to 2  $\mu\text{m}$  and a decreased content of fat globules with diameters of 2 to 10  $\mu\text{m}$ . However, an opposite effect was noticed when pressurization was at 4°C (Gervilla et al., 2001). These studies demonstrated also that pressurization in this pressure range contributed to decreasing content of FFA. Pressurization at 100 to 500 MPa was shown to facilitate a decrease in physical ripening time of cream for butter production (Buchheim et al., 1996). The authors hypothesized that it is due to the shift of melting and crystallization temperatures of the main milk-fat fractions toward higher values (Buchheim et al., 1996). Verret et al. (2009) suggested that such an effect might result from crystallization of the main TAG fractions in various polymorphic forms.

Extensive studies have been conducted on the effect of pressurization on the composition and crystallization properties of pure milk fat, its fractions, and mixtures with other fats under various conditions of crystallization and by using different analytical methods (Frede and Buchheim, 2000; Staniewski et al., 2012). Many research techniques may be applied to evaluate the effect of different modification methods on milk-fat crystallization images. These may include very simple intermediate methods evaluating the final melting point of TAG in the analyzed sample or considerably more informative curves of crystallization and melting plotted by using differential scanning calorimetry (DSC), which additionally enables precise evaluation of changes in the content of the solid phase of fat in the function of temperature changes (Breitschuh and Windhab, 1998; Lopez and Ollivon, 2009; Fredrick et

al., 2011; Rønholt et al., 2012). The latter may also be analyzed with the use of nuclear magnetic resonance (Herrera and Hartel, 2000; Foubert et al., 2004; Martini et al., 2005). Special significance in the analysis of fat crystallization image (including milk fat) is, however, ascribed to microscopic methods that include optical microscopy and—increasingly often—also electron microscopy. In turn, polymorphic changes may be analyzed with the use of X-ray diffraction (Van Aken et al., 1999; Lopez et al., 2001; Martini and Herrera, 2002; Lopez et al., 2006).

Nevertheless, multiple processes ongoing in nature lead to the formation of “apparently” unordered structures. Quantitative comparison of such objects may be difficult, and their descriptive characteristics are often imprecise and biased. This is the case with microscopic images of fats crystallization, and use of mathematical algorithms with specific parameters characterizing the structure may allow for their precise description. One of such parameters may be the fractal dimension.

Fractals have a self-similar pattern, which means they have the same structural characteristics irrespective of observation scale (Mandelbrot, 1982). Although they are ideal mathematical structures, it is possible to apply the concept of fractal geometry in the case of natural objects occurring in nature. To some extent, these structures may demonstrate some fractal characteristics, and the determined fractal dimension (often called “apparent” dimension) may well characterize these structures (Peleg, 1993). Depending on the research methods applied, it may characterize the rate of filling of an observed volume by the studied substance or the rate of filling of a surface by a ragged line, i.e., of analyzed object (Leman et al., 2005; Smoczyński and Baranowska, 2013). The fractal dimension expresses a correlation between the mass of the fractal object and its dimension. The only difference between the ordinary correlation between mass and size is the exponent, which in the case of fractal objects is an integer.

The aim of this study was to determine the effect of pressurization on crystallization properties of milk fat based on the fractal analysis of the crystallized sample.

## MATERIALS AND METHODS

### *Experimental Material*

The experimental material was anhydrous milk fat. The milk for milk-fat production came from stall-feeding cows. The milk fat was obtained from “extra” type butter produced in one of the dairy plants located in northeastern Poland. The butter was melted at a temperature 55 to 60°C, and then the milk fat was decanted from above plasma and filtrated (at 60°C)

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