

Casein Micelles Physical Properties of Zaraibi Goat's Milk in Semi- Intensive Production System

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Abstract— Casein micelles physical properties of Zaraibi goat milk collected from three locations in and around Cairo (L1, L2 and L3) were studied using Atomic Force Microscope. AFM images were analyzed using SPIP software.

The highest micelles size average was 225.06 ± 101 nm for L2. Peak breadth value was 250 nm. Surface area averages were $4.14 \times 10^4 \pm 4.78 \times 10^4$, $4.78 \times 10^4 \pm 3.83 \times 10^4$ and $4.17 \times 10^4 \pm 2.44 \times 10^4$ nm² for L1, L2 and L3, respectively. The mean volumes of particles in each category were $5.58 \times 10^5 \pm 9.01 \times 10^5$, $9.30 \times 10^5 \pm 15.85 \times 10^5$ and $6.18 \times 10^5 \pm 6.76 \times 10^5$ nm³ for L1, L2 and L3, respectively. The hairy outer layer values were 74674.291, 64551.333 and 58268.302 nm for L1, L2 and L3, respectively. Casein micelles of goat milk are compacted with a value close to 1. The average particles hardness was 0.97 ± 0.01 . Micelles oriented with right and acute angles for L1 and both L2 and L3, respectively. Particles roundness mean is 0.8. Micelle roughest surface (25.76 ± 3.64 nm) was reported for L2. The study confirmed that AFM is a powerful tool for imaging the structure of micro-molecules as casein micelles. The measured features of goat milk casein micelles structure can be useful in setting the geometrical parameters that help in improving the texture of dairy products.

Index Terms— goat milk, casein micelle, image analysis, geometrical parameters, microstructure.

I. INTRODUCTION

Dairy goats and sheep farming are a vital part of the national economy in many countries, especially in the Mediterranean and Middle East region [1], and are particularly well organized in France, Italy, Spain, and Greece [2]. However, large scale industrialization of the dairy goat and sheep sectors in many countries is limited by low volume and seasonal cycle of individual milk production, around 50 kg annually [1]-[3]. Information on composition and characteristics of goat and sheep milk is essential for successful development and marketing. Goat milk differs from cow or human milk in possessing better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition [4] – [5] – [6].

A micelle model other than cows' seems to be useful in obtaining new concepts on the micelle structure and size. In fact, there are a few data available on milks other than cows' which are not sufficient to undertake comparative studies,

even with the important contribution of [7] on the minerals of milks, and of [8] on the micelle size determinations.

Several reports of the size distribution of casein micelles in bovine milk have been published [9], but the size of the casein micelles in goat milk determined by sensitive techniques has not been reported recently. The structural organization of goat milk casein micelles has been the subject of a few studies [9]. For methodological reasons, a direct approach of the micelles structure is not easy because of its complexity, the great number of molecular species involved and the way in which they are interacting. However, useful and revealing information can be obtained indirectly using image analysis of micrographs of transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM). Microstructures analysis of goat milk casein micelles has remained up till now rather poorly studied. Thus, the aims of this study were to compare casein micelles (CM) microstructure of goat milk obtained from different districts in and around Cairo, set goat milk casein micelles geometrical properties, explore the potential of AFM for studying goat milk casein micelles and elucidate the surface morphology and size distribution of goat milk casein micelles.

II. METHODOLOGY

Fresh samples in triplicate of goat's milk were collected from three farms (L1, L2 and L3) exist under semi intensive production system in and around Cairo to investigate microstructure of goat milk casein micelles

A. Sample preparation

Raw goat milk was stored for 24h and centrifuged (three times) at 2000g at 4°C for 20 min. to remove fat. The skim milk (pH 6.79 ± 0.05 at 20°C) was stored at 4°C overnight. 3-4 ml droplets each were equilibrated for 1–2 h at room temperature. Samples were placed on previously cleaned mica disks and dried in an open air. Sample disks were kept for 24 h in closed Petri dishes at ambient temperature before imaging by AFM.

