



**HAL**  
open science

# Influence of casein on the formation of whey protein microparticles obtained by dry heating at an alkaline pH

Elise Schong, Marie-Hélène Famelart

## ► To cite this version:

Elise Schong, Marie-Hélène Famelart. Influence of casein on the formation of whey protein microparticles obtained by dry heating at an alkaline pH. *Food Research International*, 2019, 122, pp.96-104. 10.1016/j.foodres.2019.04.001 . hal-02098680

**HAL Id: hal-02098680**

**<https://hal.science/hal-02098680>**

Submitted on 22 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **Influence of casein on the formation of whey protein microparticles obtained**  
2 **by dry heating at an alkaline pH**

3 **Elise Schong, Marie-Hélène Famelart\***

4 STLO, UMR 1253, INRA, Agrocampus Ouest, 35000 Rennes cedex, France

5 \*Corresponding author; Tel: +33.223.48.53.43; Fax: +33.223.48.53.50; email address: marie-

6 helene.famelart@inra.fr

7 **ABSTRACT**

8 Dry heating (DH) at 100 °C for 36 h of a whey protein isolate powder conditioned at pH 9.5  
9 leads to the formation of stable, large and porous whey protein microparticles (PMs),  
10 resulting from the crosslinking of proteins inside the powder. These PMs could be used as  
11 high-viscosity food ingredients. Casein, present as a contaminant in whey protein powders,  
12 has been shown to become incorporated into the PMs. In this study, we investigated the  
13 effect of adding increasing amounts of sodium caseinate to whey protein powders on the  
14 formation of PMs during DH at 100 °C for 36 h. In addition, we studied PM formation during  
15 DH of a micellar casein-enriched milk protein powder (Casmic). The browning index of the  
16 dry-heated powders, and the size and water content of the microparticles were also  
17 characterized. We confirmed that sodium caseinate was incorporated into the PMs. The  
18 highest PM D[4,3] values (270 µm) were observed for powders with around 40% caseinate.  
19 Powders without added caseinate displayed D[4,3] values of 150 µm. The yield of conversion  
20 of proteins into PMs increased from 0.6 to 0.8 g/g with caseinate addition, whereas the  
21 amount of water entrapped in the PMs decreased from around 30 to 20 g/g. PMs were also  
22 formed by DH of the Casmic powder, but these particles were smaller, with sizes of around  
23 80 µm. In conclusion, our study shows that the process of DH at pH 9.5 could be applied to  
24 all milk proteins to obtain PMs with functional properties that could be used in the food  
25 industry.

26 **KEYWORDS:** whey protein; microparticle; dry heating; aggregation; casein

## 27 1. Introduction

28 Whey proteins (WPs) display a range of useful functional characteristics, such as  
29 rheological, foaming, emulsifying and gelation properties (Dickinson, 2015; Kharlamova,  
30 Chassenieux, & Nicolai, 2018; T. Nicolai & Durand, 2013). A lot of work has been dedicated  
31 to the study of WP aggregation, with the aim of increasing the functional properties of these  
32 proteins. Most of these studies involved heating a solution of WPs under a range of  
33 physicochemical conditions and studying the size, structure and properties of the resulting  
34 aggregates (Taco Nicolai, Britten, & Schmitt, 2011). During the last two decades, an  
35 increasing number of studies have investigated the effect of directly heating protein powders  
36 in the presence of sugar to induce the Maillard reaction (O'Mahony, Drapala, Mulcahy, &  
37 Mulvihill, 2017; Schong & Famelart, 2017). Whereas very few changes in protein structure  
38 are observed during the glycation of proteins under gentle dry heating (DH) conditions  
39 (Fenaille, Morgan, Parisod, Tabet, & Guy, 2003), DH treatments using high temperatures  
40 and/or over long time periods can induce significant WP aggregation (Gulzar, Bouhallab,  
41 Jeantet, Schuck, & Croguennec, 2011; Hiller & Lorenzen, 2010; Schmitt, Bovay, & Frossard,  
42 2005).

43 Our previous studies have shown that DH for over 10 h at 100 °C of a freeze-dried or a  
44 spray-dried WP powder conditioned at pH 9.5 with a water activity ( $a_w$ ) of around 0.2 leads to  
45 the formation of WP microparticles (Famelart, Schong, & Croguennec, 2018; Schong &  
46 Famelart, 2018). By dissolving the dry-heated powder in water and observing the protein  
47 microparticles (PMs), we have shown that these PMs are very large and that their size and  
48 shape are influenced by the size and shape of the powder particles. The DH treatment led to  
49 crosslinking of proteins inside the powder particles so that the powder was no longer soluble  
50 when dispersed in water. Due to the porous structure of the powder, the PMs formed after 36  
51 h of DH were able to entrap a huge amount of water (20-40 g water/g of dry PM), with a yield  
52 of formation of over 0.5 g of PM/g of powder. The PMs produced by DH of the spray-dried

53 powder were spherical with a hollow structure, and were smaller and more homogeneous in  
54 size than the those produced from DH of the freeze-dried powder (Famelart et al., 2018;  
55 Schong & Famelart, 2018). The very high viscosity values obtained in our previous study  
56 indicates that these PMs could be used as high-viscosity or food thickening agents (Schong  
57 & Famelart, 2018). The suspension of particles formed by DH of the spray-dried WP powder  
58 at 32 g/kg gave a viscosity of around 17 Pa.s at a shear rate of  $1 \text{ s}^{-1}$  and a temperature of 20  
59 °C. This viscosity value is much higher than that obtained for other WP particles in previous  
60 studies (Dissanayake et al., 2012; Inthavong, Kharlamova, Chassenieux, & Nicolai, 2016;  
61 Loveday, Su, Rao, Anema, & Singh, 2012; Zhang, Arrighi, Campbell, Lonchamp, & Euston,  
62 2016).

63 More recently, we studied the effect of lactose content on PM formation from freeze-dried  
64 powders (Schong & Famelart, 2019). Although freeze-dried flakes are much larger than  
65 spray-dried powder grains, freeze-dried powders were used as a model for spray-dried  
66 powders in this study because it was more convenient to produce powders with different  
67 lactose compositions by freeze-drying than by spray drying. Using this model, we showed  
68 that increasing the lactose content led to increases in the rate of protein crosslinking and PM  
69 formation, and to higher yields of conversion of WP into PMs to more than 0.9 g/g of powder.  
70 In contrast, increasing the lactose content led to a decrease in the amount of water  
71 entrapped in these PMs. It has been shown that dry-heated powder particles are able to  
72 swell when placed in water, leading to particle sizes in suspension being slightly larger than  
73 those in the powder. However, powder particles exposed to longer DH times and those  
74 heated in the presence of higher lactose contents swelled less when placed in water, due to  
75 the higher degree of crosslinking induced by DH. DH at 100 °C for 36 h without added  
76 lactose, but in the presence of the trace amounts of lactose present in the WP powder  
77 (around 6 g per kg powder), gave the same yield of conversion of WP into PM and led to the  
78 same amount of water entrapped in the PMs as DH for only 3 h with addition of lactose at a  
79 concentration of 0.42 g/10 g protein. Nevertheless, evaluation of the browning index of the

80 dry-heated powder showed that the formation of the PMs did not correlate with the progress  
81 of the Maillard reaction. Indeed, the mechanism of formation and the nature of the protein  
82 crosslinks are still speculative. We have found that the PMs are resistant to reduction  
83 (unpublished results). Similar findings were reported by Gulzar et al. (2011), who found that  
84 WP aggregates produced by DH at pH 4.5 or 6.5 were resistant to reduction, whereas  
85 aggregates produced at pH 2.5 were not. Isopeptide and lysinoalanine bonds have been  
86 implicated in the formation of the crosslinks as their formation is favored by high pH values  
87 and severe heating (Gerrard, 2002). Furthermore, large protein aggregates have been  
88 reported in the presence of lactose as a result of the Maillard reaction: these aggregates are  
89 made of covalent bonds resistant to reduction and have been reported to involve the  
90 degradation products of Maillard intermediates, such as dicarbonyl compounds (Friedman,  
91 1996; Hiller & Lorenzen, 2010; Lan et al., 2010; Miller, Meade, & Gerrard, 2003; Zamora &  
92 Hidalgo, 2005). These dicarbonyl compounds are highly reactive crosslinkers of lysine and  
93 arginine residues and give rise to advanced glycation end products (Gerrard et al., 2005;  
94 Miller et al., 2003). Their formation is enhanced at an alkaline pH (Martins & Van Boekel,  
95 2005; Wei, Liu, & Sun, 2018). However, the large number of reactants and the complexity of  
96 the pathways involved in the Maillard reaction, together with an inability to dissociate the  
97 PMs into smaller protein chains for analysis, mean that it appears impossible to determine  
98 the exact nature of the PM bonds.

