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# Structure, gelation, and antioxidant properties of curcumin-doped casein micelle powder produced by spray-drying

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## Abstract

The encapsulation of curcumin in micellar caseins (MC) and the production of powder was done by spray-drying. Nearly 97 % curcumin was retained and the yellow powder showed typical high casein powder morphology. The hygroscopic properties were determined, slight differences reflected less available hydrophobic sites when curcumin was bound to casein, favoring interactions with water in curcumin-enriched MC powders. No difference was detected on the internal MC structure *via* SAXS. Antioxidant activity of doped-curcumin powder presented 88 % of active curcumin. During 60 days at 40 °C storage, the antioxidant activity of curcumin measured by ABTS and FRAP was preserved with a percentage of  $82 \pm 2.0$  % and  $84 \pm 1.1$  %, respectively. Curcumin doped powders presented similar features to classical casein powders (rehydration and gelling abilities). It was demonstrated that curcumin encapsulation in MC under its powder form helped in protecting its antioxidant activity without influencing the techno-functional properties of MC. This study allowed the incorporation of curcumin *via* the MC matrix as an active food ingredient available in a powder state usable as classical milk powder in several food formulations.

## 29 **Keywords**

30 Micellar casein, Bioactive, Curcumin, Spray-drying, Powder, Antioxidant

## 31 **1. Introduction**

32 In the food industry, encapsulation is used to deliver a wide range of bioactives such as vitamins,  
33 enzymes, antioxidants and  $\omega$ -3 fatty acids<sup>1,2</sup> within small capsules or particles made of lipids  
34 (emulsions, liposomes), polysaccharides (alginate, gum beads) or proteins (milk proteins such as  
35 whey or caseins, zein or soybean proteins). Encapsulation protects the bioactives from stability  
36 problems induced by pH and temperature fluctuations, enzymatic degradations and oxidative stress  
37 <sup>2</sup>. An important step in the encapsulation process is the selection of a suitable matrix dependently  
38 from the bioactive ingredients properties such as its solubility in water, its sensitivity to oxygen or  
39 light, desired characteristics of final products and encapsulation methods.

40 Milk proteins are natural vehicles for bioactives that are widely used in the food industry for their  
41 nutritional and functional properties<sup>3</sup>. Caseins, the major milk proteins, are Generally Recognized as  
42 Safe (GRAS) and have excellent emulsifying properties allowing them to form micro- and nano-  
43 capsules. They also present mild flavor and high nutritional value, along with various shielding  
44 capabilities, essential for protecting sensitive payload and controlling bioaccessibility of the bioactive  
45 compounds<sup>3,4</sup>. In milk, caseins are under the form of micelles (micellar caseins or MC) made of  
46 hydrophilic and hydrophobic domains that are able to interact with various actives. MC consists in  
47 colloidal particles of around 100 nm radius built by interactions between about 100 casein molecules  
48 and 800 nanoclusters of amorphous calcium phosphate<sup>5</sup>. The outstanding stability of caseins and  
49 casein micelles during heating, freezing and drying processes make them valuable in encapsulating  
50 food bioactives. Moreover, they possess exceptional surface-active, self-assembly and gelation  
51 properties and high water binding capacity under defined conditions<sup>6,7</sup>. MCs have been already used  
52 as delivery systems for vitamin D<sub>3</sub>, for beta-carotene,  $\omega$ -3 fatty acids, tea catechins, curcumin, etc.<sup>8-</sup>  
53 <sup>12</sup>. Sodium caseinate has been also already used as an encapsulation matrix for curcumin but without  
54 the ability of gelling<sup>13</sup>.

55 In a previous paper, we demonstrated that curcumin interacted with MC without altering the  
56 micellar structure of caseins and also without modifying their acid gelation properties<sup>14</sup>. Curcumin,  
57 also known as 1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-heptadiene-3,5-dione, is a polyphenol  
58 extracted from the rhizomes of turmeric (*Curcuma longa*) that possesses several biological activities  
59 such as antioxidant, antimicrobial, anti-cancer, anti-inflammatory and wound healing properties<sup>15–18</sup>.  
60 However, curcumin has a low solubility in water and a short biological half-life<sup>19</sup>. Thus, the use of this  
61 promising lipophilic molecule has been hindered by its poor water solubility and low bioavailability  
62 after oral administration<sup>20</sup>. Furthermore, its antioxidant activity presents a low stability: it decreases  
63 rapidly in the presence of light and oxygen. These drawbacks lead to two main limitations in its use in  
64 food formulations: (i) a high difficulty to incorporate curcumin into aqueous media (the medium of  
65 most food formulations) and (ii) a low bioavailability of curcumin, which is typical of lipophilic  
66 substances<sup>21</sup>. A variety of approaches were pursued to overcome these limitations, which include  
67 synthesis of curcumin analogues, the use of adjuvants that inhibit curcumin metabolism and thus  
68 enhance its bioavailability (e.g. piperine), and the development of improved delivery platforms for  
69 the turmeric compound, including liposomal, nanoparticulated and phospholipid complex  
70 formulations of curcumin<sup>22–24</sup>. And so, encapsulation seems to be an efficient method that  
71 challenged food formulators into developing new efficient encapsulation systems.

72 Milk proteins are often used in food industry under a powder form to improve their preservation  
73 during storage and transport and because a powder is easier to handle than aqueous dispersions.  
74 Most food powders are produced by spray-drying due to its low cost, good availability, continuous  
75 production and easiness of industrialization. This food ingredients microencapsulation technique is  
76 efficient against deterioration, volatile losses or premature interaction with other ingredients.  
77 Recently Liu *et al.* (2015) demonstrated that the solubility of curcumin could be enhanced by spray-  
78 drying microencapsulation in whey protein isolate<sup>25</sup>. The improvement of curcumin solubility in  
79 water by complexation with WPI was demonstrated at 5 wt% by saturating the solution with

80 curcumin up to  $59.5 \pm 2.7 \text{ mg}\cdot\text{mL}^{-1}$ . Despite limited contents in curcumin ( $1.13 \pm 0.17 \text{ mg}\cdot\text{g}^{-1}$ ), the  
81 antioxidant activities of WPI-curcumin particles were high.

82 Providing functional dairy ingredients enriched with curcumin under a powder form should be useful  
83 to improve powder preservation during storage and processing stability of a food formulation. To the  
84 best of our knowledge, producing by spray-drying a curcumin-doped MC powder able to preserve the  
85 antioxidant activity of curcumin had not been studied yet. Consequently, this study was first focused  
86 on producing curcumin-doped MC powders and characterizing their main physicochemical properties  
87 such as their color, their hygroscopic properties and their structure. Then, the antioxidant power of  
88 curcumin in spray dried MC powders was evaluated both by ABTS and FRAP assays and compared to  
89 the antioxidant activity of fresh curcumin in phosphate buffer (medium used for curcumin  
90 encapsulation in MC) and non-spray-dried curcumin encapsulated in MC at the same concentration.  
91 The main techno-functional properties of these powders, like rehydration and gelation behaviors,  
92 were finally investigated.

## 93 **2. Material and methods**

### 94 **2.1. Material**

95 The native-like micellar caseins (MC) used in this study (Promilk 872 B, Ingredia Dairy Ingredients,  
96 Arras, France) were obtained by milk microfiltration and diafiltration against milk ultrafiltrate then  
97 spray-dried. MC contained 95.0 % (w/w) dry solids and, on a dry weight basis, 87.0 % total proteins  
98 (about 80.0 % MC), 7.3 % minerals, 5 % lactose, and 0.8 % fat. Curcumin (66.0 % purity), glucono-  
99 delta-lactone, ethanol (96.0 %) and sodium azide were purchased from Sigma-Aldrich (Saint-Quentin  
100 Fallavier, France).

### 101 **2.2. Preparation of solutions**

102 Stock solutions of curcumin were prepared in ethanol (96 %) at  $1 \text{ mg}\cdot\text{mL}^{-1}$  and stored protected from  
103 light at 4 °C. Phosphate Buffered Saline (PBS) solution was prepared with ultrapure water and  
104 contained for one liter: 8.00 g NaCl, 0.20 g KCl, 1.44 g  $\text{Na}_2\text{HPO}_4$ , 0.24 g  $\text{KH}_2\text{PO}_4$ . In order to prevent

105 bacterial growth, sodium azide (0.02 % w/v) was added to PBS buffer. PBS buffer filtration through  
106 0.20  $\mu\text{m}$  polyethersulfone membrane (Millipore) was performed before use. Native MC was  
107 rehydrated overnight in PBS at 7 °C; then ethanolic curcumin solution (1  $\text{mg}\cdot\text{mL}^{-1}$ ) was mixed with  
108 native MC dispersions either for 1 h or overnight: these durations were chosen to evaluate later on  
109 the time effect on curcumin antioxidant activity.