B. Samples scanning by AFM

AFM (Veeco Instruments Nano-scope, Multimode-V5)

was operated in repulsive tape mode. Nanoprobes cantilevers made of silicon (EFM 50, Digital Instruments) with a spring constant of 1–5 Nm⁻¹ and a resonance frequency of 60–100 KHz were used with oscillation amplitude of 50–70 nm. The cantilever is oscillated at or slightly below its resonance frequency with amplitude ranging from 20 to 100 nm. Scanning of the samples was performed at 0.3 Hz rate with 256 x 256 pixels.

C. Image analysis of casein micelles

Images were analyzed by the Scanning Probe Image Processor (SPIP) Software (Version 6.0.1 (BETA), Denmark) which enables the user to manipulate lateral calibration and Unit Cell Detection to account for the magnification differences in each image. The image contains a waffle pattern with a repeat distance of 10 nm and step-heights of 100 nm.

It is suitable for demonstration of Y and Z calibration. 1000 nm was used as reference pitch value to get the proper correction parameter.

D. Detection and quantification of casein micelles particles

The particle & pore analysis module using the polygon measure shape was employed. Several geometrical parameters (breadth, length, diameter, perimeter, area, roundness, compactness, orientation, volume, net volume, mean height, mean depth and solidity) were obtained by the system.

Area: The area is calculated from the shapes periphery, i.e. the closed polygon that surrounds the feature. The area is calculated using:

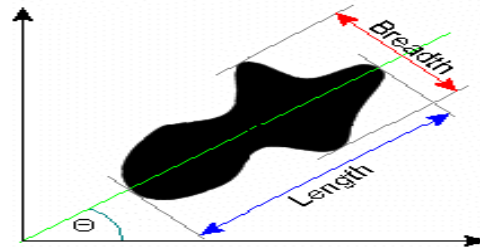
$$\text{Area}(\text{polygon}) = \frac{\sum_{i \in \text{shape}} (\chi_i + \chi_{i-1}) \cdot (y_i - y_{i-1})}{2}$$

Where the x and y are the point spacing in directions of the image, respectively

Diameter: The diameter (or Heywood diameter) is expressed as the diameter of a circle having an area equivalent to the shape's area

$$\text{Diameter} = \sqrt{\frac{4}{\pi} \cdot \text{Area}}$$

Length: Length is defined as the longest cord along the angle Θ given by the moment's axis to the x-axis.



Breadth: Or width is defined as the longest cord perpendicular to the angle Θ given by the moment's axis to the x-axis.

Perimeter: For polygon shapes the perimeter is calculated from the shape's contour as:

$$p = \sum_i \sqrt{(\chi_i - \chi_{i-1})^2 + (y_i - y_{i-1})^2}$$

Volume: Volume is the maximum of the found material volume and void volumes:

$$\text{Volume} = \text{Max} \{ \text{material volume} + \text{void volume} \}$$

Where the material volume equals the volume of all points having a Z value higher than the mean contour height

$$\text{Material volume} = \sum (Z(x, y) - Z_{MSH}) \cdot dx dy$$

Void Volume equals the volume of all points having a Z value lower than the mean contour height. This value will always be positive

$$\text{Void Volume} = \sum_{\{Z(x,y) \in \text{Shape} \mid Z \leq Z_{MCH}\}} (Z_{MCH} - Z(x, y)) \cdot dx dy$$

Where dx and dy are the point spacing in the χ and y directions of the image, respectively.

Roundness: Roundness describes the shape's resemblance to a circle. The roundness factor of a shape will approach 1.0 when the closer shape resembles a circle.

$$\text{Roundness} = \frac{4 \cdot \text{Area}}{\pi \cdot \text{Length}^2}$$

Compactness: Compactness is a measure expressing how compact a feature is. From the formula below, a circle will have a compactness of 1.0, whereas elongated and irregular shapes results in values less than 1.0.

$$\text{Compactness} = \frac{\text{Diameter}}{\text{Length}} = \frac{\sqrt{\frac{4 \cdot \text{Area}}{\pi}}}{\text{Length}}$$

