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Fractal and physico-chemical analysis of cows' milk fat globules after lipolysis

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Summary
The study investigated the effect of interface structure and bile salts on the process of milk fat globules hydrolysis under the influence of pancreatic lipase. The subject of this study were fat globules of standardized pasteurized milk, and lipid droplets of homogenized milk and milk homogenized with the addition of phosphatidylcholine. Titratable free fatty acids released, particle size distribution and changes in the emulsion system were monitored during hydrolysis of the investigated samples of milk. The presence of the phospholipid affected the formation of a lipid droplets membrane with various properties and deceleration of the lipolysis process in the presence of bile salts. The addition of bile salts resulted in intensification of the lipolysis process in the case of all samples of milk examined. Results achieved confirm the significant role of the structure and available area of the interface in the in vitro digestion process of milk fat under the influence of lipase. The fractal image analysis is a novel and useful approach to monitor changes in the emulsion system and may provide additional information on the lipolysis process.

Keywords
cows' milk fat globules; lipase; bile salts; fractal analysis

Lipids are an extremely complex group of chemical compounds. Apart from providing energy that is indispensable for vital processes, they are the basic constituent of biological membranes and play many biologically-significant functions that have not been fully recognized yet. For this reason, specific nutritional guidelines related to this group of compounds are still inexplicit and imprecise [1]. Better knowledge on the functions and properties of these compounds, their bioavailability and role in nutrition are of great significance especially in view of such health issues as cardiovascular diseases and obesity. Digestion of lipids in the gastrointestinal tract of man is a complex process involving physico-chemical and enzymatic transformations that may include emulsification, hydrolysis, and micellarization followed by absorption by enterocytes [2]. The enzymatic transformations are initiated in the stomach under the influence of gastric lipase and continued in the duodenum and intestine. The main enzyme responsible for the digestion of lipids is pancreatic lipase co-acting with colipase at the water-lipid phase boundary (interface), where conformation changes of the enzyme are likely to occur thus enabling the unveiling of the catalytic triad for the substrate [3]. A significant role in the process of the digestion of lipids is also ascribed to bile salts. Their primary role consists in the emulsification and removal of hydrolysis products from the reaction site, but they may also modify the structure of fat-water interface [4, 5].

One of the significant sources of lipids in nutrition, especially in the neonatal period, is milk. Milk lipids occur in the form of fat globules that are highly ordered and complex structures with diameters ranging from 0.2 μm to 15 μm, with a mean diameter of 4 μm. When seen from the inside, the fat globules are constituted by the lipid core composed of triacylglycerols that is surrounded by a monolayer of polar lipids and proteins, next by a protein layer, i.e. the so-called protein coat occurring in the inter-membrane space and, finally, by the real, external lipid bilayer coating

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the whole fat globule and composed of protein, glycoproteins, enzymes, non-polar and polar lipids, and phospholipids [6–8]. The quality and properties of this interface constituted by phospholipids and proteins have a significant impact on the availability of triacylglycerols for lipase.

During technological processing of milk, the structure of milk fat globule membrane (MFGM) undergoes substantial changes. The process of homogenization (pressure treatment of milk) results in the formation of globules of much smaller sizes and altered structure of the membrane. In turn, the diminished sizes of globules lead to an increase in the total surface area of globules. The native structure of the MFGM is disrupted and the newly-formed structure becomes partly covered by fragments of the membrane but also by casein micelles or whey proteins. This results in changes in milk protein configuration and in the milk fat globule membrane, which may influence lipase access to the globule interior and susceptibility of milk fat to hydrolysis by lipase. It is, therefore, crucial to explain how processes of milk treatment, homogenization in particular, affect digestion and bioavailability of lipids [9–11]. Many investigations conducted in this respect were addressing fat digestibility in newborns and the role of lipase in this process [12–14] as well as the susceptibility of artificially produced emulsions to digestion by lipase [15–17]. In contrast, relatively sparse information is available on the lipolysis of natural emulsions, especially of milk fat [18–21]. Those investigations point to a complex mechanism of the lipolysis process and to the significant role of the size of fat globules and, consequently, of the available interface as well as the structure and organization of this interface and, finally, the presence of additional substances, like colipase or bile salts, in this process.

Many processes and structures regarded as irregular may be characterized with the use of fractal geometry. Fractals are characterized by the following properties: they are self-similar, they are not determined by any mathematical formula but by a recurrent dependency, and their size is not an integer [22].

