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Moisture sorption and stickiness behaviour of hydrolysed whey protein/lactose powders

S. A. Hogan · D. J. O'Callaghan

Abstract The potentially negative effects of low molecular weight disaccharides, especially lactose, on spray-drying efficiency and storage stability of dairy powders are often counterbalanced by the presence of intact milk proteins. Hydrolysis of proteins, however, may impair such protective effects and contribute to a loss in production performance. Hydrolysed or non-hydrolysed whey protein/lactose (WP/L) dispersions were spray dried, in order to examine the effects of protein hydrolysis on relaxation behaviour and stickiness of model powders. Whey proteins included a non-hydrolysed, whey protein isolate control and three hydrolysed whey protein powders (WPH), with DH values of 8, 11 and 32, where DH = degree of hydrolysis. Hydrolysis of whey proteins increased moisture sorption in WP/L powders. Moisture sorption was higher in powders containing hydrolysed proteins. Whey proteins delayed the time-dependent onset of lactose crystallization, and this effect was greatest in powders containing WPH32. Glass-rubber transition (T_{gr}) temperatures of WP/L powders were not affected by protein hydrolysis but were dominated by the lactose fraction. Powders containing hydrolysed whey proteins were more susceptible to sticking compared to intact proteins. Surface coverage by proteins or peptides was lower in powders containing hydrolysed WP, and this would have contributed to the greater susceptibility of these powders to sticking. Results suggest that hydrolysis of WP affected the relaxation behaviour of WP/L powders and altered the rate at which lactose underwent viscous flow behaviour.

Keywords Whey protein isolate · Hydrolysis · Lactose · Moisture sorption · Stickiness/relaxation

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1 Introduction

It has been estimated that some 186 million tonnes of whey were generated from cheese production in 2008 (Affertsholt 2009) with annual growth of 2% per annum in the 6 years preceding that. Whey protein powders, produced as valuable by-products of cheese and casein manufacture, are used extensively in a wide range of food formulations because of their nutritional and functional attributes. Their range of application can be further increased by hydrolysis, which alters functional properties such as solubility, viscosity, surface activity, organoleptic qualities, digestibility and biological activity. Whey protein hydrolysates (WPH) and isolates (WPI) are considered 'high-end' products enjoying double-digit growth in recent years (Affertsholt 2010).

Hydrolysed proteins have smaller molecular mass and less secondary structure than intact proteins. Control of the molecular size of resultant peptides constitutes an essential step in the development of protein hydrolysates for dietary use. Extensively hydrolysed proteins can help lower immunological reactivity, and can be used in formulas for hyper-allergic infants and in the nutritional management of individuals unable to digest non-hydrolysed protein. The high biological value peptides generated by hydrolysis are readily absorbed, provide a valuable source of amino acids, including branched-chain amino acids (leucine, isoleucine and valine) and provide a general protein supplement in a wide variety of diets. Claims have also been made for their use in physical performance and recovery, anti-cancer and bioactive properties and their action as an appetite suppressant and weight management aid (Korhonen 2006; Ordman 2008; Smithers 2008).

Because such nutritional components are incorporated as spray-dried powders into food systems, it is of interest to study the effects of processing on the physical properties of powders, especially with regard to sticking during drying and storage stability. It is well established that physical changes in food powders are closely related to the glass transition (T_g) phenomenon (Chuy and Labuza 1994; Roos 1995). The presence of moisture accelerates physical change in powders because of its profound effect in lowering T_g. Moisture, along with low molecular weight sugars, most notably lactose, are the main sources of ingredient instability in spray dried milk powders.

Previous work has provided some understanding of the effects of milk protein on the physical behaviour of lactose in spray dried powders (Haque and Roos 2004; Hogan and O'Callaghan 2010; Silalai and Roos 2011). Powders with higher protein/lactose ratios were less susceptible to sticking. It was demonstrated that stickiness was related both to T_g and to the temperature increment by which T_g must be exceeded before sticking occurs ($T-T_g$) and that powder composition affected T_g and $T-T_g$ in different ways.

Netto et al. (1998) reported that the T_g of protein hydrolysates (without lactose) is dependent on the average molecular weight and suggested that such hydrolysates may be as important as sugars in lowering the overall T_g of food powders. Hydrolysate powders tend to be more hygroscopic and thermoplastic compared to powders containing intact proteins and hence have poorer stability. Mounsey et al. (2012) reported that although intact and hydrolysed sodium caseinate/lactose interactions were weak, stickiness was affected by protein hydrolysis. Notwithstanding previous work, information on the effects of hydrolysis on functional characteristics of whey protein powders remains limited.

The objectives of this study were to characterize the moisture sorption, and stickiness behaviour of powders composed of intact or hydrolysed WPI and lactose, in order to gain insight into the relationships between ingredient types, and their effects on drying, state transitions and relaxation behaviour of powders. Such knowledge should contribute to the development of advanced processing and spray drying control systems in order optimize efficiency during the production, storage and transport of dairy powders.