### 110 **2.3. Spray-drying of MC-curcumin mixtures**

111 The concentrate was prepared by mixing 8 L of rehydrated MC (15.5 % dry matter) with 290 mL of  
112 ethanolic curcumin solution (1  $\text{mg}\cdot\text{mL}^{-1}$ ) for 1 h before spray-drying followed by centrifugation (5000  
113 rpm, 10 min) to remove the potential free curcumin. No undissolved curcumin (Supplementary data  
114 Appendix A) was observed and so all curcumin were considered as encapsulated. Curcumin and MC  
115 mixtures were prepared so as not to exceed 2 % (v/v) addition of ethanol, hence protein ethanol-  
116 induced denaturation was avoided. MC suspensions in ethanol were used as control. Spray-drying  
117 was performed using a pilot-scale spray drier MicraSpray 150 (Anhydro, Soeborg, Denmark). A  
118 peristaltic pump was used to deliver the liquid through the bi-fluid nozzle into the spray-drying  
119 chamber with a feed flow-rate of 46.5  $\text{mL}\cdot\text{min}^{-1}$  and a nozzle pressure of 1 bar. Native MC were  
120 dispersed in PBS buffer (pH 7.4) and stirred overnight at 7 °C in order to get maximal protein  
121 hydration and stabilization. The mixture was atomized into heated air to facilitate the rapid removal  
122 of water as the droplets were mixed with the hot air in the drying chamber at an inlet temperature of  
123 180 °C. The powder particles were then separated from the drying air at an outlet temperature of  
124 90 °C.

125 To evaluate the antioxidant activity of encapsulated curcumin during storage, MC-curcumin powder  
126 were stored in Petri dishes, sealed with parafilm away from oxygen and light, at 40 °C up to 2  
127 months. A potassium acetate ( $\text{CH}_3\text{COOK}$ ) saturated saline solution was employed to set powder  
128 water activity at 0.24 during storage.

129       **2.4.       Comparison of casein and curcumin-casein powders from dry state to**  
130       **aqueous suspension**

131       **2.4.1.   Powder colorimetry**

132   Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes,  
133   France) employing the parameters L\*(lightness), a\*(redness), b\*(yellowness), C\*(chroma) and H\*  
134   (hue angle) defined in the CIEL\*a\*b\* system <sup>26</sup>. All measurements were taken as the average of at  
135   least three points on the center and the periphery of each powder layer of 1 cm high. The total color  
136   difference ( $\Delta E$ ) between Control-MC and MC-curcumin powders was calculated using the following  
137   equation 1 <sup>26</sup>:

138                                   
$$\Delta E = \sqrt{(L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2} \quad (\text{Equation 1})$$

139

140   with 0 and i indices respectively designating Control-MC and MC-curcumin powders.

141       **2.4.2.   Particle powder morphology by environmental scanning electron microscopy (ESEM)**

142   The morphology of powder particles was characterized with an environmental scanning electron  
143   microscope (ESEM, Quanta 650 FEG, FEI, USA). Using environmental condition is particularly  
144   interesting, as no coating is needed. Analyses were performed in the following conditions: 7 KeV  
145   beam energy, LSD detector, and 130 Pa chamber pressure. Powders were gently deposited on an  
146   adhesive tape and excess powder was removed by air flow, prior to analysis.

147       **2.4.3.   Hygroscopic properties**

148   Dynamic vapor sorption was used to monitor the moisture sorption capacity of spray-dried Control-  
149   MC and MC-curcumin powders in the water activity ( $a_w$ ) range from 0 to 0.95. Measuring the water  
150   sorption behavior provides information about the physical and chemical stability of the sample in  
151   humid atmospheres. Water sorption isotherms were determined gravimetrically using a DVS  
152   apparatus (Surface Measurement Systems, London, UK) equipped with a Cahn microbalance. The  
153   changes in sample weight over time at 20 °C and at any desired  $a_w$  were recorded. About 70 - 80 mg

154 sample was loaded onto the quartz sample pan. The sample mass was allowed to reach gravimetric  
155 equilibrium ( $dm/dt < 0,001$ ) before proceeding to the next humidity step. The mass change at each  
156 step was recorded which allowed the moisture sorption isotherm to be generated.

157 The isotherm profiles were fitted using the Guggenheim-Anderson-de Boer (GAB) model (equation 2)  
158 using Origin software for  $a_w$  between 0 and 0.7.

$$159 \quad m = m_{0GAB} \frac{C_{GAB} K_{GAB} a_w}{(1 - K_{GAB} a_w)(1 - K_{GAB} a_w + C_{GAB} K_{GAB} a_w)} \quad (\text{Equation 2})$$

160 The GAB model is commonly used to fit type II water vapor adsorption isotherms (typical of food  
161 solids<sup>27</sup>). The GAB model permits to determine the first monolayer moisture content ( $m_0$ ) and  
162 considers also the multilayered water molecules adsorption (up to  $a_w$  equaled to 0.7). The energy of  
163 interaction between the first and the further adsorbed water layers is determined by the sorption  
164 constant  $C_{GAB}$ . The interaction between multilayered water molecules and condensed water is  
165 represented by the specific GAB constant  $K_{GAB}$ .

#### 166 **2.4.4. Small Angle X-Ray Scattering of spray dried MC-curcumin microparticles**

167 Small Angle X-ray Scattering (SAXS) spectra were recorded on a SAXSess mc<sup>2</sup> apparatus (Anton Paar,  
168 Austria), using a line-collimation system. This instrument was associated with a ID 3003 laboratory X-  
169 Ray generator (General Electric) equipped with a sealed X-Ray tube (PANalytical,  $\lambda_{Cu\ K\alpha} = 0.1542$  nm)  
170 operating at 40 kV and 50 mA. A translucent beam stop allowed the measurement of an attenuated  
171 primary beam at  $q = 0$ . Sample powders were introduced into a Special glass capillary (1.5 mm, WJM-  
172 Glas), before being placed inside an evacuation chamber at 20 °C. The scattering of the X-ray beam  
173 was detected by a CCD camera (Princeton Instruments, 309 mm sample-detector distance). Using  
174 SAXSQuant software (Anton Paar), 2D images were integrated into 1D scattering intensities  $I$  as a  
175 function of the magnitude of the scattering vector  $q$  ( $q = (4\pi/\lambda) \sin \theta$ ; where  $2\theta$  is the total scattering  
176 angle). All data were calibrated by normalizing the attenuated primary intensity, before being  
177 corrected for the background scattering from the empty capillary. The scattering data, obtained with

178 a slit collimation, exhibited instrumental smearing. Obtained intensities were then scaled into  
179 absolute units using water as the reference material.

## 180 **2.5. Curcumin retention and determination of its antioxidant activity**

### 181 **2.5.1. Curcumin extraction procedure**

182 In order to determine the curcumin retention level in MC, an extraction from the powder was  
183 performed. Spray-dried MC-curcumin powder was rehydrated in PBS (50 g.L<sup>-1</sup>, pH = 7.4) overnight at  
184 7 °C. 10 mL diethyl ether was added to 5 mL rehydrated powder and the mixture was agitated  
185 vigorously for 10 min. It was then centrifuged for 5 min at 1050 x g in order to separate organic and  
186 aqueous phases. Diethyl ether from supernatant was recovered and evaporated to recover curcumin  
187 under nitrogen atmosphere to avoid oxidation. 1 mL ethanol 96 % was then added to solubilize  
188 extracted curcumin. The concentration of extracted curcumin was determined by measuring its  
189 absorbance at 420 nm using a spectrophotometer (Shimadzu UV1280, Japan). The curcumin  
190 retention level after spray-drying was calculated by dividing the curcumin content extracted from  
191 spray-dried powder by the curcumin content in the spray-dried concentrate, both being expressed  
192 on dry basis (equation 3). Curcumin in initial feed solution was 0.189 mg.g<sup>-1</sup>.

$$193 \text{ Extracted Curcumin retention level (\%)} = \frac{\text{Curcumin in spray dried powder mg/g}}{\text{Curcumin in feed solution mg/g}} \times 100 \text{ (Equation 3)}$$

194

### 195 **2.5.2. ABTS assay**

196 In order to compare the antioxidant activity of curcumin encapsulated in spray-dried MC-curcumin  
197 powder after extraction to the one of curcumin in native (not spray-dried) MC dispersions, a fresh  
198 free curcumin solution was prepared in ethanol at the same concentration as encapsulated curcumin  
199 after extraction (incubation in dark). The concentration of curcumin after extraction was determined  
200 by reporting the absorbance measured at  $\lambda = 734$  nm to the corresponding concentration on the  
201 calibration curve  $A_{\text{cur ETOH}} = f([\text{curcumin}])$ .

