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## Encapsulation of curcumin in milk powders by spray-drying: Physicochemistry, rehydration properties, and stability during storage

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38 **ABSTRACT**

39 Curcumin is a natural polyphenol that has strong antioxidant, anti-inflammatory and  
40 anti-carcinogenic properties, however, it is sensitive to light, oxygen and heat, and also  
41 presents very low solubility in water. Several studies have focused on curcumin  
42 preservation and bioavailability due to its health importance, especially by  
43 encapsulation. Milk may constitute a good matrix for the encapsulation of curcumin,  
44 because it contains a significant proportion of casein micelles which are amphiphilic  
45 and porous, making them suitable for carrying hydrophobic substances such as  
46 curcumin. This work aims at evaluating the efficiency of milk for encapsulating  
47 curcumin, releasing it in an aqueous medium during powder reconstitution, and  
48 improving its preservation during storage. Curcumin was encapsulated by spray-drying  
49 at pilot scale, extracted by different solvents, and quantified by UV/Vis.  
50 spectrophotometry. Physicochemical characteristics (particle size distribution, water  
51 content, water activity, and color) and rehydration properties (wettability, dispersibility,  
52 and solubility indices) of curcumin-loaded milk powder were characterized. A powder  
53 storage study was also conducted to evaluate the stability of curcumin over time at  
54 different temperatures (10, 25 and 50 °C). Drying conditions were set to obtain a  
55 product of water activity close to 0.2. A method for curcumin extraction and dosage was  
56 also validated using diethyl ether as extraction solvent. Milk powder loaded with  
57 curcumin showed no significant differences in most characteristics compared to raw  
58 milk powder, except that its wettability was decreased by the presence of curcumin.  
59 Moreover, the good encapsulation yields and the stability of curcumin antioxidant  
60 activity during storage showed the suitability of skim milk for curcumin encapsulation.

61 **Keywords:** curcumin; antioxidant activity; encapsulation; skim milk; storage stability;  
62 color.

63

## 64 **1. Introduction**

65 Curcumin is a bioactive polyphenol extracted from turmeric rhizome (*Curcuma*  
66 *longa*) [1] that has anti-inflammatory, antimicrobial, anti-amyloid, and antitumor  
67 properties. Polyphenols present antioxidant activity, hence they are prone to induce  
68 beneficial effects on human health [2]. Curcumin is known to present a very low  
69 solubility in water ( $2.99 \times 10^{-8}$  M), a characteristic that limits its use in food products,  
70 especially in aqueous solutions [1, 3]. Besides, its sensitivity to environmental  
71 conditions, including elevated temperatures, light, extreme pH, moisture, and oxygen,  
72 makes curcumin susceptible to degradation during processing and storage [2]. Thus,  
73 several studies have been carried out aiming at developing techniques for its protection  
74 in food products [2, 4, 5, 6, 7, 8, 9]. Microencapsulation is a commonly used technique  
75 to stabilize and protect bioactive compounds in microscopic particles. With this  
76 technique, a micro/nano environment is created, incorporating unstable ingredients  
77 within a carrier matrix and developing a protection to the external environment [10].  
78 Encapsulation provides protection against degradation reactions and prevents the loss of  
79 flavor, nutrients, and/or bioactive compounds. In addition, it can be used to limit the  
80 release of components during food processing and storage, while controlling the release  
81 rate in physiological (gastric, intestinal) conditions [7, 11, 12, 13, 14, 15, 16]. Numerous  
82 methods of microencapsulation exist: spray-drying, spray chilling/cooling,  
83 coacervation, extrusion, fluidized bed coating, liposome entrapment, and molecular  
84 inclusion [2]. Spray-drying surely represents the most commonly used encapsulation  
85 technique, owing to its many advantages:

- 86 - it is economical: around ten times cheaper than the lyophilization process;
- 87 - versatile: various configurations of air and spray flows exist (co-current,  
88 counter-current, or combined flows), and the spray can either be produced by a  
89 centrifugal atomizer or an atomizing nozzle;

90 - and it is suitable for meeting the quality and efficiency constraints of various  
91 industrial fields such as pharmaceuticals, cosmetics, and food.

92 Generally, spray-dried powders have good techno functional properties like  
93 good rehydration ability, high flowability and long shelf-life [17, 18]. Spray-drying is  
94 especially useful for encapsulating various heat-sensitive food ingredients such as  
95 polyphenols, flavonoids, anthocyanins, prebiotics, phytosterols and polysaccharides,  
96 because the temperature increase in the matrix remains limited throughout the drying  
97 process [2, 10, 19]. Indeed, during most of the drying time, the surface of the particle  
98 remains wet and the local temperature of the surrounding air does not exceed its wet  
99 temperature that is around 50 °C in classical spray-drying conditions [18].  
100 Consequently, the alteration of heat-sensitive compounds remains limited [20].

101 Milk is an interesting matrix candidate for encapsulation by spray-drying [7],  
102 owing to its structural and physicochemical properties: binding of ions and small  
103 molecules, surface-active and self-assembly properties, possibility of gelation, pH-  
104 dependent gel-swelling behavior, possibility to control matrix rehydration kinetics and  
105 thus bioactive release, interactions with macromolecules to form complexes and  
106 conjugates with synergistic combinations of properties, various shielding capabilities  
107 that are essential for protecting sensitive encapsulated compounds, biocompatibility and  
108 biodegradability, possibility to control the bioaccessibility of the bioactive and promote  
109 its bioavailability [7, 8].

110 The protection efficiency of milk powders towards encapsulated bioactive  
111 compounds is mastered by their main physicochemical properties, such as chemical  
112 composition, particle size distribution, and particle shape factors. In particular,  
113 granulometric characteristics (that can be deduced from particle size distribution) of a

114 powder is an important parameter because it influences many functional properties such  
115 as flowability and rehydration capacity [17, 18, 21, 22, 23].