2 Materials and methods

2.1 Materials

Whey protein isolate 'Isolac' (91% protein w/w) and three whey protein hydrolysate powders with DH values of 8, 11 and 32 (where DH=degree of hydrolysis or number of peptide bonds cleaved enzymatically and expressed as a percentage of the original number of peptide bonds) were purchased from Carbery Ingredients Ltd. (Enniskeane, Co. Cork, Ireland). Edible-grade α-lactose monohydrate (Lactopure, 94.2% w/w) was obtained from DOMO (Borculo, The Netherlands).

2.2 Preparation of spray-dried powders

Four powders containing whey protein (intact or hydrolysed) and lactose, with a protein/lactose ratio of 1/1.54, were produced at Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland from 10 kg dispersions containing 11% (w/w) protein and 22% (w/w) lactose. Protein powders were reconstituted in RO grade water at 60 °C with shearing for 20 min at 6,000 rpm using a Silverson® mixer (Silverson Machines Ltd., Chesham, UK). Lactose was added with constant mixing for 10 min, and the temperature was brought up to 70 °C in order to ensure complete solubilisation. Samples were subsequently spray dried in a pilot-scale drier (Anhydro drier Model Lab 3; APV Anhydro AS, Søborg, Copenhagen, Denmark) with pressure nozzle atomisation, and inlet and outlet temperatures of 185 and 85 °C, respectively, and final drying in an external fluidized bed. Powders were produced singly, packed in plastic-lined, paper bags and stored at 15 °C prior to analyses. All analyses were carried out within 3 months of manufacture. Whey protein/lactose powders were denoted WPI/L, WPH8/L, WPH11/L and WPH32/L to reflect the degree of hydrolysis of the whey fractions.

2.3 Powder characterisation

Free moisture content was determined by weight loss following overnight drying of 2 g powder at 102 °C. Protein ($N×6.38$) was determined by macro-Kjeldahl (IDF 2001). Ash content was determined after overnight incineration at 550 °C. Lactose content was determined by difference, and was assumed to be in the amorphous state. Chemical analysis of powders was carried out immediately after manufacture. Powder particle size was determined by laser light scattering using a Malvern Mastersizer S with dry powder accessory (Malvern Instruments Ltd., Worcs. England). All analyses were carried out in duplicate.

2.4 Moisture sorption

Moisture sorption isotherms were determined by weighing ca. 2 g powder onto aluminium dishes, and then holding at 19 ± 3 °C, in evacuated desiccators containing saturated salt solutions of P_2O_5, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, giving relative humidities (RH) of 0%, 11%, 22%, 33%, 44%, 55% and 66%, respectively. Powders were equilibrated for a minimum of 21 days, after which no change in weight was observed.

Time-dependent crystallisation of lactose was observed by loss of moisture, bound by amorphous lactose, using a gravimetric sorption analyser (Sorption Test Systems SPS 11-10 μ Projekt Messtechnik, Ulm, Germany). Samples (ca. 100 mg) were equilibrated (as defined by the conditions of the test, i.e. weight change less than 0.01% within 80 min) sequentially at 2% and 75% RH at 22 °C and changes in mass recorded automatically. Powders were first equilibrated at 2% RH (the effective lower limit of the sorption analyser) in order to ensure that powders had similar water activities prior to humidification at 75% RH.

2.5 Glass-rubber transitions (T_{gr})

Glass-rubber transition temperatures (T_{gr}) were determined by a thermo-mechanical technique, using a standard laboratory rheometer, described by Hogan et al. (2010) and Schuck et al. (2012). Powder samples (ca. 2 g) were compressed (30 N) between a base plate (Peltier) and a 40-mm-diameter steel parallel plate and heated, via the Peltier plate, from 20 to 100+ °C at a constant rate (2 °C min^{-1}). T_{gr} was identified by a significant increase in the rate of change in normal force and determined by extrapolation, to the temperature (x-axis), of the intersection of tangents to pre- and post-inflection areas of the curve.

2.6 Stickiness

A miniature powder fluidization bed technique, previously described by Hogan et al. (2009), was used to determine the sticking behaviour of spray dried powders. Powders (ca. 0.4 g) were suspended at a series of fixed temperatures (dry bulb temperatures, equivalent to spray drier outlet temperature) in a stream of air saturated by bubbling through water at controlled temperatures. The miniature fluid beds (×6) were held in an insulated, aluminium heat exchange column heated with a central element. The temperature of the water bath was gradually increased (0.8 °C min^{-1}), thus raising the relative humidity of fluidising air in contact with suspended powder particles, until the powder became sticky. Sticking points were recorded by noting the saturated air temperature or dew point (water bath temperature) at which powders, in each fluidized bed, ceased to fluidize and/or by the appearance of 'rat holes' [channels formed by air flowing through a solid (sticky) powder mass]. RH was determined from the dry bulb temperature and the dew point. Stickiness curves were generated by plotting the air temperature versus RH, at which fluidisation in each bed ceased.

2.7 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to determine the surface elemental composition of whey protein/lactose powders. Measurements were performed using a

Kratos AXIS 165 spectrometer with a 150 W Al K_α 1486.6 eV X-ray source. Pass energies were 20 and 160 eV for narrow regions and survey spectra, respectively. Dwell times were 100 and 50 ms for narrow regions and survey spectra, respectively. Analyses were carried out at the Materials and Surface Science Institute, University of Limerick, Ireland. Detailed descriptions of XPS, formerly known as ESCA (electron spectroscopy for chemical analysis), are available elsewhere (Fäldt et al. 1993; Gaiani et al. 2006).