In a logarithmic system, the dependency between the mass of an object and its size is always linear and may be expressed as follows:

$$M(R) \sim R^D \quad (1)$$

where M is mass, R is size, and D is fractal dimension of the object.

In the case of fractal objects, the power coefficient does not have to be an integer [23]. Fractal analysis may often be a useful tool for mathematical description of structures or processes displaying chaotic and irregular shape or an apparently unordered character [24–28]. The application of the fractal analysis enables unbiased and mathematical determination and comparison of differences in properties of the structures of the analysed objects.

The aim of this research was to determine the effect of changes in the native structure of the milk fat globule membrane on the lipolysis process of milk fat under the influence of pancreatic lipase. Experiments were conducted with pasteurized and normalized milk, homogenized milk and milk homogenized with the addition of phosphatidylcholine and in the presence of bile salts. Fractal image analysis was applied as a novel tool to monitor changes in processes of flocculation, coalescence or dissociation of fat globules in the course of the process.

MATERIAL AND METHODS

Materials

Fresh cows' milk was purchased at a local dairy plant in Poland and immediately pasteurized at a temperature of 72 °C for 30 s. Lipase from porcine pancreas, Type II (crude preparation that contains the lipase-colipase complex, and has also amylase and protease activity), bile salts (containing a mixture of sodium salts of cholic acid (~50%) and deoxycholic acid (~50%)) and phosphatidylcholine (60%, from egg yolk) was obtained from Sigma Aldrich (Saint Louis, Missouri, USA). All other reagents were of analytical grade.

Preparation of milk

After pasteurization, milk was centrifuged in a skimming centrifuge, and the resultant skim-milk and cream were used to standardize fat content to 4% (milk A). After preliminary analyses, such a substrate concentration was selected that assured good saturation of the enzyme and optimal conditions for its activity (data not shown). Next, part of the standardized milk was used to prepare homogenized milk (milk B and C). In order to produce homogenized milk B, milk was homogenized under the pressure of 400×10^5 Pa (temp. 60 °C ± 2 °C) using a two-stage high-pressure homogenizer NS 1001L Panda 1K (GEA Niro Soavi, Parma, Italy). In the case of milk with phospholipid, phosphatidylcholine was added to the pasteurized milk in the quantity of 0.5% and solubilized with the use of a blender (approx. 0.5 h). Next, milk was homogenized under conditions as above (homogenized milk C). Batches of milk prepared in this

way were analysed on the same day. The content of fat in milk was determined with the butyrometric method.

Hydrolysis of fat globules with lipase

Hydrolysis of fat globules with porcine pancreatic lipase was determined by titration of milk samples. To this end, 150 ml of the analysed milk sample were transferred to a conical flask. In the case of the samples with bile salts, the salts were added at this stage. After preliminary incubation in a water bath at a temperature of 20 °C for 10 min, pH value was adjusted to 7.3 using 0.1 mol·l^{-1} sodium hydroxide. Next, lipase was added. The final concentration of the enzyme and bile salts reached 1.33 mg·ml^{-1} and 5 mg·ml^{-1} of milk samples, respectively. The amount of bile salt (approx. 12 mmol·ml^{-1}) was in the physiological range reported for the fed state [29]. The quantity of liberated fatty acids was monitored for 1 h by titration with 0.1 mol·l^{-1} sodium hydroxide to pH 7.3 at a temperature of 20 °C with the use of a Titro Line easy titration unit (Schott Instruments, Mainz, Germany), a magnetic stirrer and a pH-meter (Mettler Toledo, Schwerzenbach, Switzerland). For each type of milk, a control sample was prepared with the addition of water instead of enzyme, and the difference between the experimental sample and control sample was calculated. The activity of lipase was expressed in micromoles of fatty acids released from one millilitre of a milk sample. Before hydrolysis, after 30 min and 60 min, samples were collected for determination of particle size distribution and for microscopic observations. Determinations were repeated three times for different milk samples and results were presented as mean ± standard deviation.

Measurement of particle size

Particle size distribution was determined by measurement of laser light dispersion using a Mastersizer 3000 (Malvern Instruments, Malvern, United Kingdom). Milk samples were instilled to a measuring cell till obscuration has been achieved at 10–15%. The refraction index for water and milk was 1.33 and 1.46, respectively. The particle size distribution was used to determine mean particle size $d_{(3,2)}$ and available surface of globules in milks.

