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The change of contact angles for wettability measurements

Schematic of MPI and its agglomerate rehydration in water, based on the change of particle size and SSA as a function of time.

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1	Rehydration behaviours of high protein dairy powders: the
2	influence of agglomeration on wettability, dispersibility
3	and solubility
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23 Abstract

Five common high protein dairy powders and their agglomerates produced by 24 fluidised bed granulation were evaluated and compared for their rehydration 25 26 characteristics in this study. Wettability of powders was measured by immersion wetting time, capillary rise wetting and contact angles methods, while dispersion and 27 solubilisation processes were quantified by the change of particle size and the 28 sediment height after centrifugation. The results showed that these high protein dairy 29 powders generally had poor wettability, especially for whey protein isolate and the 30 31 caseinates, which formed an impermeable layer separating the water surface and powders just after they contacted the water. However, the casein-micellar dominant 32 powders exhibited prolonged dispersion due to strong interactions inside the micellar 33 34 structures. The agglomerates with large particle size and high porosity are expected to exhibit increased wettability. However, agglomeration only caused the external 35 structural modification and thus is difficult to accelerate the dispersion process of 36 37 micellar casein, which can be explained by the milk protein isolate rehydration mechanism. The micellar structure inhibits the release of materials into surrounding 38 liquid phase, which is mainly responsible for the extended rehydration time. 39

Keywords: high protein dairy powders, agglomeration, rehydration, wettability,
dispersibility

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45 **1. Introduction**

The production of milk protein is growing rapidly worldwide due to its 46 advantageous nutritional and functional properties. As milk protein consists of casein 47 and whey protein, different milk protein materials can be produced using different 48 manufacturing processes (Oftedal, 2013). For example, milk protein is obtained from 49 skimmed milk by ultrafiltration to remove lactose and minerals; subsequently micellar 50 casein can be achieved by microfiltration to further separate whey protein; therefore, 51 whey protein is also produced from the permeate (Chandan, 2011; Kilara, 2011; 52 O'Mahony & Fox, 2013). In addition, some non-micellar caseinate, e.g. sodium 53 caseinate and calcium caseinate, can be produced from acid casein by adding alkali 54 solution (sodium hydroxide or calcium hydroxide respectively) (Farrell, Brown, & 55 Malin, 2013; Pitkowski, Nicolai, & Durand, 2009). These milk protein materials are 56 57 widely used in dairy products and infant formula, or used as emulsifiers and stabilisers in food and beverages (Chandan, 2011; Moughal, Munro, & Singh, 2000). 58 However, whatever the type of milk protein, liquid materials are usually spray-dried 59 into the powdered forms for the ease of handling, storage and transportation (Ann 60 Augustin & Clarke, 2011; Selomulya, et al., 2013). In that case, the various milk 61 62 protein powders are necessarily required to be rapidly and completely rehydrated again before use, as complete rehydration is a prerequisite for expressing the 63 functionality of the dried ingredients. The literature has already reported that micellar 64 casein powders were difficult to disperse in water and whey protein powders also 65 have very poor wettability (Gaiani, et al., 2006; Gaiani, Schuck, Scher, Desobry, & 66 Banon, 2007; Schuck, et al., 2007). Comprehensive assessment is still needed for 67 68 the rehydration characteristics of these common milk protein powders. Consequently,

it is of interest to investigate their rehydration ability and understand their rehydrationmechanism.

It is commonly believed that the rehydration process mainly consists of three 71 sequential stages, which are wetting, dispersing and solubilisation. Wetting is the 72 first step where the particles contact liquid while dispersing and solubilisation are the 73 critical phases where primary particles start to release materials from the particle 74 surface into the liquid (Forny, Marabi, & Palzer, 2011; Ji, Fitzpatrick, Cronin, Crean, 75 & Miao, 2016; Richard, et al., 2013). Once any of these three processes is limited, 76 the time for the whole rehydration is prolonged. Casein-dominated powders are 77 believed to be poorly-dispersible due to the strong interactions among the micellar 78 structures. Hence, they usually take a longer time to totally dissolve in water 79 (Baldwin & Truong, 2007; Havea, 2006; Schokker, et al., 2011). However, whey 80 81 protein powders demonstrate poor wetting behaviour where the material floats on the surface of the solution, which is considered to be the rate-limiting factor for whey 82 protein rehydration (Gaiani, Scher, Schuck, Desobry, & Banon, 2009). Therefore, it is 83 necessary to characterise the individual behaviours of milk protein powders during 84 wetting, dispersion and solubilisation processes as different milk protein powders 85 86 exhibit completely different wettability, dispersibility and solubility (Schuck, Jeantet, & Dolivet, 2012). 87