2.8 Scanning electron microscopy

Scanning electron microscopy was carried out using a field emission scanning electron microscope (Zeiss Supra Gemini, Darmstadt, Germany). Powders were lightly coated with a chromium target at 100 mA for 30 s and imaged at 1 kV.

3 Results

3.1 Powder characterization

Spray-dried whey protein/lactose powders had average protein and lactose contents of 37.0 and 57.1% (w/w), respectively (Table 1). Moisture contents following drying were in the region of 1.7% (w/w). Fat contents of WPI/L and WPH8/L powders were significantly lower than WPH11/L and WPH32/L powders, with average values of 0.4 and 3.8% (w/w), respectively. Mineral contents of powders containing more extensively hydrolysed proteins were also higher. The higher fat and mineral contents of WPH11/L and WPH32/L powders reflect the manufacturing process of these commercial whey protein hydrolysates. Higher fat contents resulted from the use of whey protein concentrate as the protein source for hydrolysis and higher mineral contents due to addition of alkali to neutralize hydrolysis-induced drop in pH. Volume average diameters [$D_{4,3}$] of WPI/L powders ranged from 81.9 μm for WPI/L powders to 55.3 μm for WPH11/L powders.

Table 1 Compositions and physical characteristics of whey protein/lactose powders

Powder	Protein	Lactose[a]	Fat	Minerals	Moisture	Particle size[b]	Average molecular weight[c]
(g.100 g^{-1})						(μm)	(Da)
WPI/L	38.7	58.0	0.3	1.3	1.7	81.9	–
WPH8/L	37.8	58.4	0.5	1.2	2.3	74.2	10,073
WPH11/L	36.7	55.9	4.1	1.4	2.0	55.3	8,464
WPH32/L	34.7	55.9	3.5	2.9	3.0	64.6	1,569

[a] Calculated by difference

[b] Volume average diameter [$D_{4,3}$]

[c] Average molecular weight of protein fractions provided by manufacturer

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3.2 Moisture sorption experiments

Moisture sorption of WP/L powders increased with relative humidity up to 55% RH (Fig. 1), where crystallisation of lactose was evidenced by a decrease or discontinuity due to the release of moisture bound previously by amorphous lactose. The amount of moisture sorbed by powders prior to lactose crystallisation increased with the extent of protein hydrolysis, with WPH32/L sorbing significantly more moisture than the other powders. WPH8/L and WPH11/L powders desorbed greater amounts of water upon crystallisation than the other two powders.

Time-dependent crystallisation of lactose at 75% RH (Fig. 2) was significantly delayed in WP/L powders compared to amorphous lactose and was delayed most significantly in WPH32/L powders. The crystallisation behaviour of amorphous lactose powder was included in this experiment for the purposes of comparison. Prior to exposure to air at 75% RH, powders were equilibrated at 2% RH, in order to ensure similar water activities. Amorphous lactose was the first to crystallize 83 min after start of RH ramp to 75% at a moisture content of 11% (w/w; i.e. crystallisation moisture content=delta mass+powder moisture content at start of experiment: average value 1.3% w/w) and an RH of 69%. Whey protein/lactose powders crystallized at 160, 136, 160 and 184 min and had moisture contents of 21.4, 21.3, 22.3 and 25.9% (w/w) for WPI/L, WPH8/L, WPH11/L and WPH32/L, respectively.

3.3 Glass-rubber transitions

Glass-rubber transitions (T_{gr}) values of whey protein mother powders (without lactose), established by thermo-mechanical analysis, decreased with increasing a_w but did not show significant differences due to hydrolysis of whey proteins, and no clear effect could be observed due to differences in average molecular weight of protein species (data not shown). WPH32 mother powders were more fragile for any given equilibrium RH compared to WPI, i.e. showed a greater rate of change in normal force around the glass-rubber transition (Fig. 3a). In contrast with whey
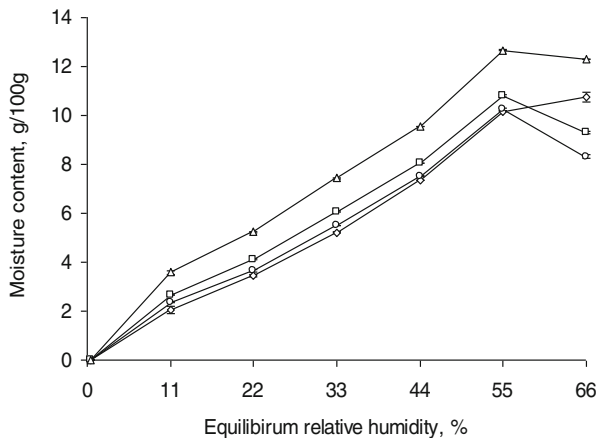