99 The WP powder used in these previous studies was 90% protein. About 18% of these  
100 proteins were caseins, present as contaminants of the WP powder. It has been shown that  
101 these caseins were transferred into the PMs together with the WPs (Schong & Famelart,  
102 2019). The aim of the current study was to further our understanding of the mechanism of  
103 PM formation by investigating the effect of replacing WPs with casein on the formation of  
104 PMs. As in our previous study investigating the effect of lactose content on PM formation,  
105 freeze-dried powders were used in the current study as a convenient model for predicting the  
106 behavior of caseins on spray-dried powders. Herein, we report on the changes in the

107 properties of the powder particles and PMs induced by adding sodium caseinate to WP  
108 before freeze-drying and DH at 100 °C for 36 h. To gain an insight into the effect of casein  
109 structure on its transfer into the PMs, a sample of micellar casein-enriched milk protein was  
110 also studied after undergoing the same heating process.

## 111 **2. Materials and Methods**

112 Most of the materials and methods used in this study have been described in more detail  
113 previously (see Schong & Famelart (2019)).

### 114 *2.1. Materials*

115 The commercial WP powder was obtained from a confidential source and was the same  
116 powder as that used in our previous studies (Famelart et al., 2018; Schong & Famelart,  
117 2018, 2019). The solid matter and protein contents of the WP powder were  $965.6 \pm 0.3$  and  
118  $911 \pm 7$  g per kg powder, as determined respectively by the desiccation method (International  
119 Dairy Federation, 2010), and by the Kjeldahl method (International Dairy Federation, 2004,  
120 2014, 2016) using 6.38 as the conversion factor. Approximately 82% of this protein was WP  
121 and around 18% was casein, present as a mix of  $\alpha$ s1,  $\alpha$ s2,  $\beta$  and  $\kappa$  caseins, as determined  
122 by polyacrylamide gel electrophoresis (Schong & Famelart, 2019). The free-lactose content  
123 of the powder was  $5.92 \pm 0.16$  g per kg powder as determined by ion chromatography  
124 (Gaucheron, 2003). Thus, the lactose to protein ratio was around  $6.5 \cdot 10^{-3}$  g/g.

125 The commercial sodium caseinate (NaCas) powder was obtained from Lactalis  
126 (Bourgarré, France) and contained  $936.9 \pm 3.4$  g of solid matter,  $878.06 \text{ g} \pm 2.1$  of protein  
127 and  $2.99 \text{ g} \pm 0.03$  g of free-lactose per kg powder. Thus, the lactose to protein ratio for the  
128 NaCas powder was around  $3.4 \cdot 10^{-3}$  g/g.

129 The commercial micellar casein-enriched milk (Casmic) protein powder (Promilk 852B)  
130 was obtained from International dairy ingredients (Arras, France) and contained  $936.7 \pm 0.5$   
131 g of solid matter,  $779.8 \pm 0.2$  g of protein (among which 93% was micellar casein) and  $29.42$   
132  $\pm 1.16$  g of lactose per kg powder. The proportion of casein and WP in this powder was  
133 determined by Kjeldahl analysis of non-protein and non-casein nitrogen. The lactose to  
134 protein ratio was around  $38.10^{-3}$  g/g, and was thus 6–11 fold higher than that for the two  
135 other powders.

## 136 *2.2. Preparation of the dry-heated powders*

137 The NaCas powder was mixed with the WP powder at levels of 0, 20, 25, 33, 50 and  
138 100% by weight. The casein content as a percentage of the weight of total protein was  
139 calculated for each mixture based on the composition of the powders as 18, 34, 38, 44, 58  
140 and 99%, respectively. These samples were then referred to as “Cas18” to “Cas99”. The  
141 lactose to protein ratio decreased progressively by a factor of two from the Cas18 powder to  
142 the Cas99 powder. Similarly, the dry matter content in the solution showed a 3% decrease  
143 from 965.6 to 936.9 g/kg. A sample of 100% of Casmic, containing 93% casein, was also  
144 prepared.

145 Solutions containing 150 g powder/kg were prepared by stirring overnight at 4 °C. The pH  
146 of these solutions was then adjusted to pH 9.5 by addition of  $5 \text{ mol.L}^{-1}$  and then  $1 \text{ mol.L}^{-1}$   
147 NaOH. The solutions were then rapidly frozen and freeze-dried. The powders were finely  
148 crushed, adjusted to an  $a_w$  of  $0.25 \pm 0.01$  for two weeks and dry-heated at 100 °C for 36 h.  
149 Only one solution and powder were prepared for each mixture of casein and WP, but two  
150 samples of each powder solution were dry-heated and further analyzed.

151 2.3. Analysis

152 2.3.1. Powder

153 The granular size distribution of the unheated freeze-dried powder after crushing was  
154 analyzed as described previously in Schong & Famelart (2019) using the QICPIC analyzer  
155 and the dry disperser RODOS/L module (Sympatec GmbH, Clausthal-Zellerfeld, Germany).  
156 Two measurements of the distribution of the “diameter of a circle of equal projection area” of  
157 the particles were taken for each sample and the mean volume diameter (D[4,3]) was  
158 deduced. Two measurements were performed for each unheated sample.

159 Samples of powder particles were transferred to a glass lamella and observed on a light  
160 microscope (Olympus BX51, Rungis, France) with a x10 objective. Photographs (10 per  
161 sample) were taken using a Qiclick CCD camera (Q Imaging, Microvision Instruments, Evry,  
162 France) and the Archimed software program (Microvision Instruments).

163 The intensity of the Maillard reaction was estimated by monitoring changes in the  
164 browning index (BI) of the powders. Briefly, the BI was deduced from L\* (whiteness), a\*  
165 (redness/greenness) and b\* (yellowness/blueness) measurements made using a CR-300  
166 Chromameter (Minolta, Roissy, France). The BI was obtained according to Maskan (2001) as  
167 follows:

168 
$$BI = \frac{[100 (x - 0.31)]}{0.17}$$
 equation 1

169 
$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$$
 equation 2

170 Three measurements were performed for each dry-heated powder.

171 2.3.2. Proteins and protein microparticles

172 Powders were reconstituted to a final concentration of 10 g of powder.kg<sup>-1</sup> in 0.2 g.kg<sup>-1</sup>  
173 sodium azide in deionized water and adjusted to pH 6.5 (suspension S0). One S0  
174 suspension was prepared for each dry-heated powder.

175 Samples (1 mL) of the S0 suspension were acidified by addition of 300 µL of 0.5 M  
176 acetate-acetic acid buffer (pH 4.6), and then centrifuged for 15 min at 10000 g. The resulting  
177 supernatants, containing residual native whey proteins (NWP), were then frozen until  
178 analysis. Casein is not soluble at pH 4.6 and was partitioned into the pellet during this  
179 fractionation process. The levels of native β-Lactoglobulin (β-Lg) and α-Lactalbumin (α-lac),  
180 present in the supernatants were quantified by reversed phase high performance liquid  
181 chromatography (RP-HPLC) as described in Schong & Famelart (2019). NWP were  
182 quantified as the sum: β-Lg + α-lac. One sample was prepared for each S0 suspension and  
183 three RP-HPLC injections were done for each preparation.

184 The size distribution of PMs with sizes of 0.02–2000 µm was measured in S0 suspensions  
185 at 20 °C using a Mastersizer 2000 equipped with the HydroSM module (Malvern Instruments,  
186 Worcestershire, UK), as described by Famelart et al. (2018). The D[4,3] was then deduced.  
187 Two samples were taken from each S0 suspension and measurements on each sample  
188 were carried out in triplicate. Changes in the D[4,3] of the powder particles in suspension  
189 were evaluated by determining the size ratio (SR) from the D[4,3] of the PMs/D[4,3] of the  
190 powder particles.

