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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 ± 2.0 % and 84 ± 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 ω -3 fatty acids^{1,2} 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 ². 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 properties³. 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^{3,4}. 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 phosphate⁵. 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^{6,7}. MCs have been already used
52 as delivery systems for vitamin D₃, for beta-carotene, ω -3 fatty acids, tea catechins, curcumin, etc.⁸⁻
53 ¹². Sodium caseinate has been also already used as an encapsulation matrix for curcumin but without
54 the ability of gelling¹³.

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 properties¹⁴. 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^{15–18}.
60 However, curcumin has a low solubility in water and a short biological half-life¹⁹. Thus, the use of this
61 promising lipophilic molecule has been hindered by its poor water solubility and low bioavailability
62 after oral administration²⁰. 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 substances²¹. 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^{22–24}. 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 isolate²⁵. 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 Na_2HPO_4 , 0.24 g KH_2PO_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 μm 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 (CH_3COOK) 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* system²⁶. 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 (ΔE) between Control-MC and MC-curcumin powders was calculated using the following
137 equation 1²⁶:

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

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 solids²⁷). 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 mc² 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 2θ 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⁻¹, 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⁻¹.

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

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)²⁸. The ABTS^{•+} 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^{•+} solution was diluted with 96 % ethanol to obtain
208 an initial absorbance of 0.70 ± 0.02 at 734 nm as measured by a UV-Spectrophotometer (Shimadzu
209 UV1280, Japan). 10 μ L of free curcumin were mixed with 1 mL ABTS^{•+} 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 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 FeCl_3 solution²⁹.

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 nm²⁹.
223 In order to measure the absorbance of the samples, 30 μ 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 (ΔBS). 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 ± 0.80 , a redness a^* of -
269 0.64 ± 0.01 and a yellowness b^* of 5.75 ± 0.20 , whereas MC-curcumin powder appeared yellow and
270 showed a lightness L^* of 95.16 ± 1.00 , a redness a^* of -4.65 ± 0.07 and a yellowness b^* of $62.64 \pm$
271 0.90 (Table 1). The high ΔE 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 ± 0.19 ; nevertheless its low chroma value C^* of
274 5.79 ± 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 ± 0.16 corresponding to yellow slightly green; its chroma
276 value C^* of 22.62 ± 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 μm . Sadek *et al.* (2014) demonstrated that particle structure was first
281 governed by the composition of milk matrix³⁰. For example, the presence of caseins in the matrix is
282 responsible for wrinkled, non-spherical and dense powder structures^{30,31}. 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 macrocapillaries²⁷.

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_{GAB} values related
296 to monolayer water-binding energy were of the same order of magnitude and equaled to 8.39 ± 1.17
297 and 9.54 ± 1.35 for Control-MC and MC-curcumin respectively. A very close K_{GAB} value (1.05 ± 0.02)
298 was obtained for both powders. High protein content dairy powders gave similar GAB constants^{32,33}.
299 The slight differences in m_0 and C_{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 water³⁴. 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 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 structure¹⁴. 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 structure¹⁴. 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 ± 0.01
335 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 literature³⁵. 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 ± 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 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^{36–38}. 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 ± 1.1 and 31.1 ± 0.7 μm 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 μm and from 47.6 to
386 58.5 μm 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 μm (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 μm 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)³⁹. Moreover, this phenomenon was also observed for milk protein concentrate powders⁴⁰.
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^{31,33}. 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 (Δ BS) all along the sample height. 150 min
411 after GDL addition, an increase of Δ 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, Δ 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 Δ 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 Δ 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 addition¹⁴.

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 ± 2.0 % and 84 ± 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.

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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 ΔBS averaged between 15 and 45 mm sample height of Control-micellar casein and curcumin-doped micellar casein powders reconstituted at 20 °C.