Fig. 1 Moisture sorption isotherms of whey protein/lactose (WP/L) powders. WPI/L (*diamond*), WPH8/L (*circle*), WPH11/L (*square*) and WPH32/L (*triangle*). Moisture contents were expressed on a wet weight basis
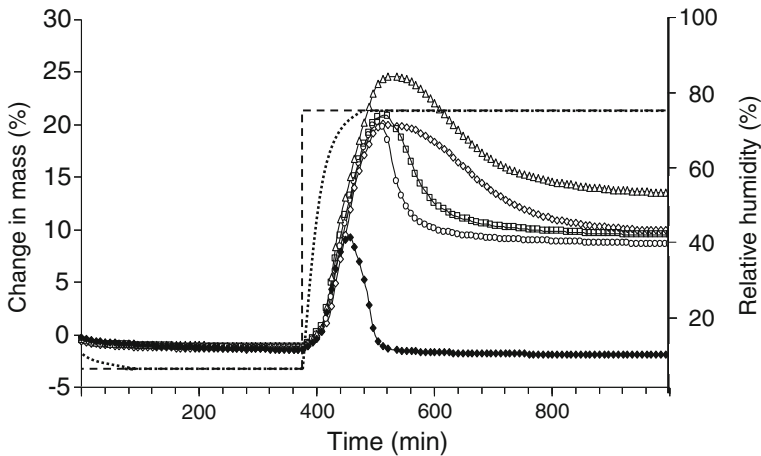
Fig. 2 Change of mass against time during equilibration (2% RH) and sorption (75% RH) cycles for whey protein/lactose (WP/L) powders held at 22 °C. Equilibrium conditions, as defined by the conditions of the test (change in mass of less than 0.01% over 80 min), were established for all powders prior to RH step change. WPI/L (*white diamond*), WPH8/L (*white circle*), WPH11/L (*white square*), WPH32/L (*white triangle*) and amorphous lactose (*black diamond*). RH set point (*long dashed lines*), RH actual (*short dashed lines*)

protein mother powders, T_{gr} curves of WP/L powders at each RH were more constant with respect to shape (Fig. 3b) and did not show a faster rate change in normal force as a function of protein hydrolysis. The rate of change in normal force around the glass-rubber transition increased in all powders with increasing RH.

3.4 Stickiness behaviour

Stickiness curves show a profile of temperature and RH conditions required for powders to cease fluidising in a stream of air, at defined temperature and RH. The sticking point, thus generated, reflects powder behaviour under conditions comparable to those of a spray drier outlet or fluidized bed. The RH at which sticking of whey protein mother powders and whey protein/lactose powders occurred decreased with higher air temperatures (equivalent to spray drier outlet temperatures; Fig. 4). Whey protein mother powders were significantly less susceptible to sticking than the whey protein/lactose powders from which they were manufactured, i.e. higher RH conditions were required at a given temperature for sticking to occur. In all cases, hydrolysis of whey proteins increased the susceptibility of powders to sticking. Sticky curves of whey protein/lactose powders were closer together with respect to RH than whey protein mother powders, suggesting that the effects of protein hydrolysis were less pronounced in powders containing lactose.

Sticking occurs at a temperature above T_{gr} by an increment referred to as $T-T_{gr}$. Values for $T-T_{gr}$ can be established by superimposing a stickiness curve onto a T_{gr} curve plotted as a function of RH, measured at a series of equilibrium RH (Fig. 5). $T-T_{gg}$ of WP/L powders decreased with increasing levels of hydrolysis with values of 50, 46, 39 and 38 °C for WPI/L, WPH8/L, WPH11/L and WPH32/L, respectively. As in the case of whey protein mother powders, T_{gr} of whey protein/lactose powders decreased with increasing RH but did not show any significant effect of protein hydrolysis (Fig. 5 lower curves).
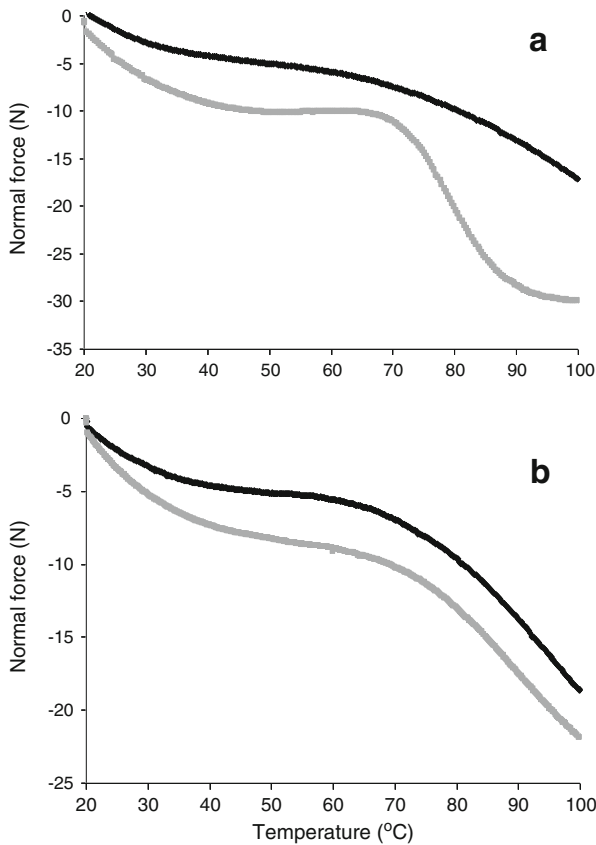
Fig. 3 Representative glass-rubber transition curves of powder equilibrated at 11% RH. **a** Whey protein mother powders: WPI (*black line*), WPH32 (*grey line*) and **b** Whey protein/lactose powders: WPI/L (*black line*), WPH32/L (*grey line*)