191 PMs were isolated by centrifuging around 2 g of each S0 suspension at 10 000 g for 15  
192 min at 20 °C. Pellets containing sedimented PMs were then weighed in the wet state to  
193 obtain the wet weight (WW) and then after drying using a Savant Speed Vac Concentrator  
194 SVC-100H (Hicksville, USA) to obtain the dry weight (DW). The dry yield of conversion of the  
195 powder dry matter into PMs (DY) was then determined as the dry weight of the pellet  
196 obtained from 1 g of dry-heated powder. The wet yield of conversion (WY) was determined

197 as the wet weight of the pellet obtained from 1 g of dry-heated powder. As the dry matters of  
198 the WP and NaCas mixtures and of the Casmic powder were made up of about 94% and  
199 83% protein, respectively, the DY and WY provided good estimation of the yield of  
200 conversion of proteins into particles. The water content (WC) of the PMs, determined as the  
201 weight of water per 1 g of dry pellet, was calculated as follows:

$$202 \quad WC = \frac{(WW-DW)}{DW} \quad \text{equation 3}$$

203 Measurements were performed four times for each suspension.

204 S0 suspensions were diluted 10-fold in distilled water and observed by confocal laser  
205 scanning microscopy (CLSM) using an inverted TE2000-E microscope (Nikon, Champigny-  
206 sur-Marne, France) with a x20 oil-immersion objective. Proteins were probed with Nile Blue.  
207 Ten images per sample (512 x 512 pixels<sup>2</sup>, representing 636 x 636 μm<sup>2</sup>) were acquired at a  
208 distance of 15 μm away from the bottom lamella.

#### 209 *2.4. Statistical analysis*

210 The effects of powder composition on the D[4,3] and on the residual NWP content were  
211 analyzed by analysis of variance (ANOVA) and, in case of significance, by the Tukey's  
212 multiple comparison test for paired comparisons of means. A P value of less than 0.05 was  
213 used as the level of significance. All statistical analyses were performed using the R software  
214 package.

### 215 **3. Results and discussion**

#### 216 *3.1. Powder characterization*

217 The BI of the powders before (0 h) and after DH for 36 h is shown in Fig. 1. For the WP  
218 powder without added casein (Cas18 sample), the BI was 12 before DH and 45 after 36 h of

219 DH. These values are in accordance with those described previously: BI scores of 7-16  
220 before DH and 27-54 after 36 h of DH were obtained for the same powder after same  
221 treatments by Famelart et al. (2018) and Schong & Famelart (2018, 2019). The BI of all  
222 powders containing a mixture of WP and NaCas was around 10 before DH and increased by  
223 a factor of four after DH. The highest BI after DH was obtained for the powders with around  
224 40% casein; the BI then decreased as the NaCas content of the powders increased. This  
225 decrease in BI may be explained by a reduction in the lactose to protein ratio when WPs  
226 were replaced by NaCas; however, the reason for the increase in BI between powders with  
227 18% casein and those with 44% casein is unknown. One hypothesis is that the alkaline pH  
228 applied before DH induced WP denaturation and unfolding, leading to an increased exposure  
229 of the lysine residues and their glycation via the Maillard reaction. Glycation levels in caseins  
230 would be expected to be higher than those in WPs as caseins are unstructured proteins and  
231 do not need to be unfolded to be able to react with lactose. This could explain the initial  
232 increase in BI with increased casein content. The BI of the Casmic powder increased by a  
233 factor of 12 after DH, probably because this powder had a higher lactose to protein ratio  
234 promoting a more intensive Maillard reaction.

235 The granular size distribution of the unheated powder is shown in Fig. 2A. The size  
236 distribution of the powder particles made of WP and NaCas showed a slight shift towards  
237 larger sizes with the addition of NaCas. This shift was confirmed by the significantly higher  
238  $D[4,3]$  values observed for the powder particles with increasing casein content (the grey  
239 points in Fig. 2C). As with the BI, a maximum for the  $D[4,3]$  value was observed in powders  
240 containing around 40% casein. This increase in  $D[4,3]$  was probably due to higher resistance  
241 of powders with higher NaCas contents (or lower lactose contents) to crushing of the freeze-  
242 dried powder during preparation for DH. However, it is unclear why this increase in  $D[4,3]$   
243 was not linear with increasing casein content. This change in size of the powder flakes may  
244 be influenced by the freeze-drying process. Firstly, freeze-drying begins with freezing of the  
245 solution leading to separation of the protein solution into, first, liquid water in the form of ice

246 crystals and, second, a concentrated solution of proteins and solutes. Ice is subsequently  
247 removed during the sublimation step, together with the water adsorbed onto the solutes  
248 (Franks, 1998). Concentrated proteins and solutes may then interact, be denatured, and  
249 either precipitate in the crystalline state or stay as an amorphous solid that can also proceed  
250 to glass transition at low temperatures. We assumed that the structure of the freeze-dried  
251 cake before the crushing step was made up of pores the size of ice crystals embedded in the  
252 protein amorphous concentrate, and that the crushing would lead to breakage preferentially  
253 through these pores. Thus, the structure of the cake would be expected to have  
254 consequences on the size of the powder flakes: a larger crystal size distribution would lead  
255 to a cake with larger pores, a denser amorphous protein phase, and to smaller flakes than  
256 would be observed with smaller crystals. However, this hypothesis cannot be confirmed as  
257 we do not know the sizes of the crystals in the different frozen mixtures. Secondly, although  
258 the increase in size of the powder flakes appeared to correlate with the small decrease in  
259 lactose content resulting from substitution of WP by NaCas, reduced amounts of lactose are  
260 very unlikely to lead to an increase in powder flake size as decreasing the lactose content  
261 has been shown previously to decrease flake sizes (Schong & Famelart, 2019). Thirdly,  
262 protein powders are more rigid and brittle at temperatures below the glass transition  
263 temperature ( $T_g$ ) and softer and more viscous-like at temperatures above the  $T_g$ . The  $T_g$   
264 values of NaCas, Casmic and WP at an  $a_w \sim 0.3$  are 61, 72 and 84 °C, respectively (Schuck,  
265 Jeantet, & Dolivet, 2012). The lower the  $T_g$  of the powder, the closer the powder would be to  
266 the transition towards a softer material, and the softer the material would be. Thus, we would  
267 expect Cas18 to be harder than Cas99, but the observed flake sizes contradict this  
268 explanation. Furthermore, differences in behavior between WP and micellar casein, with  
269 relevant consequences on powder morphology, have been reported previously during spray-  
270 drying (Sadek et al., 2014). Powder particles of micellar casein are wrung, and invaginated,  
271 whereas those of WP are totally spherical, and present a large vacuole of occluded air and a  
272 smooth surface. These differences could be ascribed to the fact that when proteins are  
273 concentrated to above a critical concentration during droplet drying, casein or caseinate

274 undergo a sol-gel transition (Bouchoux et al., 2009) and globular proteins undergo a glass  
275 transition (Brownsey, Noel, Parker, & Ring, 2003; Parker, Noel, Brownsey, Laos, & Ring,  
276 2005), leading to development of solid-like properties. Storage moduli of 200-300 Pa for  
277 casein gels and > 10000 Pa for  $\beta$ -Lg and bovine serum albumin gels have been reported at  
278 concentrations of 100-200 g/kg for casein and > 400 g/kg for globular proteins (Bouchoux et  
279 al., 2009; Brownsey et al., 2003; Parker et al., 2005). Thus, the differences in powder  
280 morphology and size may have been caused by differences in the physicochemical  
281 properties of the proteins, such as their transition concentration or gel firmness. However,  
282 casein gels formed by concentration are softer than WP gels, a finding which again is  
283 contradictory to our observation that flake size increases with increasing casein content. In  
284 conclusion, the mechanism underlying the changes in flake size with altered composition of  
285 the mixtures remains unclear. The observed changes in flake size were small, with the size  
286 of powder flakes ranging between 5 and 500  $\mu$ m and the D[4,3] values ranging between 60  
287 and 100  $\mu$ m for all powders; however, any changes in flake size or structure could have  
288 major consequences on the formation of PMs.

