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MICROSTRUCTURE, RHEOLOGICAL AND GEOMETRICAL PROPERTIES OF FAT GLOBULES OF MILK FROM DIFFERENT ANIMAL SPECIES

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The variation between different Egyptian milk species (cows, buffaloes, sheep, goat and camel) in microstructure, shape, size and volume distribution as well as rheological properties, of fat globules was determined. Milk samples were scanned with SEM. The electron micrographs obtained were analyzed using an image analysis computer program. Results showed fat globules of different milk species as an oval shape in a regularity of spatial arrangement and encased in a lipoprotein membrane. Fat globules of different species vary considerably in diameter from those <1 μm to ~18 μm. Buffalo's globules were the greatest in size (8.7 μm), whereas close size values were found between cow and sheep globules (3.78 and 3.76 μm) as well as goat and camel globules (3.2 and 2.99 μm). On the contrary, the size of buffalo fat globules ranging from 0.1–4.0 μm was obviously less (23.0%) than that of camel (80.6%), goat (73.3%), cow (68.4%) and sheep (55.3%).

Fat globules exhibited different patterns according to their sizes and milk type. Small globules, as in camel's milk, were less spherical than the large ones, as in buffalo's milk (at α=0.05). An inverse proportional relationship (r=-0.8) was found between spherical diameter and compactness. Most of the fat globules in buffalo's, cow's and goat's milks oriented with obtuse angle, while those for sheep's and camel's milks oriented with acute angles. Changes in distribution of fat globules volume, perimeter, surface area, width and length were similar to that of size and significantly (p<0.001) affected by milk species.

INTRODUCTION

Many animals are kept to produce milk for human consumption. The most important are cows, buffaloes, sheep (ewes), goats and camels. These animals form the basis of commercial milk production in various parts of the world. The strong commercial importance of cow's and buffalo's milks have caused them to be studied more extensively than goat's, sheep's and camel's milks [Kelly, 2001; Haenlein & Abdellatif, 2004].

In recent years, the growing consumption of dairy products made from sheep's, goat's and camel's milks has required more knowledge of the raw materials [Haenlein, 2001, 2004; Soryal, 2000; Boyazoglu & Morand-Fehr, 1999; Jandal, 1996; Mehaia, 1993; Farah *et al.*, 1990; Abou-Lehia, *et al.*, 1989; Mann, 1988; Loewenstein *et al.*, 1980]. Fat constitutes one of the most important fractions of milk. Fat globules play an eminent role in the technology and properties of dairy products. Size of fat globules is of particular importance in such processes as separation of milk, churning of cream, cheese making as well as functionality of cheese [Rowney *et al.*, 2003; Everett & Olson, 2003; Ma & Barbano, 2000; Gunasekaran & Ding, 1999; Metzger & Mistry, 1995]. It determines the amount of protein absorbed per unit of interface area, and products emulsion stability and their optical and rheological properties (colour and viscosity) [Walstra, 1995; Walstra & Jenness, 1984] as well as conductivity and elastic constant [Clausse, 1983].

On the other hand, fat globules; vary in their size, distribution, microstructure and rheological properties between milk species [Mehaia, 1995; Abd El-Hamid & Khader, 1982]. Identifying these characteristics for fat globules of different species will enable dairy plants to produce high quality products, *e.g.* cheese making principles are similar for milk of all species but some modifications required to account for variations in milk components such as fat globules of different species. A reduction in the size of milk fat globules in cheese milk has been directly associated with a decrease in free oil formation, and this is related to the fat-water interfacial membrane and the emulsion stability of the globule [Oberg *et al.*, 1993; Cano-Ruiz & Richter, 1997]. Measurement of the size and shape of fat globules in cheese is necessary to determine the impact on cheese functionality [Rowney *et al.*, 2003]. Fat in cheese plays a role of preventing the protein from completely coalescing. In the cheese curd, wherever there is a fat globule, it prevents the chains of para-casein micelles from joining together into thicker strands. At this stage the fat is evenly distributed throughout the curd providing a smooth texture [Oberg *et al.*, 1993]. Additionally, fat globule membrane appears to have a high ability to bind water in cheese and so, to allow a significant increase in cheese yield [Goudédranche *et al.*, 2000]. To reduce fat in yogurt, total solid is lowered to 9–10%. This adversely affects physical and sensory properties of yogurt. Syneresis, weak body, lack of flavor and poor texture and mouthfeel are common defects of non-fat and low-fat yogurt [Trachoo, 2003]. All of the fat droplets play an important role at the air interface, helping to provide that smoothness. The process of freezing and aeration of the mix

