Impact of spray-drying conditions on the particle size of microparticulated whey protein fractions

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ORIGINAL PAPER

Impact of spray-drying conditions on the particle size of microparticulated whey protein fractions

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Abstract The particle size of microparticulated whey proteins is decisive for the sensory properties and applicability of these products in foodstuffs. The impact of spray-drying conditions on the particle size of whey microparticles was studied. Solutions of 10% (*w*/*w*) whey proteins in native mixed form (ratio of α-La/β-Lg, 20:70) and isolated form were microparticulated in a scraped surface heat exchanger with a denaturation degree of 90% or higher and were dried using a lab-scale spray dryer. The effect of drying temperatures on the particle size was evaluated for the powder form as well as after reconstitution in distilled water to the original solids content, following a multilevel factorial design with variation of the inlet (180–210 °C) and outlet (75–105 °C) temperatures in the dryer. A slight increase in particle size was observed in the powders independent of the inlet and outlet drying temperatures, which was reversible after dissolution in water. Residual water content of the powder was reduced, independent of the protein composition, as the outlet temperature during drying was increased. Scanning electron microscope micrographs of the obtained powders showed spherical particles with morphological differences depending on the protein composition of the solutions. The results were validated at pilot scale using a spray dryer with centrifugal disc atomisation.

Keywords Microparticulation · Spray drying · Whey protein concentrate · α-lactalbumin · β-lactoglobulin

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1 Introduction

Heat treatment of whey protein concentrate (WPC) can promote the unfolding of the protein if the temperature is high enough, which can result in irreversible aggregation of the proteins and the formation of stable gel structures if the protein concentration is above the critical concentration for gelling. When this operation is performed under shearing, e.g. using a scraped surface heat exchanger (SSHE), the heat-induced aggregation can be controlled. In this case, the formation of a continuous network of denatured protein is avoided. However, whey protein microparticles (MWP) are the result of this operation, a process known as microparticulation. Depending on processing conditions, it is possible to produce spherically shaped MWP with high creaminess and water-binding capacity that can be used as fat replacer due to the "ball bearing" effect (Paquin et al. 1993), which results in a pleasant mouth feeling, similar to the creaminess of milk fat, or as a functional ingredient for targeted structure modification in many food products. Singer and Dunn (1990) found that MWP with a mean particle size of 0.1–3.0 μm and dispersed in water are perceived as smooth and rich. Spiegel (1999) found after microparticulation of WPC with a SSHE that the threshold between a smooth and a slight mealy mouthfeel for MWP lies around a particle size of 20 μm. Sensorial detectability, however, also depends on the food system containing MWP. The major proteins of whey, β-lactoglobulin and α-lactalbumin, are meant to possess different functional properties when present in isolated form, compared with the native mixture present in whey (Rojas et al. 1997). The enhanced nutritive profile of the individual whey fractions, especially in the case of pure α-La (Lönnerdal and Lien 2003), makes their processing interesting for the food industry and offers new applications for whey ingredients. However, little is known about the microparticulation of pure protein fractions.

WPC in liquid form, whether native or heat treated, are microbiologically stable only for a short time due to the high concentration of protein and lactose, factors which parallel to the high water activity enable rapid growth of bacteria. Therefore, they are rapidly incorporated in processed foodstuffs, or spray dried in order to extend their shelf life, enable an easy handling and reduce transport costs (Landström et al. 2000). It remains still unknown which effects are caused on the particles during drying, especially on their particle size or their solubility after reconstitution.

In few investigations studying microparticulation of whey protein and their functional properties, spray drying has been applied as an intermediate step for conservation of the material. Iordache and Jelen (2003) used spray drying of microfluidised WPC at different pH values with inlet and outlet temperatures of 125 and 65 °C. Tobin et al. (2010) used respectively 187 and 87 °C for spray drying of microparticles of whey protein and inulin obtained with a SSHE. Dissanayake et al. (2012) studied the functionality of dried microfluidised WPC at 180 and 80 °C and low pH. Among the mentioned studies, no observations have been made so far on changes in solubility, particle size or morphology of the aggregates, as it has been already done, e.g. for native whey proteins (Straatsma et al. 1999; Nijdam and Langrish 2005; Anandharamakrishnan et al. 2007; Gaiani et al. 2010). Little, if any, is known on the behaviour of MWP under spray-drying conditions. The questions are (1) whether MWP grow in size through agglomeration when liquid droplets are converted and shrink into powder particles, and (2) whether such agglomerates disintegrate upon

redispersion. In this study, we determined the influence of the drying conditions on the particle size distribution of MWPs in isolated and native mixed form with optimum sensory characteristics. A multilevel factorial design was applied in order to investigate the effects of the inlet and outlet air temperatures on the mean particle size $d_{50,\ 3}$ in powder as well as in the reconstituted form. Optimum drying temperatures for microparticulated whey proteins were obtained with a laboratory spray dryer, and used for validation in pilot scale. The rest humidity of the powders from laboratory and pilot trials was measured and their morphology was analysed.