116 The ability to reconstitute milk from powder is an essential property for  
117 industrials and consumers using powdered dairy ingredients or products. The  
118 International Dairy Federation has defined three reconstitution indices, namely  
119 solubility, dispersibility, and wettability indices [24, 25], that describe different steps of  
120 powder rehydration and depend on powder composition and affinity between water and  
121 its components, but also on particle size, shape, and structure (porosity) [18, 26, 27].

122 During the storage of dairy powders, several changes can be observed mainly  
123 depending on storage temperature and powder water content. Dairy powders are  
124 susceptible to undergo agglomeration and caking, which are known to alter various  
125 powder characteristics like particle size distribution, rehydration ability, and color [26,  
126 27, 28]. When employed as an encapsulation matrix, physicochemical and techno  
127 functional properties of powdered milk must be kept as much as possible in order to  
128 ensure and maintain maximum protection of the active material [2, 28, 19].

129 The objective of the current work was to produce a milk powder encapsulating  
130 curcumin and study the evolution of degradation of curcumin powder during storage at  
131 different temperatures. Then, spray-drying was performed to produce milk powders in  
132 which curcumin is encapsulated. Physicochemical characteristics (particle size  
133 distribution, moisture content, colorimetric properties) and rehydration behavior  
134 (accessed by the wettability, dispersibility, and solubility indices) were evaluated for the  
135 fresh powder. Encapsulation efficiency was also determined just after spray-drying.  
136 Last, curcumin-loaded milk powder was stored at different temperatures (10, 25, and 50  
137 °C) to determine the stability of curcumin upon aging.

138

## 139 **2. Material and methods**

### 140 2.1. Material

141 Skim milk powder “Lait spray 0 %”, used to prepare concentrates to be spray-  
142 dried, was purchased from Ingredia (Arras, France); its moisture content was 3.5 %  
143 (w/w). Curcumin powder ( $\geq 94$  % purity) was purchased from Sigma-Aldrich (Saint-  
144 Quentin Fallavier, France). All other chemicals, of analytical grade, were provided by  
145 Carlo Erba (Milan, Italy).

146

### 147 2.2. Production of milk powders by spray-drying

148 The production of milk with and without curcumin was conducted by spray-  
149 drying 810 g skim milk concentrates at 30 % dry extract. Curcumin was solubilized in  
150 ethanol at maximum concentration ( $1 \text{ mg}\cdot\text{mL}^{-1}$ ). The curcumin concentration in skim  
151 milk concentrates was set at 0.083 mg/g milk powder (dry basis, d.b.) as the  
152 concentration of ethanol in spray-dried concentrates should not exceed 2 % (w/w)  
153 according to the spray-drying pilot specifications. The employed spray-dryer was a  
154 Micra Spray MS150 (Anhydro, Soeborg, Danemark) with a co-current flow single  
155 tower equipped with a bi-fluid nozzle (60 100 + Buffer Air Fluid 120, Spraying  
156 System). The operating parameters were defined according to Perrone et al. [29] that  
157 have studied the influence of drying parameters on water activity. The objective is to  
158 obtain powders with a water activity lower than 0.2, which makes them less sensitive to  
159 biochemical alterations during storage [29]. In this work, spray-dried skim milk  
160 powders with curcumin and without curcumin will be noted LC and LS respectively.  
161 Immediately after spray-drying, powders were stored at 10 °C in sealed polypropylene  
162 bags. The spray-drying yield (%) was calculated by dividing the dry mass of produced  
163 powder by the dry mass of spray-dried milk (810 g).

164 In order to regulate the outlet air temperature at 90 °C, the inlet air temperature  
165 and the product flow rate needed to be adjusted, depending on weather conditions, as  
166 inlet air humidity was not regulated (Table 1). A fan power of 70 % and a spray  
167 pressure of 1 bar were maintained for all drying experiments.

168 According to Table 1, even if spray-drying conditions were close, significant  
169 differences were denoted between the two spray-drying assays. Globally, the inlet air  
170 temperature and the concentrate flow-rate of the second assay (LS2 and LC2) were a  
171 little higher. In general, when the inlet flow is higher, the outlet temperature tends to be  
172 lower, which could be seen in the second assay.

173

### 174 2.3. Curcumin extraction and dosage

175 First, it was found in preliminary studies (data not shown) that ethanol is the best  
176 solvent for curcumin extraction; moreover, it is readily available and less dangerous  
177 than other organic solvents. A wavelength of 425 nm was chosen for performing  
178 curcumin quantification by UV-visible spectrophotometry.

179 Curcumin extraction was achieved according to the following procedure: 0.1 g  
180 curcumin-loaded skim milk powder was solubilized in 5 mL distilled water using a  
181 Stuart SB3 rotator for 50 min at 40 rpm at  $25 \pm 1$  °C. Then, 10 mL diethyl ether was  
182 added, and the mixture was allowed to stir in the same conditions. The mixture was then  
183 centrifuged at 5 088 x g for 5 min with a Hereaus Megafuge 8R centrifuge (Thermo  
184 Fisher Scientific) and the supernatant was recovered and placed under a dinitrogen  
185 atmosphere until complete evaporation of diethyl ether. Finally, 1 mL ethanol was  
186 added. The curcumin solution was diluted hundredfold before analysis by UV-Visible  
187 spectrophotometry at 425 nm. Curcumin ethanolic solutions up to 0.1 mg/mL were  
188 analyzed in the same conditions for calibration purposes.