Orientation: Gives the angle of the axis of momentum. To obtain the orientation we find the line which best fits all the points in the object, actually only the points describing the contour are used. This line is the "axis of momentum". Having the moment axis it's simply a matter of calculating the angle to the x-axis.

$$M_x = \sum_{x,y} x^2 - \frac{[\sum_{x,y} x]^2}{\text{Area}}$$

$$M_y = \sum_{x,y} y^2 - \frac{[\sum_{x,y} y]^2}{\text{Area}}$$

$$M_{xy} = \sum_{x,y} (x \cdot y) - \frac{\sum_{x,y} x \cdot \sum_{x,y} y}{\text{Area}}$$

$$\Theta = \tan^{-1} \frac{M_x - M_y + \sqrt{(M_x - M_y)^2 + 4 \cdot M_{xy}^2}}{2 \cdot M_{xy}}$$

Solidity: A measure describing the resemblance of the shape's area with its convex area

$$\text{Solidity} = \frac{\text{Area}}{\text{Convex Area}}$$

Mean contour height: Mean contour height is the average Z value (relative to Z=0) of the shape's contour points. The local Z values are calculated using bi-linear interpolation at each contour point. In the case of user defined shapes (manually drawn) and Ellipse/Circle shapes the contour is sampled at less than $\sqrt{2}$ pixel spacing's by adding temporary contour points.

Mean height:

Mean height is the average of Z values (relative to the mean contour height) of all points inside the shape having $Z \geq Z_{MCH}$.

Mean Height

$$= \frac{\sum_{\{Z(x,y) \in \text{Shape} | Z \geq Z_{MCH}\}} (Z(x,y) - Z_{MCH})}{\sum 1}$$

Mean depth: Mean depth is the average of Z values (relative to the mean contour height) of all points inside the shape having $Z \leq Z_{MCH}$.

Mean Depth

$$= \frac{\sum_{\{Z(x,y) \in \text{Shape} | Z \leq Z_{MCH}\}} (Z_{MCH} - Z(x,y))}{\sum_{\{Z(x,y) \in \text{Shape} | Z \leq Z_{MCH}\}} 1}$$

Roughness: The roughness average, Sa, is defined as:

$$S_a = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |Z(x_k, y_l)|$$

E. Statistical analysis.

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

III. RESULTS AND DISCUSSION

A. Casein micelles microstructure and size

To understand the important role of casein micelles in the formation and stabilization of dairy products, native casein micelles were investigated using AFM in tapping mode. The fine structure of the casein micelles was observable with AFM. It is typical, when using AFM to observe a 3-dimensional spherical object such as a casein micelle, that the central region of the image is darker than the periphery. Casein micelles physical properties of goat milk collected from three different locations in and around Cairo were analyzed and representative images are shown in Fig. 1.

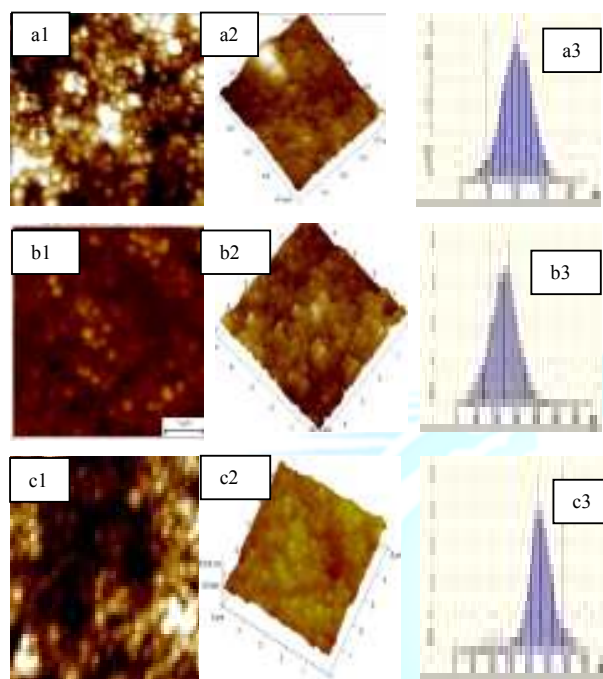


Fig 1. Topographs of AFM casein micelles of Zaraibi goat milk samples obtained from Ras Suder in Sinai (a), Cairo University in Giza (b) and 10th of Ramadan (c) farms.

