

# Spray-drying of protein/polysaccharide complexes: Dissociation of the effects of shearing and heating

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1	Spray-drying of protein/polysaccharide complexes: dissociation of the
2	effects of shearing and heating
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## 23 Abstract

24 The aim of this study was to dissociate the effect of atomization from that of heating during the spray-drying of Low Methoxyl (LM) pectin/sodium caseinate complexes. The properties 25 of these complexes were studied by measuring turbidity, particle size distribution, 26 zeta-potential, as well as surface hydrophobicity of caseinate within the formed complexes. 27 The results showed that the spraying step had a significant effect on the charge and the size of 28 29 the complexes. In fact, the application of atomization resulted in the dissociation of caseinate/pectin aggregates especially for high pectin concentrations. Besides, the analysis of 30 31 the surface hydrophobicity of caseinate indicated that complexation with high concentrations of pectin is able to protect the structure of the protein against heat denaturation. This study 32 allowed a better understanding of the influence of atomization and heat treatment (during the 33 dehydration step) on the molecular interactions within caseinate/pectin complexes. 34

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Keywords: LM pectin, sodium caseinate, atomization, complexes, thermal stability

# 37 **1. Introduction**

In the past few decades, spray-drying technique has been widely used for encapsulation of food 38 39 ingredients, which focus on protecting and controlling the release of active compounds. During this drying process, the solvent, that is most often water, will be rapidly evaporated, and the 40 active compounds will be entrapped with encapsulating material (Gharsallaoui, Roudaut, 41 Chambin, Voilley, & Saurel, 2007). There are four stages involved in spray drying, namely (i) 42 atomization of the feed solution, (ii) contact of spray with the hot gas, (iii) evaporation of 43 moisture and (iv) particle separation. The loss of free water results in a reduction of water 44 activity and thus extend the shelf life of food products. However, the spray-drying process is 45accompanied with thermo-mechanical stresses on the active compounds through exposure to 46 high temperatures, distribution of air-water interfaces, shearing, and dehydration (Ben Amara, 47Eghbal, Degraeve, & Gharsallaoui, 2016). During the spray-drying process, the pressure is 48 49 mainly from two stages, the stage of atomization (distribution of air-water interfaces, shearing) and the stage of heating (high temperature, dehydration). Atomization is a very important and 50 complex step (Foerster, Gengenbach, Woo, & Selomulya, 2016; Wittner, Karbstein, & Gaukel, 5152 2018), which is same for other microencapsulation technologies like spray-cooling or spray-chilling. Heat treatment can easily change the structure of thermal sensitive material, 53 especially for protein. This process may cause reversible or irreversible denaturation of proteins, 54 55 which involves the disruption and possible destruction of both the secondary and tertiary structures. When proteins denatured, they can lose solubility to aggregation due to the exposure 56 of hydrophobic groups and some bio-functional proteins (enzymes) can lose their biological 57 function due to the loss of the active site. 58

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The research concerning the formation of complexes between proteins and polysaccharides

has received more and more attention (Devi, Sarmah, Khatun, & Maji, 2017; Li & de Vries,
2018). In fact, adding polysaccharides to form protein-polysaccharide complexes can somehow
protect protein against denaturation during spray-drying process as shown in our previous works
(Ben Amara, Eghbal, Degraeve, & Gharsallaoui, 2016; Ormus, Oulahal, Noël, Degraeve, &
Gharsallaoui, 2015).

Sodium caseinate is a kind of milk proteins containing  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -, and  $\kappa$ -caseins. These four 65 proteins have a strong tendency to associate with each other to form supra-molecular aggregates 66 (Surh, Decker, & McClements, 2006). Pectin is a natural polysaccharide found as a component 67 of cell walls in fruits and some roots, which is widely used in the food industry for its thickening 68 and gelling properties. It is composed by a chain of  $\alpha$ -(1-4)-linked D-galacturonic acid. 69 According to the degree of methyl esterification, pectin can be classified as high-methoxyl (HM) 70 and low-methoxyl (LM) pectin. Compared to HM pectin, LM pectin requires less sugar to form 7172 gels, and can form gels in a wider range of pH (from 2.6 to 7.0). The system of pectin and caseinate is extensively investigated since over twenty years (Cheng & McClements, 2016; 73 Matia-Merino, Lau, & Dickinson, 2004; Turgeon, Schmitt, & Sanchez, 2007) and it was recently 74 75explored in our previous studies (Eghbal, Degraeve, Oulahal, Yarmand, Mousavi, & Gharsallaoui, 2017; Eghbal, Yarmand, Mousavi, Degraeve, Oulahal, & Gharsallaoui, 2016). According to these 76 studies, complex coacervation of caseinate and LM pectin could happen at pH 3. At this pH, 7778 positively-charged caseinate and negatively-charged pectin can form insoluble complexes due to electrostatic interactions. A greater understanding of how caseinate interact with pectin is 79 essential to control structure, functionality and applicability of this system. 80