2 Materials and methods

2.1 Preparation of solutions

Isolated fractions of β-Lg and α-La with a protein content of 90% and 94% (*w*/*w*), and a purity of 98% and 92%, respectively, were prepared from whey protein isolate (protein content, 96.3% (*w*/*w*); WPI 895, Fonterra, New Zealand) following a selective aggregation and membrane separation method as described by Toro-Sierra et al. (2011). Dispersions of 10% (*w*/*w*) isolated protein were prepared in concentrated milk serum. This serum was produced by filtration of concentrated skim milk with 28.8% (*w*/*w*) solids from skim-milk powder (Alpavit, Heising, Germany) using an ultrafiltration membrane with 10 kDa nominal molecular weight cut-off (material: polyether sulfone; DSS Silkeborg AS, Silkeborg, Denmark). The concentrated milk was adjusted to 13% (*w*/*w*) lactose and 660 ppm Ca^{2+} with addition of de-ionised water and $CaCl_2{\cdot}H_2O$ (Merck, Darmstadt, Germany). The protein powder was dispersed under stirring in concentrated milk serum and allowed to fully rehydrate over night at 4 °C. After protein dispersion, further addition of calcium chloride to a Ca:Protein mass ratio of 1:15 was performed, in order to promote aggregate formation (Spiegel and Huss 2002; Vardhanabhuti and Foegeding 2008). Prior to microparticulation, solutions were allowed to equilibrate at ambient temperature and pH was adjusted to 4.6 using 1 M HCl. Preliminary experiments found that pH 4.6 was adequate for production of microparticulated whey proteins with an average particle diameter smaller than 20 μm for all fractions.

Additionally, a solution with a protein concentration of 10% and 13% (*w*/*w*) lactose was prepared by mixing a commercial whey protein powder (Bayolan P35, BMI, Landshut, Germany) in water in order to obtain microparticles from whey protein in the native mixture of α-La and β-Lg. The powder contained 36% (*w*/*w*) protein and 50% (*w*/*w*) lactose as determined by the Dumas Method (FP-528, LECO Instrumente GmbH, Mönchengladbach, Germany) and RP-HPLC (further details are reported by Toro-Sierra et al. 2011). This mixture contained α-La and β-Lg in a ratio of 20:70 (*w*/*w*).

2.2 Microparticulation

Batches of 2-L whey protein solution in isolated form or as a mixture of α-La and β-Lg (20:70) were microparticulated using a SSHE (Type VWK01/60-400, Schröder Kombinator, Lübeck, Germany). These were particulated and used as starting material for drying at lab scale using the SSHE in a closed loop batch as described by Spiegel (1999), including successive heating and cooling in one single exchanger, the

complete loop having a total volume of almost 2 L. Batches of 15 L were produced for evaluation of drying with a pilot spray dryer, using the optimum drying temperatures obtained from the lab-scale trials. For this purpose, the SSHE was used in continuous operation, comprising two heating units, a holding section and a cooling unit. The volumetric flow was 30 $L.h^{-1}$ for both batch and continuous production with the SSHE, and the rotation speed of the blades was set to 1,200 rpm, the highest possible, which corresponds to an approximate shear rate of 628 s^{-1} (Spiegel 1999). Hot pressurised water was used as heating medium and tap water for cooling. The protein dispersions were heated for a denaturation degree of at least 90% with following conditions: for WPC containing isolated α-La at 90 °C for 20 min, isolated β-Lg at 90 °C for 5 min and native mixtures of both at 95 °C for 6 min. After heating, solutions were cooled down with tap water and stored at 4 °C in screw-cap glass bottles until spray drying.

2.3 Quantification of the degree of denaturation

The degree of denaturation (DD), defined indirectly as the ratio of native protein in the heated solution to total native protein in the non-heated solution, was determined using Eq. (1) with C_0 and C_t being the concentration of native whey protein before and after heating, as measured by RP-HPLC following the procedure described by (Toro-Sierra et al. 2011).

$$DD = 1 - \frac{C_t}{C_0} \tag{1}$$

2.4 Drying of the microparticulated solutions

Spray drying of the microparticles in laboratory scale was performed using a spray dryer BÜCHI B-290 (Büchi Labortechnik AG, Flawil, Switzerland) with a spray nozzle (diameter 1.5 mm) for atomisation. Air humidity was monitored with a humidity sensor FHA 646 E1 (Ahlborn, Holzkirchen, Germany). The variation of inlet and outlet temperatures during drying in lab scale was done following a multilevel factorial design as described further in Section 2.9. Air-inlet temperatures were directly programmed at the device, and the corresponding air outlet temperature for a defined experiment resulted from regulation of feed flow between 0.4 and 1.1 $L.h^{-1}$ by the peristaltic feed pump. The air to feed ratio was set to maximum with a nozzle air flow of 742 $L.h^{-1}$, while the flow of drying air was 35 $m^3.h^{-1}$. Each separate experiment was started with distilled water until constant inlet and outlet temperatures were reached. The feed was switched from water to the whey protein solution and the outlet temperature was adjusted with a slight variation of the feed flow due to the differences in viscosity between both products. The temperature of the feed was kept constant at 30 °C with a water bath. After constant drying conditions were reached, a volume of 100 mL was dried, as measured with a graduated glass beaker. After passing the cyclone, the powder was collected in screw-cap plastic cups, sealed and stored in a dry and cool place until analysis. Only free flowing powder was sampled; all particles adhering to the walls of the dryer were discarded. A complete cleaning cycle with additional drying with hot air was performed between each experiment.