189 The results are given in milligrams of curcumin per gram of skim milk powder  
190 (d.b.) (mg/g), according to the following equation:

$$191 \quad \frac{m_c}{m_p} = [\text{Curc}]_{\text{powder}} \quad (\text{Equation 1})$$

192 And the proportion of spray-dried curcumin found in analyzed powder was  
193 calculated according to:

$$194 \quad \%curcumin = \frac{[\text{Curc}]_{\text{powder}}}{[\text{Curc}]_{\text{conc}}} \cdot 100 \quad (\text{Equation 2})$$

195 Where  $m_p$  designates the mass of skim milk powder (d.b.) (g),  $m_c$  the mass of detected  
196 curcumin (mg),  $[\text{Curc}]_{\text{conc}}$  the curcumin concentration in the feed concentrate (0.083  
197 mg/g dry mass of skim milk concentrate), and  $[\text{Curc}]_{\text{powder}}$  the curcumin concentration  
198 found in powders (in mg/g dry mass of skim milk powder).  
199

## 200 2.4. Physicochemical properties of powders

### 201 2.4.1. Particle size distribution

202 The particle size distribution was measured using a Mastersizer 3000 laser  
203 diffraction granulometer (Malvern Instruments), supplied with the Aero S dry  
204 dispersion unit. To obtain a correct obscuration (1 %), samples were dispersed at 1.8 bar  
205 air pressure, 100 % air pressure, 40 % feed rate, and 2.7 mm hopper length. The particle  
206 size estimator was chosen as the volume mean diameter and the mean particle size d50  
207 was defined as the diameter for which 50 % of analyzed particles have lower size [30].  
208

### 209 2.4.2. Moisture content and water activity

210 The moisture content was determined by the AOAC method 925.10 [31]. The  
211 water activity was determined with the water activity analyzer HygroPalm apparatus  
212 (HP23-AW-A, Rotronic, Croissy-Beaubourg, France).  
213

### 214 2.4.3. Colorimetry

215 Powder color was characterized with a Microflash 200D apparatus (Data color  
216 International, Lawrenceville, USA). For each sample, 10 g powder was poured in a Petri  
217 dish (leading to a powder layer of approximately 1 cm high) and color measurement  
218 was performed according to the three colors coordinates L, a\*, b\* of CIEL\*a\*b\*  
219 system. The L\* value represents sample luminosity, varying from black (0) to white  
220 (100), the a\* value represents the color varying from red (+) to green (-) and the b\*  
221 value represents the color varying from yellow (+) to blue (-) [28]. The chroma, that  
222 defines the degree of color saturation, was calculated with the following formula:  $C^* =$   
223  $\sqrt{(a^{*2}+b^{*2})}$ . Also, the hue angle (H\*), derived from the two coordinates a\* and b\*, was  
224 determined as  $\arctan(b^*/a^*)$ . All measurements were taken as the average of at least  
225 three points on the center and the periphery of the Petri dish filled with powder samples.

226

### 227 2.5. Powder functionalities

228 The rehydration properties of milk powders are essential techno-functional  
229 properties for application purposes. The rehydration behavior and kinetics of spray-  
230 dried skim milk powders were characterized.

231

#### 232 2.5.1. Rehydration behavior

233 The water solubility index was determined by the methodology described in [25,  
234 18]. The wettability and dispersibility indices were measured according to [18]. The  
235 solubility was calculated from the volume of sediment (mL) remaining after two  
236 centrifugations. The procedure consists in adding 10 g powder in 100 mL distilled  
237 water, stirring the mixture during 90 s at 3 800 rpm, letting it at rest for 15 min, then  
238 stirring it briefly with a spatula and pouring it into the centrifuge for 5 min at 160 x g.

239 Fill the tube with water up to the 50 ml mark. Disperse the sediment in the water and  
240 centrifuge again at 160g for 5 min. Note the quantity of sediment (ml). The insolubility  
241 index (II) is equal to the volume of sediment (ml) remaining after the second  
242 centrifugation from 50 ml of  
243 reconstituted liquid. The solubility index (SI), expressed as a %, is equal to:

$$244 \quad SI = 100 - [2 \cdot II] \quad (\text{Equation 3})$$

245 Wettability analysis consists in measuring the time required for a powder sample  
246 to cross water surface in given conditions: 100 mL distilled water was added into a 400-  
247 mL beaker and then 10 g skim milk powder was gently poured at the water surface.

248 The dispersibility index is the percentage of powder mass which can pass  
249 through 200- $\mu$ m mesh size sieve. 10 g skim milk powder was added to 100 g distilled  
250 water, then the mixture was vigorously stirred with a spatula for 15 s. After that, the  
251 mixture was sieved, and the dry extract of the filtrate was determined after drying at 105  
252 °C for 7 h in an oven. The dispersibility index (DI), expressed as a %, is equal to:

$$253 \quad DI = \frac{(100+w) \cdot X_{DM}}{w \cdot \frac{100-X_{RM}}{100}} \quad (\text{Equation 4})$$

254 Where w is the weight of the powder used,  $X_{RW}$  is the residual free water content of the  
255 powder (% w/w),  $X_{DM}$  is the dry matter of the filtrate after sieving (% w/w). Express the  
256 result to one decimal place.  
257

## 258 2.6. Powder aging during storage

259 The stability of encapsulated curcumin during storage was also evaluated.  
260 Powder samples were stored at 10, 25, and 50 °C for 2 months in hermetically-sealed  
261 plastic pots at 0.43 water activity by using a saturated solution of potassium carbonate  
262 [32]. Qualitative analysis of curcumin by UV/visible spectrophotometry at 425 nm was  
263 performed on aged powders after the extraction step.

264

## 265 2.7. Statistical analysis

266 Spray-dried powders were produced in duplicate and all the analyses were  
267 triplicated. Statistical analysis (ANOVA to check the occurrence of significant  
268 differences among samples and Tukey's HSD test to identify what samples were  
269 significantly different) was carried out with STATISTICA 7.0 software (StatSoft) at  $p <$   
270 0.05.

271

## 272 **3. Results and discussion**

### 273 3.1 Spray-drying

274 Spray-dried powder amounts and yields obtained in the first and second spray-  
275 drying assays were represented in table 2.