81 In this study, we separated the atomization process and heating process in spray-drying by 82 dissociating the atomizer from spray-drier and replacing heating process with water bath heat treatment. To the best of our knowledge, the separate effects of atomization and heating steps during spray-drying on the properties of protein/polysaccharide complexes were not reported in literature. Through testing the physicochemical properties of caseinate/pectin complexes before and after atomization and heat treatment, we hoped to investigate the effect of atomization and heat treatment on the formation of protein/polysaccharide complexes.

# 89 **2. Materials and methods**

#### 90 2.1. Materials

LM pectin (Unipectine<sup>™</sup> OF 305 C SB) was purchased from Cargill (Baupte, France). The degree of esterification was from 22% to 28% and the degree of acetylation was from 20% to 23%. Sodium caseinate was purchased from Fisher Scientific (United Kingdom). Protein content in sodium caseinate determined by the Kjeldahl method was 93.20% (nitrogen conversion factor N=6.38). Analytical grade imidazole (C<sub>3</sub>H<sub>4</sub>N<sub>2</sub>), acetic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), 8-Anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma-Aldrich Chimie (St Quentin Fallavier, France).

# 98 **2.2. Solution preparation**

Imidazole-acetate buffer solutions (5 mM) were prepared by dispersing imidazole and 99 acetic acid into distilled water and then adjusting the pH to 3.0. The stock solutions were 100 prepared by dispersing LM pectin and sodium caseinate powders to unadjusted imidazole-acetate 101 102 buffer and stirring for at least 2 h at room temperature, and then adjusting the pH to 3.0. The pH was adjusted by using HCl (0.1 or 1.0 M) or NaOH (0.1 or 1.0 M). Complexes solutions were 103 prepared by mixing the stock solutions and then diluted to the desired concentration by 104 imidazole-acetate buffer (pH 3.0). The final sodium caseinate concentration was constant at 5.0 105 g/L, and the LM pectin concentration was 0.5, 2.0 and 6.0 g/L. After mixing, the complexes 106 solutions were stirred for at least 2 h at room temperature and the pH was checked again. 107

# 108 **2.3. The atomization process**

109 The atomizer (Mini spray-dryer B-290) was manufactured by BUCHI (Switzerland). The 110 atomizer nozzle was disassembled to prepare the complex solutions after atomization. The

nozzle consists of three inputs: an air inlet and two inputs for the liquid as well as an outlet. An 111 atomizer pump that set at a flowrate of 0.5 L/h was used to feed the nozzle with the previously 112113 prepared complex solutions. In order to study the effect of the shearing applied during the spray-drying process, the sodium caseinate/LM pectin complex mixtures were subsequently 114 sprayed at a rate of 0.5 L/h through the nozzle of the atomizer (complex after spraying) and 115 recovered in containers until the tests are carried out. All the atomization experiments were 116 carried at room temperature, the suspensions of complexes before and after spraying were also 117 maintained at room temperature. 118

## 119 **2.4. Heat treatment**

During spray-drying, the suspensions of complexes are exposed to high temperatures during a very short time, which makes it possible to dry the sprayed micro-droplets rapidly. For separately studying the effect of the heat treatment, the various mixtures obtained (in 10 mL tubes) were heated in a water bath at 80 °C for time intervals set at 1 min (T1), 2 min (T2), 3 min (T3) and 4 min (T4). Complexes that underwent heat treatment were compared with unheated complexes (T0).

#### 126 **2.5. Turbidity measurement**

The turbidity of all complexes suspensions at pH 3 were measured by using a UV/Vis spectrophotometer (Jenway 3705, Villepinte, France) at 600 nm in plastic cuvettes (1 cm path length). Each suspension was diluted 3 to 6 times with imidazole-acetate buffer at pH 3 until the optical density value of each diluted suspension was less than 1.5. The solution of (5.0 g/L) sodium caseinate was used as a control during the measurements, and every suspension was analyzed at least three times.

#### 133 **2.6. Particle size distribution**

Particle size distributions of sodium caseinate/LM pectin complexes were assessed by a laser diffraction instrument (Mastersizer 3000, Malvern Instruments, Malvern, UK). To avoid multiple scattering effects, the complexes were injected into the measurement chamber where it was diluted with imidazole-acetate buffer (5 mM; pH 3) prior to the measurements. The complexes were continuously stirred throughout the measurement to ensure the samples were homogeneous. The volume mean particle diameter ( $D_{43}$ ) was calculated by the software from the three injections of three separate samples with five readings per sample.

141 **2.7. Zeta potential measurement** 

142 The electric charges ( $\zeta$ -potential) of sodium caseinate/LM pectin complexes were 143 determined using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). If necessary, the 144 samples were diluted with pH 3 imidazole-acetate buffer. The mean  $\zeta$ -potential (ZP) values (±SD 145 (standard deviation)) were obtained from the instrument, standard deviation of measurements 146 that are repeated three times for each suspension.

147 **2.8. Measurement of surface hydrophobicity** 

For the surface hydrophobicity study, the 8-anilino-1-naphthalenesulfonic acid (ANS) 148 fluorescent probe was used (Bi, Tang, Gao, Jia, & Lv, 2016). For each suspension studied, 149 increasing volumes of the probe (20 mg/L) ranging from 10 to 30 µL were added to 4.0 ml of the 150suspension. The maximum fluorescence intensity due to the binding of the ANS molecules to the 151hydrophobic sites of the protein was measured by carrying out a scanning of the wavelength 152between 420 nm and 650 nm for an excitation wavelength of 380 nm. The excitation and 153emission bandwidths were set at 5.0 nm. The scan rate was 500 nm/min. Fluorescence intensity 154 measurements were performed with a LS 55 spectrofluorometer (PerkinElmer, Courtaboeuf, 155

France). All measurements were performed at room temperature using a quartz cuvette (4 faces, 1 cm path length). The addition of the probe was achieved until no increase of the fluorescence intensity was observed. The maximum fluorescence intensity was plotted against the concentration of ANS. The initial slope of the curve of maximum fluorescence intensity (arbitrary unit, U.A) versus ANS concentration (mg/L) was calculated and used as an index of surface hydrophobicity ( $S_0$ ). For each suspension, the test was repeated three times.

#### 162 **2.9. Statistical analysis**

All assays were measured at least in triplicate. Means and standard deviations were calculated and differences between means were determined with the Least Significant Difference (LSD) test at p < 0.05 significance level (Statgraphics Centurion XV).

# 167 **3. Results and discussion**

#### 168 **3.1. Effect of atomization on the properties of caseinate/pectin complexes**

169 The effect of atomization (shearing) on the properties of caseinate/pectin complexes was studied at three different ratios (sodium caseinate concentration kept constant: 5.0 g/L; LM 170pectin concentration changed: 0.5, 2.0 or 6.0 g/L). Before atomization, the turbidity of 171complexes increased (Fig. 1a) with increasing pectin concentration while the average size 172 reached a peak (Fig. 1b). This indicated that, with addition of pectin, caseinate could combine 173more and more pectin to form complexes leading to a growing size until a pectin concentration 174 of 2 g/L and turbidity increase. When pectin amount continued to increase, the particle size 175decreased leading to the formation of numerous small complexes resulting in the still increase of 176 turbidity. 177

As shown in Fig. 2a, the  $\zeta$ -potential values of complexes changed from positive to negative 178with increasing pectin concentration, due to the constant amount of positively charged caseinate 179 molecules neutralized by the increase amount of negatively-charged pectin. At the low (0.5 g/L) 180 and high (6.0 g/L) concentrations of pectin, the caseinate/pectin complexes were highly charged, 181 182 and at intermediate (2.0 g/L) concentration of pectin, the complexes were more neutrally charged. These results also confirmed that the size decrease at high (6.0 g/L) concentration of pectin was 183 due to electrostatic repulsions. There was a slight decrease of the protein hydrophobicity only at 184 high pectin concentration (6.0 g/L) (Fig. 2b), which means that hydrophobic sites of caseinate 185 would be masked by the pectin chains. 186

After atomization, the turbidity of complexes was almost constant at low (0.5 g/L) and intermediate (2.0 g/L) concentrations of pectin. At high concentration (6.0 g/L) of pectin, the turbidity of complexes significantly decreased (p < 0.05) after atomization (Fig. 1a). The average

size of complexes significantly decreased (p < 0.05) at all the 3 tested ratios (Fig. 1b), which is in 190 agreement with other published studies (Serfert, Schröder, Mescher, Laackmann, Rätzke, Shaikh, 191 192 et al., 2013). The partial dissociation of complexes was due to shear forces during their passage in the nozzle and an increase in their overall charge favoring electrostatic repulsions, especially 193 at high concentration (6.0 g/L) of pectin (Fig. 2a). In addition, there was a significant increase of 194 the hydrophobicity S<sub>0</sub> (particularly at 2.0 g/L and 6.0 g/L of pectin), which could indicate 195 196 molecular rearrangements and unfolding of the structure of the protein caused by exposure of hydrophobic sites (Fig. 2b). 197

#### 198 **3.2.** Effect of heat treatment on the properties of caseinate/pectin complexes

Heat treatment had a significant effect on the turbidity and the size of the complexes (Fig. 3). With the increase of the heat treatment time, the turbidity value of complexes increased (p < 0.05) from  $0.98 \pm 0.05$  to  $2.87 \pm 0.28$  cm<sup>-1</sup> at the lower concentration of pectin. It was almost constant (~ 4.22 cm<sup>-1</sup>) at the intermediate concentration of pectin. However, at the higher concentration of pectin, the turbidity value of complexes increased (p < 0.05) from  $5.29 \pm 0.09$  cm<sup>-1</sup> to  $6.78 \pm$ 0.04 cm<sup>-1</sup> until the 4<sup>th</sup> min where we had a decrease up to  $6.05 \pm 0.17$  cm<sup>-1</sup> (Fig. 3a).

205 As shown in Fig. 3b, the size of complexes formed at low concentration of pectin (0.5 g/L) was constant as a function of heating time (55.77  $\pm$  4.53 µm). Combined with the increased 206 turbidity (Fig. 3a), it can be concluded that heat treatment caused the formation of more 207 numerous particles which had the same size as the caseinate/pectin complexes at low pectin 208 concentration. At intermediate concentration of pectin (2.0 g/L), the size increased from  $264.17 \pm$ 209 26.36 to  $380.80 \pm 35.05 \ \mu m \ (p < 0.05)$  with increase of heat treatment time. Combined with the 210 stable turbidity (Fig. 3a), it clarified that heat treatment caused the aggregation of complexes at 211 this pectin concentration. The most interest part was the significant decrease (p < 0.05) of 212

particle size from 220.46  $\pm$  38.08 to 104.16  $\pm$  29.62 µm at high concentration of pectin (6.0 g/L), with increase of heating time. This result indicated that heat treatment caused dissociation of aggregates. This also explained the decrease of turbidity after 4 min of heating time (Fig. 3a). High concentration of LM pectin may have induced bridging flocculation of protein aggregates (Jones, Lesmes, Dubin, & McClements, 2010). It can be assumed that, after heating treatment, bridging flocculation was suppressed.

At low concentration of pectin, slight decrease (p < 0.05) in  $\zeta$ -potential (Fig. 4a) and 219 increase in surface hydrophobicity (Fig. 4b) indicated that the structure of complexes changed. 220 Anionic groups of pectin and hydrophobic sites in caseinate molecules could be exposed 221 following heat treatment. The change of the electrostatic charge from positive to negative and a 222 slight increase in the hydrophobicity at intermediate concentrations of pectin indicated that the 223 molecular rearrangement allowed to "protect" the protein from heat denaturation. At high 224concentration of pectin, surface hydrophobicity was almost constant as the increasing time, 225 which confirmed the protective effect of pectin against heat treatment. According to Jones, 226 Decker and McClements (2010), the ability of LM pectin to increase the thermal denaturation 227 228 temperature may be due to differences in the electrostatic interactions between carboxyl groups on the pectin molecules and charged groups on the folded and unfolded protein surfaces. 229

# **3.3. Combined effect of atomization and heat treatment on the properties of complexes**

To simulate the whole spray-drying process, the caseinate/pectin complexes were atomized and subsequently heated. Combined effect of atomization and heat treatment was evaluated by measuring the properties of atomized complexes treated by heating at 80 °C water bath for 0 to 4 min.

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As shown in Fig. 5, with increasing heat treatment duration, at low concentration of pectin

(0.5 g/L), the turbidity of atomized complexes slightly increased (p < 0.05) from 1.05 ± 0.17 cm<sup>-1</sup> to 2.08 ± 0.03 cm<sup>-1</sup> and the particle size was constant (44.52 ± 5.73 µm). The increase in number of particles could be due to the aggregation of free or partially complexed caseinate molecules. Compared to the properties of caseinate/pectin complexes directly treated by heating without atomization at low pectin concentration (Fig. 3), the atomized complexes had a smaller size and the turbidity of atomized complexes as function of heat treatment duration increased more slightly.

At intermediate pectin concentration (2.0 g/L), with the increase of heating duration, 243 compared to the stable turbidity of caseinate/pectin complexes without atomization (Fig. 3a), 244 heat treatment caused the slight decrease (p < 0.05) in turbidity of atomized complexes from 4.42 245 $\pm 1.00$  cm<sup>-1</sup> to 2.93  $\pm 0.12$  cm<sup>-1</sup> (Fig. 5a), and significant increase (p < 0.05) in particle size from 246  $115.70 \pm 34.17$  to  $379.22 \pm 24.72 \ \mu m$  (Fig. 5b). Thus, we can assume that the association 247 between small complexes could be accentuated by electrostatic charge neutralization (Fig. 5c). 248 Compared to the caseinate/pectin complexes directly treated by heating without atomization, 249 atomized complexes formed at intermediate pectin concentration were more sensitive to heat 250 251 treatment.

Atomized complexes formed at high pectin concentration (6.0 g/L) showed a constant particle size ( $32.61 \pm 4.62 \mu m$ ) and an increased turbidity from  $3.85 \pm 0.06 \text{ cm}^{-1}$  to  $8.97 \pm 0.97$ cm<sup>-1</sup> with the increase of heat treatment duration (Fig. 5). Similar results were also found in our previous research (Amara, Eghbal, Degraeve, & Gharsallaoui, 2016). The increase of turbidity could be due to thermal dissociation of aggregates, which was already deconstructed by shearing, and formation of new complexes with free pectin chains. Highly negatively charged complexes could be protected from aggregation by electrostatic repulsions (Fig. 5c).

# 260 **4. Conclusions**

In this study, caseinate/pectin complexes were prepared at pH 3 at three different caseinate/pectin ratios. To evaluate the separate effect of mechanical and thermal steps on the complexes during the spray-drying process, atomization and heat treatment were dissociated and applied to the caseinate/pectin complexes. The properties like turbidity,  $\zeta$ -potential, particle size and surface hydrophobicity of complexes were measured.

The results indicated that atomization caused dissociation of aggregates into smaller size 266 complexes at all pectin concentrations. At low concentration of pectin, heat treatment caused the 267 aggregation of excess caseinate. At intermediate concentration of pectin, heat treatment 268 intensified aggregation of complexes. At high concentration of pectin, heat treatment favored the 269 dissociation of the aggregates. Complexes after atomization were more sensitive to heat 270 treatment, but a high concentration of pectin could protect the complexes against heat 271aggregation. These results could help to develop new delivery systems based on spray-dried 272 protein/polysaccharide complexes. 273

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## 333 Figure Captions

Fig. 1. Effect of atomization on turbidity (a) and particle size (b) of caseinate/pectin complexes at three different concentrations of pectin (the concentration of caseinate was kept constant at 5.0 g/L; pectin concentrations was varied from 0.5 to 6.0 g/L; pH 3).

Fig. 2. Effect of atomization on ζ-potential (a) and surface hydrophobicity (b) profiles of
caseinate/pectin complexes at three different concentrations of pectin (the concentration of
caseinate was kept constant at 5.0 g/L; pectin concentrations was varied from 0.5 to 6.0 g/L; pH
3).

**Fig. 3.** Turbidity (a) and particle size (b) of caseinate/pectin complexes at three different concentrations of pectin as function of heat treatment duration from 0 to 4 min at 80 °C (**T0**: unheated control, **T1**: 1 min, **T2**: 2 min, **T3**: 3 min, **T4**: 4 min; the concentration of caseinate was kept constant at 5.0 g/L; pectin concentrations was varied from 0.5 to 6.0 g/L; pH 3).

Fig. 4. ζ-potential (a) and surface hydrophobicity (b) of caseinate/pectin complexes at three
different concentrations of pectin as function of heat treatment duration from 0 to 4 min at 80 °C
(T0: unheated control, T1: 1 min, T2: 2 min, T3: 3 min, T4: 4 min; the concentration of
caseinate was kept constant at 5.0 g/L; pectin concentrations was varied from 0.5 to 6.0 g/L; pH
3).

Fig. 5. Combined effect of atomization and heat treatment (80 °C) on turbidity (a), particle size
(b) and ζ-potential (c) of caseinate/pectin complexes at three different concentrations of pectin
(T0: unheated control, T1: 1 min, T2: 2 min, T3: 3 min, T4: 4 min; the concentration of

- 353 caseinate was kept constant at 5.0 g/L; pectin concentrations was varied from 0.5 to 6.0 g/L; pH
- **3**).