For the validation of the experiments in pilot scale, optimum drying temperatures from the lab-scale trials were used for drying of 15 L batches of microparticulated whey proteins using a spray dryer Production Minor (GEA Niro, Sorbok, Denmark) equipped with a centrifugal disc for atomisation of the samples. The drying air intake was set to the maximum with 411 $m^3.h^{-1}$ as well as the atomisation with 24,000 rpm. The experiments were replicated three times.

2.5 Particle size measurement

Particle size distribution of liquid samples before drying and after resuspension of powders in water was measured using a laser spectrometer LS 230 (Beckman Coulter, Krefeld, Germany). For the latter analysis, the obtained powders were dissolved in distilled water to the solids content of the initial solution and allowed to stir for 2 h at ambient temperature using a magnetic stirrer. The particle size distribution of dried samples in powder form was determined using a Malvern Mastersizer 2000 equipped with a Scirocco dry dispersing unit (Malvern Instruments GmbH, Herrenberg, Germany). For all measurements a real diffraction index of 1.41 for protein and 1.33 for water was used. All samples were measured in triplicate. Mean values of $d_{50,\ 3}$, i.e. the volume based median particle size, with confidential intervals of 95% are reported.

2.6 Scanning electron microscopy

The powder samples were fixed on aluminium rods using double-sided adhesive tape. Excess particles were removed by directing a jet of dry air to the surface of the rods. The samples were coated with gold in a SCD 005 sputter coater (BAL-TEC, Witten, Germany) and examined with a Jeol JSM-5900 LV scanning electron microscope (Jeol Gemany GmbH, Eching, Germany) operating at an accelerating voltage of 10 kV. A similar procedure was applied for the samples after resuspension, with the difference that a portion of the suspension containing the dissolved powders was diluted 1:10 with distilled water. A few drops of the solution were deposited on a glass slide and allowed to dry over night in a dessicator-containing silica gel. The slides were coated with gold and handled in a similar way as the powder samples.

2.7 Determination of solid content in suspensions of microparticulated protein

The solid content of the solution was determined before drying using a microwave oven including an analytical scale with a precision of 0.2 mg (AVC80, CEM, Kamp-Lintfort, Germany). An approximate amount of 3 g were given to a special adsorbent pad and dried until constant weight was reached. Each sample was measured at least in triplicate.

2.8 Determination of the powder moisture content

The bulk moisture content of the spray dried microparticulates was determined using the Karl Fischer method with a titration apparatus (Titro-Line KF, Schott, Mainz, Germany). Each powder was measured in triplicate. The measurements were valid if

the relative error between them was lower than 0.5%. The average bulk moisture of the powder for all inlet air temperatures is reported.

2.9 Statistical analysis

A multilevel factorial design was applied for evaluation of the influence of temperature on particle size during spray drying of microparticulated whey protein. Two experimental factors (inlet air temperature, outlet air temperature) were evaluated at four levels (inlet—180, 190, 200 and 210 °C; outlet—75, 85, 95 and 105 °C). The experiments were divided into three fully randomised blocks. Each experiment was run in triplicate. Statistical analysis was done using STATGRAPHICS Plus 5.0 (StatPoint Technologies, Warrenton, VA). Significant correlation of data was verified using Durbin—Watson statistics ($p<0.05$). All confidential intervals are stated at the 95% confidence level.

3 Results and discussion

3.1 Characterisation of the starting product

Batches of microparticulated whey proteins were prepared using a surface scraped heat exchanger. An averaged composition of all microparticulated batches is listed in Table 1. The composition of each concentrate resembled the characteristics of a WPC35, i.e. 35% (*w*/*w*) protein in dry mass. A denaturation degree of the whey proteins higher than 90% was reached using the processing conditions mentioned therein. Contrary to the results of Spiegel (1999) for microparticulated WPC with high lactose levels, the obtained microparticles had mean particle sizes lower than 20 μm, resulting in high creaminess and smoothness, as assessed after each experiment. The influence of drying conditions on particle size distribution according to a multilevel factorial design was performed by drying portions of at least 100 mL of the

Table 1 Processing conditions and characterisation of microparticulated whey proteins

Product	Composition				Processing conditions (SSHE)	$d_{50,3}$ (μm)	Denaturation degree	
	Protein ($g.L^{-1}$)	Lactose ($g.L^{-1}$)	pH	Solids (% *w*/*w*)			$DD_{\alpha\text{-La}}$	$DD_{\beta\text{-Lg}}$
MP α-La (ratio of α-La/β-Lg, 100:0)	98	136	4.6	28.5%	90 °C/20 min at 1,200 rpm	6.43	94%	
MP β-Lg (ratio of α-La/β-Lg, 100:0)	102	128	4.6	28.5%	90 °C/5 min at 1,200 rpm	16.51		96%
MP WPC (ratio of α-La/β-Lg, 20:70)	94	138	4.6	29%	95 °C/6 min at 1,200 rpm	2.98	87%	94%

SSHE scraped surface heat exchanger, *MP α-La* microparticulated α-lactalbumin, *MP β-Lg* microparticulated β-lactoglobulin, *MP WPC* microparticulated whey protein concentrate, *DD* degree of denaturation

microparticulated solutions listed in Table 1 for each drying temperature combination.