276 Powder yields were higher in the first spray-drying assay. In the second spray-  
277 drying, environmental conditions led to a little reduction of the outlet air temperature,  
278 which favored powder adhesion to spray-dryer walls [20, 27, 33]. Most losses during  
279 spray-drying were due to particles adhering to the spray-dryer walls, but there was also  
280 a proportion of very small particles that were not retained by the cyclone. In fact, there  
281 is a relationship between the size of the particles and losses in the exhaust air: the  
282 smaller the particles, the harder they can be recovered with a cyclone [33, 34].

283

### 284 3.2 Physicochemical properties

285 Physicochemical characteristics mean particle size, moisture content, water  
286 activity, and colorimetric parameters (lightness  $L^*$ , chroma  $C^*$  and hue angle  $h^*$ ) of  
287 skim milk powders with and without curcumin were determined (Table 3).

288 Mean particle diameters of spray-dried powders were smaller than those found  
289 in the literature for milk powders, even on the same pilot-scale plant [18, 21, 25, 30].

290 This could result from the presence of ethanol in skim milk concentrates, which was  
291 used to solubilize curcumin. Indeed, viscosity of concentrates containing a small  
292 proportion of ethanol is expected to be lowered, which is known to enhance the liquid  
293 jet breakup, leading to smaller droplets and thus smaller particles [30, 32]. All spray-  
294 dried powders presented very close mean diameters, showing that the presence of  
295 curcumin did not impact the size of spray-dried particles.

296 All produced powders presented water contents close to 5 % (w/w), which is a  
297 sign of a good storage ability of powders [18]. No significant difference was denoted  
298 among powders with or without curcumin, whatever the spray-drying assay. Fang [2]  
299 found that spray-dried powders present lower moisture content at higher temperatures,  
300 which was also observed in the present study. The atmospheric conditions during the  
301 second atomization were warmer and wetter, which also contributed to produce a wet  
302 and adherent powder.

303 The spray-drying conditions significantly influenced the water activity of the  
304 samples, as the powders produced during the first spray-drying assay had a slightly  
305 lower water activity (0.12) than those of the second spray-drying assay (0.14). This  
306 difference can be attributed to the higher feeding flow-rate of concentrate of the second  
307 spray-drying assay, which led to lower temperature and probably a higher relative  
308 humidity of outlet drying air [33, 34].

309 The introduction of curcumin in food powders is expected to change their color  
310 by increasing their yellowness [35]. This was checked by colorimetric analysis (Table  
311 3). Powder luminosity was significantly different for skim milk with and without  
312 curcumin and for the two spray-drying assays. The presence of curcumin increased  
313 powder lightness but also its chroma (representing the degree of color saturation),  
314 showing that powders with curcumin were brighter and more colored. The main color of

315 all powders was greenish-yellow, even in the absence of curcumin. For skim milk  
316 powders free from curcumin, this can be explained by the presence of riboflavin, a  
317 yellowish-green colored milk compound.

318

### 319 3.3. Powder functionalities

320 Most food additives and ingredients are presented in powder form and must be  
321 rehydrated before use; hence, water-powder interactions and more generally the ability  
322 to reconstitute a powder in water are essential properties for the formulation of many  
323 food products [18, 33]. Rehydration indices of produced skim milk with and without  
324 curcumin are displayed in Table 4.

325 Skim milk powder in the absence of curcumin was poorly wettable (wettability  
326 times ranging between 30 and 35 min), but upon curcumin addition, powder became not  
327 wettable (wettability times superior to 45 min). Wettability was the only factor that  
328 showed significant difference between skim milk powders with and without curcumin  
329 whatever the spray-drying assay. Dispersibility and solubility of LS and LC differed in  
330 the first spray-drying assay but were similar in the second one. This shows that a small  
331 change in spray-drying conditions may have a tremendous impact on powder  
332 functionalities. Indeed, it can be guessed that modifying the evaporation rate (mainly by  
333 changing concentrate feed rate, drying air temperature or flow-rate) may have an impact  
334 on the location of curcumin within skim milk particles, as more curcumin at the surface  
335 of particles may affect their rehydration capacity, owing to the hydrophobicity of this  
336 bioactive compound. This explanation is in accordance with the results of the first  
337 spray-drying assay, where skim milk powder without curcumin exhibited lower  
338 wettability and solubility but a better dispersibility. Moreover, it can be guessed that  
339 curcumin addition modified powder surface structure. According to Murrieta-Pazos et

340 al. [27], the surface of skim milk powders is mainly covered by proteins. Due to the  
341 interaction of curcumin with casein, modification of casein micelle conformation  
342 probably occurred [34, 35, 36], which induced a change of the interaction sites of  
343 caseins at the surface of particles and affected interactions of powder with water.

344 It is considered that a powder is dispersible when dispersibility index exceeds  
345 85 % [18]. Therefore, spray-dried powders of the current study had low dispersibility.  
346 Generally, a soluble powder is not very dispersible because it dissolves easily when  
347 being in contact with water, so it does not separate into small particles but solubilizes  
348 quickly in water [18, 33]. The high solubility of spray-dried powders is thus consistent  
349 with their poor dispersibility. Also, producing powders at pilot scale results in low mean  
350 particles size (see the d50 values around 15  $\mu\text{m}$ , which is relatively small for food  
351 powders) [37], and powders constituted by small particles have a tendency to be  
352 cohesive [38] and thus hard to disperse.

353

#### 354 3.4 Storage study

355 Sample color was followed during storage (Table 5 and Figure 1) with a view to  
356 correlate colorimetric changes to the evolution of curcumin content in powders (Figure  
357 2). Indeed, curcumin is yellow, and its alteration leads to color loss [39] or the  
358 formation of degradation products having a more brownish/reddish color [40, 41].

359 As previously discussed from table 1 results, for all spray-dried powders, the  
360 lightness at time zero was high. Furthermore, color saturation was higher in skim milk  
361 powders containing curcumin, due the yellow color by curcumin. Also, the main color  
362 of all powders was similar: greenish-yellow.