Mean particle size $d_{(3,2)}$ is defined as

$$d_{(3,2)} = \sum n_i d_i^3 / \sum n_i d_i^2 \quad (2)$$

where n_i is the number of droplets of diameter d_i.

Results represent mean values from at least five measurements of three milk samples.

Light microscopy

Before and during lipolysis, samples of the analysed milk were transferred onto glass slides, covered with a cover glass and observed under a light microscope (Carl Zeiss, Jena, Germany) at a magnification of 63×. A series of images (10–12) was recorded using a Moticam Pro CCD camera (Motic Asia, Hong Kong, China). The obtained photographs from three milk samples were used for image analysis and for determination of fractal dimension.

Fractal analysis

The series of photos taken served to determine fractal dimension. Image analysis was conducted with the use of NIS-Elements software (Nikon, Tokyo, Japan). After enhancing contrast of the images, measurement of surface area and diameter of fat globules was carried out. A minimum of 100 measurements were carried out for each milk sample, and then fractal dimension was computed from the dependency

$$\log A \sim \log P^{2/D_L} \quad (3)$$

(A is area, P is perimeter, D_L is fractal dimension of the perimeter) and slope of the straight line in this dependency. The results of three repetitions for different milk samples were combined to obtain the fractal dimension.

RESULTS AND DISCUSSION

Hydrolysis of milk fat under the influence of lipase

Milk was evolving along with man in pursuit of assuring the best nutrition to the progeny, so the native structure of the milk fat globule membrane should assure optimal digestion and, thereby, bioavailability of milk fat. In this research we investigated the impact of the changes in the native structure of the membrane on the lipolysis process of milk fat under the influence of pancreatic lipase. One of the objectives was to gain insight into the role of milk homogenization on the digestion of milk fat. Therefore, we used pasteurized and homogenized milk (most often consumed) and crude enzyme preparation. The native membrane of fat globules is mainly composed of phospholipids and proteins. After homogenization, the size of globules decreases and consequently the newly-formed interface is subject to a significant increase. The quantity of membrane material may not be enough to coat the newly-formed enlarged surface, hence adsorption is observed principally in the case of surface-active components of milk,

mainly casein micelles and, to a lesser extent, for whey proteins. The composition of the newly-formed membrane changes. After phosphatidylcholine addition to milk before homogenization, it may, as a surface-active substance, take part in the formation of a novel, altered interface. Fig. 1 presents results on hydrolysis of the three types of milk with porcine pancreatic lipase with/without bile salts addition.

Results obtained demonstrate that phosphatidylcholine added during homogenization affected the formation of the milk fat droplet membrane with properties differing from those of both the native membrane and the membrane formed upon homogenization of raw milk. In milk A, without bile salts addition, the quantity of released fatty acids was the lowest and, after one hour of incubation, reached approx. 14.5 μmol·ml^{-1}, which corresponds to hydrolysis of approx. 10% the initial amount of fat. In the first 5–10 min, a slight delay of hydrolysis could be observed, probably linked with the occurrence of the lag phase. This delay is usually explained by the protecting role of MFGM. Small changes in MFGM occurring in the stomach in the gastric phase under the influence of gastric lipase or small amounts of free fatty acids can modify the interface and allow the access of lipase-colipase system to the triglyceride core [13, 18].

In the case of homogenized milk B and C, the quantity of liberated fatty acids did not differ. Their linear increase might be observed and, after a hour, their concentration reached approx. 31 μmol·ml^{-1} for homogenized milk B and approx. 34 μmol·ml^{-1} for homogenized milk C, which was approx. twice as much as in the case of milk A.

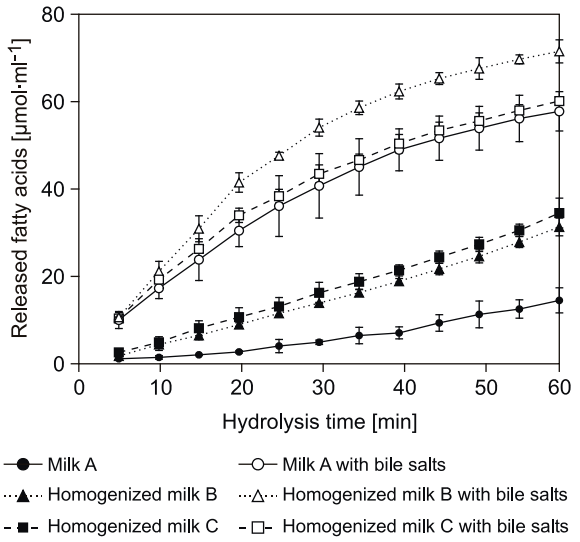

Fig. 1. The quantities of fatty acids released from cows' milks by lipase.