3.2 Influence of drying temperature on particle sizes of MWP

In the case of spray drying of microparticulated WPC (ratio of α-La/β-Lg, 20:70), isolated β-Lg or α-La, it was determined that a slight particle growth occurs across all evaluated inlet and outlet temperatures, when compared with the particle sizes of the aggregates in liquid form (Tab. 1), which are indicated with an arrow for the corresponding solutions in Fig. 1. There was no significant variation ($p>0.05$) of the particle sizes of the obtained powders with neither air-inlet nor air-outlet temperatures. The powder sizes were 13.40±1.56, 17.40±0.80 and 8.83±1.34 μm for microparticulated whey protein concentrate (MP WPC), microparticulated β-lactoglobulin (MP β-Lg) and microparticulated α-lactalbumin (MP α-La), respectively. The greatest difference between the aggregates in liquid and dried form was recorded for MP WPC with 11.25 μm, followed by MP α-La with 2.40 μm and MP β-Lg with 0.89 μm. The first two of them exhibit clearly skin formation as presented in Fig. 2a, c. During spray drying of solutions containing native whey proteins, casein or lactose, Gaiani et al. (2010) found no significant difference in the powders, with $d_{50,\ 3}$ around 8 μm for all obtained powders. Their experiments were conducted in a similar manner to our study, using a Büchi B290 lab-scale dryer. From our results we can conclude then, that a significant difference between the powder particles was found when the protein source was changed. However, as described by Kim et al. (2009), the obtained particles were smaller than 20 μm, which is typical for lab-scale devices. It has also been noticed, that the particles recover their original size after re-dispersion in water (Figs. 3 and 4), which coincides with the observations of Tobin et al. (2010) for microparticulated WPC dried with an air outlet temperature of 87 °C. It can be inferred that the aggregation observed in dry state is not of covalent nature and any slight difference between the size of the original and the reconstituted dispersion may be an effect of the rehydration capacity of the powders.

According to Filkova and Mujumdar (1995), the predicted Sauter diameter of the droplets based on the data from Table 1 varies between 130 and 190 μm for MP WPC, between 65 and 90 μm for MP β-Lg and between 160 and 230 μm for MP α-La. Compared with the measured size of the powder particles, it is possible that many protein aggregates are part of a single droplet during drying.

The optimum temperatures for spray drying of microparticulated whey proteins, using a spray dryer with nozzle in lab scale, are represented in Table 2. The temperature intervals were selected based on the following criteria: (a) in the present investigation low temperatures led to increased water content in the powders (Section 3.4) which resulted in higher stickiness and reduced the overall amount of free flowing powder. Therefore higher air outlet temperatures were favoured; (b) air inlet temperatures at the minima of the response surfaces (Fig. 1) were preferred. The reported temperature intervals were used for validation in pilot scale using a rotational disc for atomisation, as described in Section 3.6.

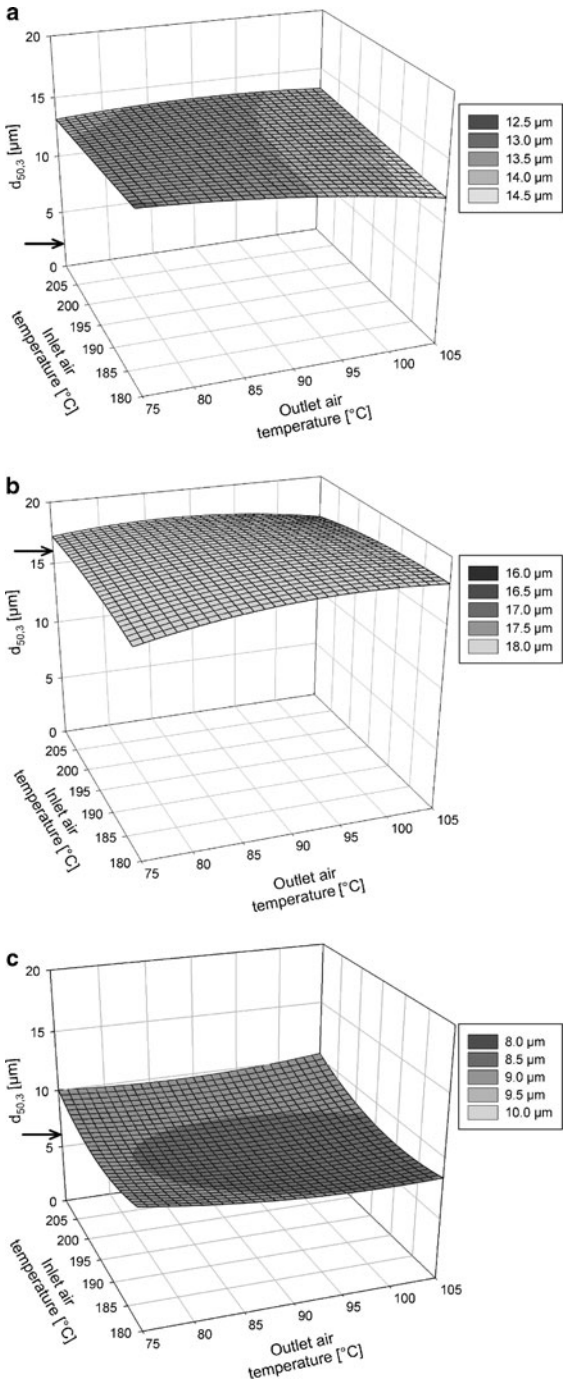

Fig. 1 Effect of inlet and outlet air temperature on the volume-based median particle size $d_{50,\ 3}$ of **a** microparticulated WPC, **b** microparticulated β-lactoglobulin and **c** microparticulated α-lactalbumin in lab scale. Atomisation of the liquid with spray nozzle. The *arrow* indicates the mean particle size of the suspensions before spray drying