Agglomeration is a particle size enlargement process that creates granulates by adding a binder and forming bridges to link primary particles together. The process is used to change the structural and physical properties by increasing the size of the particles and the voids between particles, and also by decreasing the bulk density of powders (Rajniak, et al., 2007). Hence, the modified structure is believed to influence

the rehydration characteristics of powders. For example, the wetting phase is 93 affected by large particles with large pores, which allow water to penetrate into 94 particles more easily (Hogekamp & Schubert, 2003). Dispersibility is also related to 95 96 the particle size and the density of powders (Goalard, Samimi, Galet, Dodds, & Ghadiri, 2006). Some studies reported that the agglomeration process played a 97 beneficial role in the wetting behaviour of milk protein isolate powders but no 98 significant improvement for dispersion (Gaiani, et al., 2007; Ji, Cronin, Fitzpatrick, 99 Fenelon, & Miao, 2015). Therefore, it is of interest to find out if the applomerated 100 101 powders can positively affect the rehydration behaviours for the cases of other protein powders. The rehydration process of milk protein can be described generally 102 in the following mechanism: wetting of the powders; detachment of powders into 103 104 primary particles; release of materials from particles into the aqueous phase and simultaneous continuous erosion of the surface layer until the collapse of particles 105 and their complete dissolution (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 106 2009). The agglomerated powders may also have an additional step which is the 107 dissolution of the solid bridges linking the particles with the resulting granules 108 dispersing into primary particles (Forny, et al., 2011). To the best of our knowledge, 109 few reports have investigated this mechanism for the rehydration kinetics of milk 110 protein and compared with its agglomerated form. 111

The objective of this study is to investigate the effect of agglomeration on the rehydration properties of high protein dairy powders (protein content >80%). Milk protein isolates (MPI), whey protein isolates (WPI), micellar casein (MC), sodium caseinate (SC), calcium caseinate (CC) are used as the model systems. The results will be used to exhibit the rehydration characteristics and also to better explain the rehydration mechanism of milk protein powders and their agglomerates.

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119 2. Materials and methods

120 2.1 Materials

The composition of the milk protein powders used in this study is showed in 121 Table 1. MPI and SC were supplied by Kerry Ingredients (County Kerry, Ireland). 122 123 WPI was supplied by Davisco Food International (Le Sueur, MN, USA). CC was produced by Teagasc (County Cork, Ireland). Skim milk (Kerry Ingredients, County 124 Kerry, Ireland) was used to produce MC by a pressure driven process with 100 kDa 125 molecular weight membranes and then the obtained retentate was vacuum 126 evaporated to increase the solid content to approximately 38%. The concentration 127 process was performed at 65 °C. Finally, the MC powders were obtained by a spray 128 drying process, where the inlet and outlet temperatures were 180 °C and 85 °C, and 129 the drying air-flow rate was 750 $m^3 h^{-1}$. Before the measurements, all the powders 130 were dried in a vacuum oven (Jeiotech, Seoul, Korea) at 45 °C overnight to obtain the 131 final moisture content of about 1.5% and then kept in the desiccators. 132

133 2.2 Agglomeration process

The agglomeration process of all these milk protein powders was carried out by a top-spray fluid bed granulator (VFC-Lab Micro flo-coater, Vector Corporation, Lowa, USA). 50 g of each model powder was fed into the product vessel. As different milk powders have different fluidisation behaviours in the fluidised bed, the appropriate upward flowing air stream from 30 L·min⁻¹ to 250 L·min⁻¹ was adjusted for the fluidisation of each powder (MPI: 200 L·min⁻¹; WPI: 70 L·min⁻¹; MC: 250 L·min⁻¹; SC:

30 L·min⁻¹; CC: 40 L·min⁻¹). Meanwhile, the adjustable amount of 15% lactose 140 solution binders, based on the different granulation behaviours of these milk protein 141 powders, were injected by a peristaltic pump (1 mL·min⁻¹). (25 g liquid was used for 142 MPI, MC and CC granulation process, while WPI and SC needed 20 g and 10 g 143 binders respectively.) The air pressure on the nozzle was 1 Bar. When the lactose 144 binders had been used up, the agglomerates were dried by air for another 15 145 minutes at 50 °C. After that, all agglomerated powders continued to be dried in the 146 vacuum oven together with the standard powders to ensure similar moisture content. 147