202 The antioxidant activity of curcumin in ethanol, curcumin in phosphate buffer, curcumin extracted  
203 from rehydrated native MC after 1 h and overnight contact times and finally curcumin extracted from  
204 spray-dried MC-curcumin powder was determined according to a method adapted from Re *et al.*  
205 (1999)<sup>28</sup>. The ABTS<sup>•+</sup> radical cation was generated by making react 7 mM ABTS with 2 mM aqueous  
206 potassium persulfate solution in a 1:1 ratio (v/v) and the mixture was allowed to stand in the dark at  
207 20 °C for 12 – 16 h before use. The resulting ABTS<sup>•+</sup> solution was diluted with 96 % ethanol to obtain  
208 an initial absorbance of  $0.70 \pm 0.02$  at 734 nm as measured by a UV-Spectrophotometer (Shimadzu  
209 UV1280, Japan). 10  $\mu$ L of free curcumin were mixed with 1 mL ABTS<sup>•+</sup> solution and the resulting  
210 absorbance at  $\lambda = 734$  nm was read at 20 °C after 5 min incubation in the dark; the same was realized  
211 for extracted curcumin. Analyses were carried out in triplicate. Based on the absorbance measured,  
212 the concentration of active curcumin was determined and the amount in mg was calculated. The  
213 curcumin antioxidant activity (AA) percentage was then calculated by comparing the amount of  
214 active curcumin in mg to the total amount of initial MC encapsulated curcumin in mg.

### 215 **2.5.3. FRAP assay**

#### 216 **2.5.3.1. Reagent preparation**

217 Reagents included 300 mM acetate buffer pH 3.6, 16 mL of glacial acetic acid  $17.43 \text{ mol.L}^{-1}$  of acetate  
218 buffer solution, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in  
219 water. Working FRAP reagent was prepared as required by mixing 50 mL acetate buffer, 5 mL TPTZ  
220 solution and 5 mL  $\text{FeCl}_3$  solution<sup>29</sup>.

#### 221 **2.5.3.2. FRAP assay samples**

222 50 mL freshly prepared FRAP reagent was heated to 37 °C and a reagent blank was read at 593 nm<sup>29</sup>.  
223 In order to measure the absorbance of the samples, 30  $\mu$ L curcumin solution (extracted from native  
224 or spray-dried MC-curcumin powders) were added to 1 mL FRAP reagent and the absorbance was  
225 measured at 593 nm after 5 min incubation in the dark.

226 **2.6. Characterization of techno-functional properties of Control-MC and MC-**  
227 **curcumin powders**

228 **2.6.1. Particle size distribution and rehydration kinetics of native MC and spray-dried Control-**  
229 **MC and MC--Curcumin powders.**

230 Particle size distributions were determined using a Mastersizer 3000 laser granulometer (Malvern  
231 Instruments, UK) equipped with Aero S dry dispersion unit. To obtain a correct obscuration (1 %),  
232 samples were dispersed at 1 bar air pressure, 40 % feed rate and 3 mm hopper gap and  
233 Fontainebleau sand was added to improve powder flowing by reducing cohesiveness. Distinct sand  
234 particles distribution was easily suppressed from granulometric MC profiles because the peak  
235 corresponding to the sand particle size distribution did not cover the sample peak. The particle and  
236 air refractive index 1.45 and 1 respectively. Five measurements were conducted for each sample. The  
237 median value of the particle size distribution,  $d(50)$ , was used, which means that 50 % of particles  
238 have a lower diameter.

239 The rehydration experiments of spray-dried Control-MC and MC-curcumin powders were performed  
240 with the same laser granulometer but with the Hydro MV wet dispersion unit at 20 °C. An  
241 appropriate amount of sample was dispersed in water to reach between 15 % and 20 % obscuration  
242 with a particle refractive index of 1.45 (refractive index of casein) and a dispersant refractive index of  
243 1.33 (refractive index of water). The rehydration profiles were constructed by 3 000 measures with a  
244 measure frequency of 10 s and a rehydration duration of 8.5 h.

245 **2.6.2. Acid gelation properties of spray-dried Control-MC and MC-curcumin powders**

246 **2.6.2.1. Rheological properties of acid gels**

247 Glucono-delta-lactone (GDL) (3 % (w/v)) was gently stirred in Control-MC and MC-curcumin  
248 suspension obtained after rehydration (5 % (w/v)) for 3 min and a 20 mL aliquot was immediately  
249 transferred into the gap of coaxial cylinders (DIN C25) of a Kinexus rheometer (Malvern Instruments,  
250 UK). The sol-gel transition and the development of the gel structure was followed using 0.1 % shear

251 strain and 1.0 Hz frequency at controlled temperature of 20 °C. The time at which the elastic  
252 modulus ( $G'$ ) became greater than 1 Pa was considered as the gelation point. Each experiment was  
253 triplicated.

#### 254 **2.6.2.2. Multiple light scattering for the monitoring of milk gelation**

255 The aggregation of the MC-curcumin dispersions after GDL addition was followed by multiple light  
256 scattering measurements using a Turbiscan Classic MA2000 apparatus (Formulaction, France)  
257 operating with a pulsed near infrared light source at 850 nm. Backscattered intensities all along the  
258 sample, more precisely between 15 and 45 mm above the bottom of the test tube, were then  
259 recorded every 5 min after GDL addition (corresponding to time zero). With Turbisoft software  
260 (Formulaction), the first scan recorded at time zero was subtracted from following scans in order to  
261 highlight temporal evolution of backscattered intensity ( $\Delta BS$ ). The entire Turbiscan device was  
262 thermostated at 20 °C. Turbiscan measurements were acquired 3 min after GDL addition and sample  
263 stirring. Assays were performed in triplicate.

### 264 **3. Results and discussion**

#### 265 **3.1. Spray -dried Control-MC and MC-curcumin powders colorimetry**

266 The color of spray-dried Control-MC and MC-curcumin were determined by the CIEL\*a\*b\* system. As  
267 expected since curcumin is mainly used as a food colorant, its presence modified the powder color.  
268 Control-MC powder was indeed white and showed a lightness  $L^*$  of  $94.04 \pm 0.80$ , a redness  $a^*$  of -  
269  $0.64 \pm 0.01$  and a yellowness  $b^*$  of  $5.75 \pm 0.20$ , whereas MC-curcumin powder appeared yellow and  
270 showed a lightness  $L^*$  of  $95.16 \pm 1.00$ , a redness  $a^*$  of  $-4.65 \pm 0.07$  and a yellowness  $b^*$  of  $62.64 \pm$   
271  $0.90$  (Table 1). The high  $\Delta E$  value of 57.04 indicated that the colors of Control-MC and MC-curcumin-  
272 doped powders were markedly different. Control-MC powder color should be yellow to slightly green  
273 based on its corresponding hue angle  $H^*$  of  $96.42 \pm 0.19$ ; nevertheless its low chroma value  $C^*$  of  
274  $5.79 \pm 0.12$  explains the very low color saturation and so the powder appears white. MC-curcumin

275 powder presents a hue angle  $H^*$  of  $96.36 \pm 0.16$  corresponding to yellow slightly green; its chroma  
276 value  $C^*$  of  $22.62 \pm 0.90$  reflects a high color saturation and so the powder appears yellow.

### 277 **3.2. Comparison of powder particle morphology by ESEM**

278 ESEM images of powders did not evidence any significant size or shape modification resulting from  
279 curcumin addition (Figure 1). All particles were wrinkled and non-spherical and the size of the largest  
280 population was about 30  $\mu\text{m}$ . Sadek *et al.* (2014) demonstrated that particle structure was first  
281 governed by the composition of milk matrix<sup>30</sup>. For example, the presence of caseins in the matrix is  
282 responsible for wrinkled, non-spherical and dense powder structures<sup>30,31</sup>. Since the matrix here was  
283 constituted of caseins, Control-MC and MC-curcumin particles morphology presented in Figure 1 was  
284 in great agreement with expected shape features.

### 285 **3.3. Hygroscopic properties of Control-MC and MC-curcumin powders**

286 Furthermore, the sorption isotherms of spray-dried Control-MC and MC-curcumin were established  
287 and fitted using the GAB model (Figure 2). Type II isotherms were obtained for all samples. Type II is  
288 conventionally divided into three zones. The first one corresponds to the strongly bound water  
289 monolayer and usually gives an idea of the hygroscopic character of the sample. The second zone  
290 ranging from 0.3 to 0.6  $a_w$ , gathering the linear region, is related to capillary adsorbed water which is  
291 more loosely bound. The third zone, is linked to free water present in macrocapillaries<sup>27</sup>.