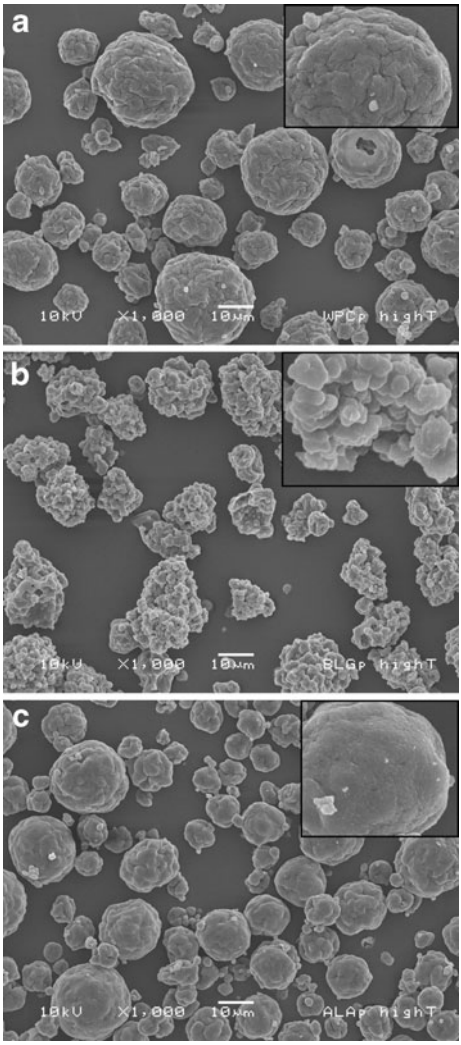

Fig. 2 SEM micrographs of the spray-dried microparticulated whey protein fractions in lab scale with an air outlet temperature of 105 °C. **a** MP WPC, **b** MP β-Lg and **c** MP α-La. *Abbreviations: MP WPC* microparticulated whey protein concentrate, *MP β-Lg* microparticulated β-lactoglobulin, *MP α-La* microparticulated α-lactalbumin

3.3 Particle size of the aggregates after reconstitution in distilled water

Reconstitution of powders in water to the initial dry mass content of the solutions after spray drying was performed in order to analyse the reversibility of the aggregation observed in powder form when compared with the original liquid suspensions. It has been observed that after dissolution of the lab-scale dried powders and stirring for 2 h, the mean diameter of the particles was not significantly different ($p>0.05$) from the values of the original suspension before they were spray dried (Figs. 3 and 4). As illustrated in Fig. 1, the increase in particle size was found to be independent of drying temperature. Therefore, the

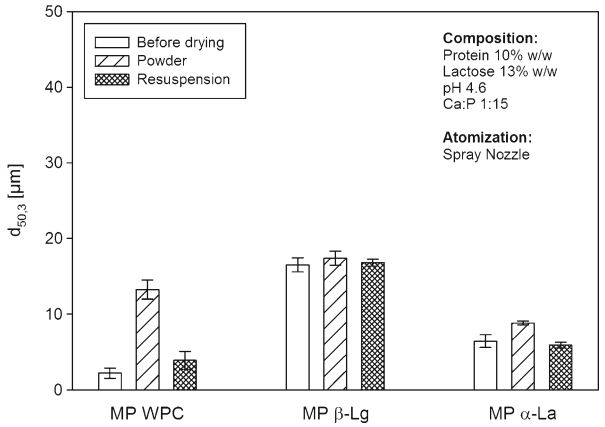

Fig. 3 Mean particle size of the microparticulated whey proteins as an average of all measured inlet and outlet air temperatures. For each whey protein fraction, the values for microparticles in liquid form before spray drying (*left*), in powder form (*center*) and in liquid form after reconstitution in water (*right*) are reported. *Abbreviations: MP WPC* microparticulated whey protein concentrate, *MP β-Lg* microparticulated β-lactoglobulin, *MP α-La* microparticulated α-lactalbumin

represented data in Fig. 3 correspond to the mean value of all results from the 16 possible temperature combinations.