363 During storage, a decrease in powder lightness was observed for all samples and  
364 this darkening phenomenon was more intense at 50 °C. At 10 and 25°C, the color

365 saturation remained unaffected in the course of storage, but at 50 °C color saturation  
366 increased for powders without curcumin, while it slightly decreased for powders with  
367 curcumin. Moreover, the hue angle showed a decrease at 50 °C, indicating a shift of  
368 powders without curcumin to orange-yellow, while it remained similar all along the  
369 storage for powders with curcumin. It can be guessed that at 50 °C, the decrease in  
370 luminosity, increase in saturation and increase in angle hue corresponds to the formation  
371 of brown pigments, due to the occurrence of the Maillard reaction. In fact, Maillard  
372 reaction corresponds to a group of chemical reactions initiated by the condensation of  
373 an amino group with a reducing sugar and resulting in the formation of brown  
374 compounds [33].

375         The formation of brown pigments is evidenced by photographs displayed in  
376 Figure 1: after 60 days storage at 50 °C, the color change was more pronounced for  
377 skim milk powder without curcumin, hence the limitation of color change for LC  
378 powders may be attributable to the presence of curcumin. This observation should be  
379 put in relation with the antioxidant power of curcumin that improved powder  
380 preservation during high-temperature storage, presumably by decreasing the kinetics of  
381 the Maillard reaction.

382         Skim milk powders with curcumin tended to remain a little more yellow than  
383 powders without curcumin [42], due that a significant proportion of curcumin was still  
384 present after 60 days storage at 50 °C, as evidenced by curcumin quantification (Figure  
385 2).

386         During storage, curcumin was more altered when stored at high temperature, as  
387 expected for this thermolabile compound: about 83 %, 72 %, and 58 % residual  
388 curcumin were found after 60 days storage at 10, 25, and 50 °C, respectively (Figure 2).  
389 This demonstrates the importance of adequate conditioning and storage conditions to



390 avoid changes in powder characteristics and the amount of residual bioactive material  
391 [26, 27, 28, 38, 39]. Encapsulation in dairy matrices may be a way to limit its  
392 alteration, owing to the interaction with casein micelles [36] that can improve curcumin  
393 stability [43].

394         The decrease in curcumin content was correlated to the decrease in yellow color:  
395 indeed, the Pearson correlation coefficients between residual curcumin content and  
396 color saturation were 0.64, 0.79, 0.82 for powders stored at 10, 25, and 50 °C,  
397 respectively, showing a marked positive correlation whatever the storage temperature.  
398 Thus, the variation in concentration of curcumin explains a large part of the evolution of  
399 powder color during storage. The stronger correlation for powders stored at 50 °C may  
400 be derived from the increase in curcumin degradation rate at this higher temperature  
401 [44].

402

403         Figure 3 illustrates the link between chroma ( $C^*$ ) and curcumin content. It can  
404 be noted that, whatever the storage temperature, both chroma and curcumin  
405 concentration tended to decrease with storage time. Besides, storage temperature had a  
406 negative effect on curcumin-loaded skim milk powder chroma and this can be put in  
407 relation with the fact that low temperatures help maintain curcumin color [43, 44]. For  
408 all temperatures, from 14 storage days, the decrease in curcumin content was  
409 accompanied by a decrease in powder chroma and this phenomenon was more evident  
410 at 10 °C. For all temperatures, between 28 and 42 days, the decrease in powder chroma  
411 was more significant compared to the other storage times. It may be guessed that  
412 curcumin alteration during storage begins with curcumin located at the powder surface,  
413 which strongly affects powder color. Then, at longer storage times, curcumin hidden in  
414 the powder bulk starts to be degraded, but without much affecting powder surface

415 appearance. Hence, the link between powder surface color saturation and curcumin  
416 content may be more relevant at relatively short storage times. At 25 °C and especially  
417 at 50 °C, for the higher storage times (approximately from 42 days), the positive  
418 correlation between chroma and curcumin content became less evident, as at these  
419 temperatures the powders were expected to undergo Maillard reaction, which results to  
420 the formation of brown pigments, thus affecting powder color independently from the  
421 degree of curcumin alteration. It can then be concluded that the relation between chroma  
422 and curcumin content was not linear due the existence of other factors affecting powder  
423 color.

424         This correlation between powder color and curcumin content is very interesting  
425 for industrials, as they can use powder color as an indicator to easily and quickly  
426 evaluate the degree of curcumin alteration upon storage.

427         This study permitted to show that the presence of curcumin in spray-dried skim  
428 milk powders did not significantly affect their main physicochemical characteristics,  
429 except color, which was obviously more yellow in curcumin-loaded powders, and color  
430 stability, which was improved by curcumin. Differences found among investigated  
431 powders were more related to small changes in spray-drying conditions. It was also  
432 suggested that Maillard reaction could be limited during storage (even at temperature as  
433 high as 50 °C) by addition of a small curcumin proportion in dairy powder formulation.  
434 Also, it was noted that curcumin alteration upon storage at high temperature remained  
435 limited and this can be explained by the protective role of the dairy matrix against  
436 curcumin oxidation. Previous studies with milk proteins have shown that milk proteins  
437 (especially casein micelles) can substantially contribute to the protection of bioactive  
438 compounds like curcumin by forming complexes with these molecules through  
439 hydrophilic and/or hydrophobic interactions [7, 13, 40, 43].

440

#### 441 **4. Conclusion**

442 This study dealing with encapsulation of curcumin in skim milk powder by  
443 spray-drying permitted to evidence the impact of small changes in spray-drying  
444 conditions, as well as the presence of curcumin on physicochemical and functional  
445 properties of resulting powders. Spray-drying yield was slightly affected by minor  
446 variations of spray-drying conditions. In the second spray-drying assay, the air outlet  
447 temperature was a little lower, which increased particle adhesion to the spray-dryer  
448 walls and slightly decreased process yield. This also impacted powder water activity:  
449 powders of the second assay had a slightly higher water activity, consistently with the  
450 lower air outlet temperature. Variations in spray-drying conditions between the two  
451 assays were so close that other physicochemical (mean particle size, span, moisture  
452 content) and functional (wettability, dispersibility, solubility) properties were similar.