148 2.3 Wettability measurements

Wetting process can be described as: firstly, the interface of solid and gas is 149 replaced by the interface of solid and water; secondly, inward diffusion of the liquid 150 through the capillary structures of the porous powder particle (Yuan & Lee, 2013). 151 Three methods were used to quantify the wettability of powders. Wetting time by 152 immersional wetting procedures can be used as an initial screening and distinguish 153 between powders with general good or poor wettability. Modified Washburn method 154 155 by capillary rise wetting was used to describe the water diffusion capacity of these powders, while contact angle in spreading wetting procedure is a widely used index 156 to evaluate the wettability by water droplet overcoming interfacial tensions between 157 the solid and gaseous phase. 158

159 2.3.1 Wetting time

160 This traditional method evaluates the wettability by measuring the time required 161 to achieve complete wetting. A set quantity of powder is gently discharged onto the 162 surface of water and allowed to immerse spontaneously without agitation. Powder

wetted in less than 60 seconds is usually considered easy to wet while powder which 163 takes longer than 120 seconds is considered non-wettable. Thus, in this study, 6 g of 164 each sample was dropped into a 400 ml beaker containing 100 ml of distilled water 165 at 20 °C (GEA Niro, 2005). The beakers were chosen as the same size with a 166 diameter of 70 mm and a surface area of approx. 38.48 cm². Wetting time is 167 recorded by a timer and all the measurements were repeated three times. Images of 168 WPI particles were also captured by an optical microscope (Olympus BX51M) just 169 after the particles contacted with water on glass slides. Images taken at different 170 171 magnifications were used to show the formation of external layers outside the particles surface, which restrained the water from further wetting of the particles. 172

173 2.3.2 Modified Washburn method

The wettability of powders can also be measured by the Washburn method 174 175 (Washburn, 1921). The detailed principles of a modified Washburn method including the instrument was described in the study of Ji, et al. (2015). It is based on a 176 capillary rise wetting procedure which quantifies the wettability by the weighing of the 177 additional mass of wetted powder as a function of time. A 2 g sample was used each 178 time in this study and was loaded into a cylindrical glass tube with an open base 179 bottom, which was covered by both a piece of filter paper and gauze. After that, the 180 tube was fixed just above the surface of water which allowed the water to penetrate 181 into the particles by capillary force. Finally, the additional mass of wetted powder 182 183 was recorded at 10 minutes. All the measurements for each sample were repeated three times. 184

185 2.3.3 Contact angle

Contact angle (θ) is a widely used primary parameter to quantify the wettability 186 of a solid surface by a liquid (Yuan, et al., 2013). A small contact angle (θ <90°) 187 represents good wettability for the solid, and a large contact angle ($\theta > 90^\circ$) 188 represents poor wetting behaviour. As the wetting behaviour is a dynamic process 189 190 that liquid penetrate into powder bed, in this study, it was followed by observing the changes of contact angles. An optical tensiometer (Attension Theta, Biolin Scientific 191 192 Ltd., Espoo, Finland) was used to measure the contact angle based on sessile drop spread wetting. A 12 µL deionised water droplet was gently dripped on the surface of 193 the powder substrate to carry out the dynamic live measurements at a temperature 194 of 20 °C. The powder substrate was prepared by a leveller to ensure a smooth 195 196 surface formed when measuring the tangent angle at the contact point of the three 197 phases. The change of contact angle was recorded as a function of time and each sample was measured five times. 198

199 2.4 Dispersibility and solubility measurements

200 2.4.1 Particle size measurements

201 In general, the dispersion process corresponds to the decrease of particle size, due to the release of materials from the surface of primary particles (Fang, 202 203 Selomulya, Ainsworth, Palmer, & Chen, 2011). Thus, the change of particle size distribution (PSD) of the suspension during agitation is an applicable method to 204 monitor the dispersion process of milk protein powders, especially for the case of 205 casein-dominant powders with poor dispersibility (Mimouni, et al., 2009). A Malvern 206 Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK) equipped with a 4 207 mW He-Ne laser operating at a wavelength of 632.8 nm was used to measure the 208 PSD in this study. The samples were diluted in a 120 ml dispersion unit, which was 209

filled with 25 °C ± 2 °C distilled water and agitated at a speed of 2000 rpm. The 210 appropriate amount of each milk protein powder was weighed out in order to reach 211 the ideal level obscuration of 8% for the machine. 5 mg of MPI, agglomerated MPI 212 and MC were precisely weighed to make sure the results of specific surface area 213 (SSA) are comparable, while WPI, SC, CC and their agglomerates were weighed 214 100 mg for the measurements. Both PSD and SSA were continuously measured by 215 216 2 minutes intervals as the particles were mixed, until D (50) reached approx.1µm (D (50) is defined as the diameter of 50% particles). Triplicate measurements were 217 218 carried out for each powder.

219 2.4.2 Sediment height

Light transmission technology together with an analytical centrifuge (L.U.M. 220 GmbH, Berlin, Germany) was used to measure the sedimentation behaviour of 221 222 samples. Initial sediment and compressed sediment can be exhibited by the varied intensities of the transmission of NIR light based on different optical density in 223 suspension along the sample cell during the centrifugation (Crowley, et al., 2015). 224 The sediment height was determined by the area of greatest optical density, which is 225 the area of lowest transmission value having subtracted the steady-state value 226 caused by the cell bottom. In this study, 1.5 g of each of the samples was rehydrated 227 into 100 mL 25 °C deionised water to create 1.5% (w/v) concentration suspension. A 228 magnetic stirring bar (length 2.5 cm) was used to agitate the suspension at 400 rpm 229 for 30 minutes, and then followed by sampling 400 μ L at a constant distance (1 cm) 230 from bottom of the beaker to fill into polycarbonate cells. Two centrifugations were 231 set up for the measurements, which included firstly 36 g for 10 minutes and then 232 followed by 168 g for a further 10 minutes. The measurements were performed every 233

10 seconds for the first 10 minutes 36 g centrifugation and then every 60 seconds for
next 10 minutes 168 g centrifugation.

236 2.5 Scanning electron microscopy

2 g of standard MPI powder were added to 100 mL distilled water to create the 237 2% (w/v) suspension. Stirring was performed by an overhead mixer (Eurostar 40 238 239 digital, IKA, Staufen, Germany) and a 4-bladed stirrer of 50 mm diameter (R 1342, IKA) for different rehydration time at 25 °C (60 minutes, 90 minutes and 150 minutes). 240 One or two drops of each MPI suspension was deposited for 5 minutes on the silicon 241 substrates, which were rinsed by 100 mM phosphate buffer (pH=7) in advance. 3% 242 glutaraldehyde solution was used to fix the chemical structures of rehydrated milk 243 protein for 15 minutes (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010a). After 244 that, distilled water was used to wash the samples to remove the fixing chemicals. 245 246 The samples were dehydrated by graded ethanol, which were 50%, 70%, 90% and 100% (Dalgleish, Spagnuolo, & Goff, 2004), and then they were further dried in a 247 desiccator with P₂O₅ until they were suitable to be observed by scanning electron 248 microscopy in a vacuum environment ($\sim 5 \times 10^{-6}$ mbar). A field emission scanning 249 electron microscope (Zeiss Supra, Carl Zeiss Microscopy GmbH, Jena, Germany) 250 was used for imaging at 1.5 kV. 251

252

253 3. Results and discussion

3.1 The wettability of high milk protein powders

According to the results of the immersion wetting procedure (Table 2), all of the 255 milk protein powders are difficult to wet with water, due to their particles floating on 256 the surface of the water and not sinking completely below the surface even after 20 257 258 minutes. It is not surprising because dairy powders with high protein content (>80%) are usually hydrophobic (Havea, 2006; Hussain, Gaiani, & Scher, 2012). Fig. 1 259 shows the WPI particles coated by layers just after contacting water. An 260 impermeable layer was formed at the powder/water interface. This may result in the 261 non-hydrated regions, where water had no access to penetrate into the particles. 262 263 The traditional wettability method based on immersion wetting process provides an initial screening of the milk protein powders. The Washburn method was used to 264 further differentiate these powders with poor wetting behaviours, as illustrated in Fig. 265 266 2. This shows that MC and CC uptake the most weight of water among all the milk protein powders, which were 0.469 g and 0.555 g, respectively. WPI and SC only 267 adsorbed less than 0.1 g water in 10 minutes because water cannot penetrate into 268 these powders spontaneously under the high contact angle (θ >90°) and negative 269 capillary pressure (Lazghab, Saleh, Pezron, Guigon, & Komunjer, 2005; Palzer, 270 Sommer, & Hiebl, 2003). Therefore, milk protein powders, especially WPI and SC, 271 are believed to very quickly reach the point at which the weight of water balances the 272 capillary pressure. 273

274 Contact angle (θ) based on the droplet spreading wetting is also a commonly 275 used index to evaluate the wettability of powders (Gao & McCarthy, 2006). It 276 considers the changes in θ values as a function of time to quantify the dynamic 277 process other than a static state (Mittal, 2006). As shown in Fig. 3A, it can be seen 278 that the contact angle of MC reduced significantly with a final angle of 20° in just 279 about 10 seconds, which showed the best wetting behaviours of all powders.

280 However, for MPI powder, the water droplet took a much longer time (280 seconds) to reach an equilibrium angle. Moreover, in Fig. 3B, CC, SC and WPI were believed 281 to have extremely poor wettability, due to the high initial contact angles only 282 283 changing by about 10° (CC & SC) and 40° (WPI) respectively, even after 300 seconds of measurement. The contact angle results were consistent with the results 284 based on Washburn measurements, except for CC, which took up the most water 285 weight by capillary force. This may be because when CC particles contacted water, 286 they seemed to quickly adsorb water and form a thick gel-like film. That may cause a 287 288 certain amount of water to combine with CC particles and the formed film was likely to prevent further water penetration. However, the precise explanation is still 289 unknown and needs further study in the future. 290

3.2 The dispersibility and solubility of high milk protein powders

The dispersibility of milk protein powders can be quantified by the light 292 scattering method, as it is used to measure the changes of particle size during 293 powder dispersion (Ji, et al., 2015). In Fig. 4A&B, MC and MPI powders exhibited 294 slow dispersion, based on the long mixing time required for the migration of particle 295 size from the original size (about 50 µm) to the size when particles were mostly 296 solubilised (below 1 µm). MC took approximately 20 minutes to reach an equilibrium 297 size while MPI needed 40 minutes to reach complete dissolution. The particle size 298 (D50) of CC powder remained at about 80 μ m, which suggests that the CC particles 299 were mostly not dissolving into water during the 90 minutes. In Fig. 4B, SC exhibited 300 very good dispersion behaviour as its particle size decreased dramatically below a 301 size of 1 µm within just 6 minutes. WPI disappeared very quickly within 2 minutes, as 302 the machine did not detect any signals of particles after 2 minutes mixing. (The 303

dissolved WPI are nanoscale, which is out of detecting range.) At the same time, it is interesting to see that the particle size of MC, MPI and CC increased over the initial couple of minutes. This is due to particles absorbing water just following the wetting stage and this swelling process leads to the increase in particle size at the first stage of dispersion. The same phenomenon was presented by others (Bhandari, Bansal, Zhang, & Schuck, 2013; Gaiani, et al., 2006).

Besides the measurement of particle size, sedimentation is also used to 310 describe the solubilisation behaviour of powders. As explained in the Methods 311 section, sediment formation is determined according to the intensity of light 312 transmission, due to the compressed sediment created the area of greatest optical 313 density (Crowley, et al., 2015). Thus, the lowest transmission value is observed and 314 used to calculate the sediment height in Table 3. It can be seen that WPI and SC 315 316 have no sediment at all after 30 minutes rehydration and no matter 36 g or 168 g centrifugation. These findings were consistent with the previous results based on the 317 rate of change of particle size, which means that WPI and SC are both powders with 318 good dispersibility from the initial particle size to the lower measurement limit of the 319 machine. MC, MPI and CC all had sediment with a height higher than 3 mm under 320 321 36 g centrifugation and about 2.5 mm height under the 168 g centrifugation, after 30 minutes rehydration. This shows that micellar casein-dominant powders, including 322 MPI and MC, require long times to be completely rehydrated because of the strong 323 interactions between the micellar structures, and this has also been concluded by 324 others (Crowley, et al., 2015; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010b; 325 Schuck, et al., 2007). The structures mainly consist of casein micelles, linked 326 327 together by calcium phosphate bridges and surrounded by a layer of casein molecules which helps to stabilize the micelle in solution (Farrell, et al., 2013; 328

McMahon & Oommen, 2013; Schokker, et al., 2011). Thus, the release of micelles 329 from powder particles is time-consuming. However, the artificial non-micellar form of 330 caseinate powders, e.g. SC, are more easily dissolved into water (Pitkowski, et al., 331 332 2009). It is surprising to find that CC showed extremely poor solubility in water at a temperature of 25 °C, which is totally different from the behaviour of SC. Some 333 papers report similar results, including calcium induced aggregation and precipitation 334 of caseinate solution due to the specific binding of calcium which results in 335 aggregation and precipitation that is considered to be hydrophobic (Guo, Campbell, 336 337 Chen, Lenhoff, & Velev, 2003; Thomar, Benyahia, Durand, & Nicolai, 2014). Consequently, CC powders may be difficult to reconstitute into water again, but this 338 still depends on pH and temperature (Moughal, et al., 2000). 339

340 3.3 The effect of agglomeration on the wettability and dispersibility of high milk341 protein powders

342 3.3.1 The effect of agglomeration on wetting behaviours

Firstly, Fig. 2, which is based on the capillary rise wetting procedure, shows that 343 all the agglomerated powders adsorbed more water than the standard powders. 344 Even if the agglomerated WPI, SC and CC formed similar impermeable films as they 345 formed on the surface of the standard powders when contacting the water, the 346 agglomerates still presented comparatively better wetting behaviours. The 347 agglomerated MC was shown to improve in wettability by the most, with a water 348 uptake of 4.9 g in 10 minutes. The agglomerated MPI also showed an increase of 349 350 about 0.8 g. The agglomeration process not only largely increased the size of milk protein particles, but also created the granules with high porosity due to the 351 formation of void structures (Turchiuli & Castillo-Castaneda, 2009). Hence, 352

agglomerated powders usually have better wettability than standard powders, as 353 liquid is more easily able to permeate between the powder particles and wet them 354 more quickly (Lazghab, et al., 2005). Similar results were observed from Fig. 3ABC, 355 356 which compared the droplet contact angles of agglomerated powders and standard powders. The agglomerated MPI took about 50 seconds to obtain the equilibrium 357 angle of 40°, which was a significant decrease in time when compared to the non-358 agglomerated powder. A similar trend was found for agglomerated WPI, which 359 displayed lower contact angles over the 300 seconds measurement time. In 360 361 comparison to WPI and MPI, the agglomeration process appeared to only slightly enhance the wettability of MC, but this is because the contact angle changed so 362 much more rapidly for MC. For agglomerated CC powder, only a small difference of 363 20° was found after water contacting particles for 300 seconds, and there was no 364 obvious difference between SC and its agglomerate for the whole process, as the 365 external layer prevented the droplet penetrating into the particles. 366

Although large particles with loose and porous structures potentially explain the 367 beneficial influence of agglomeration on the wettability of these milk protein powders 368 (Ji, et al., 2015; Yuan, et al., 2013), Table 2 shows that most of the agglomerated 369 370 powders still needed longer than 20 minutes for completely wetting (only agglomerated MPI and MC had significantly shorter wetting times of about 480 371 seconds and 320 seconds, respectively). This is because wetting behaviour depends 372 mainly on the hydrophobicity of milk protein powders, more than their physical and 373 structural properties. In other words, it is not surprising to see that agglomerated WPI, 374 SC and CC maintained long wetting times, as agglomeration can't change the native 375 376 properties of milk protein powders or completely change the difficult-to-wet powders into easy-to-wet ones (Forny, et al., 2011). 377

378 3.3.2 The effect of agglomeration on dispersion and solubilisation behaviours

As described previously, SC and WPI powders dispersed rapidly into water. The 379 effect of agglomeration on these two powders was not significant, as it is difficult to 380 see differences in the dispersion process for the agglomerates and the standard 381 powders based on the measurements of particle size changes (Fig. 4B). Same 382 conclusions are determined from Table 3, where there were no sediments detected 383 384 after 30 minutes rehydration with 36 g or 168 g centrifugation. Therefore, it can be concluded that the influence of agglomeration process on the WPI and SC is limited 385 as they already had good dispersibility and solubility. For the slow-dispersing 386 387 powders (Fig. 4A), there was no measured size increase or swelling at the beginning of the dispersion process, which means that agglomerates have shorter swelling 388 time based on their quicker penetration by water (Ji, et al., 2015). Besides that, the 389 390 rate of change of MPI and CC agglomerate size were shown as being almost the same as their non-agglomerated powders. The agglomerated MC took significantly 391 longer to disperse than the non-agglomerated MC, which means agglomeration 392 prolonged the MC dispersion process (Gaiani, et al., 2007; Schuck, et al., 2007). It is 393 commonly believed that the slow dispersion process of MPI or MC is caused by the 394 395 slow water transfer into the "skin" of inter-linked casein micelles (Mimouni, et al., 396 2010a). The agglomeration process just modifies the physical structures by binding particles together but does not accelerate the release of materials from the primary 397 particles, which is responsible for the extended dispersion time of micellar casein 398 powders. Generally, the possible ways to increase the dispersion rate are based on 399 the destruction of the micellar structure by adding mineral salts, e.g. phosphate or 400 401 citrates solutions (Schuck, et al., 2002), or by the physical approach of ultrasonication (McCarthy, Kelly, Maher, & Fenelon, 2014). The casein micelles are 402

dissociated and then quickly dissolved into water. Therefore, fluidised bed agglomeration process played no significant beneficial role on the dispersion of micellar casein powders. Similarly, the solubility of CC precipitate is also influenced by the chemical environment rather than the physical structural modification produced by agglomeration (Thomar, et al., 2014).

Consequently, it may be concluded that the effect of agglomeration on the 408 wettability and dispersibility of dairy powders are different. According to the results of 409 five dairy powders that were investigated in this study, their wetting behaviours were 410 found to be dependent on the modification of physical properties while their 411 dispersing behaviours were mainly controlled by the native dispersibility of primary 412 particles. Thus, the agglomeration process may accelerate the rehydration of 413 powders whose wetting process is their rate-limiting step, such as WPI. If for the 414 415 powders with dispersing as their rate-limiting step, such as MC, agglomeration plays no positive role and may even negatively influence rehydration. However for MPI, 416 both wetting and dispersing processes are rate-limiting steps. Hence, it is necessary 417 to evaluate the effect of agglomeration on a complete rehydration process to find out 418 whether it shortens or prolongs the rehydration time (Ji, et al., 2015). Some other 419 420 powders like SC and CC may have problems in wetting or dispersing due to the individual native hydration behaviours (Post, Arnold, Weiss, & Hinrichs, 2012). 421 Therefore, agglomeration is believed not to significantly change their rehydration 422 properties. 423

424 3.4 The mechanism for the rehydration process of MPI powders

The MPI powder exhibited how the specific surface area (SSA) and D (50) of particles changed as they dispersed and dissolved into water, which can be

observed in Fig. 5A. Different from the gradually decrease of D (50), it is interesting 427 to find that the SSA increased very slowly for the first 20 minutes but it is followed by 428 dramatic growth for the next 40 minutes. It can be attributed to the disruption of the 429 430 aggregates and the release of the primary particles with smaller size at the beginning of the dispersion process, but it only caused a slight increase in SSA. However, after 431 20 minutes of stirring time, the materials from micellar structures started to be 432 released into the surrounding water. In that case, the SSA increased significantly as 433 more and more materials dissolved. It also can be explained by Fig. 5B, which 434 435 exhibited the volume density of unagglomerated MPI particles with different size for the different stirring time. Similarly, the volume of large particles decreased slowly at 436 first 20 minutes but decreased sharply due to the collapse of the structures. 437 Therefore, the dissolved small particles appeared from 20 minutes and the volume of 438 these particles increased rapidly during the solubilisation process. 439

The dissolution mechanism of standard milk protein isolate can be described in 440 the following steps: i) powder particles come into contact with water; ii) particles start 441 to de-agglomerate from aggregates into the individual primary particles; iii) the 442 continuous release of materials from the surface of these primary particles into 443 444 aqueous phase; iv) erosion of the external layer of particles and finally full dissolution. It considers that milk protein powder produced by atomization in a spray-dryer 445 usually consists of primary particles and these particles contain internal vacuoles 446 based on the spray dry process (Bhandari, et al., 2013; Fang, et al., 2011). Thus, the 447 dispersion process of milk protein isolate is mainly contributed by the disappearance 448 of the aggregates of primary particles and also the release of micellar casein 449 450 materials (McKenna, 2000).

The SEM images in Fig. 6 clearly demonstrate the erosion of the particle 451 surface under the effect of water transfer during the rehydration process. Before 452 wetting by water, MPI particles have smooth surfaces, which can be seen from Fig. 453 454 6A. After wetting and dispersing for a period of time, the most significant difference is the increase in the roughness of the surface of the rehydrating particles. This is due 455 to water beginning to penetrate the external layer of micellar casein (Fig. 6B). For the 456 longer rehydration periods, a much rougher surface was observed and large 457 breaches appeared to indicate the further erosion of the outer skin by water (Fig. 6C), 458 459 but the particle still did not completely break down due to the strong interactions between the micellar structures. Finally, sufficient materials were solubilised and the 460 insides of micelles were exposed leading to the eventual collapse of the structures 461 (Fig. 6D). Therefore, it is believed that the compactness skin outside of micellar 462 casein particles is responsible for restraining the individual micelles into the 463 surrounding liquid phase. 464

The agglomerated MPI were found to undergo a very similar solubilisation 465 process as the non-agglomerated MPI with the only difference being at the beginning 466 of the dispersion (Fig. 5A). The size of the agglomerates decreased sharply to about 467 468 75 μ m, which is almost the same as the unagglomerated particles after swelling. It is due to the lactose solid bridges linking the particles being easily dissolved and the 469 470 agglomerates being quickly dispersed into the non-agglomerated particles. This is exhibited by the schematic of agglomerated MPI rehydration in Fig. 5A. (Forny, et al., 471 2011; Schubert, 1987). After that, there was no significant difference between 472 agglomerates and non-agglomerates during the solubilisation process. Consequently, 473 agglomerated MPI is believed to have one additional step at first, which is the 474 475 dissolution of the solid bridges.

