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Microstructural Characterization of Casein by Microscopy and Spectroscopy Techniques

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The study of the structure and mechanical properties of Caseins at nanoscale is an interesting topic to food nanotechnology [1,2,3]. The aim of this work was to study of the microstructure of Casein by SEM (scanning electron microscopy), CRYO-TEM (Cryogenic transmission microscopy), AFM (atomic force microscopy) and Raman spectroscopy. Overall morphology and topography of Casein were studied by SEM (Hitachi, SU3500 I), CRYO-TEM (JEOL COM, USA) and AFM (Bruker, Bioscope Catalyts ScanAsyst, USA). Whereas the secondary structure of Casein was studied by Raman spectroscopy (confocal Raman microscope, USA).

Fig. 1 shows SEM images of powder protein, where the Casein is like amorphous agglomerates, these morphologies correspond to its obtention process crystallization. Fig 2. Shows CRYO-TEM Casein micelle diameter was 122.60 ± 63.40 nm. Also by means of AFM, the average roughness of the Casein at their isoelectric point and diluted in phosphate buffer solution (Fig. 3), its isoelectric point (5.0) and at ±1 pH unit, Ra (arithmetic roughness) was obtained from scanning area of 5x5 μm. At pH 5 Ra for Casein was 8.023 ± 5.28 nm, while that at pH 4 was 1.56 ± 0.58 nm and pH 6 was 2.59 ± 0.84 nm. Thus the protein in its isoelectric point showed a largest Ra values due to the agglomeration of proteins. Then parameters related to secondary structure of Casein proteins were determined by FT-Raman Spectroscopy according to [4]. Fig 4 shows spectrum of protein, where the amide 1 (Fig 4a) and amide 2 (Fig 4b) bands for the Casein presented 7.90% of the low frequency component in the laminar β arrangement and 6.02% of the high frequency component in the laminar β arrangement, while for the rotation and α helix arrangement it was of 22.59% and 63.42% respectively.

This preliminary study of morphology and topography of Casein by microscopy techniques provided an initial overview to establishing the dispersion conditions and pH's to study its mechanical properties by nanoindentation with AFM.

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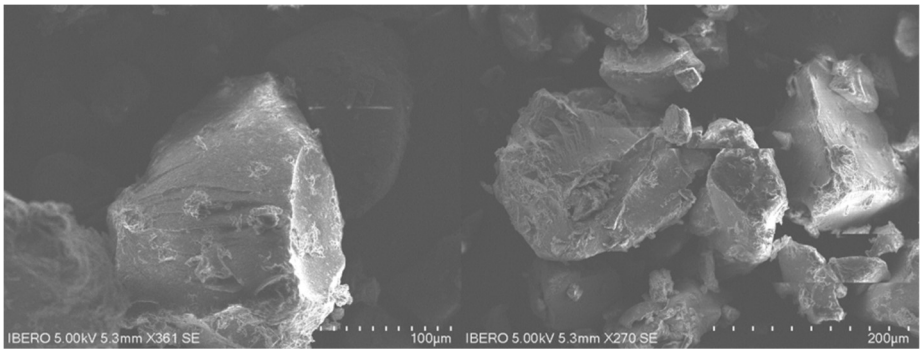

Figure 1. Scanning electron microscopy image of powder Casein.

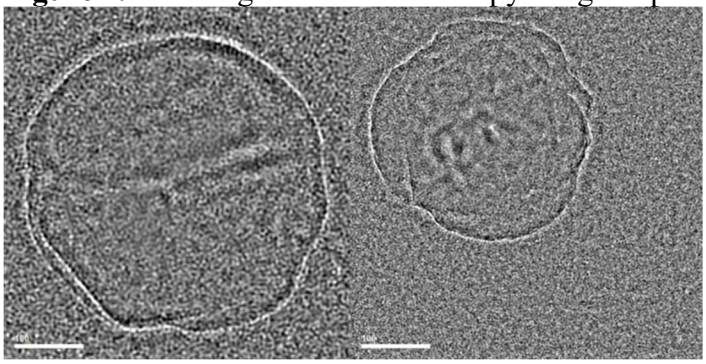

Figure 2. Cryo-transmission electron microscope images of Casein micelles. Bar corresponds to 100 nm