453 The presence of curcumin in milk did not affect the spray-drying yield.  
454 Physicochemical characteristics (mean particle size, span, moisture content, and water  
455 activity) were also unchanged due to the presence of curcumin with the notable  
456 exception of powder color. Indeed, curcumin-loaded powders were more bright (higher  
457 luminance  $L^*$ ) and had a higher saturation of yellow color (higher chroma  $C^*$  for a hue  
458 angle  $h^*$  corresponding to yellow). As regards powder functional properties related to  
459 reconstitution properties, solubility and dispersibility were unaltered by the presence of  
460 curcumin, contrarily to wettability which was markedly lowered in the presence of  
461 curcumin. Indeed, this hydrophobic compound may be found at powder surface,  
462 decreasing interactions between powder surface and water, thus making it less wettable.

463 During storage, changes in color were observed: color saturation decreased,  
464 indicating a decrease in powder yellowness. The color loss was enhanced when storage

465 temperature was increased. Color loss was correlated with the decrease in curcumin  
466 concentration, hence powder color may constitute an indicator for following the degree  
467 of alteration of curcumin encapsulated in dairy powders. For all studied temperatures,  
468 the decrease in curcumin content during storage was low (28 % loss after 6 months  
469 storage at 25 °C), showing the suitability of skim milk as an encapsulation matrix for  
470 protecting curcumin.

471 This study allowed verifying the possibility of relating, during storage, the  
472 evolutions of curcumin content and powder color saturation (chroma). It permitted to  
473 highlight the negative effect of storage temperature on curcumin content. As a  
474 perspective of this study, it would be interesting to evaluate the evolution of antioxidant  
475 activity of curcumin-loaded milk powders upon storage in order to investigate if  
476 curcumin keeps its antioxidant power when encapsulated in milk powders and stored.

477

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482

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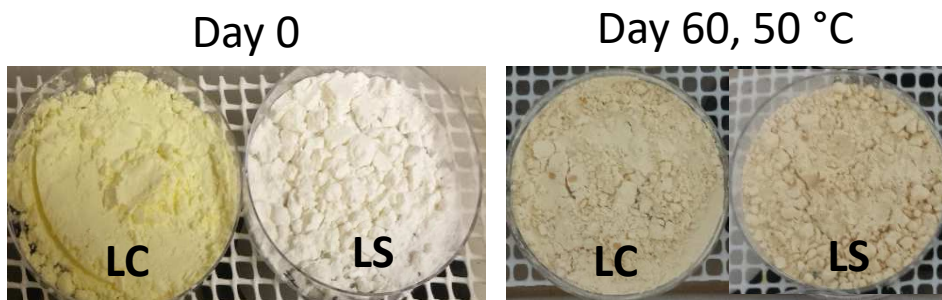


Figure 1. Skim milk powders with (LC) and without curcumin (LS), at fresh state and after 60 days storage at 50 °C.

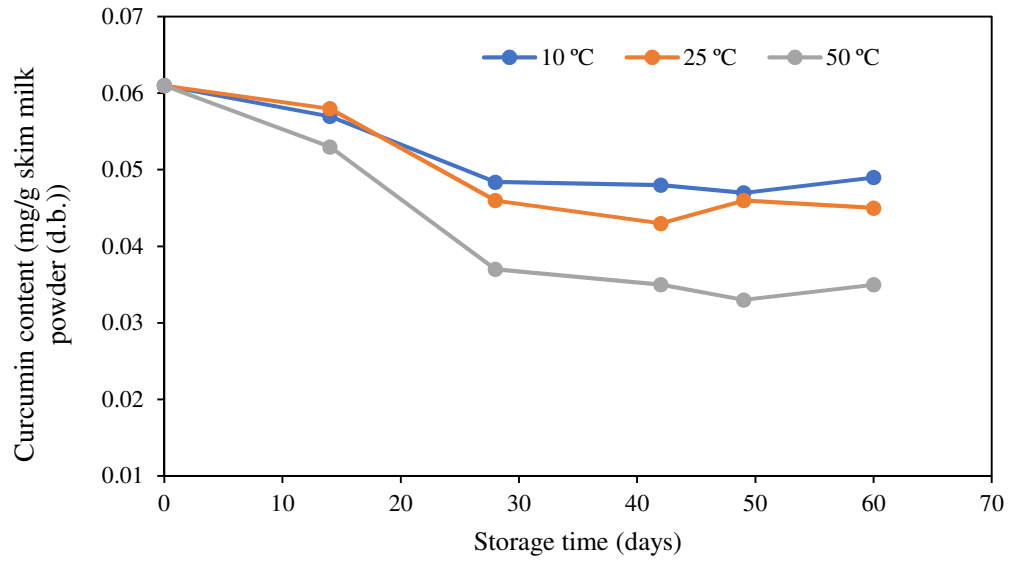


Figure 2. Evolution of curcumin content in spray-dried skim milk powders after 60 days storage at 10, 25, and 50 °C.