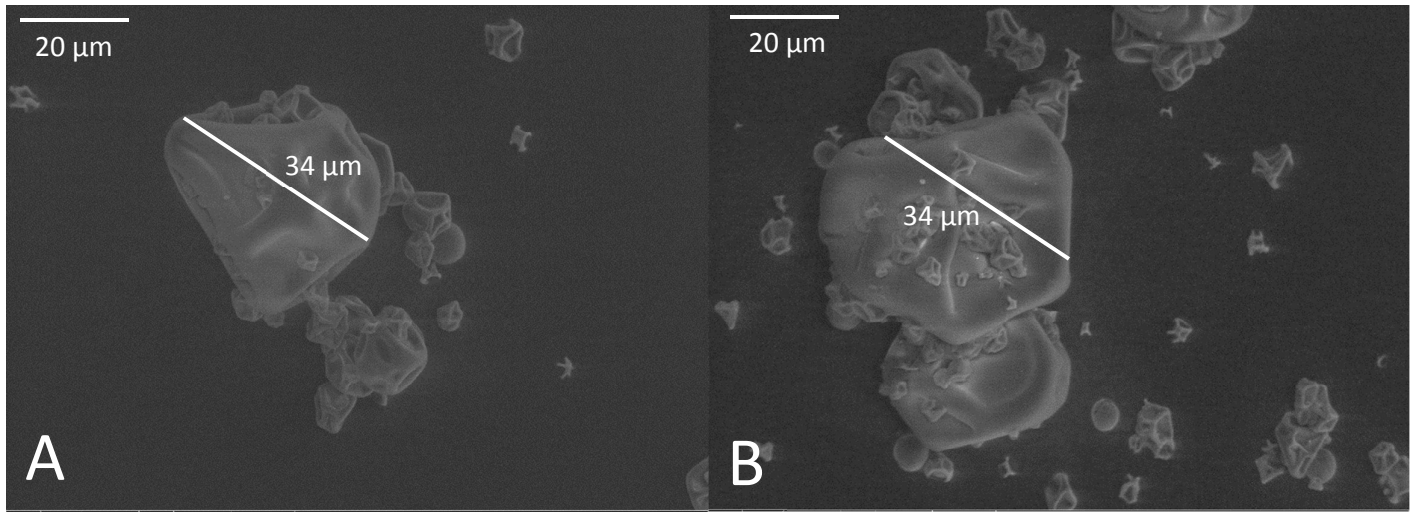


Figure 1

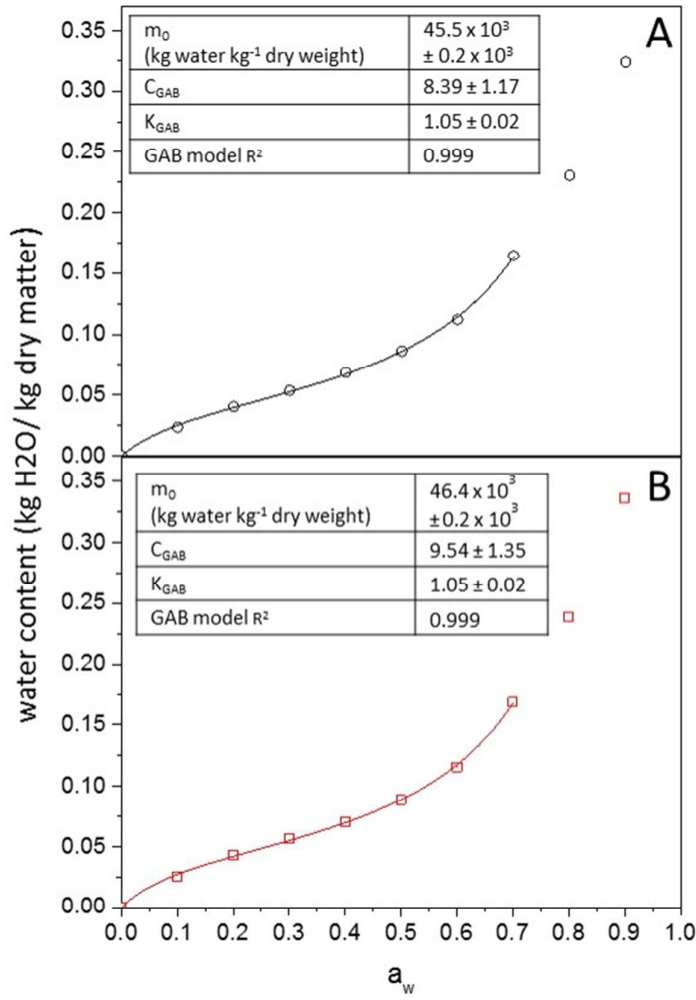


Figure 2

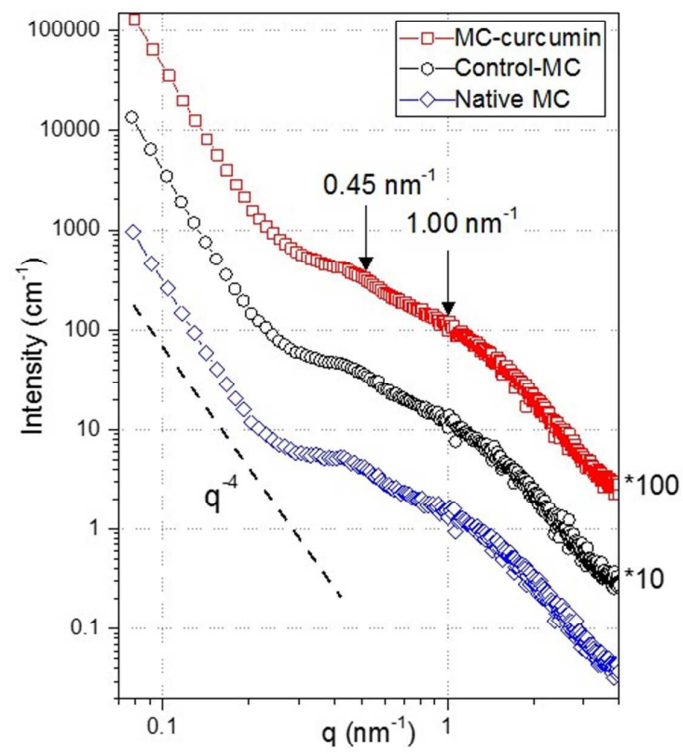


Figure 3

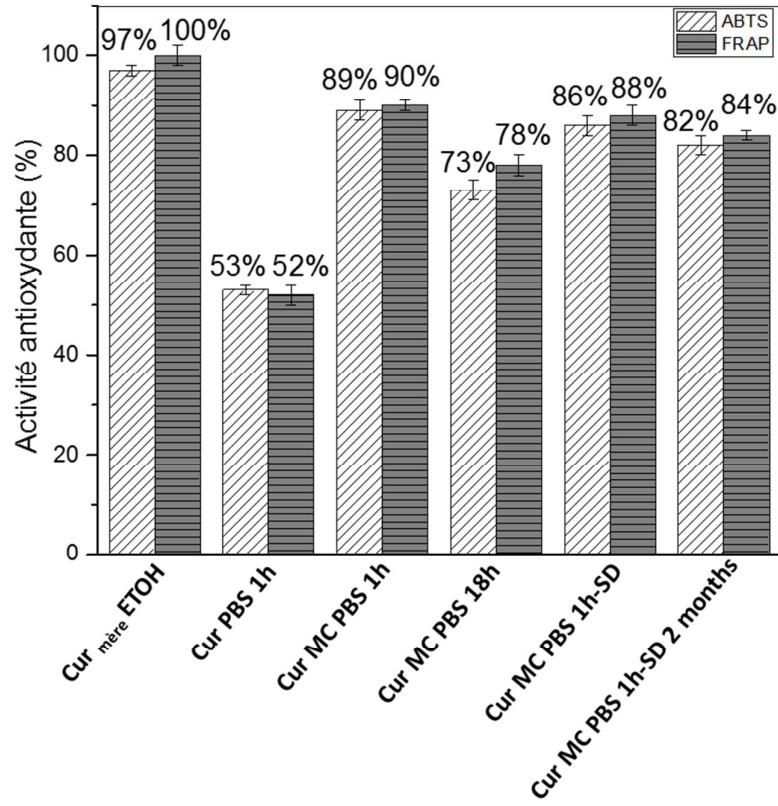