3.5 X-ray photoelectron spectroscopy

Conversion of surface elemental data to percentage coverage by food macro-constituents is normally solved by simultaneous equation according to Fäldt et al. (1993). In the present study, values for nitrogen in WP/L powders were higher than those of the reference sample (WPI), and calculations resulted in an overestimation of protein surface coverage, i.e. greater than 100%. Because nitrogen is not present in either lactose or milk fat, it is used as sole elemental indicator of proteins or peptides, i.e. as an indicator of amine bonds. An estimate of protein/peptide surface coverage of powders was therefore determined using a milk protein nitrogen conversion factor of 6.38 (IDF 2006), under the assumption that all surface nitrogen originated from proteins or peptides. In so doing, estimates of relative coverage of lactose and fat could not be made. Values of protein/peptide surface coverage ranged from 90.2% to 52.7% for WPI/L and WPH11/L powders, respectively (Fig. 6), with hydrolysis of WPI having the overall effect of decreasing protein surface coverage.
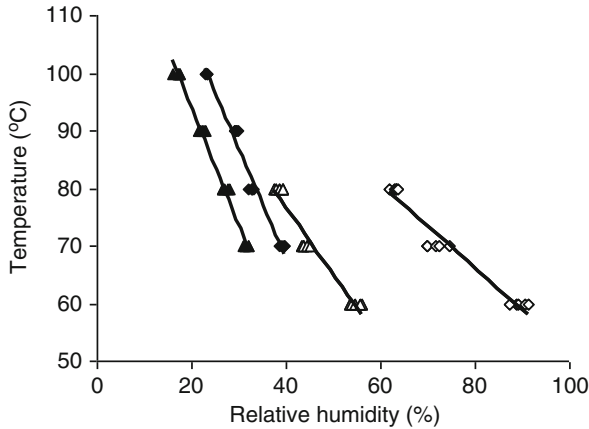
Fig. 4 Stickiness curves for whey protein mother powders: WPI (*white diamond*), WPH32 (*white triangle*) and whey protein/lactose powders: WPI/L (*black diamond*), WPH32/L (*black triangle*)

3.6 Scanning electron microscopy

WP/L powders prepared with non-hydrolysed WPI or with WPH8 or WPH11 appeared similar in shape and structure with well-defined surface morphologies, whereas, in contrast, equivalent powders containing WPH32 were smoother, with less well-defined surface features (Fig. 7). All powders appeared structurally intact following spray-drying with no evidence of broken or friable particles.

4 Discussion

The smaller average particle size of more extensively hydrolysed whey protein/lactose powders was probably not due to enhanced surface activity in these
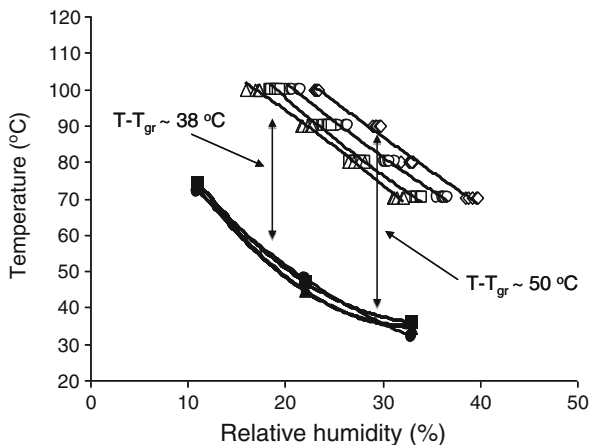

Fig. 5 Relationship between sticking temperature (*T*, *open symbols*) and glass-rubber transition temperature (T_{gr}, *closed symbols*) for whey protein/lactose powders (WP/L): WPI/L (*diamond*), WPH8/L (*circle*), WPH11/L (*square*) and WPH32/L (*triangle*). $T - T_{gr}$ values were calculated by subtraction of averaged, representative points on *T* and T_{gr} curves described by second-order polynomial regression
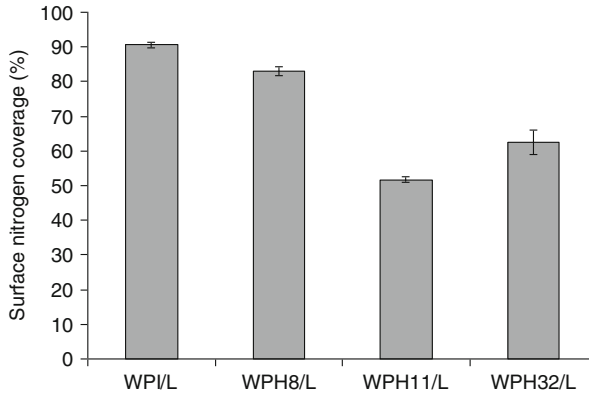
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Fig. 6 Percent surface coverage of nitrogen on whey protein/lactose (WP/L) powders