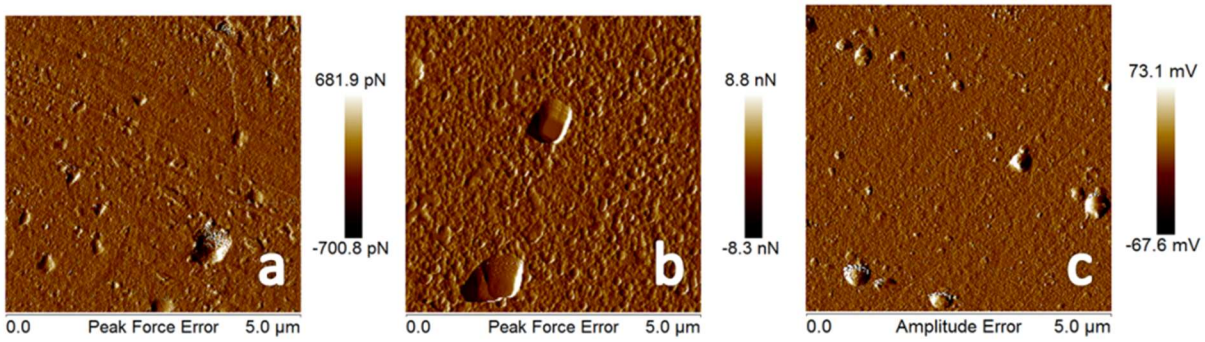

Figure 3. AFM images Casein a) pH 4, b) pH 5 and c) pH 6

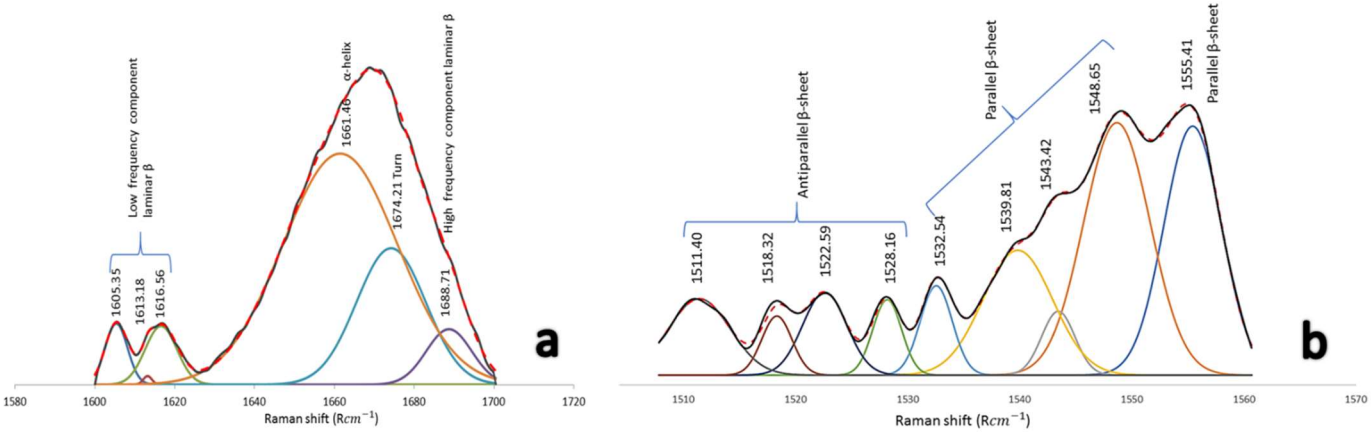

Figure 4. a) Amide I band and b) Amide II band of Casein powder.

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