292 For both spray-dried Control-MC and MC-curcumin powders (Figure 2.A-B), the experimental data  
293 were well fitted with the GAB model ( $R^2 = 0.999$  for both conditions) on  $a_w$  range from 0 to 0.7. The  
294 water monolayer values ( $m_0$ ) for Control-MC and MC-curcumin powders were close and respectively  
295 equal to  $45.5 \cdot 10^{-3} \pm 0.16 \cdot 10^{-3}$  and  $46.4 \cdot 10^{-3} \pm 0.16 \cdot 10^{-3}$   $\text{kg H}_2\text{O} \cdot \text{kg}^{-1}$  dry matter. The  $C_{\text{GAB}}$  values related  
296 to monolayer water-binding energy were of the same order of magnitude and equaled to  $8.39 \pm 1.17$   
297 and  $9.54 \pm 1.35$  for Control-MC and MC-curcumin respectively. A very close  $K_{\text{GAB}}$  value ( $1.05 \pm 0.02$ )  
298 was obtained for both powders. High protein content dairy powders gave similar GAB constants<sup>32,33</sup>.  
299 The slight differences in  $m_0$  and  $C_{\text{GAB}}$  may reflect that less hydrophobic sites are available when

300 curcumin is bound to casein, favoring interactions with water in curcumin-enriched MC powders.  
301 This is expected to lead to higher  $m_0$  and  $C_{GAB}$  values for MC-curcumin than for Control-MC powders.

### 302 **3.4. Study of micellar casein internal structure by SAXS for both spray-dried** 303 **powders.**

304 The influence of the presence of curcumin on the internal structure of spray-dried casein micelles has  
305 been studied by SAXS. Small angle scattering techniques (X-rays or neutrons) have been extensively  
306 used to study the structure of casein micelles dispersed in water<sup>34</sup>. Nevertheless, to the best of our  
307 knowledge, only one study reported SAXS data on dry casein powders. The SAXS profiles obtained for  
308 spray-dried casein powders with and without encapsulated curcumin were similar and are presented  
309 in Figure 3.

310 These scattering curves could be divided into three parts separated by two inflexion points. At low  
311 angles ( $q < 0.2 - 0.3 \text{ nm}^{-1}$ ), a  $q^{-4}$  slope was detected corresponding to the signal of a smooth interface  
312 relevant to the casein micelles surface. An inflexion point was reported at  $q = 0.45 \text{ nm}^{-1}$  related to the  
313 structure factor of calcium phosphate nanoclusters (i.e., mean distance between nanoclusters of  
314 calcium phosphate). The second inflexion point at  $1.00 \text{ nm}^{-1}$  was attributed to the form factor of  
315 these nanoclusters. In a previous paper, it was demonstrated that curcumin is encapsulated within  
316 the casein micelle without modifying its internal structure<sup>14</sup>. In the present study, the presence of  
317 curcumin inside the casein micelles during the spray-drying process did not either modify their  
318 internal structure<sup>14</sup>. Moreover, the SAXS profiles of both spray-dried powders and native MC powder  
319 did not present significant changes, shifts nor inflexion points. This indicates that the internal micellar  
320 structure was unaltered by spray-drying.

321 Knowing that the addition of curcumin did not modify neither the sorption behavior of both spray-  
322 dried Control-MC and MC-curcumin powders nor the internal structure of casein micelles, evaluating  
323 the retention level of encapsulated curcumin after spray-drying and its antioxidant activity (in  
324 comparison with free curcumin in solution) would be interesting.

### 325 **3.5. Curcumin retention and antioxidant activity**

326 After spray-drying, the MC-curcumin powder was rehydrated in phosphate buffer and curcumin  
327 concentration was determined at  $0.041 \pm 0.01 \text{ mg.mL}^{-1}$ , corresponding to  $0.184 \pm 0.01 \text{ mg.g}^{-1}$  dry  
328 matter. Based on equation 3, curcumin retention rate was calculated at  $97.35 \pm 1.02 \%$ , meaning that  
329 the encapsulation of curcumin was highly efficient and that curcumin was not released from casein  
330 micelles during the spray-drying process.

331 Furthermore, the antioxidant activity of encapsulated curcumin in spray-dried MC was quantified *via*  
332 both ABTS and FRAP assays and compared to the antioxidant activity of a freshly prepared curcumin  
333 solution in ethanol, curcumin in phosphate buffer and curcumin encapsulated in fresh native MC  
334 (with contact times of one hour and overnight) prepared at the same concentration ( $0.042 \pm 0.01$   
335  $\text{mg.mL}^{-1}$ ) and under the same conditions (incubation in dark). Data are displayed in Figure 4.

336 When curcumin is freshly prepared in ethanol, its antioxidant activity reached  $97.6 \pm 1.2 \%$  in ABTS  
337 and  $100.2 \pm 0.6 \%$  in FRAP assays as expected. When curcumin is added to phosphate buffer for 1 h,  
338 its antioxidant activity decreases to  $52.0 \pm 1.0 \%$  for ABTS assay and  $51.5 \pm 2.0 \%$  for FRAP assay. This  
339 decrease in antioxidant activity is the result of the alteration of curcumin in phosphate buffer as  
340 already reported in the literature<sup>35</sup>. In fact, when the pH is neutral, proton are removed from the  
341 phenolic group lead to the destruction of curcumin structure, lowering its antioxidant activity. The  
342 encapsulation of curcumin in MC was expected to help protecting its antioxidant activity. In fact,  
343 when curcumin was added to MC dispersions and agitated for 1 h, its antioxidant activity reached  
344  $89.0 \pm 2.1 \%$  and  $89.8 \pm 2.1 \%$  for ABTS and FRAP assays respectively. When the contact time was  
345 increased to 18 h (overnight), curcumin antioxidant activity decreased to  $73.1 \pm 2.1 \%$  and  $78.7 \pm 2.0$   
346  $\%$  for ABTS and FRAP assays respectively. The encapsulation of curcumin under its aqueous form in  
347 MC dispersions helped protecting the bioactive from degradation in phosphate buffer but its  
348 degradation nevertheless increased with time. Moreover, when curcumin was added to MC  
349 dispersion in PBS buffer during 1 h and then spray-dried, it presented a lower decrease in antioxidant

350 activity than after overnight rehydration without spray-drying. In fact, despite a limited curcumin  
351 content in the powder ( $0.181 \text{ mg.g}^{-1}$  of dry matter), ABTS and FRAP assays respectively showed  
352  $86.8 \pm 2.0 \%$  and  $88.7 \pm 2.0 \%$  antioxidant activity after spray-drying. The reported differences  
353 between curcumin in phosphate buffer and curcumin encapsulated in MC (whether it was spray-  
354 dried or not) indicated that the encapsulation process actually protected curcumin from losing its  
355 antioxidant activity and lowered its vulnerability in aqueous media. Such a lower decrease in  
356 antioxidant activity by encapsulation of bioactives was noticed in other studies as well but with  
357 emulsions or liposomes<sup>36–38</sup>. Moreover, the powder of curcumin-enriched MC was stored at  $40 \text{ }^\circ\text{C}$   
358 during two months to evaluate the antioxidant activity of curcumin after storage. During this time of  
359 storage, the antioxidant activity of curcumin measured by ABTS and FRAP was preserved with a  
360 percentage of  $82 \pm 2.0 \%$  and  $84 \pm 1.1 \%$  respectively.

361 The preservation of antioxidant activities of MC-curcumin particles also implied the effectiveness of  
362 curcumin to donate its hydrogen atom to oxygen radicals. The high retention rate of curcumin and  
363 the preservation of its antioxidant activity confirmed that micellar casein encapsulation in hydrated  
364 casein micelles followed by spray-drying is a relevant approach for drying heat-sensitive polyphenols.  
365 Indeed, the combination of short residence times with relatively low temperature surrounding  
366 droplets/particles in the course of the drying process (as long as the surface of the droplet/particle  
367 remains humid, the temperature of the surrounding air equals the humid air temperature, which, for  
368 an inlet air temperature of  $180 \text{ }^\circ\text{C}$ , does not exceed  $45 \text{ }^\circ\text{C}$ ) limits the extent of heat denaturation of  
369 bioactives.