powders as protein hydrolysis tends to increase the hydrophilicity of peptides (Mahmoud et al. 1992). It is more likely that particle size differences reflect the
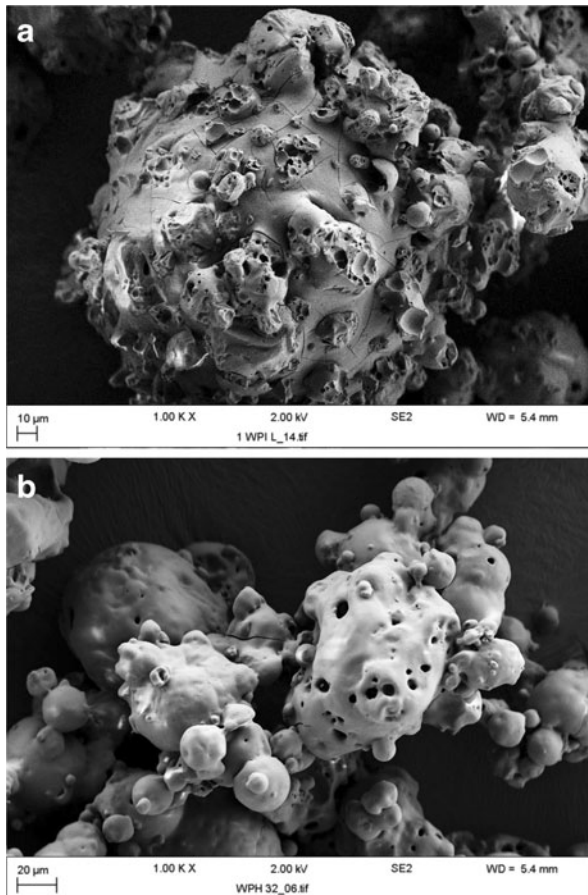

Fig. 7 Representative scanning electron micrographs of whey protein/lactose powders (WP/L). a WPI/L and b WPH32/L

lower viscosity of hydrolysed feeds prior to spray drying, which generally results in a decrease in powder particle size.

Moisture plays a critical role in determining the glass transition, crystallization behaviour and stability of spray dried powders. Berlin et al. (1968) proffered a generalised interpretation of moisture sorption characteristics of dairy powders when suggesting that macro-molecular materials (such as proteins) dominate sorption behaviour at low RH (ca. 0–20%); that the colligative properties of soluble components (such as lactose) exert their effects from about 20–60% RH, and that at vapour pressures above 60% RH, the predominant contribution to moisture sorption comes from salts. The more extensive moisture sorption observed in hydrolysed WP/L, particularly WPH32/L, compared to WPI/L, reflects the number of available water binding sites associated with lower average molecular weight peptides. Netto et al. (1998) reported an increase in moisture sorption behaviour due to protein hydrolysis and that differences in weight distribution of proteins influenced water sorption in the high a_w region. Zhou and Labuza (2007) also found that the main difference between sorption isotherms of WPI and WPH powders, without lactose, was at high a_w only. In terms of minor components, the higher salts content of WPH11/L and WPH32/L powders may have contributed to overall moisture uptake in these powders. The higher levels of milk fat in these powders probably had little or no effect as milk fat does not significantly affect isotherms even when present in large amounts (Foster et al. 2005).

Amorphous lactose undergoes crystallisation at about 40% RH at room temperature (Joupilla and Roos 1994). Although it is well established that proteins delay crystallisation of lactose in powders (Lai and Schmidt 1990; Joupilla and Roos 1994; Haque and Roos 2004), through competition for moisture and as obstacles to molecular rearrangement, hydrolysis of WPI did not alter the RH at which crystallisation of lactose occurred under the equilibrium RH conditions used. Mounsey et al. (2012) also reported crystallisation of lactose at 55% RH in casein hydrolysate/lactose powders. Lai and Schmidt (1990) used 2H nuclear magnetic resonance spectroscopy to show that changes in water mobility were directly related to the transition of lactose from the amorphous to the crystalline state.

Differences in moisture contents of powders at 66% RH reflect the extent of lactose crystallisation (Roos and Karel 1992) and/or subsequent re-absorption of moisture by non-lactose components (Shrestha et al. 2007). These would include proteins and larger peptides, still in the amorphous state. WPI/L powders appeared to behave differently in this respect. Crystallisation onset moisture contents for WPI/L (9.17% *w/w*) were very similar to those reported by Haque and Roos (2004) for whey protein/lactose powders spray-dried at a ratio of 3:1.