Iordache and Jelen (2003) observed that microparticles of microfluidised WPC presented re-aggregation as they were reconstituted in water after drying. They concluded that this effect was caused by temperature increase of the microparticles during spray drying of the samples. Although the particle size was not directly reported in this study, a decrease in the solubility of the concentrate was observed, especially for the microparticulates heated for 10 min at 90 °C and pH 4.8 or 5.8. These conditions are close to those used in our study. The authors of the study also found out that the resolubilised whey protein was sensitive to secondary heat coagulation, suggesting that some reactive sites of the thermally modified structures may have been newly reformed. The high denaturation degree of the microparticles prior to spray drying in the present study might be one of the reasons for the conservation of the particle size after reconstitution since in this case the reactivity of the protein during thermal treatment, i.e. its tendency to aggregate, is drastically reduced. Further

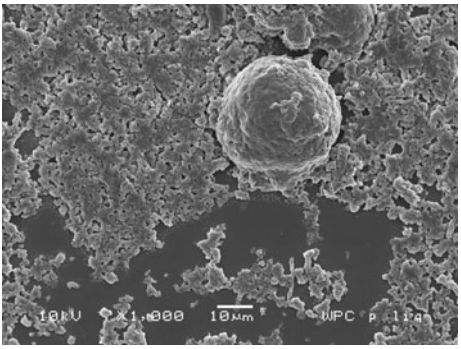

Fig. 4 SEM micrograph of the spray-dried MP WPC with an air outlet temperature of 105 °C after resuspension in distilled water (dilution, 1:10)

Table 2 Optimum temperature intervals for spray drying of microparticulated whey proteins as measured in lab scale with a nozzle as atomisation unit

Product	Air inlet temperature (°C)	Air outlet temperature (°C)
MP WPC	180–190	95–105
MP α-La	190–195	95–105
MP β-Lg	200–210	100–105

MP WPC microparticulated whey protein concentrate, *MP α-La* microparticulated α-lactalbumin, *MP β-Lg* microparticulated β-lactoglobulin

The temperatures were used for validation in pilot scale

explanation is found in the extremely reduced quantity of reactive thiol groups available for further aggregation of the whey proteins, together with the low residence time in the lab-scale spray drier. High lactose content of the protein concentrate and a low pH value (4.6) of the concentrates also contribute to the reduction of the protein reactivity during heat induced denaturation, which in other cases could aggregate and increase the size of the microparticles (Plock et al. 1998, Tolkach and Kulozik 2005).

3.4 Moisture content of the powder

The average moisture content of the samples measured by Karl Fischer titration is presented in Fig. 5 as a function of the outlet temperature. The mean value of the measured moisture level for each air inlet temperature was calculated, resulting in four single values for the four different measured outlet temperatures, instead of 16. A reduction of the moisture content in the powders with increasing outlet air temperature is shown in Fig. 5. While the moisture decreases continuously for MP β-Lg, an almost constant residual water content remains in the powder samples of MP WPC and MP α-La for outlet air temperatures above 95 °C. The measured moisture

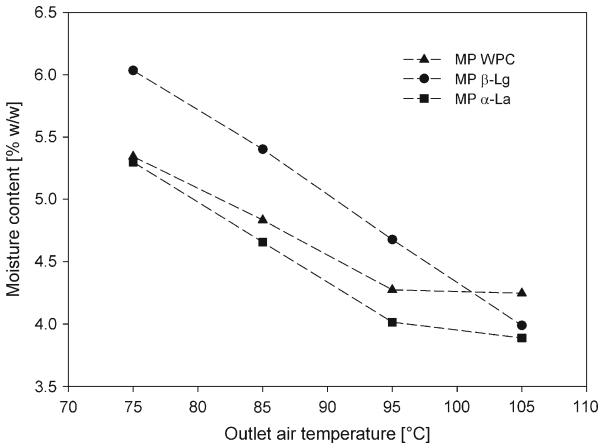

Fig. 5 Effect of outlet air temperature on the bulk moisture content of the spray-dried MP WPC. Each reported outlet temperature corresponds to the average of the four inlet temperatures. The standard error of the measurements was max. 0.5%

contents, especially those at outlet temperatures above 95 °C, are consistent with recommended quality criteria for dry dairy ingredients (Schuck et al. 2007).

The residual water content of the powders was reduced as the air outlet temperature was increased, confirming the results of Anandharamakrishnan et al. (2007). Higher outlet temperatures result in a greater driving force for heat and mass transfer, promoting a more intense removal of water from the material. The porous structure of the dried MP β-Lg particles can facilitate a better transport of vapour outside the particle, resulting in a lower residual moisture content for this product (Fig. 5). Skin formation could lead to hindered transport of water, induction of vacuole formation and in extreme cases to fracture of the surface, as observed with MP WPC and MP β-Lg (Fig. 7a, b). After cooling of the particles when exiting the dryer, condensation of the remaining vapour takes place, leading to shrivelling of the surface as depicted in Figs. 2 and 7. A more detailed discussion on the morphology of the particles is done in Section 3.5 of the present manuscript.

3.5 Morphology

A morphological characterisation of selected samples was conducted using scanning electron microscopy (SEM), as presented in Fig. 2 for microparticles dried with an inlet temperature of 200 °C and an outlet temperature of 105 °C. A difference in the surface appearance of the obtained powders has been observed for particulates containing isolated β-Lg: in this case, the powder particles seem to be composed of smaller aggregates of denatured β-Lg (Fig. 2b) resulting from the thermally induced agglomeration of the protein during microparticulation. As detailed in the upper corner of the micrographs, the structure of the obtained MP β-Lg aggregates shows a high porosity when compared with the detailed images on Fig. 2a, c from MP WPC and MP α-La, respectively. In the case of the latter samples, which contain α-La, the particles show a more compact appearance, their surface is smoother and an apparent skin formation with shrivelling is observed. The presence of some particles with cavities is also highlighted.

After resuspension of the powder, it could be confirmed that many aggregates are part of the powder particles. This is shown in Fig. 4 for MP WPC after drying and resuspension of a powder sample obtained in lab scale with an air-outlet temperature of 105 °C. Under these conditions, the agglomeration of the microparticles during spray drying appears to be irreversible. Similar observations were made with MP β-Lg and MP α-La (not shown).

According to the classification reported by Walton and Mumford (1999), powder samples from MP WPC and MP α-La (Figs. 2a, c and 7a, c) fall within the skin-forming type of materials, while samples from MP β-Lg (Figs. 2b and 7b) are characterised as aggregated particulates. Materials which are insoluble in water and therefore form a suspension tend to form agglomerates when dried. This is also a typical characteristic of the MWP, which are mainly insoluble in water and remain suspended in water or sediment according to the particle diameter. After the residual moisture is removed via saturated and non-saturated surface drying, massive or hollow particles with a very smooth surface are formed. The main difference between both morphologies is the skin (crust) formation. The agglomerated nature of the particles containing MP WPC was confirmed after resolubilisation, as represented on

Fig. 4. The formation of crust requires the presence of surface active substances (Kim et al. 2009). Although the heat treatment of whey protein can reduce their surface activity when compared with native protein (Millqvist-Fureby et al. 2001), it is clear from Figs. 2 and 7 that the microparticulated whey proteins also keep some interfacial activity, especially when α-La is present, and therefore leading to skin formation. This phenomenon can cause the hardening of the external surface of the particle and limits its growth, resulting in presence of holes at the particle surface, mainly in the particles from the pilot scale trials (Fig. 7a, c). Longer residence times inside the dryer could lead to a further increase of the droplet temperature, which directly affects the internal pressure of the droplet, causing a volume increase that can result in collapsed particles, as observed by Alamilla-Beltrán et al. (2005). The shrivelled morphology of MP WPC and MP α-La coincides also with the results presented by Millqvist-Fureby et al. (2001) for spray-dried WPC with high denaturation degree. Further studies concerning the differences in the surface properties of microparticles from the examined protein fractions should confirm these results.

3.6 Validation in pilot scale

Batches of 15 L were produced in a continuous SSHE containing two heating sections, a holding tube and a cooling section. The heating conditions and composition of the suspensions corresponded to the values of Table 1. The obtained microparticles had mean sizes similar to those produced in small scale with 4.89 μm for MP WPC, 15.77 μm for MP β-Lg and 8.26 μm for MP α-La. After spray drying of the powders an increase in the mean particle size was measured. As illustrated in Fig. 6, the difference between the particle size in liquid and dried form was much higher in the pilot scale in comparison to the results in lab scale (Fig. 3). Nevertheless, after reconstitution to the initial solid content, a reduction in particle size was observed. A slight increase of the particle size in the reconstituted suspension was noted for microparticulates of WPC and α-La, when compared with the original suspension in liquid form. Contrary to the trials in lab scale, a reversible aggregation of the

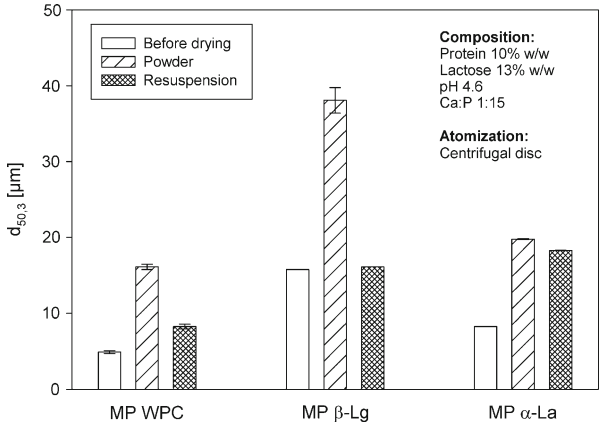

Fig. 6 Mean particle size of the MP WPC during processing and drying in pilot scale. For every whey protein fraction, the values for microparticles in liquid form before spray drying (*left*), in powder form (*center*) and in liquid form after reconstitution (*right*) are reported

microparticulates was measured only with MP β-Lg. However, the observed increase in particle size for the other suspensions did not negatively influence the sensory properties of the aggregates since $d_{50,3}$ was lower than 20 μm after dissolution of MP WPC and MP α-La in water.

Laboratory-scale spray dryers generally produce very fine particles, normally smaller than 20 μm (Kim et al. 2009), which could limit the relevance of the presented results. In order to validate our findings, the spray drying of the particulates was tested using a pilot plant equipped with a centrifugal disc. Contrary to the results in lab scale, much bigger powder particles were achieved after spray drying, and a reduced reversibility of the induced aggregation was observed after resolubilisation.