Each point represents the average of three determinations on separate milk samples ± standard deviation.

The addition of bile salts caused a significant increase in the quantity of fatty acids released from all three types of milk. In the case of milk A and homogenized milk C, the quantity of fatty acids released after one hour was at a similar level. The presence of bile salts in the course of lipolysis caused approx. 4-fold and approx. 1.7-fold increase in the quantity of liberated fatty acids in the case of milk A and homogenized milk C, respectively. In the case of homogenized milk without phosphatidylcholine addition (homogenized milk B), the quantity of released fatty acids in the presence of bile salts was higher than the respective values in both other milk samples.

The results obtained are generally consistent with findings of other authors. Berton et al. [30] showed that catalytic behaviour of human pancreatic lipase was different for native and homogenized milk fat globules and could be modulated by pancreatic lipase-related proteins 1 and 2 (PLRP1 and PLRP2). Ye et al. [21] demonstrated a lower level of lipolysis in the case of raw milk compared to reconstituted milk. Berton et al. [18] achieved a higher extent of lipolysis under the influence of human pancreatic lipase and colipase in the case of homogenized milk than in the case of raw milk. However, considering the available area of interface, authors additionally suggested that the native membrane may be more available to lipase than the membrane modified by homogenization.

Lipase is an enzyme acting at the fat-water interface. As the structure of the substrate itself did not differ between milks, hence the extent of lipolysis depended mainly on the possibility of lipase binding with the substrate, which in turn was determined by the available interface and its structural properties. One of the important steps during lipase catalysis is its adsorption at the TAG-water interface. Tensioactive proteins and phospholipids absorbed at the interface can inhibit the access of lipase to the substrate. So the composition and structure of this interface may affect the rate of lipid digestion. The lipase inhibition can be fully reversed by bile salts (depending on their concentration) in the presence of colipase. The bile salts are able to displace the molecules from the interface and to form mixed micelles [31, 32].

Our results confirm the important role of the interface quality and bile salts in the milk fat lipolysis. In the absence of bile salts, the lipase access to the substrate was limited, and both the phospholipid and milk proteins in the newly-formed membranes inhibited lipase access to the sub-

strate. The presence of bile salts caused significant increase in the amount of fatty acids released in all three milks. The addition of the phospholipid affected the formation of the membrane with diverse properties and, in this case, the amount of released fatty acids was lower as compared to the homogenized milk B. It is possible that removal of the phospholipid from the interface by bile salts was not so effective as in the case of milk proteins (caseins and whey proteins). However, still many factors may influence lipase activity, such as the thickness of the interface, substrate orientation, hydration forces, rheological properties of the film formed and also the resultant charge of the interface.

Fig. 2 presents the quantity of released fatty acids per surface area unit of the available fat in the analysed milks.

This indicates that, taking into account the available interfacial area, the hydrolysis of fat globules was the most effective in the case of the native membrane of fat globules present in milk A. The casein micelles present in the investigated milk contributed to the measured surface area, but still the differences express generally changes in the interface of the milk fat globules. In the native state, the membrane is composed of phospholipids (approx. 30–75% of the membrane), specific membrane proteins (approx. 25–70% of the membrane), cholesterol and other minor components [33, 34]. After homogenization, the percentage of phospholipids in the membrane decreases as the significant part of the newly-formed surface is being coated mainly by casein micelles and, to a lesser extent, by whey proteins. As indicated by the study, if the lipolysis of native globules of milk is more effective considering the available surface of enzyme action, this may point to probably specific synergistic interactions between the lipase-colipase system, bile salts and the membrane.

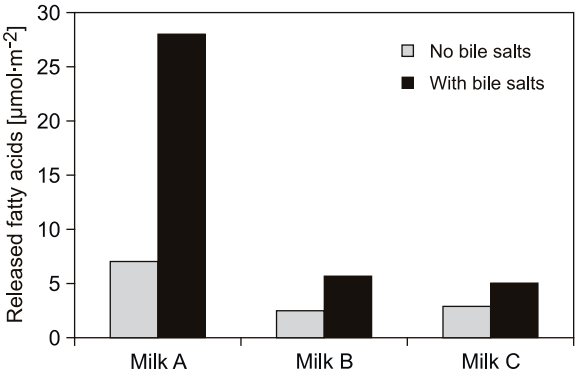

Fig. 2. The quantities of fatty acids released during lipolysis of cows' milk in the presence and absence of bile salts, expressed in relation to available surface of cows' milk fat globules.