289 Light micrographs of powder particles from samples of Cas18, Cas58 and Cas99 after  
290 DH, and from Casmic before and after DH, are shown in Fig. 3. The powder particles appear  
291 as flakes, as expected for freeze-dried powders. Although microscopy could not be used to  
292 provide accurate evaluations of the size distribution of the flakes, because of the very small  
293 number of particles observed in each sample and the risk that these small samples were  
294 unrepresentative of the bulk, we did not notice any significant size differences between the  
295 flakes of powders with different NaCas contents. The Cas99 powder flakes appeared slightly  
296 denser and more shrunken than flakes from powders with lower NaCas contents. In addition,  
297 the Casmic powder flakes appeared much more shrunken, less translucent, and much  
298 smaller than flakes from the other powders. It was of interest to note that the aspect of the  
299 Casmic flakes, and indeed that of all the other powders studied (data not shown), was not  
300 modified by the DH treatment. The size of the flakes from powders with WP and NaCas

301 appeared to be around 5–20  $\mu\text{m}$ , whereas those from the Casmic powder were  $< 10 \mu\text{m}$ .  
302 These estimates of flake size made from microscopic evaluations were smaller than the  
303 values obtained using the Qicpic method. We consider that the Qicpic method gave the most  
304 reliable estimation of flake size because using this method more than  $10^6$  particles were  
305 characterized during one measurement.

### 306 *3.2. Protein microparticle characterization*

307 Photographs of S0 suspensions of dry-heated powders were taken immediately (d0, not  
308 shown) and 5 d after their rehydration and quiescent storage (see the supplementary  
309 information and Fig. S1). The d0 suspensions of dry-heated powders appeared cloudy, due  
310 to the presence of PMs that sedimented after storage. These suspensions were yellow for  
311 the Cas18-Cas99 samples, and yellow-red for the Casmic sample. In contrast, the  
312 suspensions of powders before DH were translucent (not shown). Similar volumes of  
313 sediment appeared to be present in the Cas18 to Cas38 samples, but the volume of  
314 sediment seemed to decrease slightly in suspensions from powders with above 38% casein.  
315 A far smaller volume and browner sediment was observed in the Casmic sample. These  
316 findings show that DH of the powders for 36 h led to the formation of PMs that sedimented  
317 with time. Evaluation of the height of the sediment on the photograph revealed that these  
318 sedimented particles occupied a rather large volume: although the suspensions contained  
319 only 1% dry-heated powder, the sedimented particles occupied between 10% (Casmic) and  
320 24-17% (WP and NaCas) of the total volume.

321 The size distribution of the PMs varied between 4 and 1000  $\mu\text{m}$  for the Cas18 suspension  
322 and seemed to shift towards larger values with the addition of increasing amounts of NaCas  
323 (as shown in Fig. 2B).  $D[4,3]$  values increased as the NaCas content increased from 18% to  
324 around 40% and then decreased for samples with over 40% NaCas (Fig.2C). The size of  
325 these PMs was 2-4 fold larger than that of the powder flakes, with the Cas34-Cas44 samples  
326 having the largest PM sizes. The SR was around four for the Cas34 sample and the lowest

327 SR value, of around two, was obtained for Cas99. Swelling of the dry-heated particles when  
328 placed in the aqueous phase, leading to larger sizes in suspension than those observed in  
329 the powder, has already been reported in previous studies (Schong & Famelart, 2018): the  
330 diameter of particles obtained by DH of spray-dried powders has been shown to increase by  
331 a factor of four when placed in water. This swelling was probably due to repulsion between  
332 negative charges on proteins inside the PMs. An example of this swelling is shown for a flake  
333 from the Cas99 sample and occurred within around 20 s under the conditions used in the  
334 current study (see supplementary Fig. S2): the dimensions of the flake increased by a factor  
335 of around two, in accordance with the calculated SR of powder flakes from this sample (SR  $\approx$   
336 2). Our previous study indicated that the degree of swelling was related to the number of  
337 crosslinks between proteins inside the PM: swelling was reduced after prolonged DH times  
338 and in powders with higher lactose contents, i.e. under conditions where the number of  
339 crosslinks increased (Schong & Famelart, 2019). However, the degree of swelling may also  
340 be related to the size of the powder flakes: in the current study swelling was maximal in  
341 samples with 34-44% casein. It should be noted that the maximum BI was also observed in  
342 samples with around 40% casein.

343 The size of PMs from the Casmic suspension varied between around 2 and 1000  $\mu\text{m}$  and  
344 their  $D[4,3]$  was close to the  $D[4,3]$  of the powder flakes. In contrast to the WP-NaCas flakes,  
345 the Casmic PMs underwent only minimal swelling when placed in water, and some of the  
346 PMs appeared to be smaller than the powder particles (1-10  $\mu\text{m}$ ; Fig. 2B). As these small  
347 PMs swelled when placed in suspension, they may have been present in the powder, but  
348 were smaller than the minimum size detected by the QICPIC method ( $\sim 1 \mu\text{m}$ ). Although PMs  
349 were formed by DH of the Casmic powder, the suspended particles appeared to be smaller  
350 than the PMs observed in the other powder samples analyzed in this study. The small size of  
351 the Casmic PMs probably resulted from a high degree of crosslinking associated with very  
352 intense Maillard reactions promoted by the higher lactose content of this sample.

353 In conclusion, dry-heated powders with WP and NaCas swelled by a factor of 2-4,  
354 whereas no swelling was observed for the Casmic powder. CLSM micrographs showing the  
355 sizes of the PMs in the samples are shown in Fig. 4. No particles were visible in the powder  
356 suspensions before DH (data not shown). Angular PMs, with shapes similar to those  
357 observed for the powder flakes, were formed by DH of all the powders. The largest PMs  
358 were observed for powders with between 34 and 44% casein. PMs from the Casmic powder  
359 appeared to be much smaller than the those formed from powders containing WP and  
360 NaCas. These findings are therefore in accordance with the size measurements obtained  
361 using the Mastersizer method (Fig. 2).

362 The yields of conversion of the powder into PMs (DY and WY) and the WC of the PMs are  
363 shown in Fig. 5. The DY value of 0.6 g/g for the WP sample without added casein was the  
364 same as that reported in our previous study (0.6-0.7 g/g; Famelart et al., 2018; Schong &  
365 Famelart, 2019). The DY of PMs produced from WP and NaCas powders increased slightly  
366 with increasing NaCas content (+31% between Cas18 and Cas99). The fact that the DY  
367 increased with the caseinate content confirmed that caseinate formed part of the PMs, with  
368 the caseinate molecules being crosslinked by DH at pH 9.5. If the caseinate had not formed  
369 part of the PMs, we would have expected to see a decrease in DY with increasing NaCas  
370 content. It seems that the increase in DY with casein content (i.e. the slope of the DY-casein  
371 content curve) was most rapid between 18% and 40% casein, and slower at higher casein  
372 contents from 40 to 99%. The amount of water entrapped in the PMs (WC) from the powder  
373 without added casein was 28 g/g. This value is in accordance with the WC values of 25-30  
374 g/g found for the same freeze-dried powder in our previous studies (Famelart et al., 2018;  
375 Schong & Famelart, 2019), but is lower than the value of 49 g/g found for the spray-dried  
376 powder after the same DH treatment (Schong & Famelart, 2018). WC decreased with the  
377 addition of NaCas, but this decrease was irregular: the decrease was minimal from 18 to  
378 34% and from 50 to 99% casein, whereas it was maximal between 34 and 58% casein. The  
379 WY from the powder without added casein was 18 g/g, compared to 18-20 g/g in previous

380 studies (Famelart et al., 2018; Schong & Famelart, 2018, 2019). The WY increased between  
381 18 and 34-38% casein and then declined for powders with above 38% casein before  
382 becoming almost constant between 58 and 99% casein. In conclusion, the yields and WC  
383 values were slightly modified by the substitution of WP for Nacas. Replacing WP by NaCas  
384 promoted the formation of more PMs, but with less water entrapped in their structures. These  
385 small differences could be due to: i) changes in the size or structure of the crushed powders,  
386 with flakes produced from the Cas18 powder being smaller (Fig.2C) and those produced  
387 from Cas99 being denser and more shrunken (Fig.3); ii) differences in the amount of  
388 unfolded protein, such as casein; iii) differences in the extent of crosslinking during DH,  
389 associated with differences in lactose content. Indeed, lactose has been shown to promote  
390 the rapid formation of PMs (Schong & Famelart, 2019). However, lactose content alone  
391 cannot explain the changes observed with increasing casein content, as maximal values for  
392 the BI, particle swelling and WY were obtained for powders with around 40% casein. As  
393 discussed above, the structure of the cake would be expected to change with the size and  
394 number of ice crystals formed during freezing of the protein solution, the amount of lactose or  
395 any other solute, and with the physicochemical properties of the proteins, such as their Tg  
396 and transition concentration and the gel firmness of their concentrated solutions. As the  
397 density of the protein cake, together with the extent of the crosslinking and the protein-  
398 protein and protein-water interactions responsible for swelling, define the amount of water in  
399 the particles in suspension (i.e. the WC), it would be necessary to characterise all of these  
400 parameters to be able to fully explain the variation in properties of the PMs produced by DH.