Higher moisture availability (RH 75%) shortens the time required for moisture-induced mobilisation of lactose and re-arrangement into the crystalline form (Fig. 2). This is due to a rapid drop in glass transition temperature and a lowering of the activation energy required for viscous flow. Haque and Roos (2004) showed that the time required for lactose crystallisation increases under lower RH conditions and differs depending on the type and concentration of protein. The rate and extent of moisture sorption by WPI/L powders prior to crystallisation were similar to that observed by Haque and Roos (2004). These authors also concluded that delays to crystallisation are dependent on the interaction behaviour between lactose and protein. Ibach and Kind (2007) and Nijdam et al. (2008) also reported that proteins and

salts increase the mean path length required for migration and association of lactose molecules. Given the similarity in mineral contents of WPH11 and WPH32, it appears that the role of salts was not responsible for differences in delays to crystallisation between these two powders. The higher moisture sorption levels in WPH32/L powders prior to crystallisation suggest that the contribution of this hydrolysate may have been colligative in nature, i.e. that the effect was related to the number of available water-binding sites and that competition for moisture may have played a more significant role than any steric hindrance effect. To the authors' best knowledge, no data on the moisture sorption and time-dependent crystallisation behaviour of hydrolysed whey protein/lactose powders are available for comparison.

Glass-rubber transition temperatures of spray dried powders can be used as indicators of susceptibility to change of state. In this way, they provide information on the relaxation time of powders and their overall stability. In simple terms, the relaxation time is a measure of the time required for the energy stored in a spring (G′ or storage modulus) to shift to a dashpot (G″ or loss modulus) and dissipate in a material. Below T_g, amorphous materials exist in a non-equilibrium, glassy state and can be stored for extended periods without a significant change. Temperatures at and above T_g result in an exponential increase in molecular mobility and decreases in the viscosity of the particle surface lead to sticking, caking and collapse (Roos 1995, 2010; Foster et al. 2006). In the present study, T_{gr} can be considered as analogous to T_g (Hogan et al. 2010).

The temperature dependence of relaxation times below T_g is thought to follow Arrhenius behaviour, and relaxation times can be very long when a material is in the glassy state. Above T_g, however, this temperature dependence may deviate from normal Arhenian behaviour depending on composition (Silalai and Roos 2011). Angell (1991) developed a scheme that classifies liquids as 'strong' or 'fragile' according to the dependence of viscosity on T_g/T, and the extent of deviation from the Arrhenius temperature dependence above T_g. 'Strong' materials, such as proteins, generally demonstrate small physical changes at T_g, whereas 'fragile' materials, such as amorphous sugars, show greater changes in viscosity around T_g. Water-plasticised food components may be assumed to increase in fragility with increasing water plasticization (Roos 2011).

T_{gr} curves of WP mother powders (Fig. 3a) showed a more rapid normal force response to temperature change in WPH32 compared to WPI. A faster rate of change in the curve is indicative of more rapid development of viscous flow. Netto et al. (1998) and Zhou and Labuza (2007) reported that moisture-induced decreases in T_g of hydrolysed protein powders are greater than that for equivalent non-hydrolysed powders. Although relaxation behaviour of WP mother powders was different, it remains unclear why they did not show molecular weight effect decreases in T_{gr}. Mounsey et al. (2012) found that T_g of hydrolysed caseinate/lactose powders determined by DSC was not significantly different but that T_{gr} values did decrease with casein hydrolysis. The analytical technique used to quantify state transitions has a bearing on absolute values (Hogan et al. 2010).

The shape of T_{gr} curves in WP/L powders suggests that material relaxation was dominated by lactose. Although there is an inverse relationship between molecular weight and T_g (Levine and Slade 1986), it appears that the contribution of proteins was negligible. Glass-rubber transitions in proteins occur at much higher

temperatures than disaccharide sugars (Hogan et al. 2010) as the plasticizing effects of moisture and heat have less pronounced effect in altering the conformation of larger macromolecules.

Sticking has been attributed to the development of viscous flow induced by a decrease in particle surface viscosity around the glass transition (Downton et al. 1982). Powder particles are thought not sticky where the surface viscosity lies outside a region from 10^6 to 10^8 Pa s (Downton et al. 1982) in the case of powders containing sucrose and fructose. Areas above and below the stickiness curves in Fig. 4, represent 'sticky' and 'non-sticky' conditions, respectively, and provide information on safe operating conditions for drying (Hogan et al. (2009). In this respect, WP mother powders are considerably 'easier' to dry than WP/L powders. Hydrolysis of proteins had the effect of increasing susceptibility to sticking in all powders examined. The broad range of RH values over which WP mother powders ceased to fluidise at a given temperature provides an indication of the relative plasticity of these powders. In contrast, the clustered nature of WP/L sticky curves reflects lactose-dominated relaxation, with hydrolysis of proteins contributing to separation of the curves. The slopes of the stickiness curves also provide information on the relative fragility of the powders. The results from this study suggest that while lactose, present at constant level in the powders examined, is the main determinant of T_{gr}, interactions (direct or indirect) between protein and lactose determine subsequent viscosity-induced change.

