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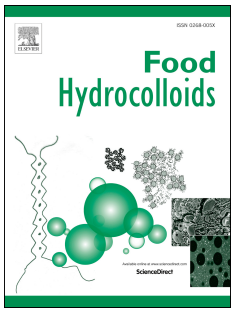

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Jennifer Burgain, Joël Scher, Jeremy Petit, Gregory Francius, Claire Gaiani

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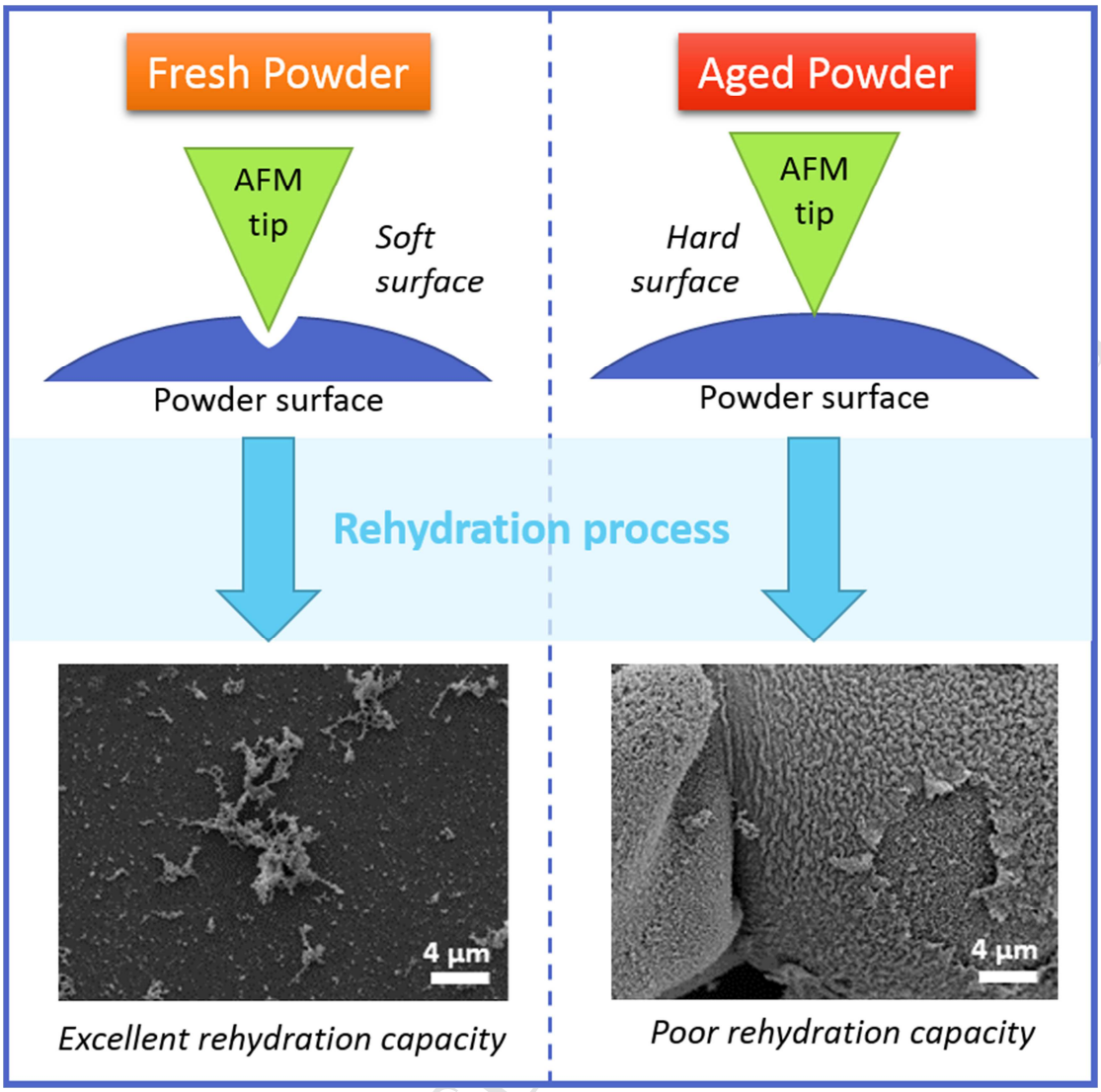

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Links between particle surface hardening and rehydration impairment during micellar casein powder storage

BURGAIN Jennifer[a,b*], SCHER Joël[b], PETIT Jeremy[b], FRANCIUS Gregory[c], and GAIANI Claire[b,d*]

[a] CNIEL, 42 rue de Châteaudun, 75314 Paris Cedex 9, France.

[b] Université de Lorraine, LIBio, Laboratoire d'Ingénierie des Biomolécules, 2 avenue de la Forêt de Haye, TSA 40602, 54518 Vandœuvre-lès-Nancy cedex, France.

[c] Université de Lorraine, LCPME, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement, UMR 7564, 54600 Villers-lès-Nancy, France.

[d] The University of Queensland, School of Agricultural and Food Science, St. Lucia, Qld. 4072, Australia.

* Corresponding author. Tel.: +33-3-83-59-58-78; Fax: +33-3-83-59-58-04.

E-mail address: claire.gaiani@univ-lorraine.fr (C. Gaiani)

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Abstract

Storage is an unavoidable critical phase regarding dairy powder reconstitution abilities, particularly for high casein content powders, which generally present a poor rehydration behavior. The ability of micellar casein powders to completely rehydrate can thus be particularly affected by storage time and temperature. To implement best practices for the optimization of storage conditions, understanding changes occurring is a crucial point. For the first time, biophysical techniques were used to investigate powder surface at the nanoscale. Atomic force microscopy revealed that particle surface became rougher during storage, associated with the formation of hollow zones (around 500 nm) holes when stored for 10 months at 40 °C. Mechanical properties of micellar casein particle surface during powder storage was quantified using AFM nanoindentation. Spatially-resolved force/indentation curves evidenced a significant stiffer surface for aged powder (Young modulus of ~20 GPa) in comparison with the fresh one (~0.2 GPa). These findings were fully consistent with the formation of a crust at the powder surface observed by high-resolution field-emission scanning electron microscopy during powder rehydration. Finally, alterations of the rehydration process can be related to modifications occurring at the particle surface during storage.

Keywords: AFM; nanoindentation; surface characterization; micellar casein powder, SEM

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1. Introduction

High-protein dairy powders are ingredients added to a large variety of products in order to improve food nutritional, functional and/or sensory properties (Kelly & Fox, 2016). The ability of dehydrated dairy ingredients to rehydrate readily in aqueous media is essential if their underlying functionality is to be exploited (Crowley, Kelly, Schuck, Jeantet & O'mahony, 2016; Fang, Selomulya, Ainsworth, Palmer & Chen, 2011; Gaiani, Schuck, Scher, Desobry & Banon, 2007; Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2009; Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2010b). Concerning high-protein powders concentrated in caseins, poor rehydration characteristics in aqueous media are often encountered. In addition, intrinsic powder properties, such as surface and bulk composition, particle structure (*e.g.* morphology, presence of pores and capillaries) and rehydration conditions (*e.g.* stirring rate, temperature, solids content), can influence rehydration behavior (Crowley et al., 2016). Micellar casein (MC) powders belongs to high protein dairy powders, they are produced by membrane filtration of skimmed milk followed by spray-drying (Schuck et al., 1994; Rollema & Muir, 2009). In these powders, whey proteins are removed and caseins are present under a micellar state (casein micelles containing colloidal calcium phosphate). Formation of inter-linked network of casein micelles at the particle surfaces during processing can explain the poor rehydration capacity of such powders. Dispersion of the powder into primary particles is the limiting step to allow complete rehydration after a reasonable period of time (Anema et al., 2006; Havea, 2006; Baldwin, 2010; Fang et al., 2012; Haque et al., 2012; Crowley et al., 2016). Moreover, the rehydration capacity decrease is linked to storage conditions (temperature, water activity, duration, etc.) and fresh powders present better rehydration properties than aged powders (Fang et al., 2011; Gazi & Huppertz 2015). For example, higher storage temperature leads to a decrease in MPC (milk protein concentrate) powders solubility (Anema, Pinder, Hunter & Hemar, 2006; Fang et al., 2011; Fyfe et al., 2011; Gazi & Huppertz

2015) and the caseins dominate the composition of insoluble material (Anema et al., 2006). Also, the quantity of insoluble material was increased at high relative humidity (Le, Bhandari & Deeth, 2011) and linked to the apparition of Maillard products (Le, Bhandari, Holland & Deeth, 2011). However, covalent cross-linking explanation as the cause of insolubility development is questionable (Gazi and Huppertz, 2015) because prolonged reconstitution times eventually lead to full solubility of MPC powders (Mimouni et al., 2010a). Finally, the composition of particle surface plays a crucial role in powder rehydration as it was supported by XPS analysis (increase in non-polar bonding) (Gaiani et al., 2006) and by Atomic Force Microscopy (AFM) where MPC established attractive forces with a hydrophobic surface (Fyfe et al., 2011). Decrease in rehydration was also attributed to compounds migration from particle core to surface during storage. MC powder is mainly composed of caseins and only residual phospholipids were found to migrate to the surface (Gaiani et al., 2006; Gaiani et al., 2007).

Dairy powder surface in depth investigation appears essential to elucidate the nature of phenomena occurring. In the pharmaceutical field, development of new techniques able to probe surface mechanical properties of the powders were recently implemented (Wu, Li & Mansour, 2010). Among these techniques, AFM nanoindentation allows measurements on individual particles while providing mechanical properties at nanometer scale. Indentation testing is a method consisting in touching the surface of interest presenting unknown mechanical properties such as elastic modulus and hardness with another material presenting knowns properties. Mechanical properties of milk protein skin layers after drying were estimated by indentation test in order to understand the mechanisms of particle formation but was not directly performed on particle powder and at a nanoscale (Sadek et al., 2015). Nanoindentation is an indentation test in which the length scale of the penetration is measured

in nanometers rather than microns or millimeters, the latter being common in conventional hardness tests (Fischer-Cripps, 2000). Nanoindentation can be performed with an atomic force microscope and the interesting advantages of the technique are to get a mapping of elastic modulus on a defined region. This particular case give local information of surface hardness. A cantilever force sensor is very reliable and sensitive which makes the AFM an ideal tool for probing by this sensor, the mechanical properties of materials with high resolution and high sensitivity. Such characterizations can be obtained by performing a force-distance curve. By analyzing the force curve approach with theoretical models, the mechanical properties (*ie* stiffness, Young's modulus) of the soft sample can be obtained (Kasas, Longo and Dietler, 2013). In the field of pharmaceutical materials, nanoindentation was for example performed on lactose (Masterson & Cao, 2008; Perkins et al., 2007) or sucrose (Liao & Wiedmann, 2004; Masterson et al., 2008; Ramos & Bahr, 2007). Pharmaceutical powders were characterized by AFM nanoindentation in order to correlate particle hardness with powder compaction performance (Cao, Morganti, Hancock & Masterson, 2010). On the best knowledge of the authors, a declination of these promising biophysical techniques to food powders was never done. Given that crust formation has been reported during process and storage, nanomechanical measurements appear as an interesting tool to evaluate very local modifications of particle powder surface. Even if the method was used from several decades for pharmaceutical application, it was never applied for dairy powders. In this context, the present study aimed at understanding how particle surface is modified at nanoscale during storage at high temperature and the consequence of these structural alterations on the hydration mechanism of particle surface during powder rehydration.

2. Material and methods

2.1. MC powders: production and storage

MC concentrate was provided by a dairy company (Ingredia, France). It was obtained from skimmed milk by tangential membrane microfiltration followed by purification through water diafiltration (Pierre, Fauquant, Le Graet, Piot & Maubois, 1992; Schuck et al., 1994). The concentrate was spray-dried at Bionov (Rennes, France) in a three-stage pilot-plant spray-dryer (GEA, Niro Atomizer, St Quentin en Yvelines, France). The inlet and outlet temperatures were fixed at 150 °C and 50 °C respectively. MC powders were packaged in sealed tins (a_w = 0.2), then stored under a controlled temperature of 40 °C for ten months. This high temperature was chosen to enhance physicochemical phenomena observed during powder storage.

2.2. Rehydration protocol

Rehydration was performed at 5 % (w/v) powder concentration in water. 0.5 g MC powder was added to 10 mL deionised water. Stirring (200 rpm) at ambient temperature was carried out for 5 min (short-term rehydration) and 60 min (long-term rehydration).

2.3. Surface observation

2.3.1. Scanning Electron Microscopy

A high-resolution field-emission scanning electron microscopy (SEM) type JEOL JSM-7100F supplied with a hot (Schottky) electron gun (JEOL Ltd., Tokyo, Japan) and having a resolution around 1 nm at 30 kV was used to investigate the surface morphology and structure of MC powders. Powder particle surface observation during rehydration was conducted according to the protocol developed by Mimouni et al. (Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2010a) with small modifications. The suspension containing powder particles (under short or long-term rehydration) was deposited on a silicon chip wafer (ProSciTech, Kirwan, Australia) that has previously been coated with poly-L-Lys (Sigma,

Castle Hill, Australia). Powder particles were able to adhere to the wafer by creating electrostatic bonds with the substrate. The suspension was kept in contact with the wafer for 5 min, then the wafer was drained and rinsed with 100 mM phosphate buffer (pH = 7). Chemical protein fixation was achieved by immersing the wafer in a solution composed of 3 % glutaraldehyde in 100 mM phosphate buffer (pH = 7) for 15 min. When the fixation was completed, the samples were gently washed in phosphate buffer and dehydrated using the following graded ethanol series: 50 %, 60 %, 70 %, 80 %, 90 % (1 time) and 100% (3 times). The elapsed time per solution was 3 min (Dalgleish, Spagnuolo & Goff, 2004). Samples were then dried by using CO_2 in a Supercritical Autosamdri-815B critical point dryer (Tousimis, Rockville, MD, USA). The silicon wafer was then mounted onto electron microscopy stubs thanks to a carbon double-sided adhesive tape. Finally, samples were coated with iridium (Q150T Turbo-Pumped Sputter Coater, ProSciTech Pty Ltd, Australia) for 2 min (~ 15 nm thick).

2.3.2. AFM: surface topography and roughness

Dairy powders were fixed onto a circular glass thanks to epoxy glue. AFM measurements were performed at room temperature using an Asylum MFP-3D atomic force microscope (Santa Barbara, CA, USA) with IGOR Pro 6.04 operation software (Wavemetrics, Lake Osewego, OR, USA). All images were acquired in liquid media, more precisely in ethanol to avoid powder rehydration during experiments. Topography images were obtained in contact mode at 1 Hz scan rate with NPG-10 gold cantilevers (Bruker AXS, Palaiseau, France). The scanned surface area was of 5 µm × 5 µm corresponding to 512 points × 512 lines. The average roughness (R_a) was calculated for MC powder particles with the following expression:

$$R_a = \frac{1}{L_x L_y} \int_0^{L_y} \int_0^{L_x} |f(x,y)| dxdy \quad (1)$$

Where f (x, y) is the surface relative to the center plane, Lx and Ly are the dimensions of the surface. Tukey test was performed using KyPlot software version 2.0 in order to determine significant differences. To this end, roughness of around ten images was used to perform statistical analyses.