401 The fact that the maximum BI, powder flake and PM D[4,3] and WY values were all  
402 observed at around 40% casein could also be explained by a specific interaction occurring at  
403 pH 9.5 between casein and WP at this ratio, with consequences on protein behaviour. This  
404 hypothesis needs to be confirmed by analysis of the proteins in solution at pH 9.5 before  
405 drying and after drying and DH.

406 The Casmic powder also formed large PMs during DH, with a higher DY than the WP and  
407 NaCas powders (Fig. 5). This higher DY indicates that more extensive crosslinking leads to  
408 retention of the micellar casein inside the powder particles during the DH and that fewer  
409 proteins were present as small aggregates or monomers in the soluble phase. These PMs  
410 entrapped much less water and displayed lower WC and WY values than those observed for  
411 the WP and NaCas powders (Fig. 5). The limited ability of the Casmic PMs to retain water is  
412 related to the fact that the D[4,3] values of the powder and PMs were very similar, thus these  
413 PMs did not swell when dissolved in water. Moreover, we have already shown that an  
414 increase in the DY of WP PMs correlates with a lower WC (Schong & Famelart, 2019).

### 415 3.3. Denaturation of whey proteins and composition of protein microparticles

416 For powders made of WP and NaCas, the level of WP denaturation before DH and after  
417 36 h of DH was almost constant, regardless of the NaCas content (Table 1). Before DH, 70%  
418 of the WPs were still native, meaning that 30% were already denatured before DH. We have  
419 already shown that adjusting the pH of the WP solution to 9.5, followed by drying and  $a_w$   
420 adjustment of the powder at 20 °C for 2 weeks, leads to WP denaturation: similar values of  
421 50-60% (Famelart et al., 2018) and 70-80% (Schong & Famelart, 2018, 2019) residual NWP  
422 were obtained before DH in previous studies.  $\beta$ -Lg was shown previously to be the main  
423 protein impacted by the pH adjustment: the native  $\alpha$ -lac and bovine serum albumin (BSA)  
424 contents before DH were 90%, whereas that of  $\beta$ -Lg was only 50% (Famelart et al., 2018).  
425 Indeed, alkaline-induced changes in WPs have been reported at pHs above 9, including  
426 irreversible unfolding and polymerization of proteins (Monahan, German, & Kinsella, 1995;  
427 Partanen et al., 2011; Taulier & Chalikian, 2001).

428 The NWP content decreased to 10-20% after 36 h of DH, and both  $\beta$ -Lg and  $\alpha$ -lac were  
429 denatured. These values are similar to those obtained in previous studies (Famelart et al.,  
430 2018; Schong & Famelart, 2018, 2019). BSA has also been shown to be denatured by DH  
431 (Famelart et al., 2018), thus we can state that most WPs are denatured by the DH treatment

432 at alkaline pH values. Finally, increasing the NaCas content did not have any effect on WP  
433 denaturation during the DH process. It was not possible to analyze the levels of NWP in  
434 samples from the Cas99 powder containing only 1% WP.

435 For the Casmic powder, the residual NWP content before DH was 100% (Table 1). One  
436 hypothesis for the lack of denaturation of the WPs in the Casmic powder is that these WP  
437 were less sensitive to the alkaline pH. Although the Casmic sample contained only 7% WP,  
438 the degree of denaturation after 36 h of DH was similar to that detected in the WP and  
439 NaCas powders, at around 90%. These findings show that WPs from the Casmic powder  
440 were also sensitive to DH at an alkaline pH.

#### 441 **4. Conclusions**

442 Our study shows that DH of a powder made of WP and NaCas conditioned at pH 9.5 led  
443 to the production of large and highly hydrated PMs. Substitution of WP by NaCas led to small  
444 changes in the formation of PMs and in their properties. The yield of conversion of protein  
445 into PMs tended to increase, whereas the amount of water entrapped in these PMs tended to  
446 decrease with the substitution of WP by casein. However, these changes were limited and  
447 not linearly dependent on the casein content. The size of the PMs was maximal in powders  
448 with around 40% casein. We showed PM formation also occurs in micellar casein-enriched  
449 milk protein powders. That fact that casein has no native structure but was still able to form  
450 PMs, throws doubt on the hypothesis that the role of the alkaline pH conditions used in the  
451 process was to unfold proteins, as suggested in Schong & Famelart (2019). Furthermore,  
452 these data suggest that it is possible to produce PMs by this process for a range of powders,  
453 including whey isolate or concentrate or a milk concentrate, as casein has only a limited  
454 effect on the yield and properties of the particles. Further studies are required to characterize  
455 the effects of this process on spray-dried powders.

456 **Acknowledgments**

457 We are grateful to the Regional councils of Brittany (grant N°13008651) and Pays de la  
458 Loire (grant n°2014-07081) for financial support and INRA for scientific coordination (J.  
459 Leonil) through the interregional project PROFIL, managed by the BBA industrial association,  
460 and to P. Hamon and F. Rousseau for their technical help with HPLC analyses and particle  
461 size analyses.

462 **References**

- 463 Bouchoux, A., Debbou, B., Gesan-Guiziou, G., Famelart, M. H., Doublier, J. L., & Cabane, B.  
464 (2009). Rheology and phase behavior of dense casein micelle dispersions. *Journal of*  
465 *Chemical Physics*, 131(16).
- 466 Brownsey, G. J., Noel, T. R., Parker, R., & Ring, S. G. (2003). The Glass Transition Behavior  
467 of the Globular Protein Bovine Serum Albumin. *Biophysical Journal*, 85(6), 3943–  
468 3950.
- 469 Dickinson, E. (2015). Microgels — An alternative colloidal ingredient for stabilization of food  
470 emulsions. *Trends in Food Science & Technology*, 43(2), 178–188.
- 471 Dissanayake, M., Liyanaarachchi, S., & Vasiljevic, T. (2012). Functional properties of whey  
472 proteins microparticulated at low pH. *Journal of Dairy Science*, 95(4), 1667–1679.
- 473 Famelart, M.-H., Schong, E., & Croguennec, T. (2018). Dry heating a freeze-dried whey  
474 protein powder: Formation of microparticles at pH 9.5. *Journal of Food Engineering*,  
475 224, 112–120.
- 476 Fenaille, F., Morgan, F., Parisod, V., Tabet, J.-C., & Guy, P. A. (2003). Solid-state glycation  
477 of  $\beta$ -lactoglobulin monitored by electrospray ionisation mass spectrometry and gel  
478 electrophoresis techniques. *Rapid Communications in Mass Spectrometry*, 17(13),  
479 1483–1492.
- 480 Franks, F. (1998). Freeze-drying of bioproducts: putting principles into practice. *European*  
481 *Journal of Pharmaceutics and Biopharmaceutics*, 45(3), 221–229.
- 482 Friedman, M. (1996). Food Browning and Its Prevention: An Overview. *Journal of*  
483 *Agricultural and Food Chemistry*, 44(3), 631–653.
- 484 Gaucheron, F. (2003). *Minéraux et Produits Laitiers*. Tec & Doc.
- 485 Gerrard, J. A., Meade, S. J., Miller, A. G., Brown, P. K., Yasir, S. B. M., Sutton, K. H., &  
486 Newberry, M. P. (2005). Protein Cross-Linking in Food. *Annals of the New York*  
487 *Academy of Sciences*, 1043(1), 97–103.
- 488 Gerrard, J. A. (2002). Protein–protein crosslinking in food: methods, consequences,  
489 applications. *Trends in Food Science & Technology*, 13(12), 391–399.

490 Gulzar, M., Bouhallab, S., Jeantet, R., Schuck, P., & Croguennec, T. (2011). Influence of pH  
491 on the dry heat-induced denaturation/aggregation of whey proteins. *Food Chemistry*,  
492 129(1), 110–116.

493 Hiller, B., & Lorenzen, P. C. (2010). Functional properties of milk proteins as affected by  
494 Maillard reaction induced oligomerisation. *Food Research International*, 43(4), 1155–  
495 1166.

496 International Dairy Federation. (2004). Milk - Determination of casein-nitrogen content - Part  
497 2: Direct method. *International Standard*, 29.2.

498 International Dairy Federation. (2010). Milk, cream and evaporated milk - Determination of  
499 total solids content (Reference method). *International Standard*, 21B.