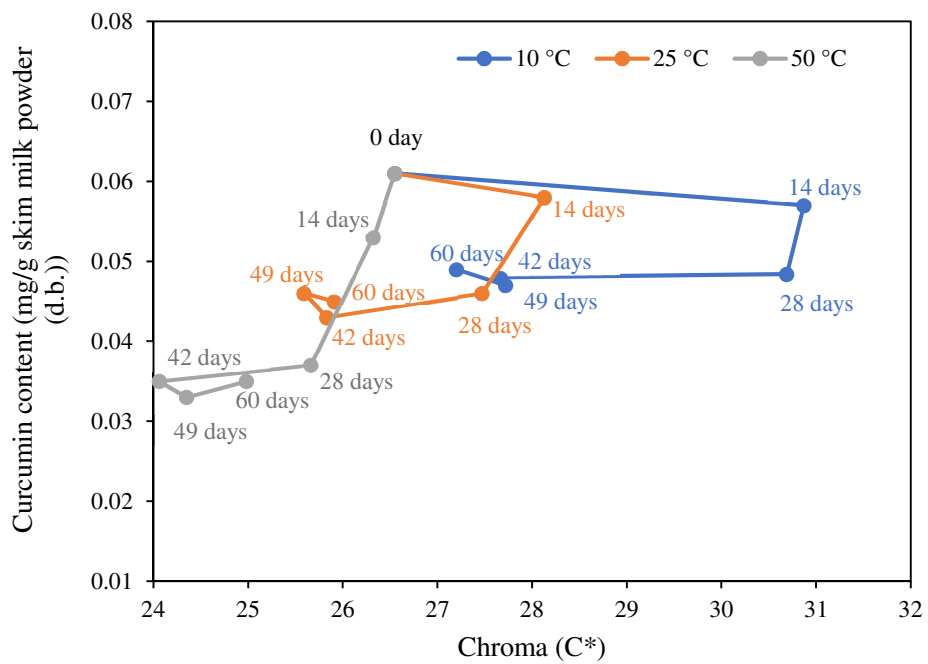


Figure 3. Relation between curcumin content in spray-dried skim milk powders and chroma (C\*) after 60 days storage at 10, 25, and 50 °C.

Table 1. Spray-drying conditions for the production of skim milk powders with (LC1 and LC2) or without curcumin (LS1 and LS2).  $T_e$  = inlet temperature;  $T_s$  = outlet temperature;  $Q_L$  = skim milk concentrate flow rate.

	$T_e$ (°C)	$T_s$ (°C)	$Q_L$ (mL/min)
LS1	161.6 ± 2.1 <sup>a</sup>	91.6 ± 1.2 <sup>b</sup>	35.0 ± 2.4 <sup>a</sup>
LC1	165.3 ± 1.2 <sup>c</sup>	92.2 ± 0.5 <sup>b</sup>	40.0 ± 0.0 <sup>b</sup>
LS2	164.4 ± 1.5 <sup>bc</sup>	88.6 ± 0.8 <sup>a</sup>	50.0 ± 1.4 <sup>c</sup>
LC2	162.8 ± 1.3 <sup>ab</sup>	88.8 ± 0.5 <sup>a</sup>	52.0 ± 0.0 <sup>c</sup>

Values in the same row followed by the same superscripted letters did not significantly differ according to Tukey's HSD test ( $p < 0.05$ ).

Table 2: Mass of spray-dried powders and yields for the two spray-drying assays.

	Powder mass (g)	Yield (%)
LS1	598,25	74
LC1	685,06	85
LS2	548,84	68
LC2	562,27	69

Table 3. Physicochemical characteristics of skim milk powders with and without curcumin obtained by spray-drying.

	d50 ( $\mu\text{m}$ )	Moisture (% (w/w))	Water activity (-)	L* (-)	C* (-)	h* (-)
LS1	14.10 $\pm$ 0.01 <sup>a</sup>	4.80 $\pm$ 0.17 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	94.12 $\pm$ 0.03 <sup>b</sup>	7.23 $\pm$ 0.04 <sup>a</sup>	100.22 $\pm$ 0.24 <sup>a</sup>
LC1	15.20 $\pm$ 0.01 <sup>c</sup>	5.58 $\pm$ 0.17 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	98.06 $\pm$ 0.04 <sup>d</sup>	26.55 $\pm$ 0.10 <sup>b</sup>	100.52 $\pm$ 0.38 <sup>a</sup>
LS2	14.91 $\pm$ 0.07 <sup>b</sup>	5.43 $\pm$ 0.39 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>b</sup>	93.39 $\pm$ 0.08 <sup>a</sup>	7.31 $\pm$ 0.09 <sup>a</sup>	100.30 $\pm$ 0.28 <sup>a</sup>
LC2	15.03 $\pm$ 0.03 <sup>b</sup>	5.23 $\pm$ 0.42 <sup>a</sup>	0.14 $\pm$ 0.00 <sup>b</sup>	97.39 $\pm$ 0.05 <sup>c</sup>	27.61 $\pm$ 0.07 <sup>c</sup>	100.46 $\pm$ 0,05 <sup>a</sup>

Values in the same column followed by the same superscripted letters did not significantly differ according to Tukey's HSD test ( $p < 0.05$ ).

Table 4. Rehydration indices (wettability time, solubility and dispersibility) of skim milk powders with and without curcumin produced in the two spray-drying assays.

	Wettability (min)	Solubility (%)	Dispersibility (%)
LS1	35.75 ± 1.66 <sup>a</sup>	89.61 ± 0.34 <sup>a</sup>	37.52 ± 1.86 <sup>b</sup>
LC1	> 45	95.77 ± 0.57 <sup>b</sup>	27.60 ± 2.43 <sup>a</sup>
LS2	31.55 ± 0.76 <sup>a</sup>	96.17 ± 0.74 <sup>b</sup>	35.52 ± 1.02 <sup>b</sup>
LC2	> 45	95.96 ± 0.76 <sup>b</sup>	33.86 ± 1.39 <sup>b</sup>

Values in the same row followed by the same superscripted letters did not significantly differ according to Tukey's HSD test ( $p < 0.05$ ).

Table 5. Evolution of powder colorimetry over 60 days storage.