Figure 4

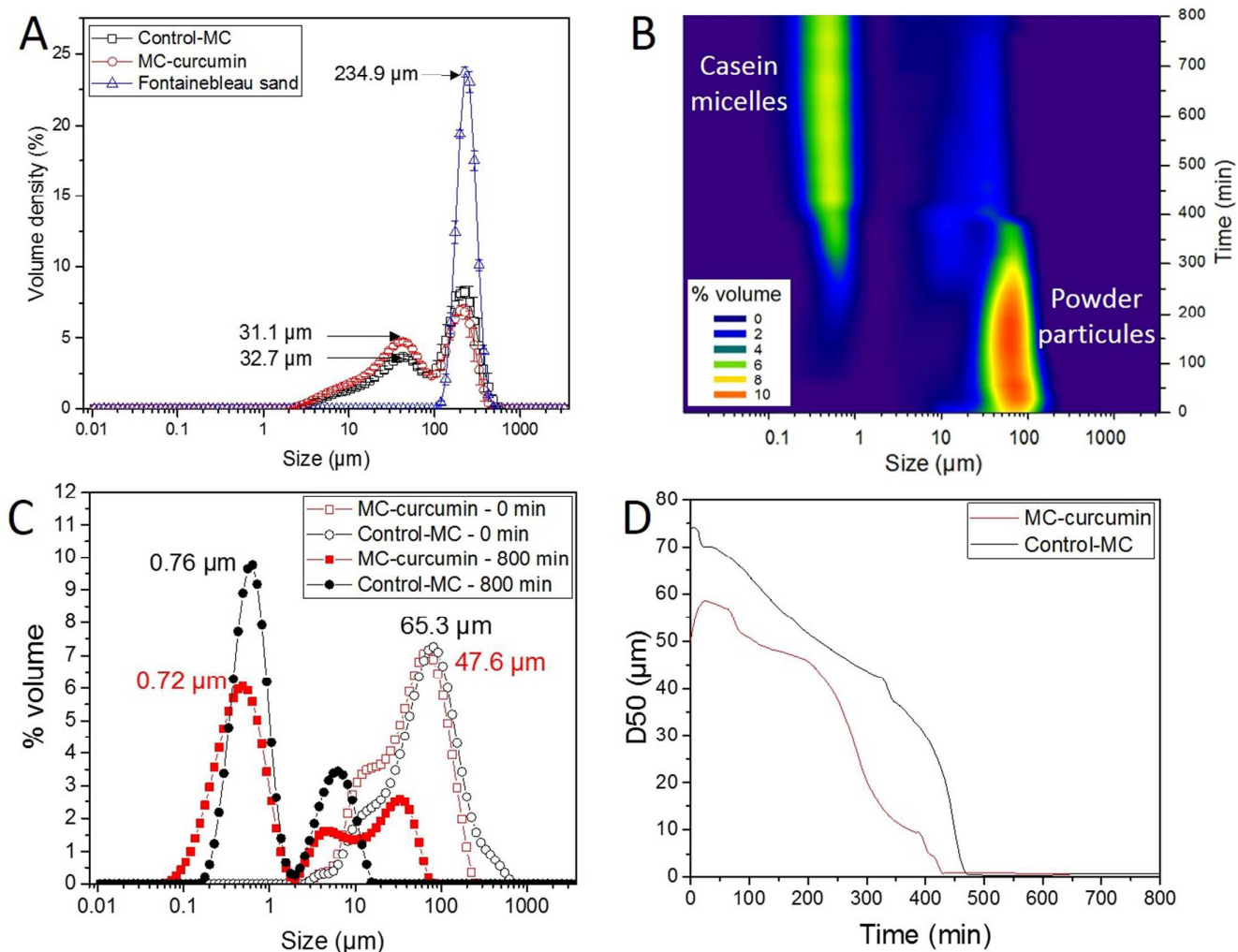


Figure 5

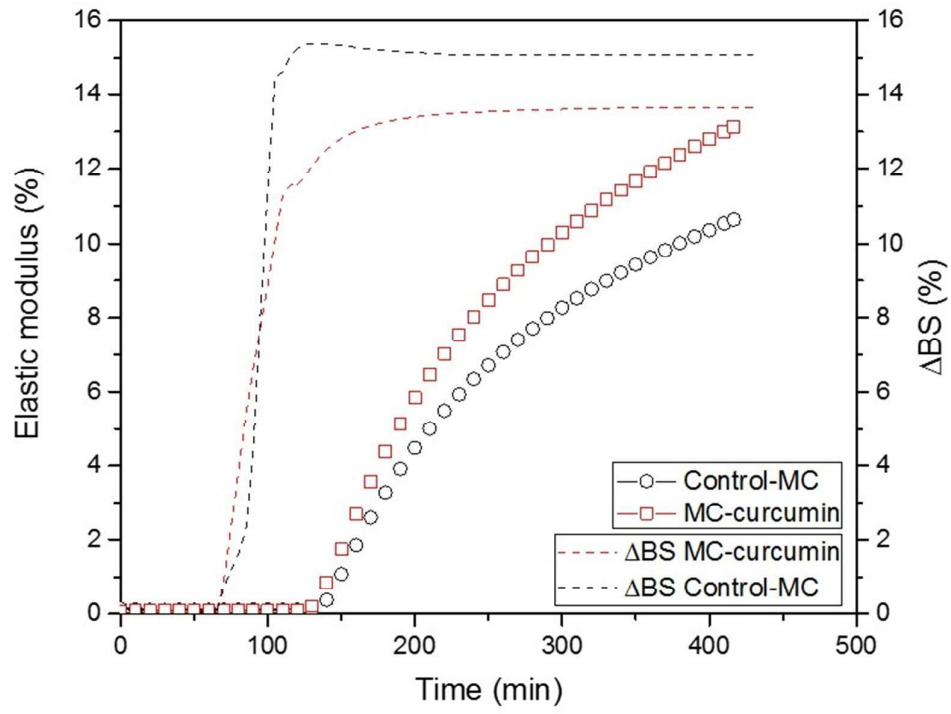




Figure 6

Table 1. Color parameters (L^* , a^* , b^* , C^* , H^* , ΔE (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 ± 0.80	-0.64 ± 0.01	5.75 ± 0.20	5.79 ± 0.12	96.42 ± 0.19	
MC-Curcumin	95.16 ± 1.00	-4.65 ± 0.07	62.64 ± 0.90	22.62 ± 0.90	96.36 ± 0.16	
ΔE (Color difference) = 57.04						