500 International Dairy Federation. (2014). Milk and dairy products - Determination of nitrogen  
501 content Part 1: Kjeldahl principle and crude protein. *International Standard*, 20–1.

502 International Dairy Federation. (2016). Milk and dairy products - Determination of nitrogen  
503 content Part 4: Determination of protein and non-protein nitrogen content and true  
504 protein content calculation (Reference method). *International Standard*, 20–4.

505 Inthavong, W., Kharlamova, A., Chassenieux, C., & Nicolai, T. (2016). Structure and flow of  
506 dense suspensions of protein fractal aggregates in comparison with microgels. *Soft  
507 Matter*, 12(10), 2785–2793.

508 Kharlamova, A., Chassenieux, C., & Nicolai, T. (2018). Acid-induced gelation of whey protein  
509 aggregates: Kinetics, gel structure and rheological properties. *Food Hydrocolloids*,  
510 81, 263–272.

511 Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., ... Xiao, Z. (2010). Temperature  
512 effect on the non-volatile compounds of Maillard reaction products derived from  
513 xylose–soybean peptide system: Further insights into thermal degradation and cross-  
514 linking. *Food Chemistry*, 120(4), 967–972.

515 Loveday, S. M., Su, J., Rao, M. A., Anema, S. G., & Singh, H. (2012). Whey Protein  
516 Nanofibrils: The Environment–Morphology–Functionality Relationship in

517 Lyophilization, Rehydration, and Seeding. *Journal of Agricultural and Food*  
518 *Chemistry*, 60(20), 5229–5236.

519 Martins, S. I. F. S., & Van Boekel, M. A. J. S. (2005). Kinetics of the glucose/glycine Maillard  
520 reaction pathways: influences of pH and reactant initial concentrations. *Food*  
521 *Chemistry*, 92(3), 437–448. <https://doi.org/10.1016/j.foodchem.2004.08.013>

522 Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave  
523 drying. *Journal of Food Engineering*, 48(2), 169–175.

524 Miller, A. G., Meade, S. J., & Gerrard, J. A. (2003). New insights into protein crosslinking via  
525 the Maillard reaction: structural requirements, the effect on enzyme function, and  
526 predicted efficacy of crosslinking inhibitors as anti-ageing therapeutics. *Bioorganic &*  
527 *Medicinal Chemistry*, 11(6), 843–852.

528 Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on  
529 protein unfolding and thiol/disulfide interchange reactions during heat-induced  
530 gelation of whey proteins. *Journal of Agricultural and Food Chemistry*, 43(1), 46–52.

531 Nicolai, T., & Durand, D. (2013). Controlled food protein aggregation for new functionality.  
532 *Current Opinion in Colloid & Interface Science*, 18(4), 249–256.

533 Nicolai, Taco, Britten, M., & Schmitt, C. (2011).  $\beta$ -Lactoglobulin and WPI aggregates:  
534 Formation, structure and applications. *Food Hydrocolloids*, 25(8), 1945–1962.

535 O'Mahony, J. A., Drapala, K. P., Mulcahy, E. M., & Mulvihill, D. M. (2017). Controlled  
536 glycation of milk proteins and peptides: Functional properties. *International Dairy*  
537 *Journal*, 67, 16–34.

538 Parker, R., Noel, T. R., Brownsey, G. J., Laos, K., & Ring, S. G. (2005). The nonequilibrium  
539 phase and glass transition behavior of  $\beta$ -Lactoglobulin. *Biophysical Journal*, 89(2),  
540 1227–1236.

541 Partanen, R., Torkkeli, M., Hellman, M., Permi, P., Serimaa, R., Buchert, J., & Mattinen, M.-L.  
542 (2011). Loosening of globular structure under alkaline pH affects accessibility of  $\beta$ -  
543 lactoglobulin to tyrosinase-induced oxidation and subsequent cross-linking. *Enzyme*  
544 *and Microbial Technology*, 49(2), 131–138.

545 Sadek, C., Li, H., Schuck, P., Fallourd, Y., Pradeau, N., Le Floch-Fouéré, C., & Jeantet, R.  
546 (2014). To what extent do whey and casein micelle proteins influence the morphology  
547 and properties of the resulting powder? *Drying Technology*, 32(13), 1540–1551.

548 Schmitt, C., Bovay, C., & Frossard, P. (2005). Kinetics of formation and functional properties  
549 of conjugates prepared by dry-state incubation of  $\beta$ -Lactoglobulin/acacia gum  
550 electrostatic complexes. *Journal of Agricultural and Food Chemistry*, 53(23), 9089–  
551 9099.

552 Schong, E., & Famelart, M.-H. (2017). Dry heating of whey proteins. *Food Research*  
553 *International*, 100(Pt 2), 31–44.

554 Schong, E., & Famelart, M.-H. (2018). Dry heating of whey proteins leads to formation of  
555 microspheres with useful functional properties. *Food Research International*, 113,  
556 210–220.

557 Schong, E., & Famelart, M.-H. (2019). Influence of lactose on the formation of whey protein  
558 microparticles obtained by dry heating at alkaline pH. *Food Hydrocolloids*, 87, 477–  
559 486.

560 Schuck, P., Jeantet, R., & Dolivet, A. (2012). *Analytical methods for food and dairy powders*.  
561 John Wiley & Sons.

562 Taulier, N., & Chalikian, T. V. (2001). Characterization of pH-induced transitions of  $\beta$ -  
563 lactoglobulin: ultrasonic, densimetric, and spectroscopic studies. *Journal of Molecular*  
564 *Biology*, 314(4), 873–889.

565 Wei, Q., Liu, T., & Sun, D.-W. (2018). Advanced glycation end-products (AGEs) in foods and  
566 their detecting techniques and methods: A review. *Trends in Food Science &*  
567 *Technology*, 82, 32–45.

568 Zamora, R., & Hidalgo, F. J. (2005). Coordinate contribution of lipid oxidation and Maillard  
569 reaction to the nonenzymatic food browning. *Critical Reviews in Food Science and*  
570 *Nutrition*, 45(1), 49–59.

571 Zhang, Z., Arrighi, V., Campbell, L., Lonchamp, J., & Euston, S. R. (2016). Properties of  
572 partially denatured whey protein products 2: Solution flow properties. *Food*  
573 *Hydrocolloids*, 56, 218–226.  
574

575 **Table 1**

576 Native residual whey protein (NWP) levels in S0 suspensions of powders before and after 36  
 577 h of dry heating (DH) at 100 °C. Data are expressed as the % of total WP in the S0  
 578 suspension.

Casein content (% w/w)	NWP	
	Before DH	After DH
18	75.4±5.1 <sup>a</sup>	14.6±2.1 <sup>a</sup>
34	77.7±7.0 <sup>a</sup>	14.4±2.7 <sup>a</sup>
38	77.2±4.3 <sup>a</sup>	13.9±1.2 <sup>a</sup>
44	71.9±0.5 <sup>a</sup>	14.3±2.4 <sup>a</sup>
58	78.6±3.7 <sup>a</sup>	10.9±1.6 <sup>a</sup>
93*	102.7±2.9 <sub>b</sub>	8.3±0.6 <sup>b</sup>

579 \* Micellar casein-enriched milk protein powder sample, all other samples are mixtures of  
 580 sodium caseinate (NaCas) and whey protein (WP) powder.

581 <sup>a,b</sup> For each column, values marked with different letters showed a statistically significant  
 582 difference in NWP content (P < 0.05).

583 **Figure captions**

584 **Fig. 1.** The browning index (BI) of powders before (grey) and after 36 h of DH at 100 °C  
585 (black) as a function of the percentage casein content (w/w). The powders analyzed  
586 contained either whey proteins with increasing amounts of sodium caseinate (circles) or  
587 micellar casein-enriched milk proteins (crosses). Plotted lines illustrate the trend in BI with  
588 increasing casein content.

589 **Fig. 2.** The granular size distribution of A) freeze-dried powder particles before dry heating  
590 (DH) and B) protein microparticles in S0 suspensions after DH of the powders. Colors from  
591 black to green represent powders with increasing amounts (w/w) of sodium caseinate  
592 (NaCas), ranging from Cas18 with a mixture of whey proteins and 18% NaCas to Cas99 with  
593 a mixture of whey protein and 99% NaCas. The red curve represents the particle size  
594 distribution for the micellar casein-enriched powder (Casmic). The distributions shown are  
595 representative of data obtained from two samples of each S0 suspension measured in  
596 triplicate; C) The mean volume diameter (D[4,3]) of powder particles (grey) and of protein  
597 microparticles (black) as a function of casein content for powders containing either whey  
598 proteins with increasing amounts of sodium caseinate (circles) or micellar casein-enriched  
599 milk proteins (crosses). For each curve, no statistical differences were observed between  
600 samples for data points marked with the same letter ( $P < 0.05$ ).