		Day 0			Day 14			Day 28		
		L*	C*	h*	L*	C*	h*	L*	C*	h*
10 °C	LS1	94.12 ± 0.03	7.23 ± 0.04	100.22 ± 0.23	94.78 ± 0.14	6.76 ± 0.39	100.99 ± 0.94	94.41 ± 0.22	6.25 ± 0.29	101.51 ± 0.66
	LC1	98.06 ± 0.04	26.55 ± 0.10	100.52 ± 0.38	95.6 ± 0.34	30.87 ± 0.15	99.13 ± 0.02	95.45 ± 0.09	30.69 ± 0.11	99.00 ± 0.18
	LS2	93.39 ± 0.08	7.31 ± 0.09	100.30 ± 0.28	94.81 ± 0.07	6.17 ± 0.06	102.21 ± 0.08	94.32 ± 0.49	6.99 ± 0.02	100.61 ± 1.00
	LC2	97.39 ± 0.05	27.61 ± 0.07	100.46 ± 0.05	98.38 ± 0.04	27.86 ± 0.22	100.35 ± 0.03	96.64 ± 0.54	28.62 ± 0.21	99.35 ± 0.45
25 °C	LS1	94.12 ± 0.03	7.23 ± 0.04	100.22 ± 0.23	94.52 ± 0.07	5.77 ± 0.11	100.82 ± 0.14	94.56 ± 0.21	6.18 ± 0.04	98.36 ± 0.21
	LC1	98.06 ± 0.04	26.55 ± 0.10	100.52 ± 0.38	97.23 ± 0.07	28.13 ± 0.05	100.17 ± 0.01	96.45 ± 0.49	27.47 ± 0.79	99.43 ± 0.50
	LS2	93.39 ± 0.08	7.31 ± 0.09	100.30 ± 0.28	94.46 ± 0.04	6.11 ± 0.03	102.81 ± 0.16	94.85 ± 0.06	7.33 ± 0.12	99.44 ± 0.15
	LC2	97.39 ± 0.05	27.61 ± 0.07	100.46 ± 0.05	98.84 ± 0.05	30.31 ± 0.36	100.03 ± 0.08	97.23 ± 0.29	31.75 ± 0.10	99.13 ± 0.02
50 °C	LS1	94.12 ± 0.03	7.23 ± 0.04	100.22 ± 0.23	91.85 ± 0.10	5.18 ± 0.02	82.76 ± 0.21	88.59 ± 0.40	17.61 ± 0.24	84.22 ± 0.08
	LC1	98.06 ± 0.04	26.55 ± 0.10	100.52 ± 0.38	94.27 ± 0.24	26.32 ± 1.09	93.57 ± 0.10	90.51 ± 0.06	25.66 ± 0.19	89.64 ± 0.14
	LS2	93.39 ± 0.08	7.31 ± 0.09	100.30 ± 0.28	92.77 ± 0.07	5.91 ± 0.11	103.91 ± 0.25	92.65 ± 0.28	13.66 ± 0.18	88.27 ± 0.03
	LC2	97.39 ± 0.05	27.61 ± 0.07	100.46 ± 0.05	97.25 ± 0.10	30.29 ± 0.84	100.09 ± 0.02	96.28 ± 1.01	27.88 ± 0.03	93.82 ± 0.09
		Day 42			Day 49			Day 60		
		L*	C*	h*	L*	C*	h*	L*	C*	h*
10 °C	LS1	91.56 ± 0.09	4.58 ± 0.07	101.75 ± 0.32	92.31 ± 0.25	4.39 ± 0.11	101.65 ± 0.19	92.13 ± 0.15	4.38 ± 0.10	101.06 ± 0.38
	LC1	92.30 ± 0.03	27.67 ± 0.25	98.67 ± 0.12	92.17 ± 0.35	27.72 ± 0.36	98.65 ± 0.086	91.93 ± 0.31	27.20 ± 0.34	99.02 ± 0.35
	LS2	90.59 ± 0.46	4.44 ± 0.06	99.98 ± 1.95	90.30 ± 0.41	4.37 ± 0.30	102.24 ± 0.72	91.37 ± 0.21	4.53 ± 0.15	101.94 ± 0.67
	LC2	93.64 ± 0.50	25.78 ± 0.04	99.41 ± 0.02	94.13 ± 0.06	25.76 ± 0.21	99.12 ± 0.84	95.00 ± 0.36	25.72 ± 0.19	99.47 ± 0.50
25 °C	LS1	94.50 ± 0.11	5.59 ± 0.09	98.19 ± 0.11	91.41 ± 0.04	9.38 ± 0.15	138.87 ± 1.14	91.83 ± 0.16	9.29 ± 0.17	136.74 ± 1.9
	LC1	95.20 ± 0.12	25.83 ± 0.07	99.23 ± 0.02	92.80 ± 0.21	25.59 ± 0.33	98.66 ± 0.21	90.90 ± 0.23	25.91 ± 0.23	98.43 ± 0.30
	LS2	94.79 ± 0.03	6.25 ± 0.03	97.63 ± 1.31	91.50 ± 0.20	5.93 ± 0.26	95.98 ± 0.38	91.03 ± 0.15	6.13 ± 0.10	95.86 ± 0.23
	LC2	95.15 ± 0.09	28.84 ± 0.13	99.09 ± 0.04	92.26 ± 0.15	27.08 ± 0.01	99.64 ± 0.12	89.86 ± 0.15	24.56 ± 0.30	99.77 ± 0.32
50 °C	LS1	85.81 ± 0.17	21.05 ± 0.13	84.31 ± 0.23	84.73 ± 0.45	21.56 ± 0.08	85.56 ± 0.42	21.66 ± 0.15	21.72 ± 0.15	86.04 ± 0.28
	LC1	87.15 ± 0.05	24.06 ± 0.01	87.98 ± 0.06	87.00 ± 0.51	24.35 ± 0.35	88.07 ± 0.98	24.97 ± 0.15	24.98 ± 0.15	88.03 ± 0.07
	LS2	88.34 ± 0.03	15.89 ± 0.06	86.50 ± 0.03	87.76 ± 0.32	15.56 ± 0.21	86.57 ± 0.09	15.80 ± 0.21	15.83 ± 0.20	86.70 ± 0.04
	LC2	87.93 ± 0.35	24.79 ± 0.14	90.73 ± 0.08	87.66 ± 0.32	24.90 ± 0.10	90.85 ± 0.02	24.84 ± 0.30	24.80 ± 0.30	90.97 ± 0.10



## Graphical Abstract:

