1 Flavor release from spray-dried amorphous matrix: effect of lactose
2 content and water plasticization

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21 **Abstract**

22 Glass-forming carbohydrates are widely used as matrix for encapsulation of nutrients and
23 bioactive compounds. In this study, encapsulation systems with lactose/whey protein isolate
24 (WPI) mixtures (4:1, 1:1, and 1:4), or WPI as wall materials and ethyl butyrate as core
25 material were prepared by spray drying. The effects of lactose content and water
26 plasticization on encapsulation efficiency and flavor release were investigated. Wall material
27 consisting of lactose/WPI (4:1) mixture had significantly ($P < 0.05$) higher encapsulation
28 efficiency. The flavor retention in powders did not have significant decrease with
29 equilibration at 0.33 a_w , while it was dramatically decreased at 0.54 a_w and 0.65 a_w as a result
30 of lactose crystallisation. Mechanical property study showed that the molecular mobility and
31 free volume of encapsulation systems with higher lactose content increased more
32 significantly with increasing water content, which accelerated the diffusion of flavor
33 molecules. Those results may use in the assessment of protection and release characteristics
34 of flavor components in formulated systems.

35 **Keywords:** Flavor encapsulation; Glass transition; Mechanical properties; Amorphous matrix;
36 Spray drying

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43 **1. Introduction**

44 In food industry, there is a growing interest for encapsulation technologies, which are
45 designed to protect the encapsulated materials and to allow controlled release. Many volatile
46 compounds are encapsulated in solid carriers to increase their protection, reduce evaporation,
47 and promote easier handling (Bae & Lee, 2008; Baranauskiene, Bylaite, Zukauskaite, &
48 Venskutonis, 2007; Beristain, Vazquez, Garcia, & Vernon-Carter, 1996; Chin et al., 2010;
49 Goubet, Le Quere, & Voilley, 1998; Jafari, He, & Bhandari, 2007; Kaushik & Roos, 2007).
50 Since flavoring components are prone to loss by evaporation, oxidation or ingredient
51 interactions, the encapsulation of flavor ingredients is among the most important applications
52 in the food industry (Baranauskiene et al., 2007). Encapsulation matrix for flavor compounds
53 can be selected from a wide variety of polymers, depending on the core materials and desired
54 characteristics of the microcapsule. Typical wall materials include proteins (sodium caseinate,
55 whey proteins, soy proteins and gelatin) and hydrocolloids (modified starch and gum arabic).
56 Among proteins, whey proteins have been shown to be an excellent encapsulating agent for
57 microencapsulation of oils/fats and volatiles (Bae & Lee, 2008; Charve & Reineccius, 2009;
58 Moreau & Rosenberg, 1996; Rodea-González et al., 2012; Rosenberg & Sheu, 1996; Sheu &
59 Rosenberg, 1995). Moreover, although a variety of methods have been proposed to
60 encapsulate flavors, spray-drying and extrusion are still the most common techniques. It has
61 been shown that wall systems of spray-dried microcapsules consisting of whey proteins
62 provide effective protection against core oxidation (Bylaitė, Venskutonis, & Maždžienė,
63 2001; Kim & Morr, 1996).

64 Previous studies have indicated that the addition of small quantities of specific low
65 molecular weight compounds to matrices can improve the storage stability of bioactive
66 (Roussanova, Murith, Alam, & Ubbink, 2010). These low molecular weight compounds act
67 as packing enhancer, leading to a reduction of the molecular hole size in the glassy state,

68 which could explain the improved glassy-state barrier properties and encapsulation
69 performance. According to Rosenberg and Sheu (1996), the addition of lactose into WPI-
70 based wall systems improves the volatile retention during spray drying and limits the core
71 extractability. This is due to lactose in its amorphous state acting as a hydrophilic sealant that
72 significantly limits diffusion of the hydrophobic core through the wall and thus leads to high
73 microencapsulation efficiency values. Furthermore, amorphous carbohydrates in the glassy
74 state are widely used as matrix for the encapsulation and stabilization of nutrients,
75 pharmaceuticals, and other bioactive compounds (Drusch, Serfert, Van Den Heuvel, &
76 Schwarz, 2006; Fäldt & Bergenståhl, 1996a, 1996b; Kaushik & Roos, 2008; Levi & Karel,
77 1995; Lim, Griffin, & Roos, 2014; Lim & Roos, 2015; Naknean & Meenune, 2010; Zhou &
78 Roos, 2012). In these applications, the glass transition temperature of amorphous matrix has
79 been used as the central physical parameter for the optimization of processing conditions and
80 storage stability (Soottitantawat et al., 2004; Townrow, Roussanova, Giardiello, Alam, &
81 Ubbink, 2010). When the temperature increases from below to above the glass transition
82 temperature, many of the physical properties of the amorphous matrix show a rapid change,
83 including increases in the free volume, molecular mobility, and dielectric coefficient. Large
84 changes in viscoelastic properties of amorphous powders also occur above the glass transition
85 temperatures (Jones, 1999; Kasapis, 2001; Royall et al., 2005). Once an encapsulation matrix
86 has gone through the glass transition and entered the metastable rubbery state, the rates of
87 deteriorative reactions and diffusion of the flavor from the particle matrix may increase
88 dramatically (Risch & Reineccius, 1995; Whorton, 1995).

89 In addition, since the presence of high molecular weight protein or carbohydrate in the
90 amorphous lactose-based systems could increase diffusion distance (Potes, Kerry, & Roos,
91 2012; Silalai & Roos, 2010, 2011), the ratios of low molecular weight compounds in
92 encapsulation systems could affect the properties of whey protein-based systems.

Furthermore, water is a highly efficient plasticizer of carbohydrates and the glass transition temperature of an amorphous carbohydrate matrix decreases strongly with increasing water content (Jouppila, Kansikas, & Roos, 1997; Jouppila & Roos, 1994; Partanen et al., 2008; Velasco, Holgado, Dobarganes, & Márquez-Ruiz, 2009; Zhou & Roos, 2012). According to Kilburn et al. (2004), the diffusional mobility of small molecules such as water, gases, or volatile organic compounds increases rapidly with increasing water content in both the glassy and rubbery states. The water content and water activity of the encapsulation matrix are key parameters for understanding the physical behavior and barrier properties of amorphous carbohydrates (Ubbink, 2003). Levi and Karel (1995) stated that water resulted in a lowering of T_g and acceleration of relaxation processes, which increased the release of 1-n-propanol in amorphous carbohydrate glasses. However, there is no further study about the roles of lactose content and water plasticization on the encapsulation properties of wall systems, and especially the relationship between the mechanical properties of amorphous matrix and their barrier properties.

Therefore, the aim of present study was to investigate the effect of amorphous lactose content and water plasticization on encapsulation properties and flavor release in dairy-based powders. How the physical and mechanical properties of wall systems affect flavor release was also investigated.

2. Materials and methods

2.1. Materials

α -lactose monohydrate (> 99% purity, **Batch No. F051621**) was kindly donated by Arla Foods Ingredients (Sønderhøj 10-12, 8260 Viby J, Denmark). Whey protein isolate (WPI), containing 71% β -lactoglobulin and 12% α -lactalbumin, was obtained from Davisco Food International (Le Sueur, MN, USA). Ethyl butyrate (EB), hydroxylamine hydrochloride, n-

hexane and aluminum oxide calcined powder ($\geq 99\%$ purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Emulsion preparation

Wall material solutions were prepared in deionized water at 40 °C for 2 h and then kept overnight on the magnetic stirrers to ensure complete hydration at room temperature (23-25 °C). Total solids concentration of wall materials was 25% (w/w), which composed of lactose/WPI mixtures or WPI. The mass ratios of 4:1, 1:1, and 1:4 for lactose/WPI mixtures were used in this study. They were defined as S1 (lactose/WPI (4:1) mixture), S2 (lactose/WPI (1:1) mixture), S3 (lactose/WPI (1:4) mixture), and S4 (WPI) according to the wall materials. Then ethyl butyrate was emulsified into the wall solutions at a proportion of 20% (w/w of wall solids). All emulsions (oil in water) were prepared in two stages. Coarse emulsion was prepared using an ULTRA-TURRAX (IKA, Staufen, Germany) operated at 10000 rpm for 1 min. Then the coarse emulsion was further homogenized using a M110-EH Microfluidizer with a 75 μm Y-type ceramic interaction chamber (Microfluidics International Corp., Newton, MA, USA) at 50 MPa for three successive homogenization steps. The homogenization process by microfluidizer was performed at room temperature with running cold water.

2.3. Spray drying

All emulsions were spray-dried by an ANHYDRO single stage spray dryer with a centrifugal atomizer (Copenhagen, Denmark) at the Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland. The inlet air temperature was around 170 ± 2 °C and the outlet temperature around 90 ± 2 °C. Spray-dried solids were kept immediately in desiccators over P_2O_5 at room temperature. Each analysis was carried out within 2 months after spray drying.

2.4. Physical properties

Loose bulk density of powders was measured using a Jolting volumeter (Funke Gerber, Berlin, Germany). Particle density (ρ_p) was measured using a Gas Pycnometer (Accupyc II 1340 Gas Pycnometer, Micromeritics Instrument Corporation, USA). Particle size distribution and specific surface area (SSA) of powder particles were determined by laser light scattering using a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Worcestershire, UK).

2.5. Chemical analysis

Protein content in powders was determined using a FP 628 Nitrogen Determinator (LECO Corporation, Lakeview Avenue, St. Joseph, Michigan, USA). Lactose content was determined using an Automatic Polarimeter (Autopol 1, Rudolph Research Analytical, Hackettstown, NJ, USA). Water content was determined using a HR83 Halogen Moisture Analyzer (Mettler Toledo International Inc., Im Langacher Greifensee, Switzerland). Water activity was measured using a water activity meter (Novasina LabMaster AW, Novatron Scientific Ltd., West Susses, UK).

In order to determine encapsulated EB content in powders, EB was extracted from powders according to the method of Kaushik and Roos (2007). 1 g of powder was dissolved in 20 mL of deionized water in glass bottles and 10 mL of hexane was added, followed by mixing with a rota mixer for 1 min. Encapsulated EB was extracted with hexane by heating the samples in glass bottles at 45 °C in a water bath for 15 min with intermittent mixing. All glass bottles were kept closed with caps and sealed with sealing film during extraction. Then the bottles were cooled to room temperature and hexane was separated from aqueous phase by centrifugation at 4000 rpm for 20 min. The amount of EB present in hexane was quantified by the method of Sheu and Rosenberg (1995). 0.5 mL of centrifuged hexane was mixed with

4.5 mL 50% ethanol solution. The colour of the solution containing EB was developed and then measured absorbance with a spectrophotometer at 525 nm. The amount of EB was determined from calibration standard curves. More details about the method of EB determination were presented in the work of Sheu and Rosenberg (1995). The EB content of each sample was carried out in triplicate.

The ratio of encapsulated EB content in powders after spray drying to initial EB content added into the emulsion was defined as encapsulation efficiency (%). The ratio of encapsulated EB content in powders during equilibration at different relative humidity (RH) to encapsulated EB content after spray drying was defined as flavor retention (%).

2.6. Water sorption and flavor release from powders at different RHs

Approximately 1 g of powders was weighed into small glass vials (25 mL). All vials were equilibrated for 216 h in evacuated desiccators over saturated salt solutions of MgCl_2 , K_2CO_3 , $\text{Mg}(\text{NO}_3)_2$, and NaNO_2 , giving RH of 33%, 44%, 54%, and 65%, respectively. All desiccators were placed at incubators with temperature of 25 °C during equilibration. 24 vials were prepared for each powder at every RH. Three vials of each powder were taken out from every desiccator at 6, 12, 24, 48, 72, 120, 168 and 216 h, respectively and the mass of samples was measured. Then water content of each powder was determined as a function of time. The residual amount of EB in the powder was determined by the method described above. The flavor retention was calculated as a function of time.

2.7. Glass transition determination

The powders were equilibrated at different RHs for 0, 6, 12, 24, 48, 72, 120, 168 and 216 h, respectively. Then the glass transition temperatures, T_g (onset), were determined using a differential scanning calorimeter (DSC Q2000, TA Instruments, Crawley, UK). To determine

T_g values, 10-15 mg of equilibrated powders was transferred to Tzero pans. The DSC pans were hermetically sealed with Tzero hermetic lids and samples were analysed. An empty pan was used as a reference. The temperature range of the scans was between -10 °C and 180 °C depending on the water content of powders. The heating rate was 5 °C/min and the cooling rate was 10 °C/min during scanning. All measurements were carried out in duplicate. T_g values were determined using TA universal analysis software, version 5.1.2 (TA Instruments, Crawley, UK) and were taken as the onset-point of the endothermic baseline shift.

2.8. Dynamic mechanical analysis

The powders were equilibrated at different RHs for 0, 6, 12, 24, 48, 72, 120, 168 and 216 h, respectively. Then a dynamic mechanical analyser (DMA Q800, TA Instruments, Crawley, UK) was used, in conjunction with a gas cooling accessory (GCA) tank, to determine the dynamic mechanical properties of powders. A rectangular stainless steel powder holder was designed to generate a defined geometry to contain powder with inner dimensions of 60 mm × 11 mm × 1 mm. A pre-weighed mass of powder mixed with aluminum oxide calcined powder at the ratio 4:1 was evenly spread within this shallow container, and the upper lid was then placed onto the top surface of the powder (Mahlin, Wood, Hawkins, Mahey, & Royall, 2009). As aluminum oxide calcined powder showed no effect on mechanical property results of dairy solids, it was added to protect dairy powder from sticking on the powder holder during the heating test. The measurements were made at a heating rate of 2 °C /min from 0 to 120 °C. DMA was operated by a sinusoidal deformation applied to the powder sample holder at a fixed strain. During dynamic heating, the samples were analysed for storage modulus (E') and loss modulus (E'') using single frequency 1 Hz. The α -relaxation temperatures, T_α , were determined using TA universal analysis software, version 5.1.2 (TA Instruments, Crawley, UK).

2.9. Statistical analysis

Measurement of protein content, glass transition, and dynamic mechanical analysis were performed in duplicate, with all other analyses performed in triplicate. Results were expressed as mean $\pm$ standard deviations (SD). One-way analysis of variance (ANOVA) was used to determine the significant differences between the mean values of each test (Microsoft Office Excel 2010, Microsoft, Inc., Redmond, WA, USA). A significance level of $P < 0.05$ was used throughout the study.

3. Results and discussion

3.1. Powder characteristics

The powder characteristics of encapsulation systems after spray drying are shown in Table 1. The EB content in the powder with wall material consisting of lactose/WPI (4:1) mixture was significantly ($P < 0.05$) higher than in the powders with wall materials consisting of lactose/WPI mixtures at ratio of 1:1 and 1:4, and WPI. As a result, the wall material consisting of lactose/WPI (4:1) mixture had significantly ($P < 0.05$) higher encapsulation efficiency than S2, S3 and S4. This might be due to the highest lactose content in S1, which could form a large extent of amorphous structure to entrap EB during spray drying. According to Roussanova et al. (2010), the density of the amorphous matrix increased when the molecular weight of a glassy carbohydrate matrix decreased. Therefore, the glass structure and increase in density of amorphous matrix might improve the barrier properties of wall materials with higher amount of lactose, and resulted in the higher encapsulation efficiency of wall materials with lactose/WPI (4:1) mixture. However, when lactose content was lower than 50% in the wall materials, the presence of lactose did not have significant impact on encapsulation efficiency of wall materials during spray drying (Table 1). Rosenberg and Sheu (1996) also showed similar results. According to their study, the

retention of EB in wall system consisting of a 1:1 mixture of WPI and lactose was not higher than in wall system consisting of WPI when the initial core material load was lower than 30% (w/w), which was in agreement with our study. Therefore, the addition of lactose in WPI-based wall systems did not have significant improvement in the barrier properties when the lactose content was lower than 50%. Additionally, the water content of powders with lower ratios of lactose of wall systems was significantly ($P < 0.05$) higher, while all powders had similar water activities. Moreover, the particle size of powders with higher amount of lactose was smaller, and the SSA values of them were larger. Furthermore, the powders with higher amount of lactose showed smaller particle density, but they showed higher loose bulk density.

3.2. Water sorption

Water sorption of powders over various water activities was plotted as a function of time, as shown in Fig. 1. Since EB in powders kept releasing during storage period, the water contents of powders shown in Fig. 1 were not only calculated according to the mass changes of powders during storage, but they were modified according to the mass change resulting from flavor release during storage. The initial water contents before equilibration are shown in Table 1. The wall system consisting of only WPI (S4) sorbed the highest amount of water in the range of 0.33-0.65 a_w . S2 with wall material consisting of lactose/WPI (1:1) mixture sorbed the lowest amount of water at 0.33 a_w and 0.44 a_w . At 0.54 a_w , loss of sorbed water was found in S1 with wall material consisting of lactose/WPI (4:1) mixture after 48 h of storage, while only small amount of sorbed water lost in S2 with wall material consisting of lactose/WPI (1:1) mixture after 72 h of storage. The rates of water sorption and lactose crystallization for powders at 0.65 a_w were higher than those at 0.54 a_w . The loss of sorbed water was observed in powders with wall materials consisting of lactose/WPI mixtures at ratio 4:1, 1:1, and 1:4 after 12 h, 24 h, and 24 h of storage at 0.65 a_w , respectively. However,

only a minor loss of sorbed water was observed in S3 with wall material consisting of lactose/WPI (1:4) mixture. In the present study, the presence of high molecular weight protein delayed lactose crystallization at 0.54 a_w and 0.65 a_w , which agreed with the previous studies (Fan & Roos, 2015; Haque & Roos, 2004). The presence of protein might result in the physical blocking or steric hindrance, which could reduce molecular diffusion and delay lactose crystallisation (Fan & Roos, 2015).

Since the increase of water content can lead to an increase in free volume by decreasing the molecular packing of the matrix molecules (van den Dries, van Dusschoten, Hemminga, & van der Linden, 2000), the difference in water content of encapsulated systems (S1-S4) during storage at various water activities might result in different rates of flavor release in the amorphous matrix. Furthermore, the structure of encapsulated matrix was destroyed as lactose crystallisation occurred when water content increased at 0.54 a_w and 0.65 a_w , which might result in a quick release of flavor from powders. In addition, the collapse of amorphous matrix might also reduce flavor molecular diffusion through the matrix and result in re-encapsulation of flavour compound when lactose crystallised (Goubet et al., 1998).

3.3. Glass transition

The glass transition temperatures of powders (S1-S3) equilibration at various RHs during water sorption were measured using a DSC and are shown in Table 2. The T_g values decreased with increasing storage time in the range of 0.33-0.65 a_w before showing lactose crystallisation (Table 2), which showed typical water plasticization of amorphous materials (Haque & Roos, 2004; Jouppila et al., 1997; Potes et al., 2012; Zhou & Roos, 2011). Moreover, the T_g values of lactose decreased when the storage RH was increased. Powder with wall material of lactose/WPI (1:4) mixture showed higher T_g values than those with wall materials of lactose/WPI mixtures at ratio 4:1 and 1:1 at 0.44-0.65 a_w . This was in agreement

with the results of Fan and Roos (2016). They indicated the T_g value of lactose/WPI mixture at ratio 3:7 was higher than those of lactose/WPI mixtures at ratios 7:3 and 1:1 at 0.33 a_w . The increasing effect of T_g values in powder with lactose/WPI (1:4) mixture might be due to the presence of high amount of protein with high molecular weight.

Phase transitions play an important role in encapsulation processes (Whorton, 1995). Levi and Karel (1995) stated that flavor retention was dependent on temperature, water content and the degree of matrix collapse. In the glassy state, flavors have very low diffusion rates in amorphous barriers. Due to the low molecular mobility of barriers, flavor release from powders in the glassy state is limited. When a glass is heated to above the glass transition, molecules gain translational mobility and enter the supercooled, liquid-like state. The increase in molecular mobility of amorphous matrix would result in the increase of evaporation of volatile flavors. Since the T_g values of powders were varied according to the storage time, storage RH and wall material compositions, the rate of flavor release was also affected by those conditions. Powders with lower amount of lactose might show lower rate of flavor release at high RH (> 44%).

3.4. Mechanical properties

Many mechanical properties of amorphous materials change dramatically at glass transition region. In this study, the mechanical properties of powders during storage over various RHs were measured using a DMA. The modulus changes of powders equilibration at 0.33-0.65 a_w are shown in Fig. 2. Since the powders consisting of lactose showed lactose crystallisation at 0.54 a_w and 0.65 a_w after storage a period (0.54 a_w : > 12 h; 0.65 a_w : > 6 h), the mechanical properties of powders were only measured before lactose crystallisation (Fig. 2). Additionally, the modulus changes of powders during storage at same RH for different time are shown in Fig. 3. Compared with the storage modulus of powders (S1-S4) after spray

308 drying (Initial powders), S1 had significantly higher storage modulus than S2, S3 and S4 at
309 the glassy state. This might be one of the reasons that the encapsulation efficiency of wall
310 material consisting of lactose/WPI (4:1) mixture (S1) was significantly higher than those with
311 lactose/WPI mixtures at ratio 1:1 and 1:4, and WPI (Table 1). The magnitudes of storage
312 modulus changes for powders were larger at higher water activity (Fig. 2 A). Moreover, the
313 magnitudes of storage modulus changes also increased with increasing storage time at same
314 water activity (Fig. 3 A1 and A2). Since increasing storage RH or storage time caused an
315 increase in the water content of powders, powders with higher water content could result in
316 larger magnitudes of storage modulus changes. According to previous studies (Roudaut,
317 Simatos, Champion, Contreras-Lopez, & Le Meste, 2004; Silalai & Roos, 2011), the
318 magnitudes of storage modulus and loss modulus changes were related to molecular mobility
319 of materials. Therefore, increasing water content of powders could increase the molecular
320 mobility of matrix, which might result in a high release rate of flavor in powders.
321 Furthermore, the storage modulus of powders with higher amount of lactose in the wall
322 materials had more significant change when water content increased (Fig. 2 A). Similar
323 results were also shown when the storage time of powders increased at same water activity
324 (Fig. 3 A1 and A2). These results indicated that increasing water content or lactose content of
325 powders could increase molecular mobility and free volume of matrix at the glass transition
326 region, which could accelerate the diffusion of flavor molecules during storage.

327 The changes of loss modulus of powders during equilibration were also measured using a
328 DMA (Fig. 2 and Fig. 3). The loss modulus of powders was small and showed minor changes
329 when the temperatures were below glass transition and they increased dramatically and
330 reached peak values with increasing temperature to the glass transition region. This indicated
331 the molecular mobility of powders increased dramatically in the glass transition region.
332 Furthermore, the peak values of loss modulus of powders were affected by the lactose content

and water content of matrix. Decreasing water content or lactose content could decrease the molecular mobility of matrix in the glass transition region.

In addition, the α -relaxation temperatures, T_α , were taken from the temperatures of loss modulus peak values at 1 Hz (Table 3). The T_α values of powders decreased with increasing water content as a result of water plasticization, which was in agreement with the study of Silalai and Roos (2010). S1 showed significantly higher T_α values than other powders after spray drying. The powder with wall material consisting of lactose/WPI (1:1) mixture (S2) showed the highest T_α values at 0.33 a_w , while powder consisting of only WPI showed the highest T_α values at 0.44 a_w , 0.54 a_w , and 0.65 a_w . The decrease of T_α values of S1 with the highest amount of lactose was larger as compared to those of S2-S4 when water content increased. These results indicated water content showed more significant effect on the structural relaxation of powders with wall materials consisting of higher amount of lactose. Since the diffusion of core materials is governed by the physical properties of the wall materials, such as the matrix structure, increasing water content of powders with wall materials consisting of higher lactose content might result in a higher diffusion of flavor molecules.

3.5. Flavor retention

The effects of water plasticization on the time-course of the flavor release in powders with different composition of wall materials are shown in Fig. 4. The retention of EB was defined as the ratio of the residual amount of EB in the powder to the initial amount after spray drying. The flavor retention in powders did not show significant change during storage at 0.33 a_w at 25 °C for 216 h, while the flavor retention of S1 showed a minor decrease with storing at 0.44 a_w . S2 showed the highest flavor retention at 0.33 a_w and 0.44 a_w , which might be due to its lowest water contents at 0.33 a_w and 0.44 a_w (Fig. 1). In addition, since the EB

content of S1 was significantly higher compared to those of other powders (S2-S4) after spray drying, S1 still had the highest EB content after storage at 0.33 a_w . However, the flavor retention of S1 dropped sharply with equilibration at 0.54 a_w . Similar result happened to the flavor retention of S1 and S2 when they were stored at 0.65 a_w . These results could be linked to lactose crystallisation of encapsulating matrix, which were in agreement with the study of Zhou and Roos (2012). They stated that the amorphous structure protected the entrapped vitamins at low a_w , but crystallization of lactose destroyed the structure. Since the T_g values of S1 at 0.54 a_w and 0.65 a_w were below the storage temperature (25 °C) (Table 2), there was a decrease in the viscosity of matrix, and an increase in the diffusivity of flavor molecules. Moreover, collapse of amorphous matrix occurred when lactose showed plasticization. Then the amorphous matrix was unable to support itself against gravity. The flavor was expelled from the crystallized matrix to the surface, and the retention of EB decreased sharply. Additionally, the rate of flavor release from S1 decreased as storage time increased at 0.54 a_w and 0.65 a_w . This might be due to the completely collapse of amorphous matrix after lactose crystallisation, and then resulted in re-encapsulation of flavour compound in the collapse matrix (Goubet et al., 1998).

In addition, the water plasticization had more significant impact on the flavor retention of powders with wall materials consisting of larger amount of lactose (Fig. 4). The flavor retention of S4 was higher than 50% even when it was stored at 0.65 a_w for 216 h (Fig. 4 D). It was due to water plasticization had more significant influence on the molecular mobility and structural relaxation of powders with larger amount of lactose (Fig. 2 and 3), which caused higher diffusion of flavor molecules in the encapsulation systems with higher amount of lactose. As it also can be seen from Table 3, the α -relaxation temperatures of powders with higher amount of lactose decreased more significantly with increasing water content. Therefore, increasing lactose content in wall materials could increase the encapsulation

efficiency at low water activities (≤ 0.33). However, the presence of lactose could result in higher molecular mobility with increasing water content, which led to accelerate the rate of flavor release at high water activities (≥ 0.54).

4. Conclusions

Amorphous encapsulated matrix using lactose/WPI mixtures as wall materials and ethyl butyrate as core materials were prepared by spray drying. The encapsulation efficiency of wall material consisting of lactose/WPI (4:1) mixture was significantly higher compared to wall material consisting of only WPI. However, wall materials with lactose/WPI mixtures at ratios of 1:1 and 1:4 did not show similar result. The different ratios of lactose/WPI in wall materials resulted in the different physical properties of encapsulation systems, including particle size, specific surface area, loose bulk density and particle density, which might affect the storage stability and flavor release of powders. The flavor retention of powders did not show significant change during equilibration at 0.33 a_w , while powder with wall material consisting of lactose/WPI (1:1) mixture had significantly higher flavor retention at 0.33 a_w and 0.44 a_w . Powders with wall materials consisting of higher amount of lactose showed dramatically decrease in their flavor retention with equilibration at 0.54 a_w and 0.65 a_w , which was as a result of lactose crystallisation. Mechanical property study indicated that powder with higher amount of lactose showed higher molecular mobility with increasing water content, and water plasticization had more significant impact on the glass transition and structural relaxation of powder with higher amount of lactose. Therefore, increasing lactose content could increase the encapsulation efficiency of wall materials during spray drying. However, this also could result in a higher rate of flavor release with storage at high water activity.

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Figure captions

Figure 1 Water sorption of encapsulation systems equilibration at 0.33 a_w (A), 0.44 a_w (B), 0.54 a_w (C), and 0.65 a_w (D) at 25 °C. Loss of sorbed water at 0.54 a_w and 0.65 a_w indicates lactose crystallization.

Figure 2 Storage modulus (A) and loss modulus (B) of encapsulation systems equilibration at 0.33 a_w and 0.44 a_w for 216 h, 0.54 a_w for 12 h, and 0.65 a_w for 6 h before showing lactose crystallisation at 25 °C. Initial powders were the powders after spray drying.

Figure 3 Storage modulus (A1 and A2) and loss modulus (B1 and B2) of encapsulation systems equilibration at 0.33 a_w and 0.44 a_w at 25 °C for different time. Initial powders were the powders after spray drying.

Figure 4 Flavor retention in powders equilibration at 0.33 a_w (A), 0.44 a_w (B), 0.54 a_w (C) and 0.65 a_w (D) at 25 °C for different time.

587 **Tables**

588 **Table 1** Powder characteristics of encapsulation systems

systems	water content (%)	water activity	d_{50} (μm)	SSA (m^2/kg)	particle density (g/cm^3)	loose bulk density (g/cm^3)	ester content (mg/g of dry solids)	encapsulatio n efficiency (%)
S1 lactose/WPI 4:1	$2.52^{\text{d}} \pm 0.03$	$0.17^{\text{ab}} \pm 0.00$	$26.20^{\text{c}} \pm 0.00$	$646.73^{\text{a}} \pm 3.27$	$1.0986^{\text{c}} \pm 0.0031$	$0.4117^{\text{a}} \pm 0.0002$	$71.04^{\text{a}} \pm 0.85$	$42.62^{\text{a}} \pm 0.51$
S2 lactose/WPI 1:1	$2.69^{\text{c}} \pm 0.02$	$0.16^{\text{b}} \pm 0.00$	$24.30^{\text{d}} \pm 0.43$	$672.93^{\text{a}} \pm 5.32$	$1.0949^{\text{c}} \pm 0.0093$	$0.3189^{\text{b}} \pm 0.0051$	$61.48^{\text{b}} \pm 3.65$	$36.89^{\text{b}} \pm 2.19$
S3 lactose/WPI 1:4	$4.07^{\text{b}} \pm 0.06$	$0.18^{\text{a}} \pm 0.00$	$27.20^{\text{b}} \pm 0.08$	$608.27^{\text{b}} \pm 5.94$	$1.1342^{\text{a}} \pm 0.0043$	$0.2763^{\text{c}} \pm 0.0010$	$60.44^{\text{b}} \pm 1.54$	$36.26^{\text{b}} \pm 0.92$
S4 WPI	$4.26^{\text{a}} \pm 0.05$	$0.16^{\text{ab}} \pm 0.01$	$30.37^{\text{a}} \pm 0.09$	$506.53^{\text{c}} \pm 3.85$	$1.1232^{\text{b}} \pm 0.0001$	$0.2466^{\text{d}} \pm 0.0011$	$62.52^{\text{b}} \pm 3.55$	$37.51^{\text{b}} \pm 2.13$

589 ¹ Values are mean $\pm$ standard deviation (n=3).

590 ^{2 a-d} Values within columns with different superscripts are significantly different at $P < 0.05$.