Topographical images reveal spherical or pseudo-spherical structures with height profiles. The bright and dark areas in the images correspond to peaks and troughs on the surface of the casein micelles. Topographs of casein micelles of goat milk showed a wide range of diameters for L1. The micellar sizes ranged from 24 – 553, 40-511 and 55-483 nm with averages of 213.58±81.69, 225.06±101 and 216.30±64.34 nm for L1, L2 and L3, respectively (Table 1).

Table 1. Maximum, minimum and average diameter (nm) of Zaraibi goat milk Casein micelles obtained from different 3 locations

Casein micelles diameter (nm)	L1	L2	L3
Max	553.040	511.204	483.331
Min	24.670697	40.546	55.396
Mean	213.58c	225.06a	216.30b
Sd	81.689	100.994	64.336
Count	157	215	200

Statistical significant differences (LSD = 1.224) were found within casein micelles diameters means as a function of location ($\alpha=0.05$). High standard deviations obtained were due to the high variation in casein micelles sizes. The corresponding change in casein micelle sizes may be attributed to variations in the raising environment conditions as climate, feeding regime, genetic properties and individualities.

B. Distribution of casein micelles size

The differences between goat milks, obtained from different locations, in casein micelles size are best appreciated by comparisons of the actual frequency distributions rather than by calculated average. The size of goat's milk casein micelles varied with the greatest proportion being in the range of 100-300 nm (Table 2). [10] found 70% of the micelles were recovered in the range of 50-100 nm, with one half of the casein micelles presented size ≥ 120 nm.

Table 2. Casein micelles size distribution of Zaraibi goat milk samples obtained from different locations.

Casein micelle diameter (nm)	Casein micelles distribution (%)		
	L1	L2	L3
0-50	0.64±0.00	0.93±6.68	0.00±0.00
50-100	7.00±14.44	13.95±14.73	3.50±16.12
100-150	11.46±13.68	7.44±13.48	7.50±14.43
150-200	26.11±2.67	16.28±17.34	27.00±15.72
200-250	26.11±12.81	21.40±14.17	32.00±14.77
250-300	17.83±12.74	19.53±14.80	20.00±15.25
300-350	5.73±4.51	9.77±17.32	7.50±12.48
350-400	2.55±4.51	5.58±9.31	1.50±9.42
400-450	1.27±12.36	3.72±14.81	0.50±0.00
450-500	0.60±38.69	0.93±3.57	0.50±0.00
500-550	0.64±0.00	0.47±0.00	0.00±0.00

Although, L1 and L2 had very small size casein micelles (<50 nm diameter), L3 was free of them as well as bigger size micelles (>500nm diameter). Significant ($P<0.001$) differences were observed in the particle size distribution. The results were in agreement with [11] and [12] who stated that casein micelles have a shape of imperfect sphere. [13] reported higher goat milk casein micelles average diameter (260 nm).

C. Casein micelles breadth, length and perimeter

As casein micelles showed imperfect spheres so they have length and breadth. Through the 3 farms, significant ($p<0.001$) influences were recognized on the breadth, length ($P<0.05$) and perimeter ($P<0.01$) of casein micelles. The average of casein micelles breadth, length and perimeter of goat milk for L1, L2 and L3 are shown in Fig.2. While, no significant differences in breadth and perimeter were found between L1 and L3 at $\alpha=0.05$, L1 was significantly different from both of them (LSD=7.205 and 23.32, respectively). Moreover, the only significant differences in length at $\alpha=0.05$ were within L1 and L2 (LSD=8.143).

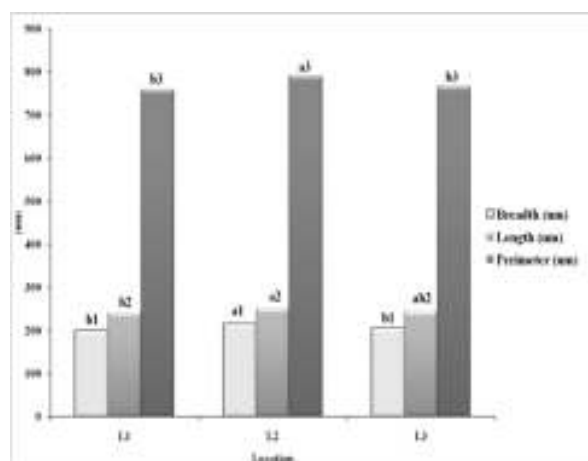


Fig. 2. The average breadth, length and perimeter (nm) of casein micelles of Zaraibi goat milk samples obtained from different locations

Distribution of casein micelles breadth, length and perimeter

To estimate particles distributions, the scattering curves were analyzed that describes the breadth, length and perimeter of the particles in the space and fits were plotted as histograms (not shown). Wide breadth ranges from 23.69 to 577.3 nm (pulled average = 206.66 ± 7.60 nm) and a peak maximum is 250 nm for L1, L2 and L3. Size distributions are expressed in particle number frequency. The breadth distribution in particle number frequency indicated the presence of 67.5%, 56.7% and 77% of the breadth values in the range of 200-300 nm for L1, L2 and L3, respectively. These micelle breadth values were close to those for cows' milk measured by [14] using transmission electron micrographs. A small proportion (0.6, 0.4 and 0.5%) of large width micelles (> 500 nm) and a large number of smaller micelles (<150 nm) as 24.2, 25.5 and 16.5% for L1, L2 and L3, respectively were observed.

Casein micelle had a length distribution of <50 nm to 600 nm with the greatest proportion (63, 50 and 71% for L1, L2 and L3, respectively) being at 200-300 nm. Large length micelles (> 500 nm) represented 2.6, 4.6 and 1.5%, while, small length micelles (< 150 nm) represented 16.5, 20.5 and 8% for L1, L2 and L3, respectively.

A large difference in casein micelle perimeter distribution was observed ($P < 0.001$). The perimeter of goat milk casein micelles ranged from 77.60 to 1952.00 nm with an average of 771.62 ± 16.52 nm. The distribution was centered on perimeter value of 1000 nm, while most (68, 60, 77.5%) of the casein was in average perimeter micelles (600-1200 nm) for L1, L2 and L3, respectively. Casein micelles perimeter correlated with the mean micelle size with a correlation coefficient = 0.968 (Table 3).

D. Casein micelles surface area, volume and net volume

As shown in Fig. 3, casein micelles surface areas were significantly ($p < 0.05$) influenced by the obtaining milk farm. Within treatments, no differences ($\alpha = 0.05$) were found between L1 and L3 (LSD= 5948). Surface area variations correlated with casein micelles sizes ($R^2 = 0.935$).

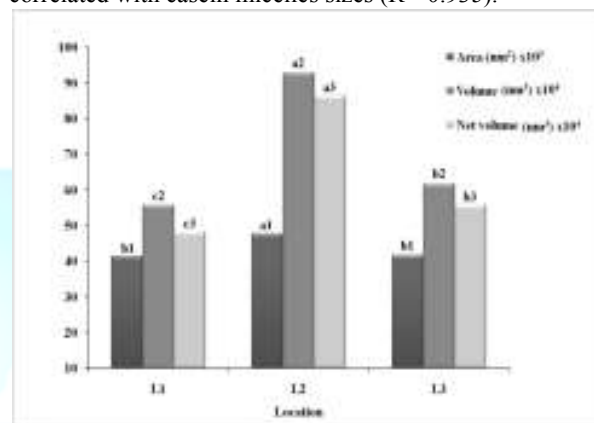


Fig. 3. The average surface area, volume and net volume of casein micelles for Zaraibi goat milk samples obtained from different locations

The maximum surface area was found with L1 (240216.61 nm^2), while the minimum was observed with L3 (183475 nm^2). Additionally, the averages were $4.14 \times 10^4 \pm 4.78 \times 10^4$, $4.78 \times 10^4 \pm 3.83 \times 10^4$ and $4.17 \times 10^4 \pm 2.44 \times 10^4 \text{ nm}^2$ for L1, L2 and L3, respectively. The location for sample collection affected significantly ($P < 0.1$) the volume of casein micelles of goat milk. However, this parameter differed ($\alpha = 0.05$) within samples locations (LSD = 18370). The mean volume of particles in each category was $5.58 \times 10^5 \pm 9.01 \times 10^5$, $9.30 \times 10^5 \pm 15.85 \times 10^5$ and $6.18 \times 10^5 \pm 6.76 \times 10^5 \text{ nm}^3$ for L1, L2 and L3, respectively. L3 showed the highest casein micelles volume ($6660323.99 \pm 675506.95 \text{ nm}^3$) followed by L1 ($6157603.51 \pm 901186.69 \text{ nm}^3$) then L2 ($15826392.52 \pm 1585869.34 \text{ nm}^3$). The smallest casein micelles volume was recognized with L1 ($716.44 \pm 901186.69 \text{ nm}^3$). The same trend of volume was observed for the net volume of the casein micelles of goat milk ($\alpha = 0.05$). A tight connection was found between volume and net volume ($R^2 = 0.997$) of casein micelles. The hairy layer (charged portion of K-casein) of the casein micelles was calculated by subtracting net volume from volume. The hairy layer values were 74674.291 , 64551.333 and 58268.302 nm^3 for L1, L2 and L3, respectively. Although, L2 had the highest volume and net volume, the outer layer was less than L1 which had less volume and net volume. As a result, L2 milk will coagulate in less time than L1.

E. Distribution of casein micelles surface area, volume and net volume

The surface area of goat milk casein micelles distribution ranged between < 10,000 to 250,000 nm² with the highest proportion 21%, 14% and 21.5% at 20,000-30,000, 40,000 - 50,000 and 30,000-40,000 nm² for L1, L2 and L3, respectively. The average volume distribution was the highest (67.5%) for L1 followed by L3 (63.5%) then L2 (56.7%) in the range of 0-500 x 1000 nm³. The net volume of casein micelles that was 1500,000 or larger constituted the higher end of particle net volume distribution. This may have affected the outer layer distribution and correlated with the particles size. The changes in number and size of particles were probably responsible for significant ($P < 0.001$) altering of the hairy layer presented on the surface of the casein micelles.

F. Casein micelles roundness, solidity, compactness and orientation

Fig. 4 shows the roundness, solidity, compactness and orientation of casein micelles of goat milk obtained from different farms. Histogram of roundness of goat milk micelles by number showed more micelles far away from being a perfect sphere (0.8). However, non significant differences were found in roundness, compactness, solidity and orientation as an influence of the sample obtaining location ($P > 0.01$). The maximum roundness for milk samples was 1.09 for L3 and minimum of 0.47 for L1 with an average of 0.79 ± 0.01 . Circularity or sphericity values of casein micelles were used to determine cheese meltability [15]. Casein micelles of goat milk are compacted with a value close to 1 (Fig. 4). The highest compactness (1.04) was noticed with L3 milk. The minimum value was 0.68 for L1 with an average of 0.89 ± 0.0612 . The highest solidity was noticed for L1 (1.016) with no significant differences within treatments at 0.01α level. The average of 0.97 ± 0.01 was obtained for hardness of the spherical particles of casein micelles (Fig. 4). Orientation is the spatial arrangement of the casein micelles. Casein micelles oriented with right and acute angles for L1 and both L2 and L3, respectively.