601 **Fig. 3.** Light micrographs of particles from powders containing whey proteins with increasing  
602 amounts (w/w) of sodium caseinate (NaCas) ranging from 18% (Cas18) to 99% (Cas99) after  
603 36 h of dry heating (36h DH), and of the particles from the powder containing micellar casein-  
604 enriched proteins (Casmic) before DH (0h DH) and after 36 h of DH (36h DH). The  
605 micrographs shown are representative of the 10 images recorded for each sample.

606 **Fig. 4** Confocal laser scanning micrographs of S0 suspensions of powders dry-heated for 36  
607 h. Suspensions were prepared from: A) powders of whey proteins with increasing amounts

608 (w/w) of sodium caseinate (NaCas), ranging from 18% (Cas18) to 99% (Cas99); and B)  
609 Casmic powder of micellar casein-enriched proteins. Bars = 200  $\mu$ m. The two micrographs  
610 shown for each powder are representative of the 10 images recorded for each sample.

611 **Fig. 5.** Dry yield (DY) of conversion of powder dry matter into microparticles, water content  
612 (WC) of the microparticles and wet yield (WY) measured as the weight of pellets from S0  
613 suspensions as a function of the percentage casein content in % (w/w). The dry-heated  
614 powders in the suspensions contained either whey proteins with increasing amounts of  
615 sodium caseinate (circles) or micellar casein-enriched milk proteins (crosses).

Fig 1

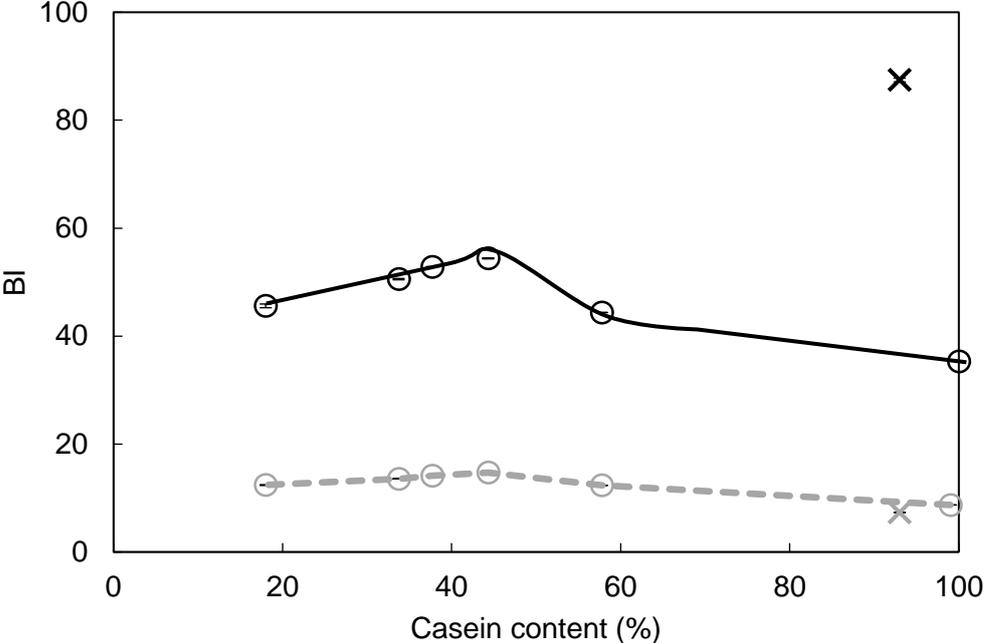


Fig. 2.

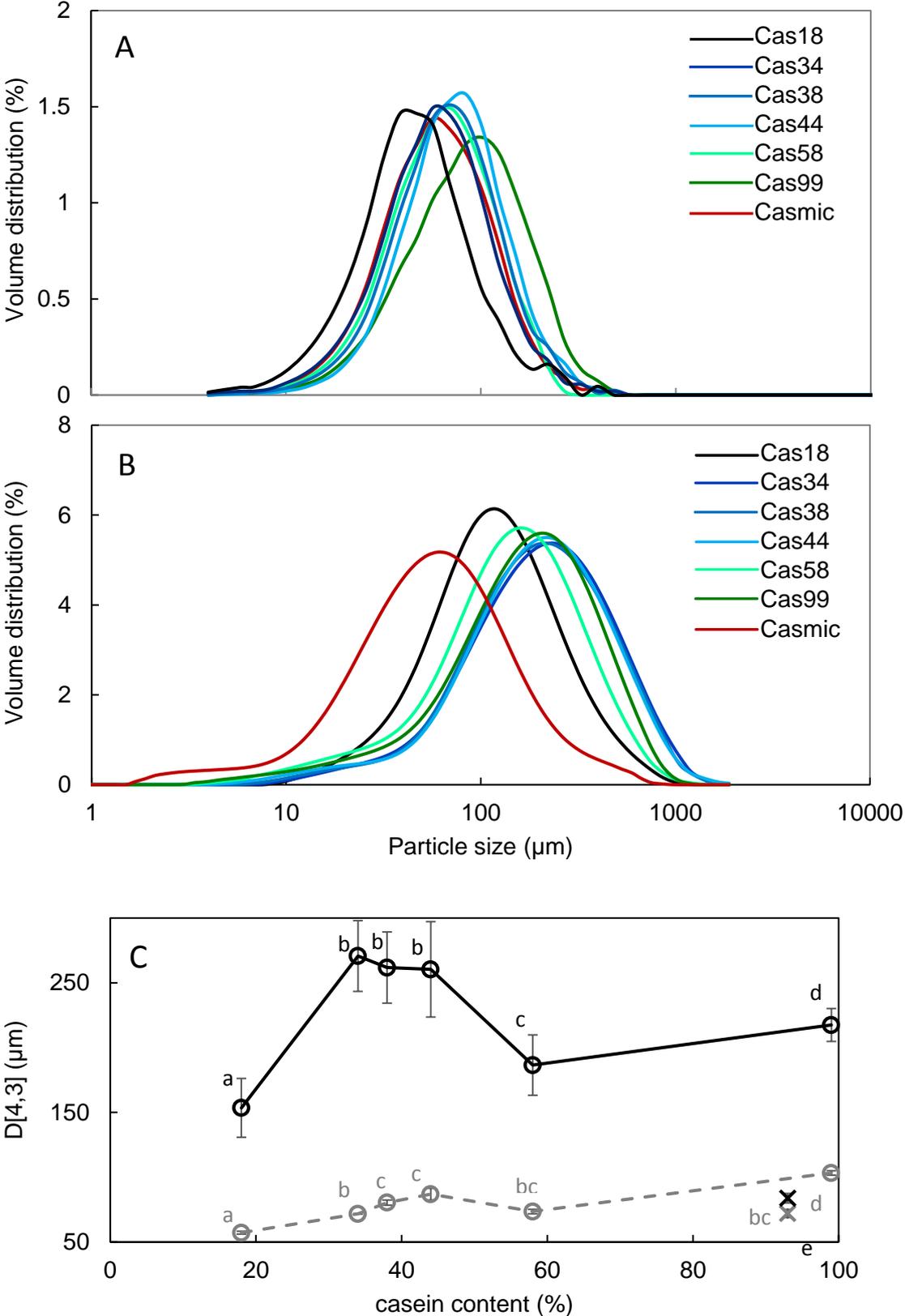
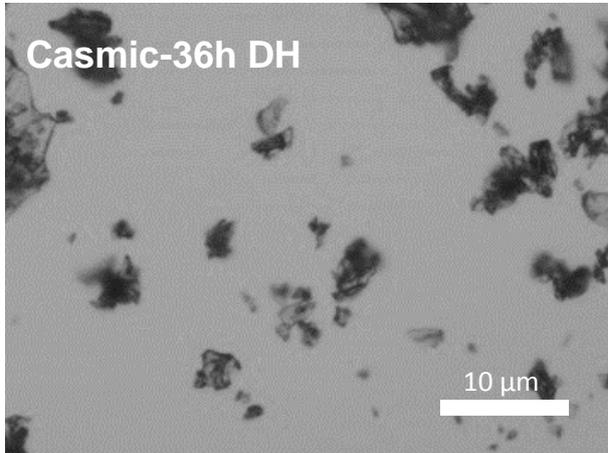
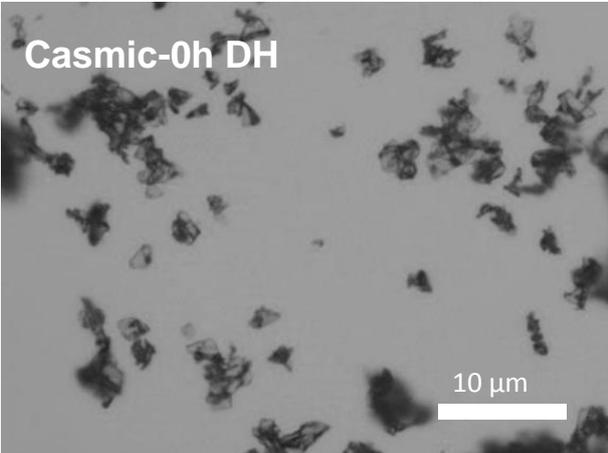
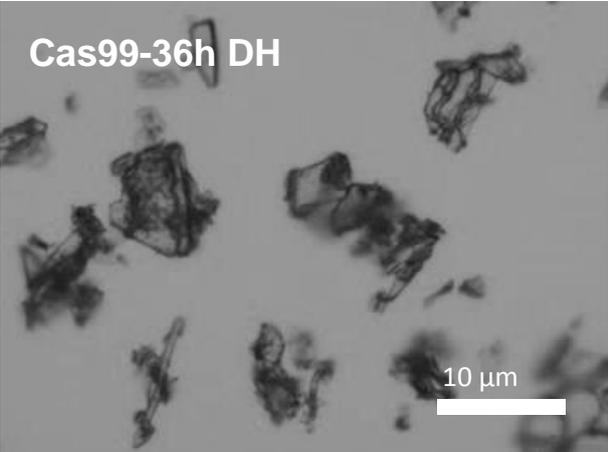
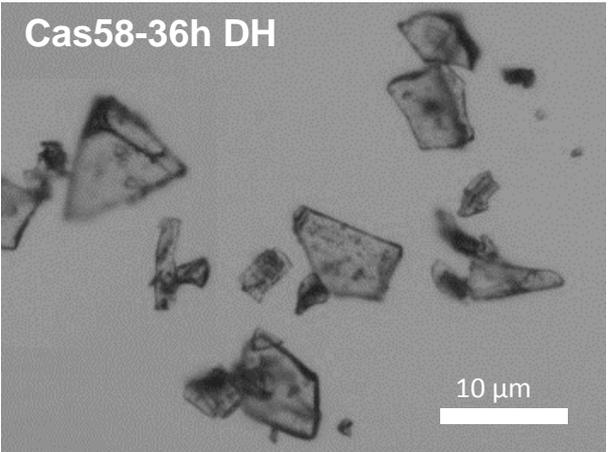
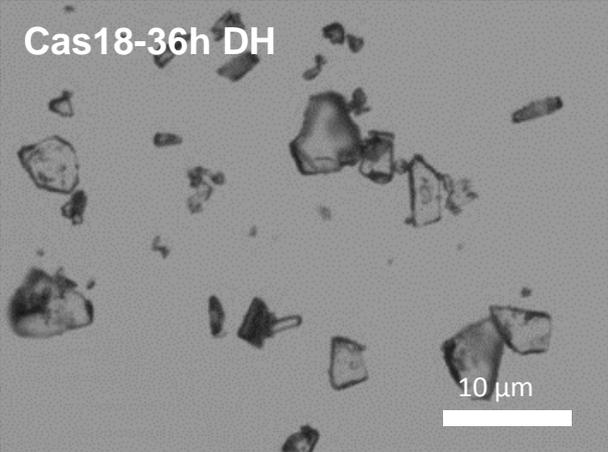