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Table 2 Glass transition temperatures ($^{\circ}\text{C}$), T_g , of powders with wall materials consisting of lactose/WPI mixtures storage at 0.33 a_w , 0.44 a_w , 0.54 a_w , and 0.65 a_w at 25 $^{\circ}\text{C}$ for different time.

water activity	time (h)	systems		
		S1 lactose/WPI 4:1	S2 lactose/WPI 1:1	S3 lactose/WPI 1:4
Initial powders	0	64 ± 0.5	57 ± 0.0	65 ± 0.0
0.33	6	55 ± 1.0	56 ± 1.0	63 ± 1.0
	12	46 ± 0.5	46 ± 0.5	45 ± 1.0
	24	46 ± 0.5	47 ± 0.0	47 ± 0.5
	48	45 ± 0.0	46 ± 0.5	49 ± 0.0
	72	41 ± 0.0	44 ± 0.5	45 ± 0.5
	120	40 ± 0.0	42 ± 0.0	42 ± 0.0
	168	39 ± 0.5	42 ± 0.0	43 ± 0.5
	216	40 ± 0.0	41 ± 0.5	43 ± 0.0
0.44	6	40 ± 0.0	41 ± 0.0	40 ± 0.0
	12	30 ± 0.0	31 ± 0.0	37 ± 0.0
	24	24 ± 0.5	28 ± 0.5	36 ± 1.0
	48	22 ± 0.0	25 ± 0.5	36 ± 0.0
	72	22 ± 0.0	25 ± 0.0	35 ± 0.0
	120	22 ± 0.5	25 ± 0.5	35 ± 0.5
	168	21 ± 0.5	25 ± 0.0	34 ± 0.0
	216	21 ± 0.0	25 ± 0.5	34 ± 0.5
0.54	6	33 ± 0.5	33 ± 0.5	38 ± 0.5
	12	13 ± 0.5	16 ± 0.0	33 ± 0.0
0.65	6	15 ± 0.5	16 ± 0.0	34 ± 1.0

¹ Values are mean $\pm$ standard deviation (n=2).

² Initial powders were the powders after spray drying.

Table 3 α -relaxation temperatures ($^{\circ}\text{C}$), T_{α} , of powders storage at 0.33 a_w , 0.44 a_w , 0.54 a_w , and 0.65 a_w at 25 $^{\circ}\text{C}$ for different time.

water activity	time (h)	systems			
		S1 lactose/WPI 4:1	S2 lactose/WPI 1:1	S3 lactose/WPI 1:4	S4 WPI
Initial powders	0	81.9 $\pm$ 0.04	78.5 $\pm$ 0.01	76.7 $\pm$ 0.02	77.3 $\pm$ 0.08
	6	73.9 $\pm$ 0.06	71.3 $\pm$ 0.02	68.2 $\pm$ 0.02	68.2 $\pm$ 0.05
	48	65.7 $\pm$ 0.10	68.2 $\pm$ 0.05	65.9 $\pm$ 0.03	61.2 $\pm$ 0.03
	120	65.7 $\pm$ 0.07	68.8 $\pm$ 0.10	65.9 $\pm$ 0.06	61.2 $\pm$ 0.07
	216	65.9 $\pm$ 0.08	68.1 $\pm$ 0.05	65.8 $\pm$ 0.09	61.3 $\pm$ 0.04
0.33	6	66.4 $\pm$ 0.05	67.3 $\pm$ 0.01	64.7 $\pm$ 0.01	67.9 $\pm$ 0.04
	48	40.0 $\pm$ 0.02	53.2 $\pm$ 0.04	63.8 $\pm$ 0.06	65.4 $\pm$ 0.05
	120	37.5 $\pm$ 0.04	50.7 $\pm$ 0.05	62.0 $\pm$ 0.04	64.1 $\pm$ 0.04
	216	36.6 $\pm$ 0.02	49.8 $\pm$ 0.03	61.1 $\pm$ 0.01	62.9 $\pm$ 0.04
0.44	6	52.4 $\pm$ 0.02	60.2 $\pm$ 0.03	63.0 $\pm$ 0.04	66.0 $\pm$ 0.01
	12	27.5 $\pm$ 0.04	31.9 $\pm$ 0.04	56.6 $\pm$ 0.03	60.3 $\pm$ 0.02
	6	28.1 $\pm$ 0.06	37.1 $\pm$ 0.03	55.8 $\pm$ 0.05	64.0 $\pm$ 0.05
0.54	6	28.1 $\pm$ 0.06	37.1 $\pm$ 0.03	55.8 $\pm$ 0.05	64.0 $\pm$ 0.05
0.65	6	28.1 $\pm$ 0.06	37.1 $\pm$ 0.03	55.8 $\pm$ 0.05	64.0 $\pm$ 0.05

¹ Values are mean $\pm$ standard deviation (n=2).

² Initial powders were the powders after spray drying.

Figure 1

Figure 2

A

B

Figure 3

A2

B2

Figure 4