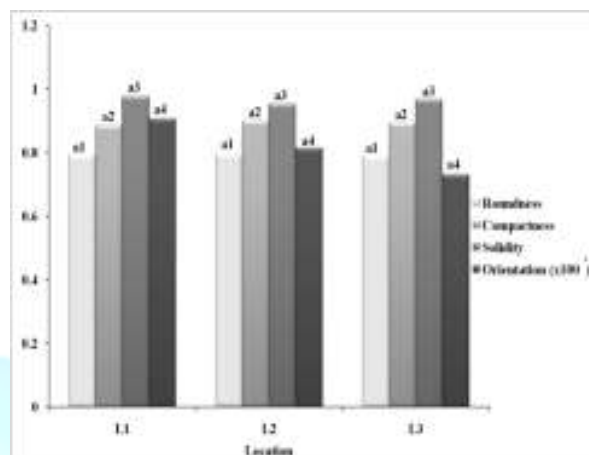


Fig. 4. The roundness, solidity, compactness and orientation of casein micelles of Zaraibi goat milk samples obtained from different locations

G. Distribution of casein micelles roundness, solidity, compactness and orientation

Differences had consequences on the relative distribution of micellar roundness. In goat milk, more than 50% of the total particles are contained in the classes 0.7-0.85. The changes in the number and size of particles were probably responsible for a significant ($P < 0.001$) alteration in the physical properties of casein micelles. 63.7, 66.99 and 56% are contained in value > 0.85 compacted micelles. In goat milk, almost all the micelles exceeded compactness of 0.6 with a maximum of 1 ± 0.056 . It appears that the narrow distribution of micelle solidity in goat milks might indicate hard particles in spite of the presence of several different micelle populations, each adjusted around different mean values. The distribution was 0.98 for all the categories. Two third (2/3) of the casein micelles of goat milk orientated with obtuse angles (63.7%), while those of L2 and L3 orientated with acute angles (50.7% and 66.5%, respectively). The only right angle was found with average orientation of casein micelles of L1, while the averages of L2 and L3 were 81.36° and 66.12°.

H. Casein micelles roughness, mean height, mean contour height and mean depth

Roughest surface casein micelle (25.76 ± 3.64 nm) was reported for L2, while the smoothest was found with L1 (22.74 ± 0.56 nm) as shown in Fig. 5.

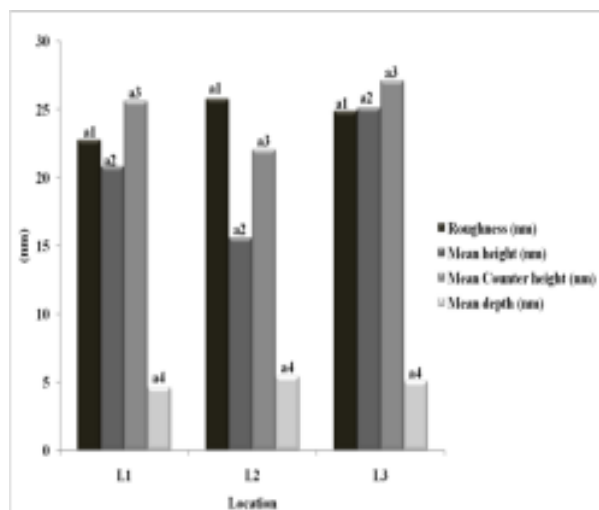


Fig. 5. Roughness (nm), mean height (nm), mean contour height (nm) and mean depth (nm) of casein micelles for different milk samples obtaining locations

Roughness analysis showed maximum values of 23.3, 29.645 and 28.262 nm for L1, L2 and L3, respectively. No significant differences were found between roughness and sample obtaining location. The surface structure of the casein micelles of goat milk is somewhat rough and irregular, which is in accordance with results from previous studies [16] - [17]. Similar observations on cow micelles were previously reported by [18]. In rough micelles, the surfaces seem to have heights or extrudes. The maximum height was found with L2 (110.738 nm) and the minimum was found with L1 (0.299968 nm) with average of 20.78 ± 10.044 , 15.583 ± 13.056 and 25.093 ± 7.486 nm for L1, L2 and L3, respectively. The mean depth measures the negative height of the surface troughs of the casein micelles. The mean depth was not affected by the farm ($P > 0.01$). The maximum (31.143 nm) and the minimum depth were found with L2 (0.208 nm) with average of 4.617 ± 3.53 , 5.41 ± 4.139 and 5.06 ± 3.457 nm for L1, L2 and L3, respectively (Fig. 5).