Fig. 3



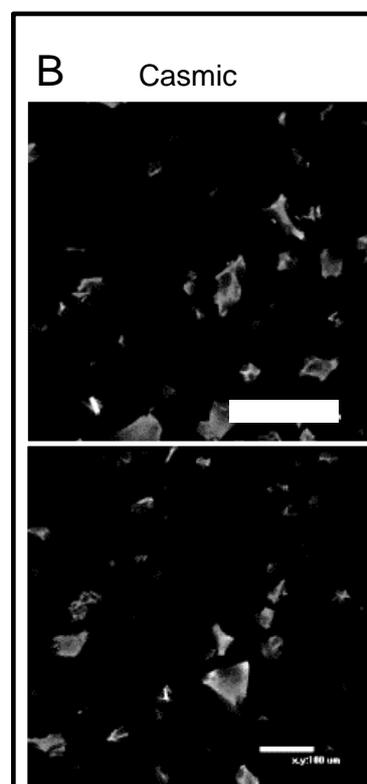
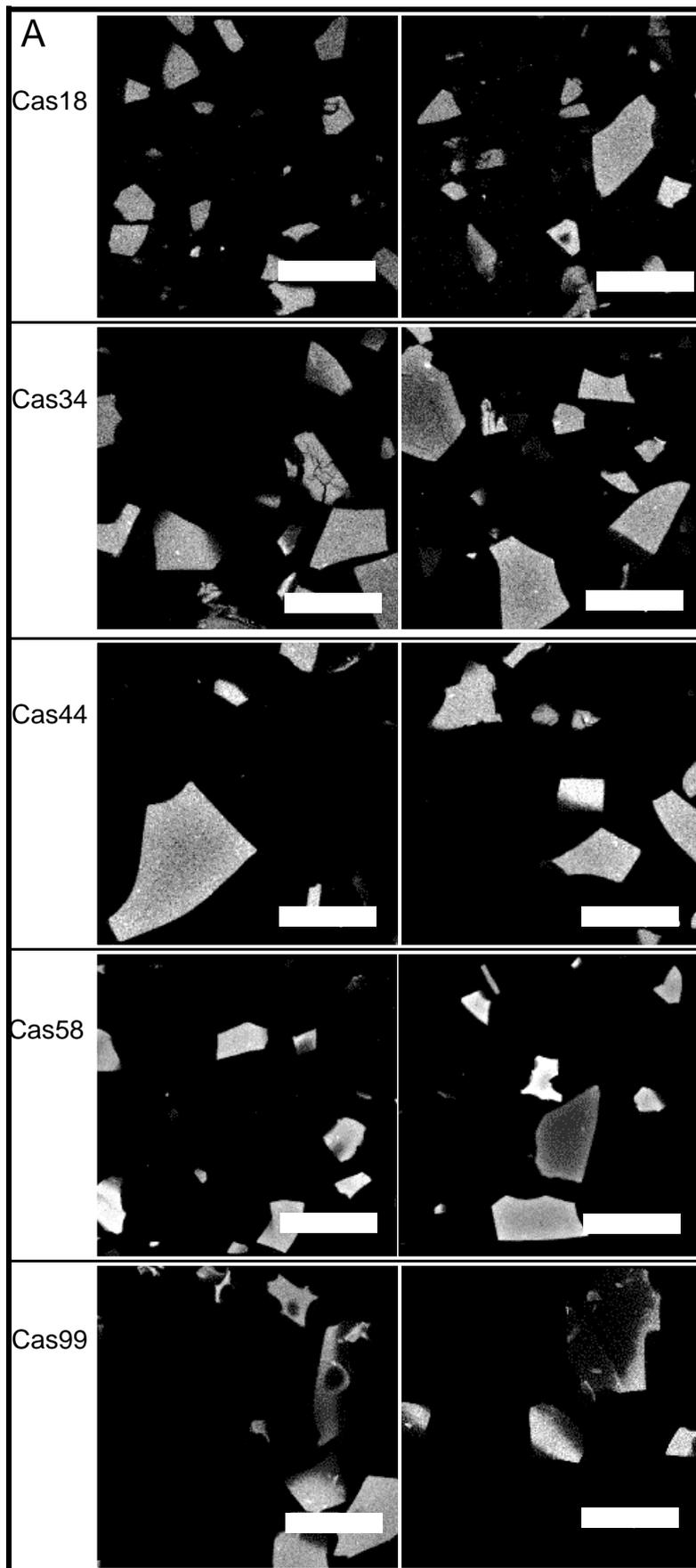


Fig. 5