370 Knowing that the obtained spray-dried MC-curcumin powder preserved its antioxidant activity and  
371 that two of the main techno-functional properties of micellar casein are its rehydration behavior and  
372 its ability to form gels, it would be interesting to evaluate the influence of curcumin addition on its  
373 rehydration behavior and sol-gel transition.

### 374 **3.6. Techno-functional properties of Control-MC and MC-curcumin powders**

#### 375 **3.6.1. Rehydration kinetics of spray-dried Control-MC and MC-curcumin powders**

376 Rehydration properties of MC powders is an essential techno-functional property for application  
377 purposes. Before studying the evolution of powder granulometry upon reconstitution in water, the  
378 particle size distribution of investigated powders has been evaluated at dry state (Figure 5.A). The  
379 mean diameters (D50, calculated after subtracting the contribution of Fontainebleau sand) equaled  
380  $32.7 \pm 1.1$  and  $31.1 \pm 0.7$   $\mu\text{m}$  for spray-dried Control-MC and MC-curcumin powders, respectively.  
381 These particles size were well consistent with the results obtained by ESEM (Figure 1).

382 Volume-based particles size distributions of Control-MC and MC-curcumin upon reconstitution at  
383  $20$   $^{\circ}\text{C}$  are displayed in Figure 5.B-C and the corresponding D50 evolution was reported in Figure 5.D.

384 Similar particle size distributions were obtained for Control-MC and MC-curcumin powders. The  
385 granulometric measurements revealed particle size increases from  $65.3$  to  $73.7$   $\mu\text{m}$  and from  $47.6$  to  
386  $58.5$   $\mu\text{m}$  for Control-MC and MC-curcumin, respectively, which could be attributed to a swelling of  
387 casein powders upon water uptake. The swelling of Control-MC (instantly) was quicker than the  
388 swelling of MC-curcumin that took around  $30$  min (Figure 5.D). This joins the hygroscopic properties  
389 of MC-curcumin powder, where less hydrophobic sites are available owing to curcumin binding to  
390 casein, favoring interactions with water in curcumin-enriched MC powders. From the time of particle  
391 swelling ( $30$  min) until around  $350$  min for Control-MC and  $250$  min for MC-curcumin, respectively, a  
392 slow particle size decreases to  $36.8$  and  $37.8$   $\mu\text{m}$  (Figure 5.D). This may correspond to a slow  
393 fragmentation of the protein particle. Then, in the last stage of casein rehydration (from  $350$  to  $470$   
394 min for Control-MC and from  $250$  to  $430$  min for MC-curcumin), D50 decreased to about  $0.7$  nm,  
395 which corresponds to the casein micelles profile. This signal corresponds to casein micelles liberated  
396 from powder particles. After the disappearance of large particles and the appearance of casein  
397 micelles (Figure 5.B), the particle size distributions remained unchanged up to  $800$  min reconstitution  
398 time and the D50 remained around  $0.76$  and  $0.72$   $\mu\text{m}$  for Control-MC and MC-curcumin powders,  
399 respectively (Figure 5.D). The dispersion stage was already evidenced for MC powders rehydrated in

400 water monitoring rheological changes during native phosphocaseinate powder dissolution (turbidity  
401 profiles)<sup>39</sup>. Moreover, this phenomenon was also observed for milk protein concentrate powders<sup>40</sup>.  
402 These authors also depicted the very slow dispersion of this powder. In the end, our results agreed  
403 well with the rehydration behavior of native micellar caseins as reported in the literature where  
404 authors depicted four phases in powder reconstitution: wetting, swelling, dispersion and finally  
405 dissolution<sup>31,33</sup>. And so, spray-dried Control-MC and MC-curcumin powders did not show any  
406 significant difference in their rehydration behavior.

407 **3.6.2. Gelation abilities of spray-dried Control-MC and MC-curcumin powders measured**  
408 **by multiple static light scattering and rheological measurements**

409 The sol-gel transition of rehydrated spray-dried MC powders acidified with GDL was followed by the  
410 temporal evolution of the relative backscattered intensity ( $\Delta$ BS) all along the sample height. 150 min  
411 after GDL addition, an increase of  $\Delta$ BS was reported for the whole sample height, corresponding to  
412 MC aggregation. Indeed, this evolution of intensity of scattered light is known to be attributed to  
413 change in particle size, which affect all particles whatever their position (height) in the sample (Figure  
414 6).

415 Indeed,  $\Delta$ BS increased rapidly 150 min after GDL addition and reached, around 250 min, 15.1 % and  
416 13.5 % for Control-MC and MC-curcumin respectively. For both powders, suspended micellar caseins  
417 started aggregating at the same time 70 min. The addition of curcumin did not significantly modify  
418 the  $\Delta$ BS percentage, and so, MC aggregation behavior was similar whether curcumin was present or  
419 not. Additionally, the temporal evolution of the elastic modulus (Figure 6) shows that gelation (sol-  
420 gel transition) occurred 145 - 150 min after GDL addition for both powders with or without curcumin.  
421 The final elastic modulus (reached 400 min after GDL addition) for MC-curcumin was slightly higher  
422 than the one obtained for Control-MC (12.8 Pa vs. 10.4 Pa, respectively). In fact, MC-curcumin  
423 presents smaller aggregated particles (Figure 5) leading to a lower  $\Delta$ BS percentage and a stronger gel  
424 formation. Thus, curcumin addition did not significantly affect neither the gelation time nor the  
425 strength of the acid gel. This evidenced that curcumin encapsulation in casein micelles did not alter

426 the gelation properties of MC. This is consistent with a previous work showing that its  
427 physicochemical properties such as size, structure and charge were not modified upon curcumin  
428 addition<sup>14</sup>.

#### 429 **4. Conclusion**

430 Micellar casein powder containing curcumin was obtained by spray-drying. MC-curcumin powder  
431 was slightly yellow and presented a wrinkled non spherical particle morphology, characteristic of  
432 high protein content powders. Curcumin addition did not modify physical properties of micellar  
433 casein such as their sorption behavior. Also, SAXS profiles demonstrated that the curcumin addition  
434 did not alter the internal structure of casein micelle even after spray-drying. Moreover, the spray-  
435 drying process did not alter the antioxidant properties of encapsulated curcumin: the obtained  
436 powders displayed high curcumin retention rates (> 95 %) and high antioxidant activity preservation  
437 (86 % for ABTS and 88 % for FRAP assays after spray-drying vs. 89 % and 90 % before spray-drying).  
438 Moreover, the antioxidant activity of curcumin measured by ABTS and FRAP was preserved during  
439 storage at 40 °C for 60 days with a percentage of  $82 \pm 2.0$  % and  $84 \pm 1.1$  % respectively. Curcumin-  
440 doped powders presented also similar features to classical casein powders in terms of reconstitution  
441 and gelation abilities. No modification on the sol-gel transition compared to undoped Control-MC  
442 was observed. This study demonstrates the interest of curcumin encapsulated in MC matrix to serve  
443 as an active food ingredient available in a powder state in several food formulations. Further studies  
444 on how curcumin would act in a more complex dairy medium (such as yoghurt or cheese), where  
445 lactic acid bacteria and proteins coexist, should be performed to enlighten on the potential of casein  
446 micelles to act as delivery systems for natural bioactives.