Changes in the size of fat globules during lipolysis

Changes in the emulsion system of the analysed milk in the course of lipolysis are depicted in Fig. 3 and Tab. 1.

The mean particle sizes $d_{(3,2)}$ in milk A, homogenized milk B and homogenized milk C were at 2.41μm, 0.50μm and 0.53μm, respectively (Tab. 1). In the case of milk A without bile salts, no significant changes were observed in the size of particles within one hour of lipolysis. The addition of bile salts caused a change in particle size distribution. After 30 min, a loss of small particles and an increase in the number of particles with sizes between 50 μm to even 300 μm could be observed in milk A. After 60 min, these changes were even more pronounced. These results are well correlated with the quantity of released fatty acids, where in the case of milk A without bile salts the extent of hydrolysis was low, whereas in the presence of bile salts the quantity of released fatty acids was 5-fold higher. In case of homogenized milk B, changes in particle size distribution could be observed even in the samples without bile salts addition. It is also consistent with results of lipolysis, where in this case the extent of lipolysis was already higher. In this case, the changes in particle size proceeded in a similar way as in milk A. Visible was also a gradual loss of particles occurring in the initial milk and an increase in the number of particles with greater sizes, however in this case the larger particles were smaller than in milk A and fitted within the range of 3–30 μm, with the maximum at approx. 10 μm. In the presence of bile salts, after 30 min of lipolysis, a loss could be observed in particles occurring in the initial milk followed by the appearance of particles with a wide range of sizes, including particles over 100 μm in size. After 60 min of lipolysis, again the prevailing group was constituted by particles with the size ranging from 3 μm to 30 μm, with a maximum at approx. 10 μm. In the case of homogenized milk with the addition of phospholipid but without bile salts, the changes were similar to those observed in homogenized milk B. The presence of bile salts contributed significantly to process intensification. A slight delay of the process was observed in homogenized milk C, as compared to milk B, which was consistent with results of lipolysis.

The course of changes in the emulsion system was generally similar in all analysed milk samples. Larger particles appeared in all the three milks,