I. Distribution of casein micelles height, depth and contour height means

Distributions obtained through mean height analysis denote the presence of small hills that may be associated with surface irregularity. 99% of the casein micelles surface mean height was in the range of 0-55 nm. Higher heights were not presented on the micellar surfaces. [19] - [20] found structure heights in the range of 0.6-3 nm. The holes contained on the surface were as deep as 20 nm. The deepest holes were found at 32 nm mean height which represented 0.5% of the total range distribution. The differences between the mean contour

height curves of the individual casein micelles of goat milk were most pronounced in the range of about 0 to 50 nm. The particles in the lowest class with mean contour height smaller than 40 nm comprised about 83, 61 and 64% of the observed total number of particles of L1, L2 and L3, respectively.

J. Equations of predicting geometrical parameters and correlations between diameter and geometrical parameters of caseins micelles of goat milk

Equations for predicting geometrical parameters were set up (Table 3).

Table 3. Statistical correlations between Zaraibi goat milk casein micelles size and its geometrical parameters obtained by AFM

Parameter	Equations	Correlation	S.d
Place	$-4.55 + 0.030 D^*$	0.202	0.055
Breadth	$-47.47 + 1.164 D^*$	0.966	0.117
Length	$33.09 + 0.972 D^*$	0.921	0.155
Perimeter	$178.61 + 2.716 D^*$	0.968	0.266
Area	$-81640.37 + 573.793 D^*$	0.935	82.590
Volume	$-4433161.90 + 23521.25 D^*$	0.659	10158.28
Net volume	$-4414595.30 + 23134.66 D^*$	0.643	10426.89
Roundness	$0.75 + 0.000 D^*$	0.059	0.001
Compactness	$0.85 + 0.000 D^*$	0.072	0.001
Solidity	$1.32 - 0.002 D^*$	-0.414	0.001
Orientation	$98.15 - 0.075 D^*$	-0.047	0.606
Mean Height	$143.45 - 0.563 D^*$	-0.552	0.321
Mean Depth	$1.79 + 0.015 D^*$	0.108	0.052
MCH	$148.42 - 0.566 D^*$	-0.574	0.305
Roughness	$19.59 + 0.022 D^*$	0.046	0.184

MCH= Mean counter height , D*= casein micelles diameter (nm) , S.d= standard deviation.

Parameters of the casein micelles profile, such as breadth, length, perimeter, area, volume, roundness, compactness, solidity, orientation, net volume, mean height, mean depth, roughness and mean contour height were multi-regression analyzed for correlations with the size of native casein micelles. As can be derived from Table 3, the size of native casein micelles highly positively correlated with breadth, length, perimeter, area and moderately with the volume. No correlations were observed with orientation, mean depth, roundness, compactness and roughness. The large variation of casein micelles induced negative correlations with solidity, orientation, mean height and mean contour height. Large micelles having the highest polymerization state would correspond to the most complex micelle structure while smaller particles could be micelles at intermediary stages of building. Further investigations on milk from other species would be useful to achieve a better understanding of the role of casein micelles in this respect [11].

IV. CONCLUSION

The study confirmed that AFM is a powerful tool for imaging the structure of micro-molecules as casein micelles. AFM can be used to analyze the images which results in a very promising technique to investigate structures and interactions between biological macromolecules under “near-native” conditions. The minimal sample preparation avoids the risk of sample contamination or undesirable denaturing effects. Moreover, AFM different modes allow obtaining good image resolution as a non-destructive technique with high sensitivity and statistical relevance. The measured features of the casein micelles structure can be useful in setting the geometrical parameters that help in improving the texture of the dairy products. The higher the hairy layer (outer glycomacro-peptide) of the casein micelles volume, the harder the coagulation of the milk as it carries the charges of the K-casein molecule. As a result, the coagulation time of the milk can be predicted depending on the volume of the hairy layer. Equations which were set could be useful for calculating the relative microstructure parameters of the casein micelles in goat milks with respect to the micelle size. Variations of the raising places of animals influence some of the casein micelles properties which in turn could affect their dairy products characteristics.

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