#### 447 **Conflicts of interest**

448 No conflict of interest to be stated.

#### 449 **Acknowledgments**

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## 454 5. References

- 455 1 Okuro PK, Furtado GF, Sato ACK, Cunha RL. Structures design for protection and vehiculation of  
456 bioactives. *Curr Opin Food Sci* 2015; **5**: 67–75.
- 457 2 Aguiar J, Estevinho BN, Santos L. Microencapsulation of natural antioxidants for food application  
458 – The specific case of coffee antioxidants – A review. *Trends Food Sci Technol* 2016; **58**: 21–39.
- 459 3 Livney YD. Milk proteins as vehicles for bioactives. *Curr Opin Colloid Interface Sci* 2010; **15**: 73–  
460 83.
- 461 4 Elzoghby AO, Abo El-Fotoh WS, Elgindy NA. Casein-based formulations as promising controlled  
462 release drug delivery systems. *J Controlled Release* 2011; **153**: 206–216.
- 463 5 Holt C. Unfolded phosphopolypeptides enable soft and hard tissues to coexist in the same  
464 organism with relative ease. *Curr Opin Struct Biol* 2013; **23**: 420–425.
- 465 6 Considine T, Patel HA, Anema SG, Singh H, Creamer LK. Interactions of milk proteins during heat  
466 and high hydrostatic pressure treatments — A Review. *Innov Food Sci Emerg Technol* 2007; **8**: 1–  
467 23.
- 468 7 Ye R, Harte F. High pressure homogenization to improve the stability of casein–hydroxypropyl  
469 cellulose aqueous systems. *Food Hydrocoll* 2014; **35**: 670–677.
- 470 8 Levinson Y, Ish-Shalom S, Segal E, Livney YD. Bioavailability, rheology and sensory evaluation of  
471 fat-free yogurt enriched with VD3 encapsulated in re-assembled casein micelles. *Food Funct*  
472 2016; **7**: 1477–1482.
- 473 9 Jarunglumlert T, Nakagawa K, Adachi S. Influence of aggregate structure of casein on the  
474 encapsulation efficiency of  $\beta$ -carotene entrapped via hydrophobic interaction. *Food Struct* 2015;  
475 **5**: 42–50.
- 476 10 Rahimi Yazdi S, Corredig M. Heating of milk alters the binding of curcumin to casein micelles. A  
477 fluorescence spectroscopy study. *Food Chem* 2012; **132**: 1143–1149.
- 478 11 Haratifar S, Corredig M. Interactions between tea catechins and casein micelles and their impact  
479 on renneting functionality. *Food Chem* 2014; **143**: 27–32.
- 480 12 Ghasemi S, Abbasi S. Formation of natural casein micelle nanocapsule by means of pH changes  
481 and ultrasound. *Food Hydrocoll* 2014; **42**: 42–47.
- 482 13 Pan K, Zhong Q, Baek SJ. Enhanced Dispersibility and Bioactivity of Curcumin by Encapsulation in  
483 Casein Nanocapsules. *J Agric Food Chem* 2013; **61**: 6036–6043.

- 484 14 Khanji AN, Michaux F, Jasniewski J, Petit J, Lahimer E, Cherif M *et al.* Structure and gelation  
485 properties of casein micelles doped with curcumin under acidic conditions. *Food Funct* 2015; **6**:  
486 3624–3633.
- 487 15 Gryniewicz G, Slifirski P. Curcumin and curcuminoids in quest for medicinal status. *Acta Biochim*  
488 *Pol* 2012; **59**: 201.
- 489 16 Esatbeyoglu T, Huebbe P, Ernst IMA, Chin D, Wagner AE, Rimbach G. Curcumin—From Molecule  
490 to Biological Function. *Angew Chem Int Ed* 2012; **51**: 5308–5332.
- 491 17 Gupta SC, Kismali G, Aggarwal BB. Curcumin, a component of turmeric: From farm to pharmacy.  
492 *BioFactors* 2013; **39**: 2–13.
- 493 18 Indira Priyadarsini K. Chemical and Structural Features Influencing the Biological Activity of  
494 Curcumin. *Curr Pharm Des* 2013; **19**: 2093–2100.
- 495 19 Pinheiro AC, Coimbra MA, Vicente AA. In vitro behaviour of curcumin nanoemulsions stabilized  
496 by biopolymer emulsifiers – Effect of interfacial composition. *Food Hydrocoll* 2016; **52**: 460–467.
- 497 20 Zou L, Zheng B, Zhang R, Zhang Z, Liu W, Liu C *et al.* Enhancing the bioaccessibility of hydrophobic  
498 bioactive agents using mixed colloidal dispersions: Curcumin-loaded zein nanoparticles plus  
499 digestible lipid nanoparticles. *Food Res Int* 2016; **81**: 74–82.
- 500 21 Borrin TR, Georges EL, Moraes ICF, Pinho SC. Curcumin-loaded nanoemulsions produced by the  
501 emulsion inversion point (EIP) method: An evaluation of process parameters and physico-  
502 chemical stability. *J Food Eng* 2016; **169**: 1–9.
- 503 22 Barui S, Saha S, Mondal G, Haseena S, Chaudhuri A. Simultaneous delivery of doxorubicin and  
504 curcumin encapsulated in liposomes of pegylated RGDK-lipopeptide to tumor vasculature.  
505 *Biomaterials* 2014; **35**: 1643–1656.
- 506 23 Sari TP, Mann B, Kumar R, Singh RRB, Sharma R, Bhardwaj M *et al.* Preparation and  
507 characterization of nanoemulsion encapsulating curcumin. *Food Hydrocoll* 2015; **43**: 540–546.
- 508 24 Tikekar RV, Pan Y, Nitin N. Fate of curcumin encapsulated in silica nanoparticle stabilized  
509 Pickering emulsion during storage and simulated digestion. *Food Res Int* 2013; **51**: 370–377.
- 510 25 Liu W, Chen XD, Cheng Z, Selomulya C. On enhancing the solubility of curcumin by  
511 microencapsulation in whey protein isolate via spray drying. *J Food Eng* 2016; **169**: 189–195.
- 512 26 MacDougall DB (ed.). *Colour in food: improving quality*. Woodhead Publishing: Boca Raton, Fla.  
513 CRC Press Cambridge, 2002.
- 514 27 Mathlouthi M. Water content, water activity, water structure and the stability of foodstuffs.  
515 *Food Control* 2001; **12**: 409–417.
- 516 28 Re R. ANTIOXIDANT ACTIVITY APPLYING AN IMPROVED ABTS RADICAL CATION  
517 DECOLORIZATION ASSAY. *Free Radic. Biol. Med.* 1999; : 1231–1237.
- 518 29 Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant  
519 power”: the FRAP assay. *Anal Biochem* 1996; **239**: 70–76.

- 520 30 Sadek C, Li H, Schuck P, Fallourd Y, Pradeau N, Le Floch-Fouéré C *et al.* To What Extent Do Whey  
521 and Casein Micelle Proteins Influence the Morphology and Properties of the Resulting Powder?  
522 *Dry Technol* 2014; **32**: 1540–1551.
- 523 31 Gaiani C, Scher J, Ehrhardt JJ, Linder M, Schuck P, Desobry S *et al.* Relationships between Dairy  
524 Powder Surface Composition and Wetting Properties during Storage: Importance of Residual  
525 Lipids. *J Agric Food Chem* 2007; **55**: 6561–6567.
- 526 32 Kelly GM, O'Mahony JA, Kelly AL, Huppertz T, Kennedy D, O'Callaghan DJ. Influence of protein  
527 concentration on surface composition and physico-chemical properties of spray-dried milk  
528 protein concentrate powders. *Int Dairy J* 2015; **51**: 34–40.
- 529 33 Gaiani C, Banon S, Scher J, Schuck P, Hardy J. Use of a Turbidity Sensor to Characterize Micellar  
530 Casein Powder Rehydration: Influence of Some Technological Effects. *J Dairy Sci* 2005; **88**: 2700–  
531 2706.
- 532 34 Ingham B, Smialowska A, Erlangga GD, Matia-Merino L, Kirby NM, Wang C *et al.* Revisiting the  
533 interpretation of casein micelle SAXS data. *Soft Matter* 2016; **12**: 6937–6953.
- 534 35 Wang Y. Stability of curcumin in buffer solutions and characterization of its degradation  
535 products. *J. Pharm. Biomed. Anal.* 1997; : 1867–1876.
- 536 36 Altunkaya A, Becker EM, Gökmen V, Skibsted LH. Antioxidant activity of lettuce extract (*Lactuca*  
537 *sativa*) and synergism with added phenolic antioxidants. *Food Chem* 2009; **115**: 163–168.
- 538 37 Capitani CD, Carvalho ACL, Botelho PB, Carrapeiro MM, Castro IA. Synergism on antioxidant  
539 activity between natural compounds optimized by response surface methodology. *Eur J Lipid Sci*  
540 *Technol* 2009; **111**: 1100–1110.
- 541 38 Sørensen A-DM, Haahr A-M, Becker EM, Skibsted LH, Bergenståhl B, Nilsson L *et al.* Interactions  
542 between Iron, Phenolic Compounds, Emulsifiers, and pH in Omega-3-Enriched Oil-in-Water  
543 Emulsions. *J Agric Food Chem* 2008; **56**: 1740–1750.
- 544 39 Gaiani C, Scher J, Schuck P, Hardy J, Desobry S, Banon S. The dissolution behaviour of native  
545 phosphocaseinate as a function of concentration and temperature using a rheological approach.  
546 *Int Dairy J* 2006; **16**: 1427–1434.
- 547 40 Mimouni A, Deeth HC, Whittaker AK, Gidley MJ, Bhandari BR. Rehydration process of milk  
548 protein concentrate powder monitored by static light scattering. *Food Hydrocoll* 2009; **23**: 1958–  
549 1965.

550

## Figure captions

Figure 1. Environmental scanning electron microscopy micrographs of spray-dried (A) Control micellar casein (B) Curcumin-doped micellar casein powders. Magnification x 1000.

Figure 2. Sorption isotherm profiles recorded at 20 °C and fitted using the GAB model obtained for (A) spray-dried control micellar casein powder (black circles), and (B) curcumin-doped micellar casein powder (red squares). Experiments were done in triplicate at 20 °C and representative profiles are shown here.

Figure 3. SAXS patterns of native micellar casein (blue diamonds), spray-dried control micellar casein (black circles) and curcumin-doped micellar casein (red squares) powders.

Figure 4. Antioxidant activity of encapsulated curcumin for native and spray-dried curcumin-doped micellar casein powders compared to fresh curcumin in ethanol and curcumin in phosphate buffer solution, quantified by ABTS and FRAP assays ( $n = 3$ ). Fresh EtOH: fresh curcumin in ethanol, PBS 1h: curcumin added to PBS (1 h contact time), MC PBS 1h: curcumin added to native micellar caseins suspensions in phosphate buffer (1 h contact time), MC PBS 18h: curcumin added to native micellar caseins suspensions in phosphate buffer (18 h contact time), MC PBS 1h-SD: spray dried MC-curcumin powder suspensions in phosphate buffer (1 h contact time). MC PBS 1h-SD: spray dried MC-curcumin powder suspensions in phosphate buffer (1 h contact time) stored 2 months at 40 °C.

Figure 5. A. Particle size distributions of spray-dried Control-micellar casein (black squares), curcumin-doped micellar casein (red circles) powders and Fontainebleau sand (blue triangles) measured in dry state. B. Evolution of particle size distributions upon reconstitution of curcumin-doped micellar casein powder in water at 20 °C. C. Particle size distribution of Control-micellar casein and curcumin-doped micellar casein powders after 0 and 800\_min reconstitution in water. (A-C). The size measurements pointed out represent D50 of the different samples D. Evolution of D50 upon time during reconstitution of Control-micellar casein and curcumin-doped micellar casein powders.

Figure 6. Evolution upon GDL acidification of elastic modulus and  $\Delta BS$  averaged between 15 and 45 mm sample height of Control-micellar casein and curcumin-doped micellar casein powders reconstituted at 20 °C.

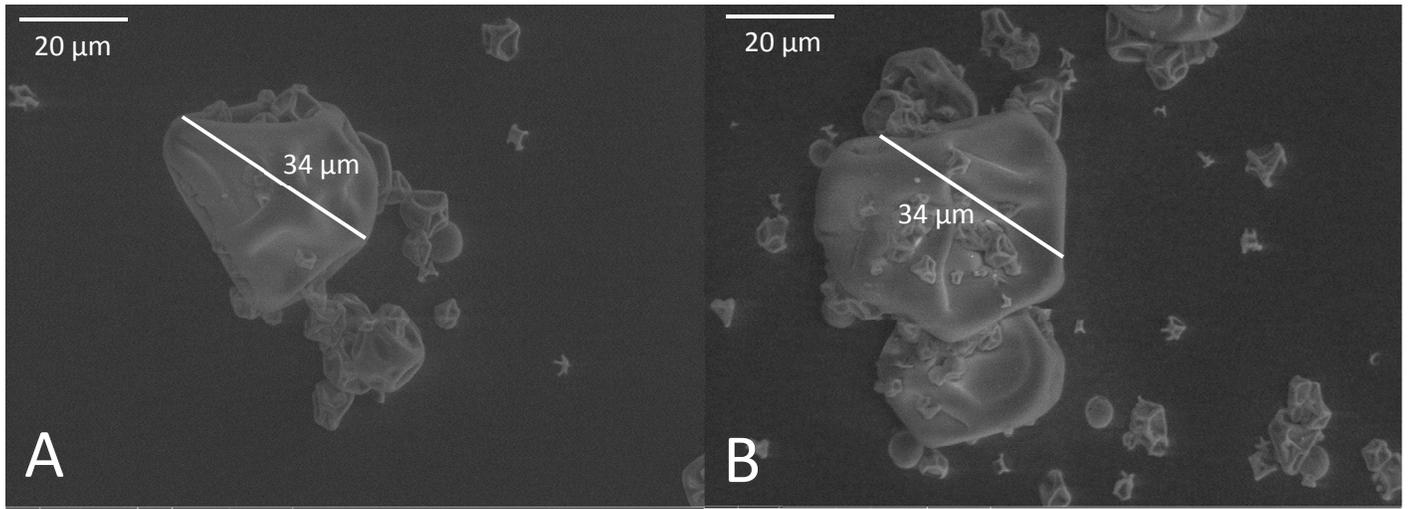


Figure 1

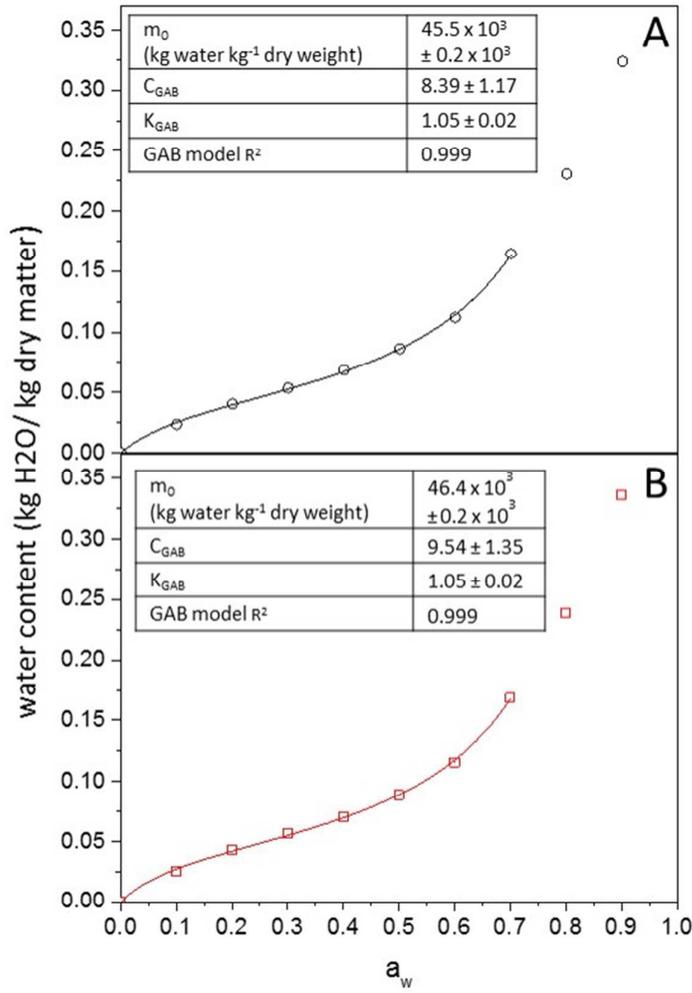


Figure 2

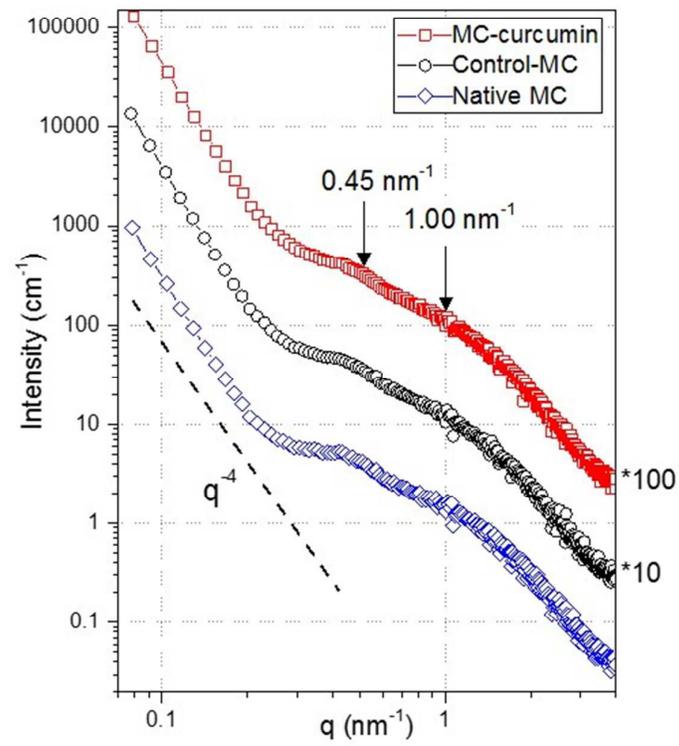


Figure 3

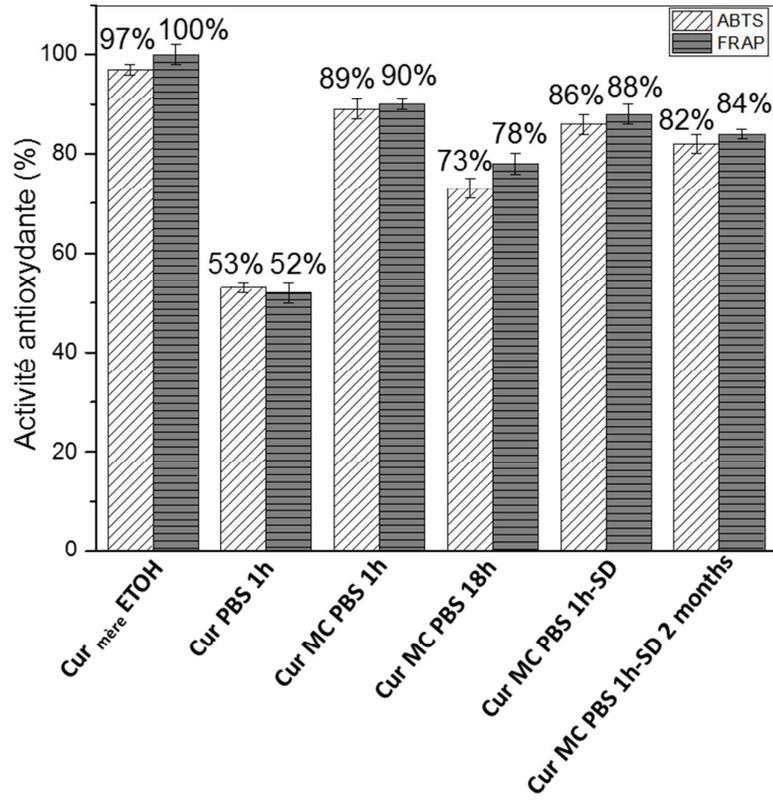


Figure 4

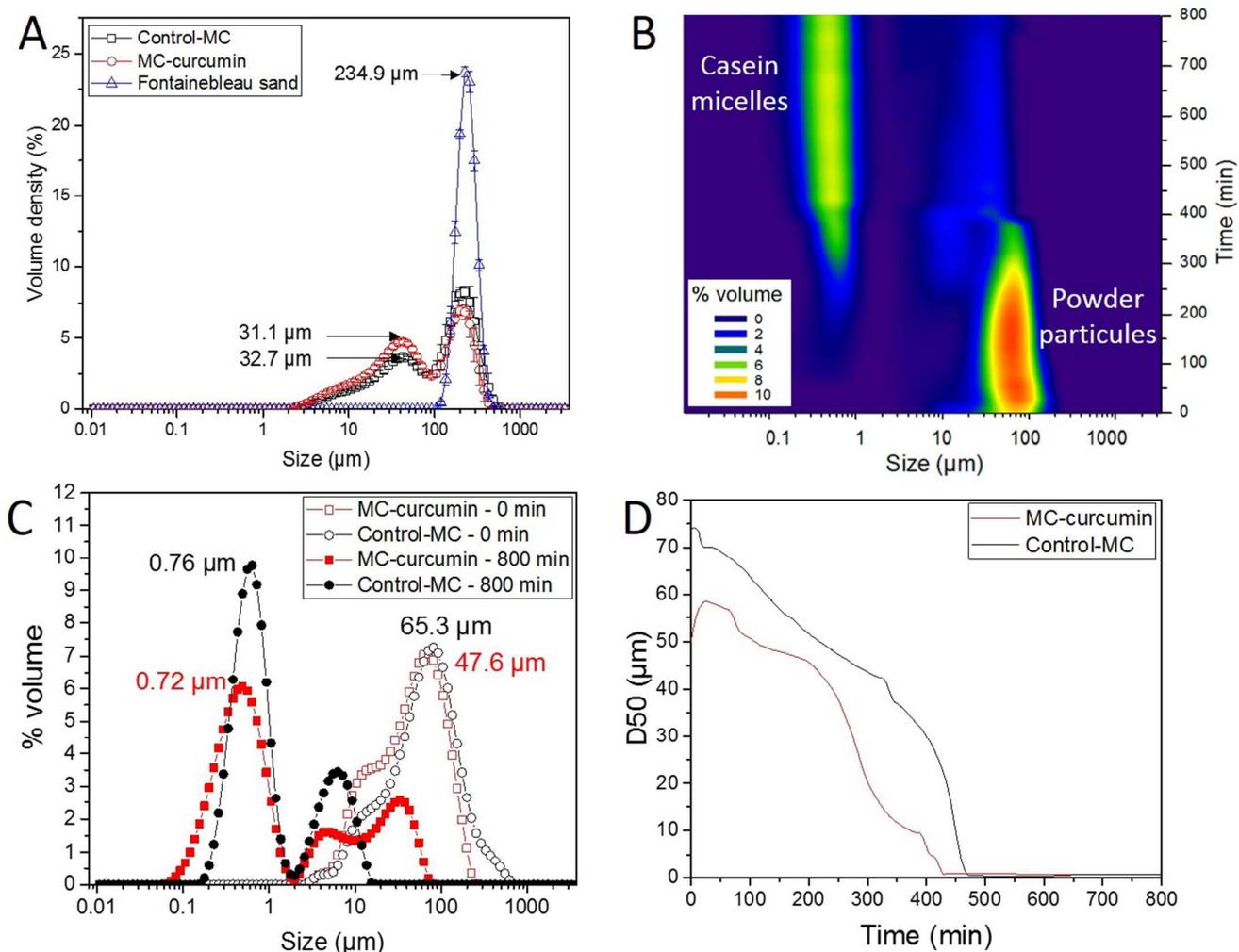


Figure 5

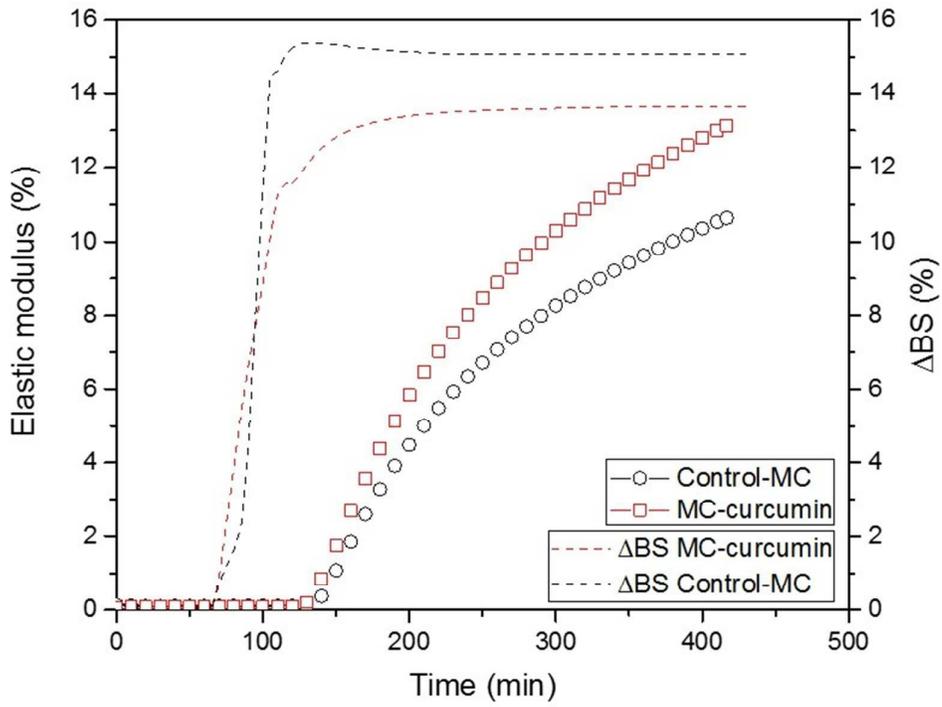


Figure 6

Table 1. Color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $H^*$ ,  $\Delta E$  (total color difference)) and pictures of spray-dried control micellar casein and curcumin-doped micellar casein powders.

	$L^*$ (lightness)	$a^*$ (redness)	$b^*$ (yellowness)	$C^*$ (chroma)	$H^*$ (hue angle)	Aspect
Control-MC	94.04 $\pm 0.80$	-0.64 $\pm 0.01$	5.75 $\pm 0.20$	5.79 $\pm 0.12$	96.42 $\pm 0.19$	
MC-Curcumin	95.16 $\pm 1.00$	-4.65 $\pm 0.07$	62.64 $\pm 0.90$	22.62 $\pm 0.90$	96.36 $\pm 0.16$	
$\Delta E$ (Color difference) = 57.04						