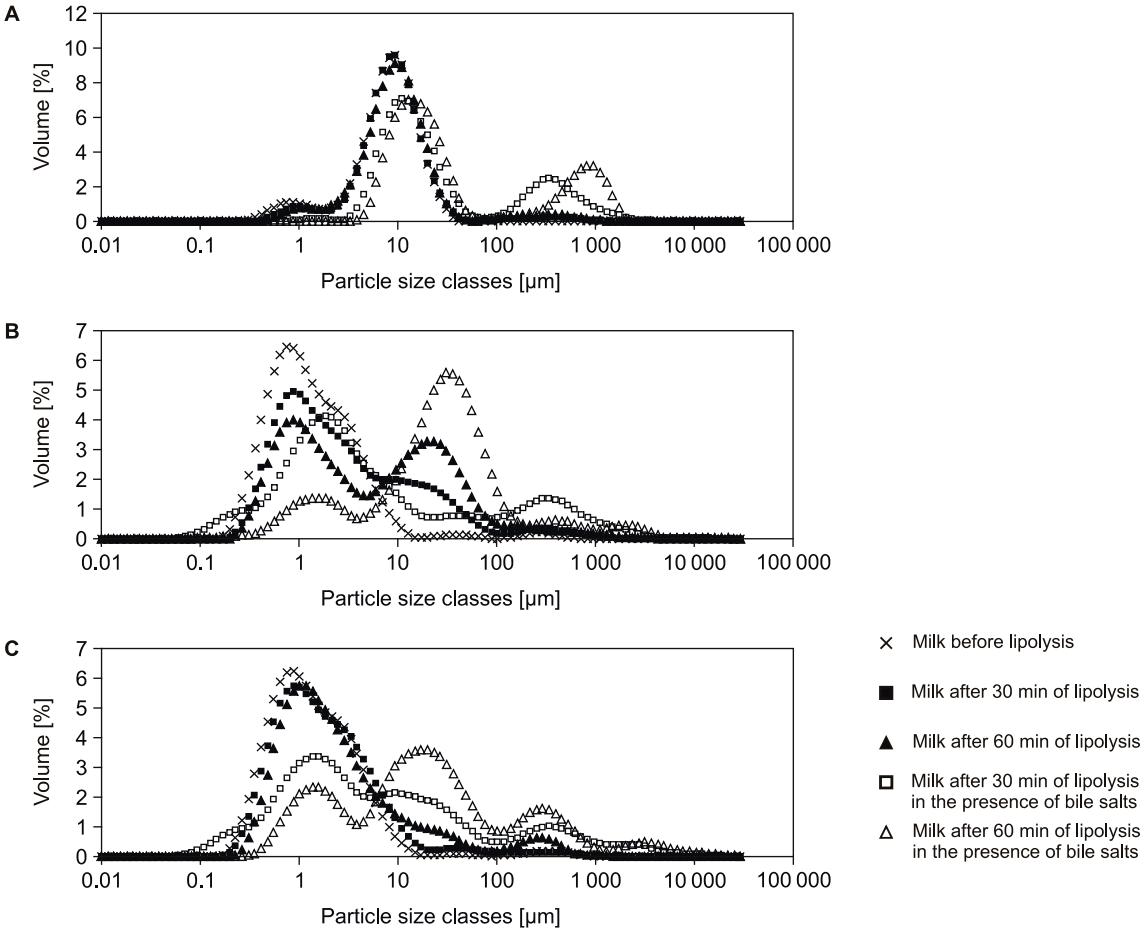

Fig. 3. The changes of particle sizes of cows' milk fat globules during lipolysis.

A – milk A, B – homogenized milk B, C – homogenized milk C. Averaged values of measurements of three separate milk samples.

Tab. 1. Physical characteristics of milk samples during hydrolysis.

Kind of milk	$d_{(3,2)}$ [μm]	Surface area [$m^2 \cdot g^{-1}$]	Fractal dimension D_L	Determination coefficient R^2
Milk A	2.41 ± 0.88	2.29 ± 0.44	1.05	0.99
after 30 min	2.64 ± 0.56	2.27 ± 0.45	1.13	0.95
after 60 min	2.69 ± 0.35	2.21 ± 0.23	1.39	0.95
with bile salts after 30 min	10.24 ± 2.73	0.57 ± 0.19	1.60	0.92
with bile salts after 60 min	27.08 ± 5.56	0.26 ± 0.01	1.74	0.91
Milk B	0.50 ± 0.05	11.99 ± 1.15	1.12	0.95
after 30 min	0.94 ± 0.17	6.26 ± 1.13	1.52	0.95
after 60 min	1.58 ± 0.11	3.59 ± 0.38	1.65	0.92
with bile salts after 30 min	1.23 ± 0.24	4.22 ± 0.18	1.41	0.92
with bile salts after 60 min	1.97 ± 0.16	2.77 ± 0.53	1.40	0.92
Milk C	0.53 ± 0.03	11.36 ± 1.04	1.12	0.96
after 30 min	0.64 ± 0.01	9.24 ± 0.09	1.66	0.96
after 60 min	0.77 ± 0.13	8.12 ± 1.26	1.59	0.94
with bile salts after 30 min	1.26 ± 0.17	3.83 ± 0.10	1.74	0.94
with bile salts after 60 min	1.83 ± 0.15	2.97 ± 0.18	1.64	0.96

Mean of at least three replications ± standard deviation.

which was probably due to the flocculation or coalescence processes. In the case of smaller sizes of initial particles, smaller aggregates were observed, whereas the larger particles yielded aggregates of larger sizes.

The changes in the emulsion system were generally proportional to outcomes of fat globules lipolysis. In addition, they confirmed the inhibition of the lipolysis process in the case of milk fat globule membrane in homogenized milk with the addition of phospholipid and in the presence of bile salts.

Fractal image analysis of the changes in the emulsion system

Changes in the emulsion system of milk fat were additionally determined with the use of fractal analysis. It consisted in the observation of fat globules in the course of lipolysis using a light microscope. The photos obtained were subjected to image analysis to enable the computation of fractal dimension (Fig. 4). Surface areas and perimeters of the observed fat globules were measured. The surface of the analysed object (A) is proportional to its perimeter P^{2/D_L},

$$A \sim P^{2/D_L} \tag{4}$$

where D_L is a fractal dimension of the perimeter. The graph of this dependency in the logarithmic system is a straight line with the inclination a

$$a = 2 \times D_L^{-1} \tag{5}$$

which enables calculating the fractal dimension of the analysed particles [35].

Fractal dimensions of the particles of the analysed milks with determination coefficients are presented in Tab. 1. Fig. 5 exemplifies a logarithmic dependency between the perimeter and surface area for raw milk. High values of determination coefficients point to good fitting of the mathematical model to the achieved analytical results.

The fractal dimensions determined in this way

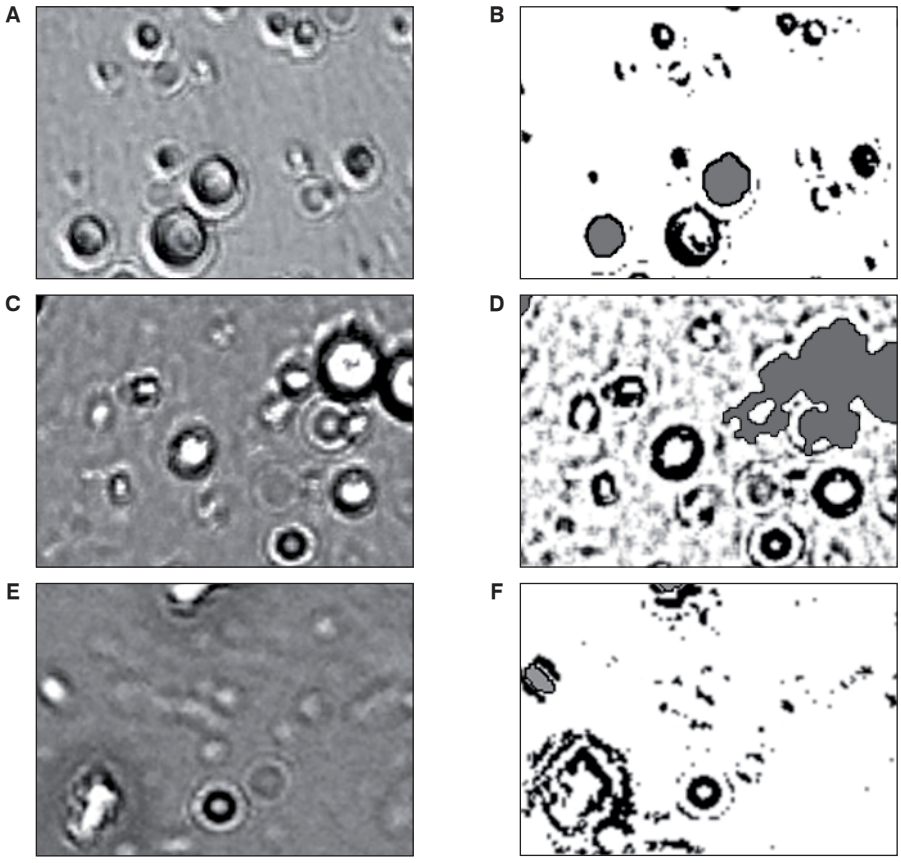

Fig. 4. Examples of images for milk before and after the image analysis process.

A – milk A before hydrolysis, C – milk A after 30 min of hydrolysis in the presence of bile salts, E – homogenized milk B after 60 min of lipolysis and in the presence of bile salts; B, D, F – the same images after the image analysis process.

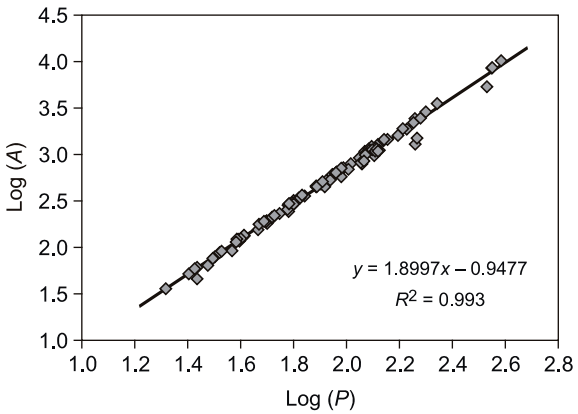

Fig. 5. The log surface area as a function of the log perimeter for the raw cows' milk.

demonstrated unevenness of the perimeter of milk fat globules. In the case of spherical shape, namely in native globules, this dimension should reach 1. When the perimeter becomes uneven, e.g. when changes occur in fat globule membrane or when particles flocculation or aggregation proceeds, this dimension is expected to increase (Fig. 4).