2.4. Surface characterization

2.4.1. XPS

Elemental composition of the surface layer of MC powder (up to 5 - 6 nm depth) was measured by X-ray Photoelectron Spectroscopy (XPS) (Gaiani et al., 2006; Rouxhet et al., 2008). Prior to analysis, the sample was outgassed under vacuum for 24 h. Spectra were obtained with a KRATOS Axis Ultra X-ray photoelectron spectrometer (Kratos Analytical, Manchester, UK) equipped with a monochromatic Al Kα X-ray ($h\nu$ = 1486.6 eV) operated at 150 W. Spectra were collected at normal take-off angle (90°) and the analysis area was 700 µm × 300 µm.

2.4.2 AFM Nanoindentation

Mechanical properties of dairy powder particle surface were obtained in three steps described in **Figure 1**.

(1) First of all, particle powders were spread onto a thin coat of epoxy glue deposited on the surface of a cleaned circular glass and the overall was let overnight to allow glue hardening.

AFM measurements were performed with a diamond tip of Berkovich type purchased from Veeco (DNISP, Veeco Instruments SAS, Palaiseau, France). Regarding the cantilever, the

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DNISP spring constant k_c was evaluated at about 300 $N.m^{-1}$ by the thermal calibration method (Levy & Maaloum, 2002). The experiments were performed at room temperature in liquid conditions, particularly in ethanol. AFM nanoindentation requires precise knowledge of the cantilever sensitivity. In order to evaluate this parameter, the calibration procedure consists in pressing the AFM tip onto an 'infinitely hard' surface, and to measure the gradient of the vertical displacement of cantilever versus the photodiode signal caused by the cantilever deflection (Clifford & Seah, 2005). Finally, once the system is perfectly calibrated, AFM nanoindentation can be performed on particle powders. A camera allows to visualize the sample and to select a particle powder. Once above the selected particle, the tip is put in contact with the surface and the measurements are imitated.

(2) Mechanical properties of particle powder surface was obtained by recording force-indentation curves. To perform nanoindentation tests, the AFM is operated in force mode and the tip is brought into contact with the surface, pushed to a maximum load (Approach curve), and then withdrawn (withdrawal curve). The voltage on the photodiode is recorded during the movement and plotted against the vertical distance to the sample. The voltages recorded can be converted into forces by Hooke's law thus providing force-distance curves. Force/volume images consisting of 16 × 16 force curves were recorded for a 20 µm × 20 µm scanned surface meaning that a pixel has a dimension of 1.25 µm × 1.25 µm.

(3) During nanoindentation measurements, force curves are recorded and converted into force versus indentation curves using appropriate treatments (Burnham & Colton, 1989). Force curves were exported as ASCII files and processed with a custom program written in Matlab (MathWorks, Inc., Natick, MA). The measured force-indentation curves were processed to estimate the Young's modulus (also known as the elastic modulus) of the sample. The Young's modulus was obtained by applying Hertz theory for elastic media (Hertz, 1882) and elasticity maps were designed.

$$F = \frac{2E \tan\alpha}{\pi (1-\upsilon^2)} \delta^2 \quad (2)$$

where F is the force, **δ** the indentation depth, *E* the Young modulus, **υ** the Poisson coefficient and α the semi-top angle of the tip.

Finally, analysis of at least three elasticity maps provided a mean elasticity value for each analyzed sample.

3. Results and discussion

3.1. Surface composition and structure of MC powders

Dairy powders surface is essential in its functional properties (Crowley, Kelly, Schuck, Jeantet & O'mahony, 2016), such as rehydration ability, which is crucial for the end use of a product. Surface composition of MC powders was determined by XPS on fresh and aged samples. XPS results are presented in **Figure S1**. Overall, no significant differences in surface composition were evidenced between analyzed samples. However, because XPS provides composition on large surface area, very local modifications cannot be revealed by this technique. Images of particle structure and surface topography were acquired by SEM. As displayed in **Figure 2**, particle sizes were heterogeneous and their surface appeared smooth in spite of the presence of dents. Regarding surface topography of fresh and aged powders, no differences were noticed, confirming previous observations reported in the literature (Fäldt & Bergenståhl, 1996; Fyfe et al., 2011). Unexpectedly, AFM topographical measurements revealed an increase in surface roughness during powder aging (**Figure 3**). These little variations were not discernible on SEM observation maybe because of the iridium coating that is thicker than 5 nm, which may have overlay differences in surface profiles of fresh and aged powders. On the contrary, AFM in contact mode is highly sensitive to very fine topographical deviations. R_a was calculated according to equation 1, and gave values of 5.1 ± 0.8 nm for the fresh powder and 7.8 ± 0.8 nm for the MC powder stored for 10 months at 40 °C. Statistical

238 analysis evidenced that roughness was significantly different ($p \leq 0.01$). In particular, as can
239 be noticed on the 3D representation (**Figure 3**), many hollows of around 500 nm diameter
240 were noticeable on particle surface after aging at 40 °C for 10 months, while slight variations
241 in roughness were observed on fresh particle powder surface.
242
243 **3.2. Nanomechanical properties of MC powder surface**
244 Nanomechanical properties of MC powder surface were explored using AFM nanoindentation
245 measurements. Force/indentation curves were analyzed using the Hertz theory to generate
246 elasticity maps (**Figure 4 - inserts**). The colorbar associates low elasticity values when the
247 region is purple and high elasticity values when the region is red. The average of elasticity
248 values obtained in three maps was used to calculate the mean elasticity of fresh and aged
249 powders (**Figure 4**). The fresh powder presented a mean elasticity of 0.16 ± 0.03 GPa which
250 is lower than values obtained for NPC skin layers (0.48 ± 1.10^{-3} GPa) by applying indentation
251 test (Sadek et al., 2015) but from the same order. These differences can be explained by the
252 system studied itself, a protein layer for Sadek et al., (2015) versus a particle powder in the
253 present study. Also drying conditions were not identical between the two systems. In
254 nanoindentation test, the length scale of the penetration is smaller than for an indentation test
255 meaning that thanks to AFM nanoindentation the extreme surface was probed. The initial
256 elasticity map revealed that surface is homogeneous as already supposed by Sadek et al.,
257 (2015) for skin layers. It was impossible to find the technique applied to systems similar as
258 ours. But, AFM nanoindentaiton was performed on pharmaceutical powders and for example
259 lactose powders exhibited values that were comprised between 0.18 and 0.51 GPa (Masterson
260 & Cao, 2008) which is not far from the elasticity values obtained on MC powders in the
261 present work. Elasticity maps recorded on stored powders were totally different: not
262 homogeneous with local harder surfaces. Nonetheless, it is important to remember that the

size of a pixel in these maps is 1.25 µm × 1.25 µm meaning that unfortunately differences in elasticity cannot be attributed to topographical modification. For future work, it could be interesting to improve resolution in order to highlight very local modifications on powder surface. Elasticity increase during storage is a consequence of particle surface hardening: indeed, fresh MC powder had a relatively soft surface at the beginning of the storage, which evolved into a stiffer surface with elasticity values of 18.85 ± 9.33 after 10 months storage at 40 °C.

3.3. Evolution of surface structure during powder rehydration

The three-dimensional organization of the material on particle surface was considerably modified during rehydration (**Figure 5**).

Short term rehydration.

Under short term rehydration, fresh MC powders globally followed the behavior described by Mimouni et al. (Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2010a). Their microstructure was characterized by a loose assembly of individual casein micelles. For aged MC powders that were stored during 6 months at 40 °C, particles appeared poorly affected by the rehydration media and casein micelles were tighter on particle surface. On fresh and 6-month aged MC powders, the remaining undissolved powder particles presented large holes of several micrometers within the surface. These holes resulted from partial material removal upon rehydration, leading to the release of surface casein micelles in the surrounding media. Slow rehydration of dairy powders is often attributed to the casein fraction, known to have a poor dispersibility, and this phenomenon may be enhanced after storage at elevated temperature (Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2010a). Finally, for prolonged storage of MC powders (10 months at 40 °C), the particle surface appeared almost unaffected by the rehydration media (no individual casein micelles can be observed) and looked like a

dry powder. Likewise, the large holes described for fresh and 6-month stored MC powders are no longer noticeable on particles stored for ten months at 40 °C.

Long term rehydration.

The long term rehydration process was conducted during 1 hour and particle surface was again analyzed. Only residual material was observed after long term rehydration of the fresh powder, while particles remained visible for powders stored at 40 °C. However, surface hydration after long term rehydration seemed to decrease with storage duration. In particular, as for short term rehydration, particles of the 10-month aged powder seem almost unaffected by the rehydration media. In the intermediate case (6-month aged powder), particles were far from fully solubilized but their surface appeared significantly hydrated with the presence of individual casein micelles. Approaching more finely the particle surface was possible by the exceptional resolution properties of a high-resolution field-emission SEM. The rehydration of a fresh MC powder allows visualizing casein micelles arranged in an open gel-like organization (Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2010a). On the contrary, the aged powder stored for 6 months at 40 °C presented a layer of tightly packed casein micelles. Finally, when the powder was stored for 10 months at 40 °C, the integrity of particle surface seemed preserved (no holes could be observed) and the previously described casein micelles assemblies were not noticeable. Only the fresh MC powder succeeded to rehydrate, whereas rehydration capacity of aged powders was markedly affected by storage.

Dynamic vapor sorption (DVS) is an interesting tool to evaluate the ability of powders to adsorb water (Schuck, Dolivet & Jeantet, 2012). The method provide information on the rehydration of a dry product through adsorption isotherm. Gaiani et al. (2006) investigated the effect of storage on surface composition, water sorption properties and powder microstructure of MC powders. A significant decreased of the monolayer water capacity was noticed by these authors on the same powders during storage for 60 days at 50 °C (around 0.0632 $kg.kg^{-1}$

313 for fresh and around 0.0524 $kg.kg^{-1}$ for aged MC powders). MC powders are the milk
314 powders able to absorb the highest quantities of water in a fresh state. In the present study,
315 water sorption properties were also evaluated (data not shown) and same tendencies were
316 obtained (around 0.0644 $kg.kg^{-1}$ for fresh and around 0.0424 $kg.kg^{-1}$ for aged MC powders).
317 This implies that the ability of aged powder to adsorb water is affected compared to fresh
318 powders which is in accordance with the SEM observation (**Figure 5**). This impossibility to
319 absorb elevated quantities of water can be related to the structural modifications of the
320 micelles observed.
321 From SEM analysis, it can be stated that the powder particle surface is not uniformly affected
322 by the rehydration media, but some parts are solubilised while other parts remain unaffected.
323 This spatial heterogeneity of particle rehydration meaning that partial removal of the crust
324 occurred during rehydration has previously been described (Mimouni, Deeth, Whittaker,
325 Gidley & Bhandari, 2010a). Additionally, heterogeneity in rehydration behavior was also
326 denoted for the first time between particles from a same batch. More precisely, the hydration
327 degree of particle surface was totally different, ranging from well to poorly hydrated (**Figure**
328 **6**). These differences between individual particles could be the result of different residence
329 time of the product in the dryer. It was shown that for a three-stage installation, depending on
330 configurations related to fine particle recycling, some powder particles could leave the tower
331 almost immediately, whilst others could remain in the installation for more than 70 min
332 (Jeantet, Ducept, Dolivet, Méjean & Schuck, 2008).
333
334 **3.4 Proposed mechanism of modification during powder storage**
335 Complete rehydration is a crucial step for the effective expression of protein functionality
336 (Crowley et al., 2015). The inhibition of water transfer inside particles is the limiting factor
337 during rehydration of powders of high protein content, especially when caseins are the

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predominant proteins. The long time required for complete rehydration was attributed to the slow dispersion of a "skin" of casein micelles present at particle surfaces (Mimouni, Deeth, Whittaker, Gidley & Bhandari, 2009). Numerous authors consider that the slow dispersion of primary particles is responsible for the extended rehydration times of casein-dominant powders (Fang, Selomulya, Ainsworth, Palmer & Chen, 2011; Gaiani, Banon, Scher, Schuck & Hardy, 2005; Richard, et al., 2013). In their work, Scokker *et al.* (2011) improved the powder reconstitutability by increasing the amount of non-micellar protein in the spray-drying feed. Two possible mechanisms contributing to the positive effect of non-micellar casein were highlighted. One of them proposes the spatial separation of the casein micelles by non-micellar casein, preventing micelle–micelle interactions which can contribute to reduce the incidence of micelle cross-linking. The present study suggests that the apparent surface structure of powder particles during rehydration results from modification occurring during storage. Up to now, exact mechanisms behind these observations were never evidenced but AFM in topographical and force mode used in the present work can help in the development of some assumptions (**Figure 7**). In fresh powders, casein micelles are spatially separated thus preventing cross-linking between caseins. After storage at high temperature, aggregation of casein micelles leads to heterogeneous surface where caseins are tighter and this phenomenon may generate the mentioned hollows that were observed on topographical measurements. From a mechanical point of view, this results in an increase of elastic modulus reflecting a harder surface for aged powders. Hardening is a time-dependent, restructuring process occurring in concentrated systems (Hogan, O'Loughlin & Kelly, 2016). The proposed mechanisms underlying are protein aggregation through covalent (intermolecular disulphide bonds) and non-covalent (hydrophobic) interactions that can results in harder surface (Zhou, Liu & Labusa, 2008). Moreover, surface hardening during storage at elevated temperature resembles to shrinkage and case-hardening phenomenon occurring during drying of food

materials (Gulati & Datta, 2015). Drying of food materials usually results in large deformations (shrinkage) due to moisture removal. Material shrinkage (or strains) results in stress development that plays a critical role in the development of food structure and final volume of the dried product.

4. Conclusion

The measurement of nanomechanical properties of MC particle surface by AFM nanoindentation revealed an increase in surface hardness during storage at high temperature, while particle surface topography was barely affected. However, particle behavior during rehydration was markedly affected by storage conditions, leading to a compact network of casein micelles for aged powders, while it was looser for fresh powders. Nanomechanics revealed particle surface modifications during storage that were not visible on SEM images but having a great influence on powder ability to rehydrate. The harder particle surface observed for aged powders can be the result of compacted micelles, a material that is further difficult to disperse, resulting in very low rehydration mechanisms. The main reason for this reduction of powder solubility could be the non-covalent aggregation of closely-packed casein micelles. Finally, the hollow structures observed on topographical images (AFM) at dry state may originate from protein aggregation and resulting to a harder surface demonstrated by nanomechanical measurements. These modification can explain why powder rehydration is tricky after storage.

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6. References

Anema, S., Pinder, D., Hunter, R., & Hemar, Y. (2006). Effects of storage temperature on the solubility of milk protein concentrate (MPC85). *Food Hydrocolloids*, 20(2), 386-393.

Baldwin, A. J. (2010). Insolubility of milk powder products–A minireview. *Dairy Science & Technology*, 90(2-3), 169-179.

Burnham, N. A., & Colton, R. J. (1989). Measuring the nanomechanical properties and surface forces of materials using an atomic force microscope. *Journal of Vacuum Science & Technology A*, 7(4), 2906-2913.

Cao, X., Morganti, M., Hancock, B. C., & Masterson, V. M. (2010). Correlating particle hardness with powder compaction performance. *Journal of pharmaceutical sciences*, 99(10), 4307-4316.

Clifford, C. A., & Seah, M. P. (2005). Quantification issues in the identification of nanoscale regions of homopolymers using modulus measurement via AFM nanoindentation. *Applied surface science*, 252(5), 1915-1933.

Crowley, S., Kelly, A., Schuck, P., Jeantet, R., & O'mahony, J. (2016). Rehydration and solubility characteristics of high-protein dairy powders. In *Advanced Dairy Chemistry* (pp. 99-131): Springer.

Crowley, S. V., Desautel, B., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A. (2015). Rehydration characteristics of milk protein concentrate powders. *Journal of Food Engineering*, 149, 105-113.

ACCEPTED MANUSCRIPT

411 Dalgleish, D. G., Spagnuolo, P. A., & Goff, H. D. (2004). A possible structure of the casein
412 micelle based on high-resolution field-emission scanning electron microscopy.
413 *International Dairy Journal*, 14(12), 1025-1031.
414 Fäldt, P., & Bergenståhl, B. (1996). Spray-dried whey protein/lactose/soybean oil emulsions.
415 1. Surface composition and particle structure. *Food Hydrocolloids*, 10(4), 421-429.
416 Fang, Y., Selomulya, C., Ainsworth, S., Palmer, M., & Chen, X. D. (2011). On quantifying
417 the dissolution behaviour of milk protein concentrate. *Food Hydrocolloids*, 25(3),
418 503-510.
419 Fang, Y., Rogers, S., Selomulya, C., & Chen, X. D. (2012). Functionality of milk protein
420 concentrate: Effect of spray drying temperature. *Biochemical Engineering Journal*,
421 62, 101-105.
422 Fischer-Cripps, A. C. (2000). Introduction to contact mechanics. New York: Springer.
423 Fyfe, K. N., Kravchuk, O., Le, T., Deeth, H. C., Nguyen, A. V., & Bhandari, B. (2011).
424 Storage induced changes to high protein powders: influence on surface properties
425 and solubility. *Journal of the Science of Food and Agriculture*, 91(14), 2566-2575.
426 Gaiani, C., Banon, S., Scher, J., Schuck, P., & Hardy, J. (2005). Use of a turbidity sensor to
427 characterize micellar casein powder rehydration: Influence of some technological
428 effects. *Journal of Dairy Science*, 88(8), 2700-2706.
429 Gaiani, C., Ehrhardt, J., Scher, J., Hardy, J., Desobry, S., & Banon, S. (2006). Surface
430 composition of dairy powders observed by X-ray photoelectron spectroscopy and
431 effects on their rehydration properties. *Colloids and Surfaces B: Biointerfaces*, 49(1),
432 71-78.
433 Gaiani, C., Scher, J., Ehrhardt, J. J., Linder, M., Schuck, P., Desobry, S., & Banon, S. (2007).
434 Relationships between dairy powder surface composition and wetting properties

ACCEPTED MANUSCRIPT

during storage: importance of residual lipids. *Journal of agricultural and food chemistry*, 55(16), 6561-6567.

Gaiani, C., Schuck, P., Scher, J., Desobry, S., & Banon, S. (2007). Dairy Powder Rehydration: Influence of Protein State, Incorporation Mode, and Agglomeration. *Journal of Dairy Science*, 90(2), 570-581.

Gazi, I., & Huppertz, T. (2015). Influence of protein content and storage conditions on the solubility of caseins and whey proteins in milk protein concentrates. *International Dairy Journal*, 46, 22-30.

Gulati, T., & Datta, A. K. (2015). Mechanistic understanding of case-hardening and texture development during drying of food materials. *Journal of Food Engineering*, 166, 119-138.

Haque, E., Whittaker, A. K., Gidley, M. J., Deeth, H. C., Fibrianto, K., & Bhandari, B. R. (2012). Kinetics of enthalpy relaxation of milk protein concentrate powder upon ageing and its effect on solubility. *Food chemistry*, 134(3), 1368-1373.

Havea, P. (2006). Protein interactions in milk protein concentrate powders. *International Dairy Journal*, 16(5), 415-422.

Hertz, H. (1882). Über die Berührung fester elastischer Körper.

Hogan, S. A., O'Loughlin, I. B., & Kelly, P. M. (2016). Soft matter characterisation of whey protein powder systems. *International Dairy Journal*, 52, 1-9.

Jeantet R., Ducept F., Dolivet A., Méjean S. & Schuck P. (2008). Residence time distribution : a tool to improve spray-drying control. *Dairy Science & Technology*, 88(1), 31-43.

Kasas, S., Longo, G., & Dietler, G. (2013). Mechanical properties of biological specimens explored by atomic force microscopy. *Journal of Physics D: Applied Physics*, 46(13), 133001.

460 Kelly, A. L., & Fox, P. F. (2016). Manufacture and Properties of Dairy Powders. In
461 *Advanced Dairy Chemistry* (pp. 1-33): Springer.
462 Le, T. T., Bhandari, B., & Deeth, H. C. (2011). Chemical and physical changes in milk
463 protein concentrate (MPC80) powder during storage. *Journal of agricultural and*
464 *food chemistry*, 59(10), 5465-5473.
465 Le, T. T., Bhandari, B., Holland, J. W., & Deeth, H. C. (2011). Maillard reaction and protein
466 cross-linking in relation to the solubility of milk powders. *Journal of agricultural*
467 *and food chemistry*, 59(23), 12473-12479.
468 Levy, R., & Maaloum, M. (2002). Measuring the spring constant of atomic force microscope
469 cantilevers: thermal fluctuations and other methods. *Nanotechnology*, 13(1), 33.
470 Liao, X., & Wiedmann, T. S. (2004). Characterization of pharmaceutical solids by scanning
471 probe microscopy. *Journal of pharmaceutical sciences*, 93(9), 2250-2258.
472 Masterson, V. M., & Cao, X. (2008). Evaluating particle hardness of pharmaceutical solids
473 using AFM nanoindentation. *International Journal of Pharmaceutics*, 362(1–2), 163-
474 171.
475 Mimouni, A., Deeth, H. C., Whittaker, A. K., Gidley, M. J., & Bhandari, B. R. (2009).
476 Rehydration process of milk protein concentrate powder monitored by static light
477 scattering. *Food Hydrocolloids*, 23(7), 1958-1965.
478 Mimouni, A., Deeth, H., Whittaker, A., Gidley, M., & Bhandari, B. (2010a). Investigation of
479 the microstructure of milk protein concentrate powders during rehydration:
480 alterations during storage. *Journal of Dairy Science*, 93(2), 463-472.
481 Mimouni, A., Deeth, H. C., Whittaker, A. K., Gidley, M. J., & Bhandari, B. R. (2010b).
482 Rehydration of high-protein-containing dairy powder: Slow-and fast-dissolving
483 components and storage effects. *Dairy Science & Technology*, 90(2-3), 335-344.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Perkins, M., Ebbens, S. J., Hayes, S., Roberts, C. J., Madden, C. E., Luk, S. Y., & Patel, N. (2007). Elastic modulus measurements from individual lactose particles using atomic force microscopy. *International Journal of Pharmaceutics*, 332(1), 168-175.

Pierre, A., Fauquant, J., Le Graet, Y., Piot, M., & Maubois, J. (1992). Préparation de phosphocaséinate natif par microfiltration sur membrane. *Le Lait*, 72(5), 461-474.

Ramos, K., & Bahr, D. (2007). Mechanical behavior assessment of sucrose using nanoindentation. *Journal of materials research*, 22(07), 2037-2045.

Richard, B., Le Page, J.-F., Schuck, P., Andre, C., Jeantet, R., & Delaplace, G. (2013). Towards a better control of dairy powder rehydration processes. *International Dairy Journal*, 31(1), 18-28.

Rollema, H. S. & Muir, D. D. (2009). 6 Casein and Related Products. Dairy powders and concentrated products, 235.

Rouxhet, P. G., Misselyn-Bauduin, A. M., Ahimou, F., Genet, M. J., Adriaensen, Y., Desille, T., Bodson, P. & Deroanne, C. (2008). XPS analysis of food products: toward chemical functions and molecular compounds. *Surface and Interface Analysis*, 40(3-4), 718-724.

Sadek, C., Pauchard, L., Schuck, P., Fallourd, Y., Pradeau, N., Le Floch-Fouéré, C., & Jeantet, R. (2015). Mechanical properties of milk protein skin layers after drying: Understanding the mechanisms of particle formation from whey protein isolate and native phosphocaseinate. *Food Hydrocolloids*, 48, 8-16.

Schokker, E. P., Church, J. S., Mata, J. P., Gilbert, E. P., Puvanenthiran, A., & Udabage, P. (2011). Reconstitution properties of micellar casein powder: Effects of composition and storage. *International Dairy Journal*, 21(11), 877-886.

ACCEPTED MANUSCRIPT

507 Schuck, P., Piot, M., Méjean, S., Le Graet, Y., Fauquant, J., Brulé, G., & Maubois, J. (1994).
508 Déshydratation par atomisation de phosphocaséinate natif obtenu par microfiltration
509 sur membrane. *Le Lait*, 74(5), 375-388.
510 Schuck, P., Dolivet, A., & Jeantet, R. (2012). Analytical methods for food and dairy
511 powders.Wiley-Blackwell, Oxford
512 Wu, X., Li, X., & Mansour, H. M. (2010). Surface analytical techniques in solid-state particle
513 characterization for predicting performance in dry powder inhalers. *KONA Powder*
514 *and Particle Journal*, 28(0), 3-19.
515 Zhou, P., Liu, X., & Labuza, T. P. (2008). Effects of moisture-induced whey protein
516 aggregation on protein conformation, the state of water molecules, and the
517 microstructure and texture of high-protein-containing matrix. *Journal of agricultural*
518 *and food chemistry*, 56(12), 4534-4540.

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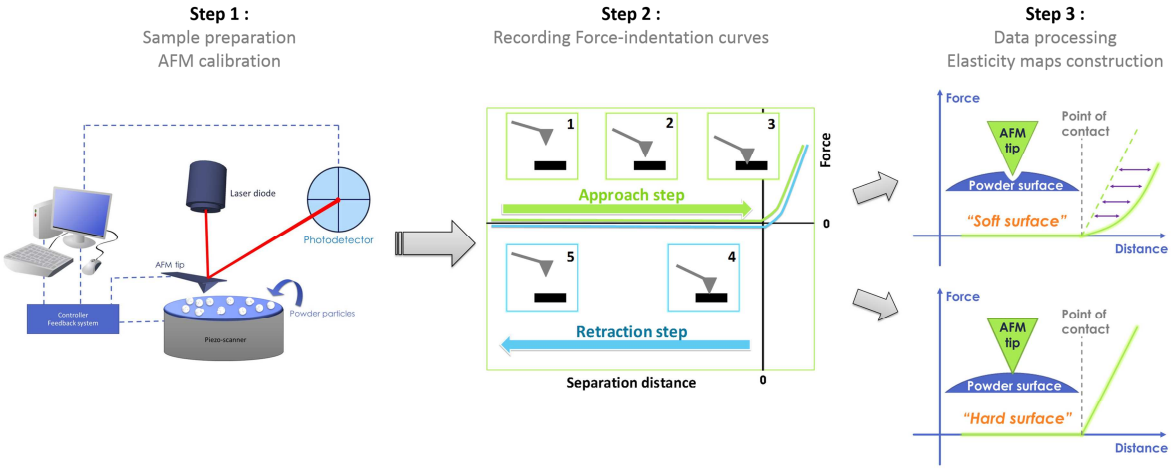

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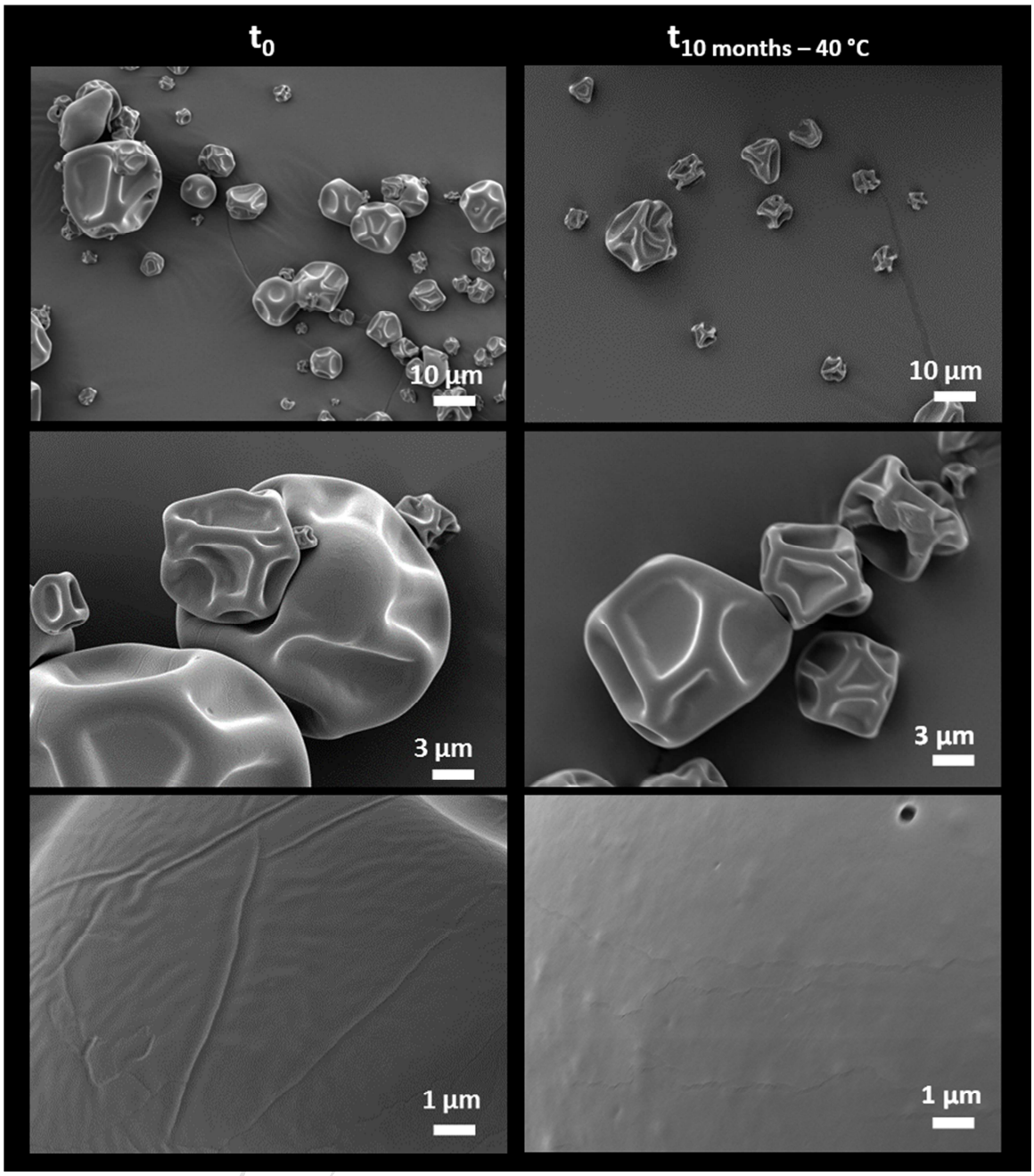
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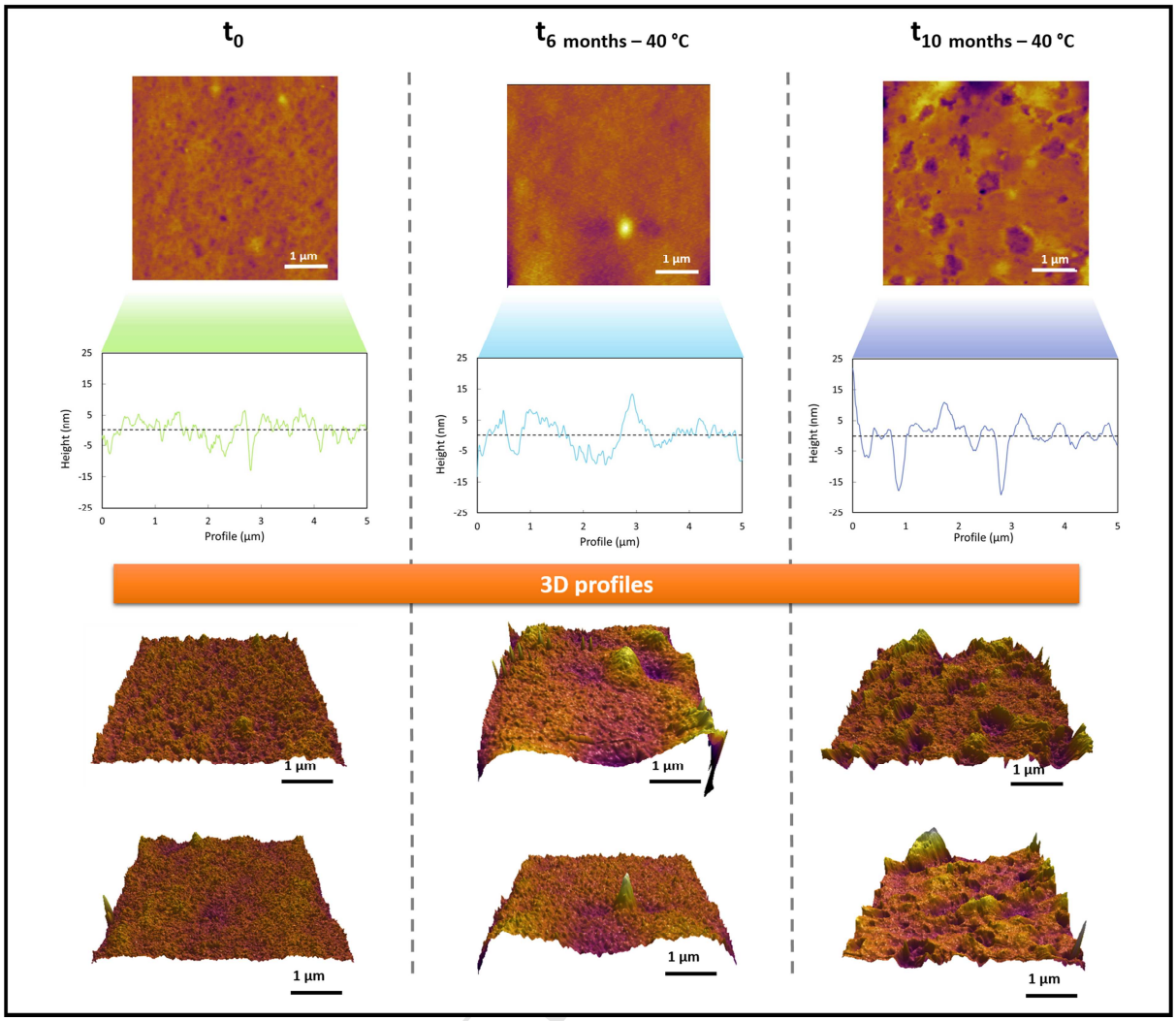

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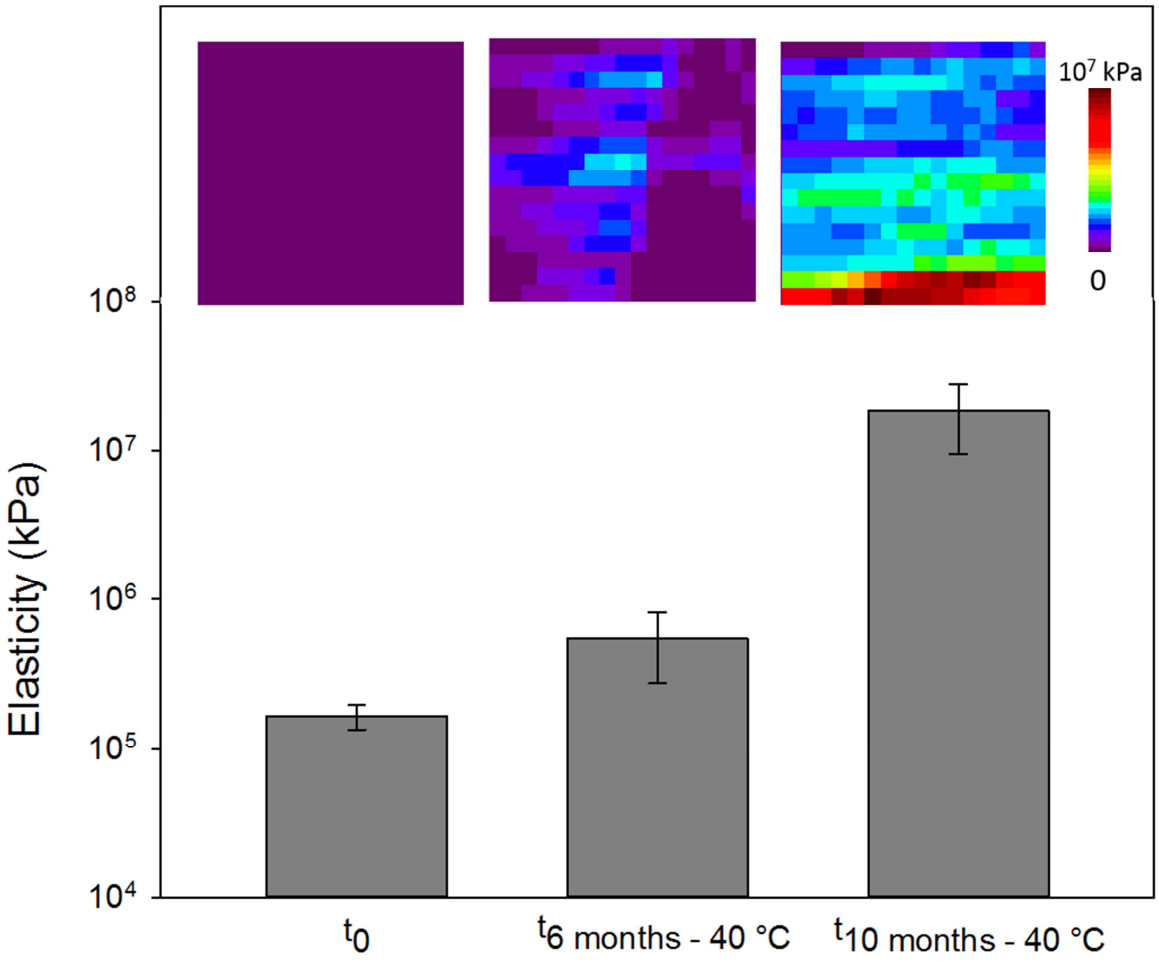
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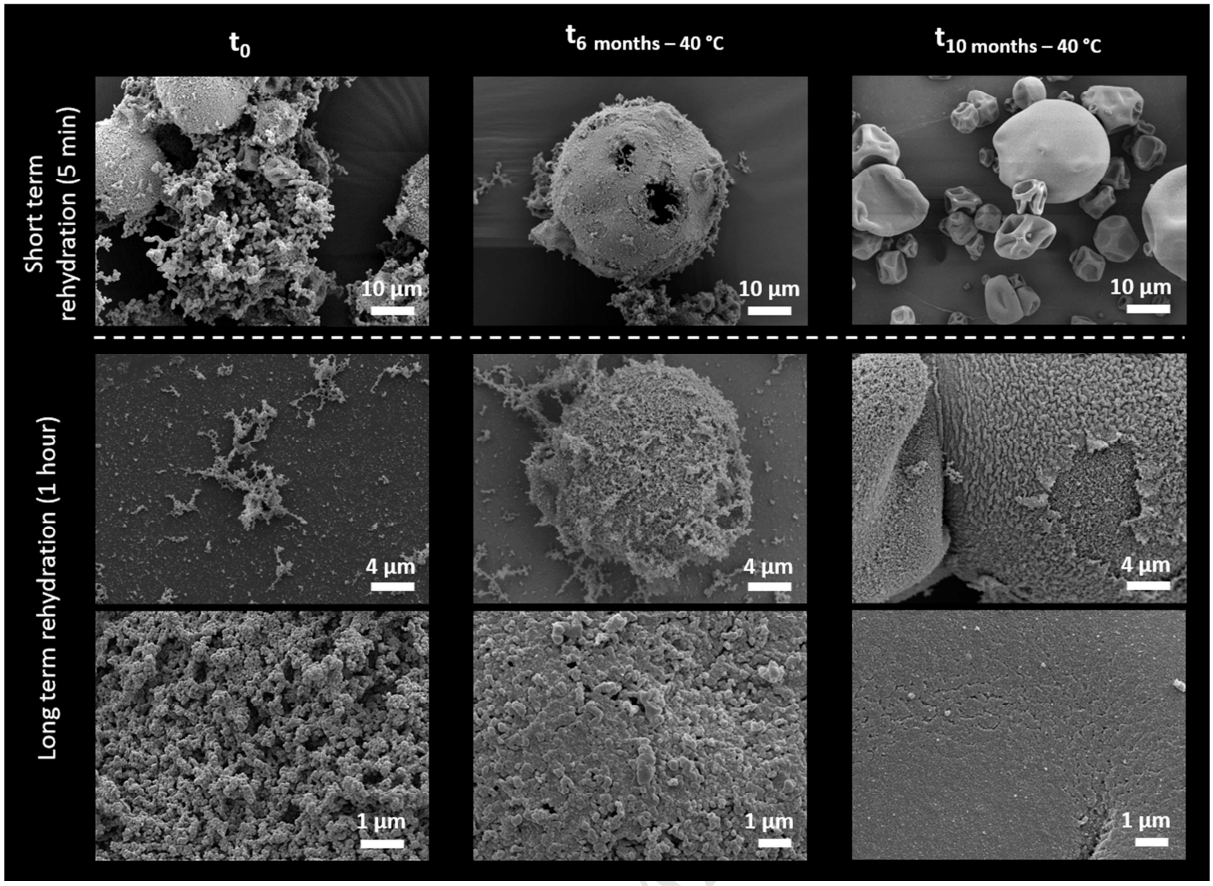

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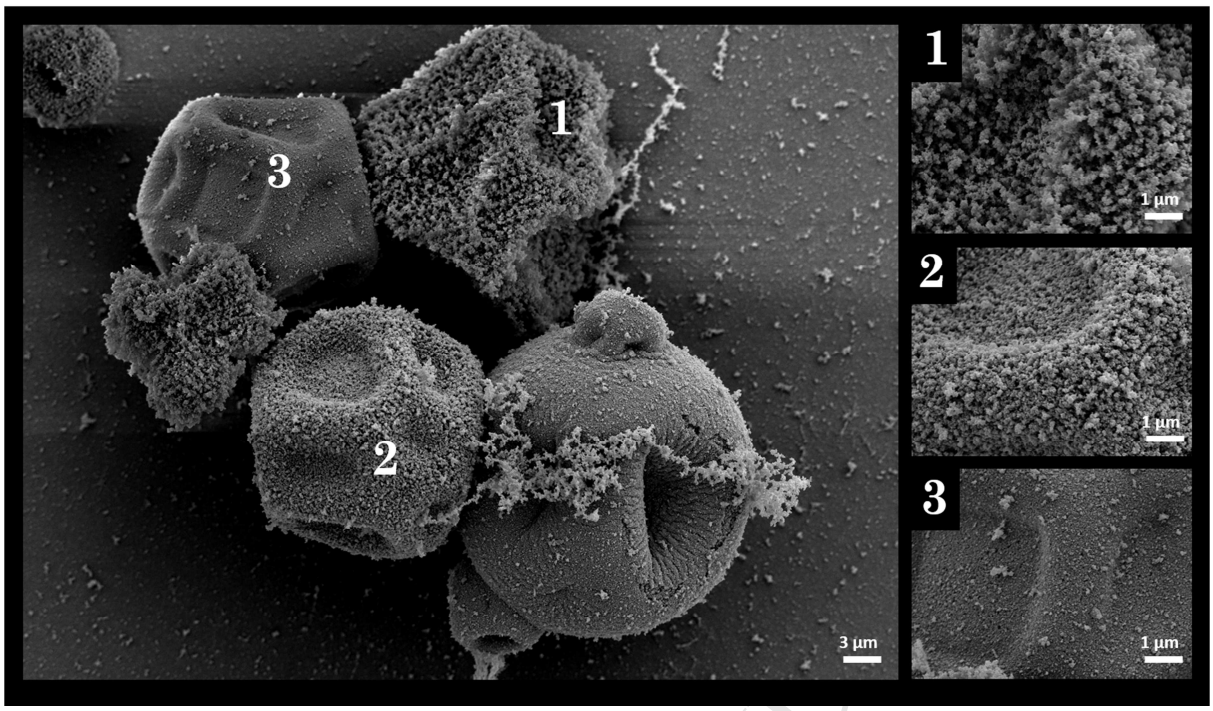

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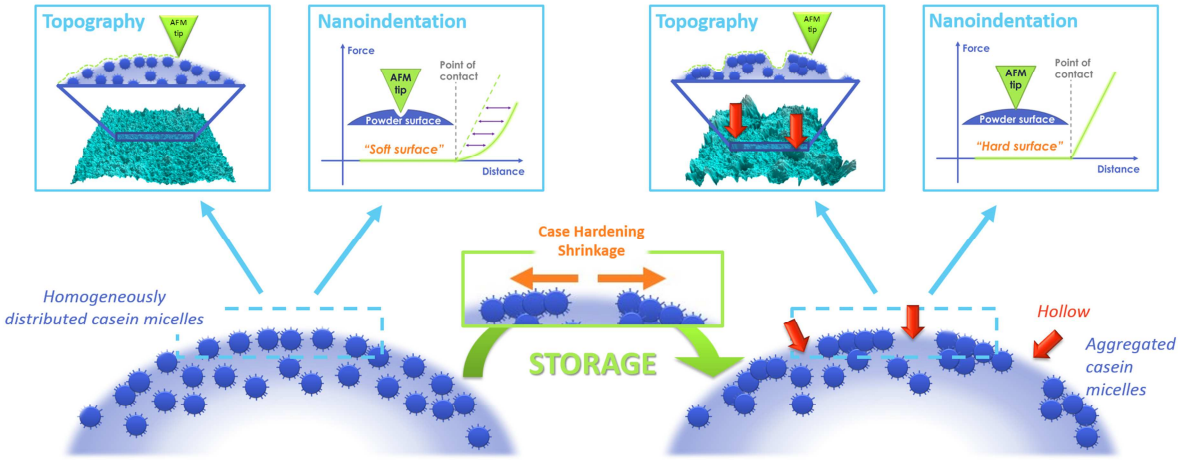

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- MC powder presents hollow zones and harder surface during storage
- Storage induce loss of rehydration ability of MC powder
- Surface hydration is heterogeneous between particles from a same powder batch

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