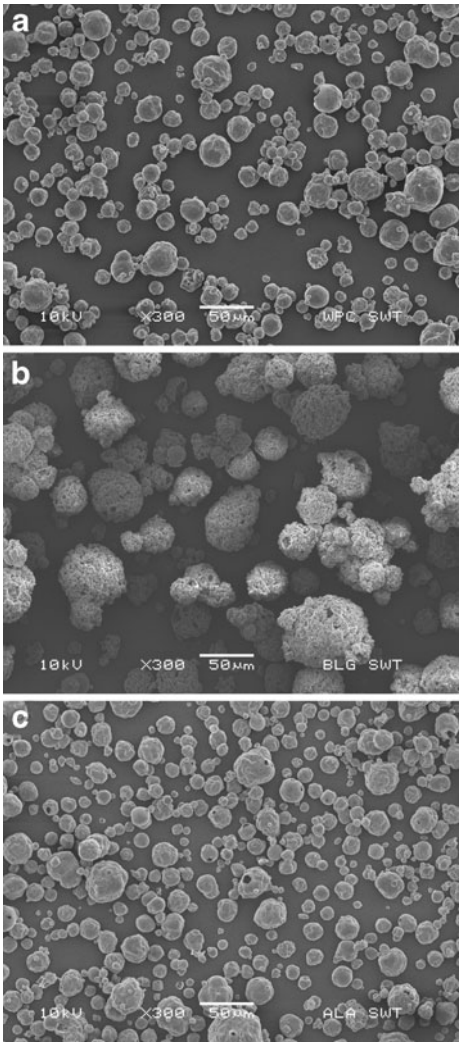

Fig. 7 SEM micrographs of the spray-dried MP WPC fractions in pilot scale. **a** MP WPC, **b** MP β-Lg and **c** MP α-La. *Abbreviations: MP WPC* microparticulated whey protein concentrate, *MP β-Lg* microparticulated β-lactoglobulin, *MP α-La* microparticulated α-lactalbumin

As described by Elversson et al. (2003), there is a linear relationship between the droplet size and the size of the powder particles. The atomisation of the liquid with a pneumatic nozzle results in smaller droplets than when a centrifugal disc is used (Filkova and Mujumdar 1995). This could be one of the reasons for the increase in the particle size in powder form between the trials in laboratory and pilot scale. The difference in residence time of the droplets for spray dryers with different dimensions might play also a decisive role in the size of the powdery particulates and their solubility. Gianfrancesco (2009) examined the mean residence time of a pilot plant with a similar size than the dryer used in our experiments in pilot scale. He found it to be around 2 min. During this time a severe hardening of the crust can occur, leading to a hindered moistening of the particles. Therefore, a higher irreversibility of the aggregation effect induced by spray drying (Fig. 6) is observed in pilot scale. In comparison, the droplets remain few seconds in the lab-scale dryer, resulting in complete different characteristics of the surface skin and better wettability. A highly porous particle as MP β-Lg could possess better hydration properties, leading to better results after resolubilisation. The disruption of the aggregates represents a decisive step during rehydration of dried milk protein concentrates (Mimouni et al. 2009), which could be easier in the case of MP β-Lg due to a better penetration of water into the powder particles.

SEM micrographs of the pilot scale powders (Fig. 7) show morphologies which are very similar to those obtained with the lab-scale drier (see Section 3.4). MP WPC and MP α-La appeared smooth and slightly shrivelled. When MP β-Lg was dried, it resulted in a porous structure composed of numerous agglomerates. Despite different spray systems, the higher amount of vacuoles present in the powder particles is remarkable, indicating a higher pressure inside the particles and possibly the formation of a much harder skin at the surface. Especially in the case of MP β-Lg (Fig. 7b), the presence of aggregated droplets is noticeable, which can be one of the reasons for increased particle sizes when compared with Fig. 2b. Here, the aggregation from single droplets during drying was not observed in such an extent as in Fig. 7b.

The residual water content of the samples obtained in pilot scale varied from 2.37% to 2.77% (*w*/*w*).

4 Conclusions

The influence of the drying temperatures on the particle size, the moisture content and the morphology of microparticulated α-La, β-Lg and WPC has been evaluated using a lab-scale spray dryer with a nozzle as atomisation system. The microparticulated whey suspensions used for the analysis had a denaturation degree higher than 90% and mean particle sizes below 20 μm. It has been observed that the drying temperature exerts no influence on the particle size of microparticulated whey proteins, as measured with aggregates from native mixture of α-La and β-Lg or their isolated forms. A slight increase in particle size was detected when the liquid and dry forms were compared. Nevertheless, the mean particle size of the microparticles was reduced to values very close to the original aggregates before drying when they were reconstituted in water. These results are valid for whey proteins with a denaturation degree higher than 90%. Higher air-outlet temperatures led to a decrease in residual

water content of the powder samples, which should be considered for the stability of the product during storage. Different atomisation systems do not influence either the irreversible aggregation of the particles or its capability of falling apart to their initial size after resuspension. These results were confirmed during validation with a pilot drying plant provided with a centrifugal disc. Atomisation using a spray nozzle has shown better results concerning conservation or minimisation of the particle size of the aggregates after spray drying, although results with the rotating disc were also satisfactory.

It should be confirmed with additional studies, if in presence of lower denaturation rates of the protein particles, further reaction and agglomeration during drying could be also avoided. The spray-drying of suspensions containing reduced lactose concentrations, higher pH values or a lower denaturation degree of the initial microparticulates, which could be prone to further aggregation during heating, should be also considered for further studies.

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