476

477 **4. Conclusion**

The rehydration properties of five common high protein milk powders and their 478 agglomerates were investigated in this study. The application of a variety of 479 measurement techniques provided a more complete insight into the rehydration 480 481 behaviour of the powders which exhibited different wetting and dispersion behaviours. Poor wettability was the rate-limiting factor for WPI and SC rehydration, 482 483 while poor dispersibility was the main problem for MC rehydration. Both MPI and CC displayed both poor wettability and poor dispersion. Agglomeration had a beneficial 484 role on the droplet penetration process, and this was more significant for WPI and 485 MPI powders. In capillary rise wetting procedure, agglomerated MPI and MC present 486 the significantly better wettability. Agglomeration had no beneficial effect on 487 dispersibility as it does not influence the structure of the primary particles. In fact, it 488 may even have slowed dispersion by adding the additional step of dissolving the 489 solid bridges between the particles. 490

491

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 Table 1 Composition and particle size of milk protein powders

 Table 2 Wetting time of milk protein powders

Table 3 Sediment height after 36 g and 168 g centrifugation for both 10 minutes

Fig.1. Optical microscopy images of WPI particles, upon which formed the impermeable hydrophobic layers when wetted by water. The particles were dispersed in water on glass slides. (Left: scale = $200 \ \mu$ m; Right: scale = $50 \ \mu$ m)

Fig.2. Weight of adsorbed water for samples (MPI, WPI, MC, SC, CC and their agglomerates) by capillary rise wetting after 10 minutes.

Fig.3ABC. A: The change of contact angle (°) as a function of time for MPI, MC and their agglomerates using the sessile drop technique in approx. 20 °C temperature. **B:** The change of contact angle (°) as a function of time for WPI, SC, CC and their agglomerates. **C:** Images of MPI, WPI and MC as examples to show how the water droplets penetrated into powders by the different time intervals.

Fig.4AB. A: Particle size D (50) measurements of dispersed particles of MPI, CC and their agglomerates for every 2 minutes in $25 \pm 2^{\circ}$ C water. **B:** Particle size D (50) measurements of SC, MC and their agglomerates. (WPI quickly dispersed into water and Mastersizer cannot detect its particle size. Even agglomerated WPI can only be measured for the first 4 minutes)

Fig.5AB A: Schematic of MPI and agglomerated MPI particles rehydration in water, based on the change of particle size D (50) and specific surface area as a function of time. **B:** Volume density (%) based size distributions of dispersing MPI particles measured as a function of time. It showed the volume concentration of both undissolved and dissolved powder particles.

Fig.6ABCD SEM images of MPI particles after different rehydration time. **A**: spray-dried particles before rehydration (1.4 kV); **B**: particles after 60 minutes rehydration (1.5 kV); **C**: particles after 90 minutes rehydration (1.5 kV); **D**: particles after 150 minutes rehydration (1.5 kV). The white arrow in panels C indicates the presence of large breaches caused by water erosion.

	MPI		WPI		MC		SC		CC	
	NA	А								
Protein (%, w/w)	86.0	82.0	90.0	84.0	84.0	80.1	88.0	86.0	87.0	81.4
Lactose (%, w/w)	1.0	6.2	1.0	6.5	2.0	7.3	0.1	2.4	0.2	6.6
Moisture (%, w/w)	4.0	5.0	5.0	5.2	5.0	6.8	5.7	5.9	5.5	6.3
Particle size D(50) (µm)	49.3 ± 1.5	188.0 ± 2.0	54.5 ± 1.8	179.0 ± 4.0	50.0 ± 1.4	220.0 ± 6.0	85.0 ± 1.0	208.0 ± 3.0	65.7 ± 2.1	194.0 ± 3.0

 Table 1 Composition and particle size of milk protein powders

*NA = non-agglomerated; A = agglomerated.

 Table 2 Wetting time of milk protein powders

		MPI	WPI	MC	SC	СС
Wetting	NA	>1200	>1200	>1200	>1200	>1200
(seconds)	А	480 ± 120	>1200	320 ± 14	>1200	>1200

*NA = non-agglomerated; A = agglomerated.

		MPI		WPI		MC		SC		CC	
		NA	А	NA	А	NA	А	NA	А	NA	А
Sediment height (mm)	36 g x 10 min	3.25 ± 0.05	2.75 ± 0.11	0	0	3.23 ± 0.12	3.65 ± 0.23	0	0	3.63 ± 0.17	3.87 ± 0.17
	168 g x 10 min	2.45 ± 0.05	1.95 ± 0.10	0	0	2.62 ± 0.10	2.78 ± 0.15	0	0	2.57 ± 0.12	2.70 ± 0.06

Table 3 Sediment height after 36 g and 168 g centrifugation for both 10 minutes

*NA = non-agglomerated; A = agglomerated.