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causes the milk fat emulsion to undergo a process called partial coalescence, in which the fat droplets form clusters and aggregates of fat that surround and stabilize the air bubbles. The same process is what creates structure in whipped cream [Goff *et al.*, 1999]. Earlier studies have shown that MFGM can act as a natural emulsifier in the reconstitution of milk fat globule emulsion [Kanno, 1989; Kanno *et al.*, 1991] and that the emulsion was stable within a temperature range of 4 to 55°C. In spite of that, a meager attention has been devoted to study these properties and variations between the different milk species. In addition, they have to be still elucidated and are difficult to deal with.

The steady improvement in the accuracy, sensitivity, and reliability of laboratory instruments together with the data storage and processing capability of modem computers has revolutionized the way the fat globules physical characteristics are established. In this respect, the scanning electron microscope (SEM) with software image analysis enables detailed measurements of photographed objects as fat globules [El-Zeini, 2001; Gunasekaran & Ding, 1999; Walstra, 1995; Kalab, 1993; Holcomb, 1991; Bunville, 1984].

The objective of this study was to use the SEM-digital image analysis technique in measuring size and volume distribution as well as rheological and geometrical properties of fat globules of Egyptian buffalo's, cow's, sheep's, goat's and camel's milks along with the evaluation of their microstructure variations.

MATERIALS AND METHODS

Fresh cows', buffaloes', goats' and sheep's milk samples were collected from the herds of Faculty of Agriculture, Cairo University, Giza. While, fresh camels milk samples were obtained from Egyptian Hogon Co., Giza, Egypt.

Four fresh milk samples for each type of milk were prepared. The 0.2 mL milk samples were fixed with 2 mL glutaraldehyde (1%) in 0.1 mol/L phosphate buffer (pH 7.2) at room temperature for 15 min with occasional stirring. Samples were diluted (1:50) with distilled water and deposited on cover slips by dipping. The cover slips with dispersed milk were mounted on stubs, so that a good conductivity pathway could be obtained from the top of the cover slips to the metal stubs, once they had been sputter coated. The cover slips were air dried, then transferred to a desiccator and held under vacuum for 24 h. The dried mounted cover slips were coated with gold using EM Scope SC500 sputter coater (Ashford, Kent, England). Three photographs, for each sample, were taken using a Scanning Electron Microscope (SEM) (JEOL – JSM-35, Tokyo, Japan). The micrographs were scanned by an Epson Scanner (Model GC. 9500). Images of at least three fields for each micrograph were analyzed by Climax Vision computer program (Climax Technologies Inc., Longueil, Qc., J4GITS, Canada). The images were digitally processed to produce binary images which were measured through the system to obtain several rheological and geometrical properties. These include: spherical diameter, sphericity (S: describes how close a shape is to a perfect sphere, for a perfect sphere S=0), intensity, compactness (describes how dense the fat globules are depending on the distance between the voxels; the smaller the distance between the voxels, the higher the compactness), roughness (measures the irregularity of the fat globules surfaces), orientation (3-dimension), perimeter and area (2-dimension). The accuracy of 2-dimension measurements was tested mathematically. The total number of fat globules in the field and frequency distribution of each parameter were also determined by the program. The percent of field occupied by globules (volume distribution in percentage) of fat were calculated according to Mc Gann *et al.* [1980] and Sirlaorkul *et al.* [1991].

Statistical analysis. A randomized block design was used to evaluate the effect of the treatment (milk species) on the dependant variables measured using subprogram MSTAT (v4c, 1989). A multiple linear regression analysis was applied and "T" test was used to analyze the differences between means at $p<0.05$.

RESULTS

Fat globule microstructure

Micrographs of a scanning electron microscope show fat globules of buffalo (Figure 1a), cow (Figure 1b), sheep (Figure 1c), goat (Figure 1d) and camel (Figure 1e) milks as spherical globules with an oval shape in irregularity of spatial arrangement surrounded by a lipoprotein membrane. The fat globules of different species vary considerably in diameter from those <1 μm to about 18 μm. Additionally, buffalo's milk fat globules had the biggest diameter (8.7 μm) compared to that of cow's (3.95 μm), sheep's (3.78 μm), goat's (3.2 μm) and camel's (2.99 μm) milks (Table 1). In cow's, sheep's and camel's milks, elongated globules of variable sizes were distinguished throughout milk serum. On the other hand, goat's milk fat globules had the best spherical structure (S=0.33) compared to those of the other milk species (Table 2). The

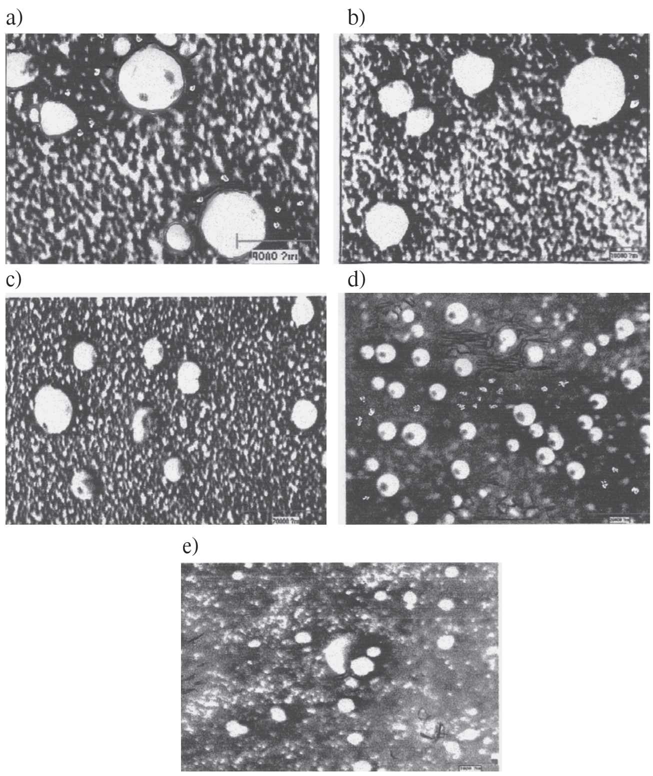

FIGURE 1. Micrographs of scanning electron microscope fat globules of buffalo (a), cow (b), sheep (c), goat (d) and camel (e) milks.

results were in accordance with Attaie & Richter [2000], Gunasekaran & Ding [1999] and Olivier & Paquin [1991].

Fat globules size distribution

The differences between the milk species in fat globules size are best appreciated by comparisons of the actual frequency distributions rather than by calculated average. Table 1 shows the fat globules size distribution of cow's, buffalo's, goat's, sheep's and camel's milk. There was a significant ($p<0.01$) influence of milk type on the spherical diameter of fat globules and particle size distribution in different species (Table 2). No significant ($\alpha=0.05$) differences were observed in the particle size of cow's and sheep's milk from one side and goat's and camel's milk from the other side.

These results were in concordance to what El-Zeini [2001], Walstra *et al.* [1999], Farah & Ruegg [1991] stated. Attaie & Richter [2000] reported close values for fat globules spherical diameters of different milk species. Jandal [1996] reported that the size of goat's milk fat globules varied from 0.1 to 10 μm with the greatest proportion being <2.0 μm while, the reverse is true for cow's milk which had spherical fat globules varying in diameter from 0.1 to 15 μm [Rowney *et al.*, 2003; Goudédranche *et al.*, 2000; Saini & Gill, 1991; Wahba *et al.*, 1988]. In addition, it can be observed from the size distribution profile (Table 1) that the distribution width in fat globules size of buffaloes milk ranging 0.1–4.0 μm (23%) was less than that of camel's, cow's, sheep's and goat's milk (80.6, 68.4, 55.3 and 73.3 %, respectively). That large number of small fat globules of these milks contributed only to a slight extent to the amount of fat in milk as well as some fat globules that were larger than 8.0 μm in camel's (0%), sheep's (1.0%), cow's (5.2%) and goat's (9.2%) milks, compared to that of buffalo's milk (44.1%). Fat globules of this size (≥8.0 μm) could have an important impact on the smoothness or hardness of cheese. Therefore, a negative correlation expressed that the number of fat globules decreased as the mean diameter increased in all various milks ($r=-0.812$). These changes in the number and size of particles were probably responsible for a significant ($p<0.001$) alteration in the physical properties of cheese such as firmness, compactness, texture and their functionality [Rowney *et al.*, 2003; Goudédranche *et al.*, 2000; Jandal, 1996; Grandison, 1986].

Fat globules length and width

The structure of fat globules showed imperfect spheres which could have length and width as imperfect rectangles (Figure 1). However, an increase in the degree of fat globules elongation is believed to be an indicator of fat globules distortion [Everett & Olson, 2003]. With milk species variation, significant ($p<0.001$) influences were recognized on the length and width of fat globules. Negative correlations were found (-0.84 and -0.77) between milk types and length and width of fat globules. The frequency distribution (Figure 2) showed wide length ranges at 4.0, 2.0, 4.0, 8.0 and 4.0 μm for 35, 52, 23, 26 and 27% of buffalo's, cow's, sheep's, goat's and camel's milks fat globules, respectively. However, the same results were obtained with width ranges except for shifting sheep's, goat's and camel's milk fat globules to 10.0, 2.0 and 6.0 μm for 52.0, 32.0 and 31.0%, respectively. Although, at $\alpha=0.05$, the lengths differed significantly among various types of milk, width differed only with buffalo's milk.

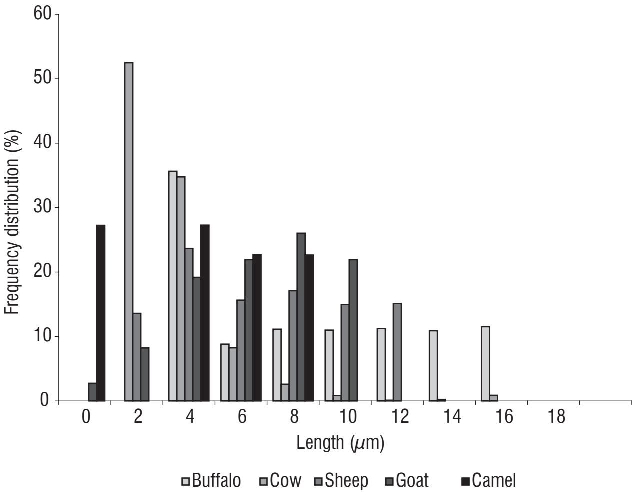

FIGURE 2. The distribution of fat globules length for different milk species.

TABLE 1. Fat globules distribution of different milk species.

Fat globule diameter (μm)	Buffalo		Sheep		Cow		Camel		Goat	
	No in groups	% Distr.	No in groups	% Distr.	No in groups	% Distr.	No in groups	% Distr.	No in groups	% Distr.
0.1–1	-	-	-	-	-	-	69	19.94	140	25.4
1–2	-	-	86	15.69	127	19.01	-	-	148	26.86
2–4	205	23.78	217	39.59	330	49.4	210	60.69	116	21.02
4–6	195	21.67	221	40.33	131	19.61	49	14.16	25	4.53
6–8	103	11.45	18	3.28	24	3.59	18	5.20	72	13.04
8–10	97	10.78	4	0.73	34	5.09	-	-	35	6.34
10–12	117	13.0	2	0.37	1	0.15	-	-	16	2.89
12–14	-	-	-	-	-	-	-	-		
14–16	-	-	-	-	21	3.14	-	-		
16–18	183	20.34	-	-	-	-	-	-	-	-
18–20	-									
Total	900		548		668		346		552	
Average (μm)	8.7		3.78		3.95		2.99		3.19	

Fat globules perimeter

Figure 3 illustrated the distribution of fat globules perimeter for different milk species. Milk type plays a significant ($p<0.001$) role in defining fat globules perimeters measured. In spite of that, no significant ($\alpha=0.05$) differences were found between cow's and sheep's fat globules perimeters on one hand and between that of sheep's and goat's milk on the other hand (Table 2). The highest perimeter maintained with buffalo's milk fat globules (27.79 μm) with 99% accuracy compared to the calculated perimeters. Moreover, the lowest value occurred with camel's milk fat globules (9.6 μm) with 97% accuracy. The correlation matrix showed 0.81 between the perimeter of fat globules and the type of milk. Attaie & Richter [2000] and Gunasekaran & Ding [1999] found similar results.

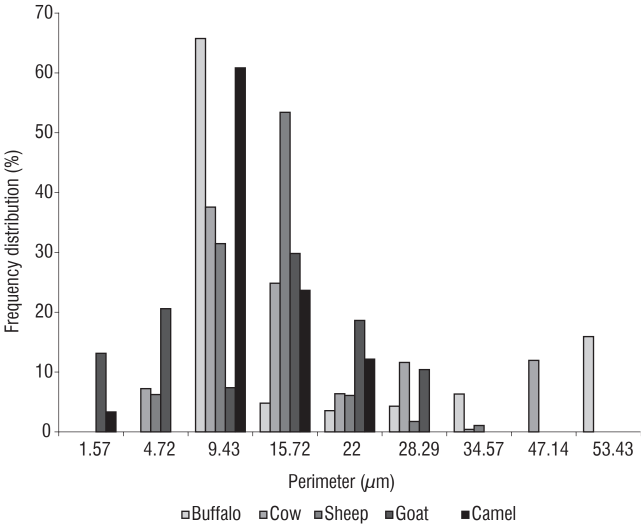

FIGURE 3. The distribution of fat globules perimeter for different milk species.

Fat globules surface area

As shown in Table 2, fat globules surface areas were significantly ($p<0.001$) influenced by the types of milk examined. Trends of changes in the surface area were similar to those observed for fat globules sizes distribution (Table 1) with the highest values reported at 17 μm (61%), 15 μm (30%), 7 μm (34%), 5 μm (60%) and 3 μm (47%) diameter for buffalo's, cow's, goat's, sheep's and camel's milks fat globules, respectively. The reproducibility's of using surface area measuring method were 93, 98, 98, 91, and 89% for the above-mentioned milk species, respectively, compared with the calculated fat globules surface area. Surface area of cow's milk fat globules did not change significantly ($\alpha=0.05$) from that of sheep's milk. However, buffalo's milk fat globules surface area differed significantly ($p<0.001$) from those of the other milk species. Rowney *et al.* [2003] reported 23.3 μm^2 and 8.8 μm^2 for fat globule surface areas of unhomogenized and homogenized cow's milk, respectively, whereas Goudédranche *et al.* [2000] recorded surface area in the range from 5 to 11 μm^2 per 100 g for cow's milk fat globules. The difference in these results may be attributed to the measuring procedures.

Fat globules volume

The changes in particle size distribution of fat globules on a volume basis are shown in Figure 4. For all milk species, the average volume was the highest for buffalo's milk fat globules (344.9 μm^3), while the lowest (14.0 μm^3) was for that of camel's milk. Statistically, milk type affected significantly ($p<0.001$) the fat globules' volume. The highest volume distribution was at 17.0 μm (75%), 15 μm (54%), 11 μm (32%), 7 μm (34%) and 5 μm (60%) diameters for buffalo's, cow's, goat's, camel's and sheep's milks fat globules, respectively.

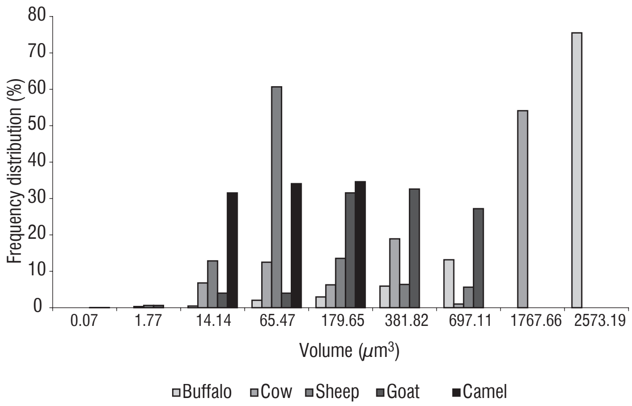

FIGURE 4. The distribution of fat globules volume for different milk species.

TABLE 2. Average rheological parameters values of fat globules of different milk species.

Property	Buffalo	Cow	Sheep	Goat	Camel	LSD (0.05)	Student T value	Probability
Spher. diam. (μm)	8.7	3.95	3.78	3.2	2.99	0.2855	4.816	< 0.001
Compactness (0–1)	0.71	0.81	0.79	0.86	0.91	0.0595	7.701	< 0.001
Sphericity (0–1)	0.59	0.91	0.89	0.33	0.81	0.0596	0.318	> 0.001
Volume (μm^3)	344.93	32.28	28.29	18.65	13.99	1.145	3.829	0.002
Surface roughness (0–1)	0.91	0.99	0.99	0.6	0.95	0.2837	1.084	0.0297
Surface area (μm^2)	58.34	12.26	11.31	8.74	7.78	1.209	4.086	< 0.001
Perimeter (μm)	27.786	13.02	11.93	11.4	9.6	1.389	4.768	< 0.001
Intensity (%)	87.43	98.5	97.05	94.53	87.36	1.558	0.425	0.677
Orientation (°)	107.46	90.79	45.16	105.69	68.07	1.59	2.116	0.053
Length (μm)	9.85	4.67	4.1	3.68	3.07	0.2382	5.386	< 0.001
Width (μm)	4.15	2.02	1.9	1.86	1.65	0.4763	4.203	< 0.001

Close results were cited by Attaie & Richter [2000], Ma & Barbano [2000] and Gunasekaran & Ding [1999].

Fat globules surface roughness

As the type of milk changed, the fat globule surface roughness significantly ($p<0.001$) altered (Figure 5). In spite of that, no correlation was found between milk type and fat globule surface roughness. The highest distribution of surface roughness was maintained for buffalo's, cow's and camel's milk fat globules (22.59%, 23.35%, 24.41%, respectively) at spherical diameters of 0.75 μm for buffalo's milk and 0.55 μm for both cow's and sheep's milk. While that of goat's (28.77%) and camel's (25.67%) milks inscribed at 0.65 μm spherical diameters. Among treatments, the least significant ($\alpha=0.05$) difference was found with goat's milk roughness (0.67) only (Table 2). El-Zeini [2001] reported similar results for fresh buffalo's milk.

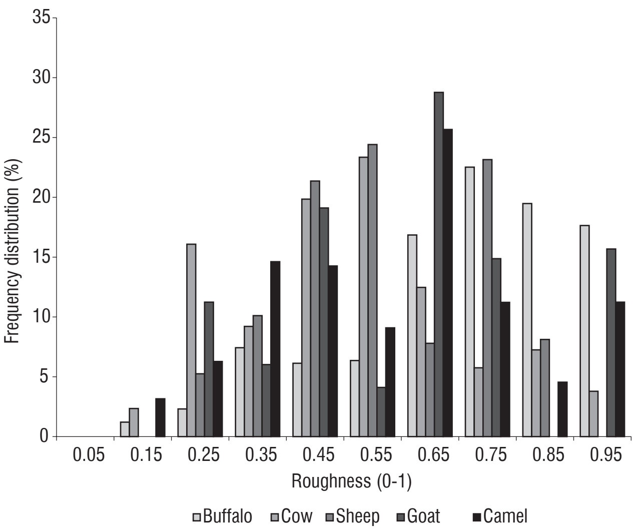

FIGURE 5. The distribution of fat globules roughness for different milk species.

Fat globules intensity

Most of the fat globules of various milk species that have been examined had high intensity values, particularly sheep's milk (Table 2). The fat globules varied considerably ($p<0.001$) in the intensity both within and between species. Image analysis of the intensity of fat globules showed a distribution with a maximum frequency; 44.0, 96.0, 100, 67.0 and 36.0% for buffalo's, cow's, sheep's, goat's and camel's milk, respectively. No correlation was found for the apparent differences in the intensity of different milk species. Among milk types, no significant differences ($\alpha=0.05$) were found for fat globules intensity between both cow's and sheep's as well as between buffalo's and camel's milks. El-Zeini [2001] reported similar results for fresh buffalo's milk.

Fat globules orientation

A remarkable difference was apparent ($p<0.001$) between mammals' milk with regard to the orientation of fat globules. More than two thirds (2/3) of the fat globules of buffalo's, cow's, goat's milk orientated with obtuse angles (77.0%, 70.0% and 67.0%, respectively), while those for sheep's and camel's milks orientated with acute angles (75.0% and 63.0%, respectively). The only right angle was found with average orientation of fat globules of cow's milk (Table 2). The impact of species variation on sheep's and camel's milk fat globules orientation was similar ($\alpha=0.05$), but the other species orientation differed to a great extent. El-Zeini [2001] reported similar results for fresh buffalo's milk.

Fat globules compactness

Figure 6 shows the distribution of fat globules compactness for different milk species. As expected, buffalo's milk fat globules compactness (0.71) significantly differed from that of the other milk species (Table 2). The highest compactness (0.91) was noticed with camel's milk, which had the lowest average spherical diameter (2.99 μm). The size of fat globules significantly ($p<0.001$) controlled their compactness on the one hand. On the other hand, milk source dramatically ($p<0.001$) interfered in the value of fat globules compactness (Table 2). There was an inverse proportional relationship ($r=-0.8$) between fat globules spherical diameter and their compactness. Within the treatment, no differences were found ($\alpha=0.05$) between cow's (0.81) and sheep's milk (0.79) as well as between goat's (0.86) and camel's milk (0.91) compactness. El-Zeini [2001] reported similar results for fresh buffalo's milk.

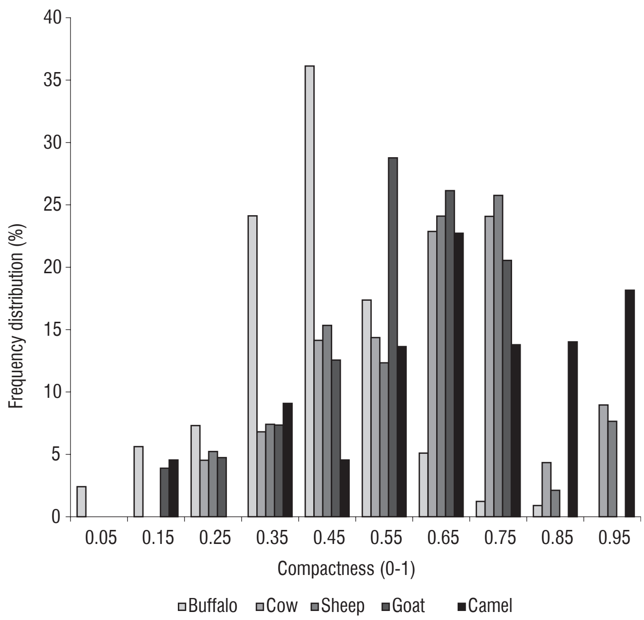

FIGURE 6. The distribution of fat globules compactness for different milk species.

Fat globules sphericity

It is likely that the overall sphericity values of fat globules differed significantly ($p<0.001$) from one species to the other. Furthermore, 31.8, 9.8 and 13.3% of fat globules of camel's, sheep's and cow's milks, respectively were imperfect spheres ($S>0.8$). The maximum sphericity for buffalo's milk was 0.75 with an average of 0.59. Moreover, 57% of goat's milk fat globules had the sphericity <0.6 with an average of 0.33 (Table 2). The results obtained implied that the small globules in cow's, sheep's and camel's milks were less spherical than the large ones as in buffalo's milk at $\alpha=0.05$. These results were in agreement with findings of Gunasekaran & Ding [1999] and El-Zeini [2001] for buffalo's milk. On the other hand, circularity and sphericity values of fat globules were used to determine cheese meltability [Gunasekaran & Mehmet, 2003].

DISCUSSION

The microstructure, rheological and geometrical properties of milk fat globules of different animal species showed a wide range of variations. The results obtained characterized each type of milk secreted by each species. The average fat globule size markedly differs between species and breeds of milk animals. Ranking the milk of the most important species of domestic livestock by average size globules gives: buffalo's milk, cow's milk, goat's milk, sheep's milk and camel's milk. Comparing breeds, milk with a high fat content as buffalo does will usually contain large globules than milks with a low fat content. Towards the end of the lactation, there is a tendency for the animal to produce smaller globules. The globules at the beginning of lactation are comparatively large. In addition, proportion of short chains fatty acids are low initially and increased until at least 8 to 10 weeks into lactation. Milk fat globules changed more by the amount and composition of dietary fat than any other dietary component. Seasonal, regional, genetic differences could be the other factors that brought about those variations. High dietary protein intake would increase the proportion of the long chain fatty acids in milk. Variations in fat globules size highly correlated with the differences in all the rheological and geometrical parameters measured in all milks tested. These differences were consistent with the observations of Palmquist *et al.* [1993].

CONCLUSIONS

Fat globules rheological and geometrical parameters measured are most beneficial for dairy manufacturers, as they enable them to predict the properties of the final product and through manipulating those parameters, an improvement could be achieved in the dairy industry. Setting those parameters provides a chance to choose the best fitting type of milk to good quality products. In addition, discovering milk adulteration through fat globules would be possible. Nonetheless, quantification of image features obtainable with Climix program could serve as an objective criterion for evaluating the effect of a number of variables of interest on fat globules and their influence on cheese and other dairy products. However, the only limitation was in the lack of the publications in this regard.

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