The relationship between stickiness and the T_g has been investigated in a number of studies (Boonyai et al. 2004; Paterson et al. 2005; Silalai and Roos 2011). Hogan and O'Callaghan (2010) found that inclusion of milk proteins increases $T-T_g$ in lactose-containing powders. Similarly, Silalai and Roos (2010, 2011) found that relaxation times of non-fat milk, solids were affected by protein content and that a longer contact time between particles may be needed for milk powders with high protein contents to stick. The present study confirms such observations in that $T-T_{gr}$ values decreased with increasing levels of whey protein hydrolysis (Fig. 6). Values observed in the present study were slightly higher than those found for hydrolysed sodium caseinate/lactose powders (Mounsey et al. 2012) using the same fluidised bed apparatus.

Recently, Zhou and Roos (2011) showed that delays to crystallisation of sugars were dependent on protein type but that T_g was not significantly affected by either type or molecular weight of proteins. Similarly, glass transition behaviour of lactose is not greatly affected by salts, but salts cause dramatic changes to water sorption and crystallization behaviour of freeze-dried lactose mixtures (Omar and Roos 2007).

Roos (2011) reported that knowledge of the macroscopic glass transition behaviour may be misleading in the prediction of food stability, and that although T_g gives information on the temperature range over which changes in material properties occur, it does not predict the extent of change during food processing and storage. Rahman (2006) and Kasapsis et al. (2003) reported that transitions determined by DSC should not be considered as fully equivalent with rheological transitions. Boonyai et al. (2007) and Hogan et al. (2010) have also shown that physico-chemical changes in powders do not necessarily correlate with enthalpy change alone.

The nature of interactions of proteins, sugars and moisture is still to be fully resolved. Adhikari et al. (2009) suggested that protein and lactose should be

considered separately with regard to T_g. López-Díez and Bone (2000) and Tzannis and Prestrelski (1999) reported significant interactions in spray dried mixtures of disaccharides and proteins via hydrogen bonding of sugar molecules, and that a phase separation occurs between protein and sugars upon crystallisation of the latter, resulting in separate, non-interacting species. Mounsey et al. (2012) found no significant differences in protein secondary structure whether present with lactose in either the amorphous or crystalline form and suggested that these components were largely immiscible or phase separated.

The dichotomy between bulk and surface compositions has been the subject of much attention (Gaiani et al. 2006; Jayasundera et al. 2009; Murrieta-Pazos et al. 2012). The preferential representation of protein and fat on powder surfaces has also been a feature of studies involving XPS. It is commonly held that surface active materials such as proteins diffuse rapidly to the air–water interface during atomisation and stabilise powders during drying. Kim et al. (2003) also proposed that the diffusion of lower molecular components (sugars and salts), towards the center of particles during spray drying, is a possible mechanism for the greater presence of proteins and fat on powder surfaces. XPS analysis in the present study (Fig. 6) suggests that diffusion of lower molecular weight protein hydrolysates away from the surface, combined with decreased hydrophobicity compared to intact proteins, leads to greater relative surface concentrations (exposure) of lactose and an associated increase in stickiness. Although surface fat could not be measured by XPS, in this instance, it was probably greatest in WPH11L and WPH32L powders, due to higher bulk fat levels and the previously reported phenomenon of overrepresentation of surface fat in milk powders (Vignolles et al. 2009). Surface protein also results in a layer of higher T_g compared to bulk T_g (Shrestha et al. 2007; Adhikari et al. 2009) thus further contributing to protection against sticking.

SEM micrographs (Fig. 7) show that the smaller average particle size of WPH11/L and WPH32/L powders was not due to broken particles, as seen previously in extensively hydrolysed protein/lactose powders (Mounsey et al. 2012). The smoother surfaces seen in WPH32/L powders may have been due to their more hygroscopic nature and resulting plasticisation of powder surfaces. Such extensive changes in surface viscosity, combined with the smaller particles sizes (greater surface area), are likely to have contributed to the greater susceptibility to sticking of hydrolysed WP/L powders.

The presence of surface fat in WPH32/L powders may also have contributed to the surface morphology observed. The fluidisation behaviour of WPH11/L and WPH32/L powders in the stickiness measurement apparatus was not, however, markedly different from the powders with lower fat levels (WPI and WPH8/L). This suggests that surface fat was not an issue at least with respect to sticking or agglomeration of powder particles under ambient or high temperature/low RH conditions (where melting of surface fat might have contributed to particle sticking). The absence of such effects suggests that surface fat did not play a significant role in the stickiness behaviours observed.

5 Conclusion

This study investigated the effects of whey protein hydrolysis on moisture sorption, state transition and stickiness behaviour of whey protein/lactose powders. Hydrolysis

of whey proteins increased the hygroscopicity of WP/L powders and caused delays to lactose crystallisation. The presence of proteins and peptides with lactose did not alter glass-transition temperatures of powders at the ratio examined. Findings suggest that whey protein hydrolysates compete with lactose for available water and alter the viscous flow behaviour of lactose in powders. Increasing the extent of whey protein hydrolysis made powders more susceptible to sticking. Coverage of proteins on powder surfaces decreased, with protein hydrolysis leading to greater exposure of lactose. It is probable that hydrolysates provide obstacles to diffusion and self-association of semi-solid lactose, in ways dependent on size, tertiary structure, the number of available hydration sites, co-solvent interaction, aggregation states, etc. This study provides information required for better understanding of relaxation behaviour in food materials and for improved control during spray drying of dairy powders.

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