Fractal dimensions calculated for milk A and for both homogenized milks before lipolysis should approach unity, and in our study they indeed reached the values of 1.05 and 1.12, respectively (Tab. 1).

In the case of milk A, the fractal dimension increased slightly after 30 min, to finally reach 1.39 after 60 min. This points to changes undergoing in the structure of fat globules and to the initiation of their flocculation and coalescence even though particle size distribution did not indicate such changes. The presence of bile salts affected an increase in fractal dimension to the value of 1.74, which indicated significant changes in globules microstructure. In that case, the graph of particle size distribution depicted also changes indicative of the flocculation and/or coalescence process of the particles.

In the case of homogenized milk B, the fractal dimension increased significantly already in the absence of bile salts. This was also consistent with results of particle size distribution. In the presence of bile salts, the size of particles after 30 min fitted within a wide range. Apart from flocculation and aggregation, there could appear a new class of particles with varied sizes and shapes. In that case, the fractal dimension attained an intermediate value and reached 1.41. Although changes were triggered in the particle size distribution after 60 min of lipolysis, the fractal dimension did not change significantly. It might result from the accumulation of lipolysis products. In this case, structures with altered, rod-like shapes were observed (Fig. 4E). This change in shape could affect a change in the value of fractal dimension.

In the case of homogenized milk C, the changes followed generally a similar course as in homogenized milk B. Worthy of notice is some delay of the process. In the absence of bile salts, the fractal dimension reached approx. 1.6. Yet in the presence of bile salts, it did not decrease as in the case of homogenized milk B. This might denote the still high contribution of products of fat globules flocculation at a simultaneously lower amount of reaction products.

Ye et al. [21] observed a more complex scheme of changes in the structure of fat globules in the case of raw and reconstituted milk. Some differences might be due to various activities of the enzyme and composition of bile salts that have a significant effect on the lipolysis process.

The application of fractal image analysis is a unique approach and, to the best of authors' knowledge, it has been used for the first time to monitor changes in the emulsion system. This methodology can be applied to any microscopic technique used to obtain the images. Using electron microscopy, one can get more precise results with greater magnification. Also the possible shape distortion of milk fat globules can be avoided by using confocal microscopy without the need for cover slips.

The fractal dimension may be a useful parameter enabling detection of subtle changes in the emulsion system. During particle size distribution analysis, the mechanical interactions and shearing forces during measurements may lead to the disruption of delicate interactions that lead to flocculation and, as a consequence, may affect the final result of the measurement. This could happen in the case of milk A lipolysis in the presence of bile salts. After 60 min of lipolysis, the fractal dimension increased, whilst the particle size distribution remained unchanged. A phenomenon of this type may gain significance in the case of larger globules where weak interactions may be broken more easily than in the case of small globules in homogenized milk. In this case, the fractal analysis may allow for less biased evaluation of the ongoing changes.

CONCLUSIONS

Results achieved in the study confirm the significant impact of structure and available area of the interface on the susceptibility of milk fat

globules to digestion with pancreatic lipase. The presence of bile salts affected significant intensification of the lipolysis process in all milk samples. The addition of phosphatidylcholine to milk during homogenization (milk C) resulted in the formation of the membrane with diversified properties and in the deceleration of the lipolysis process in the presence of bile salts, compared to homogenized milk B. Nevertheless, taking into account the available surface of globules in milk, the increase in the percentage of both protein (milk B) and phospholipids (milk C) in the membrane compared to the native structure may lead to a diminished availability of substrate for lipase. This confirms the significant role of the native structure of the membrane in the process of milk fat digestion under the influence of lipases and may even indicate the specific interaction of the membrane with lipase-colipase and bile salts system.

The enzymatic reactions proceeding during lipolysis are accompanied by processes of flocculation or coalescence. Changes observed in the emulsion system were alike for all milk samples and were proportional to the results of lipolysis. The samples of milk differed only in the sizes of the formed aggregates that were proportional to the size of fat globules leading to their formation. Gentle interactions resulting in weak bonds and flocculation process may remain unnoticed during the measurement of particle size distribution as the shearing forces appear. In that case, the application of image analysis coupled with determination of the fractal dimension may be a useful alternative enabling detection of subtle changes in the emulsion system.

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