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The effects of formulation and processing on surface characteristics and functional properties of dairy powders

Thesis Presented to the National University of Ireland for the Degree of Doctor of Philosophy

By

Grace M. Kelly, B.Sc. (Hons.)

December 2015

Under the supervision of:

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# Contents

Declaration ............................................................................................................................................. 9

Acknowledgements .................................................................................................................................. 10

Abstract................................................................................................................................................... 11

Publications ............................................................................................................................................. 19

Conference and poster presentations ..................................................................................................... 20

Chapter 1: Literature Review .................................................................................................................. 22

1.1 Introduction ........................................................................................................................................ 23

1.2 Proteins ................................................................................................................................................ 24
  1.2.1 Caseins and caseinates .................................................................................................................. 25
  1.2.2 Whey proteins .............................................................................................................................. 26

1.3 Spray-drying ........................................................................................................................................ 27
  1.3.1 Spray-drying of dairy ingredients and infant milk formula (IMF) ................................................. 29
  1.3.2 Component migration during spray-drying .................................................................................... 30

1.4 Mechanical and physico-chemical properties of dairy powders ......................................................... 32
  1.4.1 Stickiness, caking and collapse ...................................................................................................... 33
  1.4.2 Flowability ..................................................................................................................................... 36

1.5 Glass transition temperature ............................................................................................................... 39
  1.5.1 Glass transition models in food systems ....................................................................................... 40
  1.5.2 Glass transition and stickiness in spray-drying ............................................................................. 41

1.6 Crystallisation of lactose during storage ............................................................................................. 42
  1.6.1 Methods for characterisation of crystalline matter in powders .................................................. 43

1.7 Rehydration of dairy powders .............................................................................................................. 44
  1.7.1 Mechanism of powder dissolution ............................................................................................... 45
    1.7.1.1 Wettability ............................................................................................................................ 46
    1.7.1.2 Sinkability ............................................................................................................................ 47
Chapter 3: Effect of increasing protein concentration on the dissolution characteristics of dairy ingredients

Abstract

3.1 Introduction

3.2 Materials and Methods

3.2.1 Materials

3.2.2 Reconstitution of powders

3.2.3 Dissolution profile over time

3.2.4 Solubility

3.2.5 Scanning electron microscopy

3.2.6 Statistical analysis

3.3 Results and discussion

3.3.1 Composition and morphology

3.3.2 MPC dissolution profiles

3.3.3 Solubility of individual protein fractions

3.4 Conclusions
Chapter 4: Effect of type of oil and spray drying conditions on physical characteristics of a spray-dried dairy emulsion

Abstract

4.1 Introduction

4.2 Materials and Methods

4.2.1 Materials

4.2.2 Experimental Design

4.2.3 Preparation of model emulsions

4.2.4 Measurement of fat globule size and powder particle size

4.2.5 Rheological measurements

4.2.6 Determination of physico-chemical properties

4.2.7 Free fat content of powders

4.2.8 Differential Scanning Calorimetry (DSC)

4.2.9 Dynamic Vapour Sorption (DVS)

4.2.10 Confocal laser scanning microscopy

4.2.11 Statistical Analysis

4.3 Results and Discussion

4.3.1 Physical properties of powders

4.3.2 Fat globule size in emulsions and in reconstituted powders

4.3.3 Viscosity

4.3.4 Differential scanning calorimetry

4.3.5 Dynamic Vapour Sorption

4.3.6 Confocal Microscopy

4.4 Conclusions

4.5 References
## Chapter 5: Water sorption and diffusion properties of spray-dried dairy powders containing intact and hydrolysed whey protein

### Abstract

### 5.1 Introduction

### 5.2 Materials and Methods

#### 5.2.1 Materials

#### 5.2.2 Preparation of spray-dried powders and experimental design

#### 5.2.3 Viscosity

#### 5.2.4 Powder characterisation

#### 5.2.5 Powder particle size distribution

#### 5.2.6 Water sorption isotherms

#### 5.2.7 Initial rate of water sorption

#### 5.2.8 Water diffusion coefficient by Fick’s law

#### 5.2.9 Scanning electron microscopy (SEM)

### 5.3 Results

#### 5.3.1 Viscosity and powder properties

#### 5.3.2 Sorption isotherms

#### 5.3.3 Sorption kinetics and water diffusion coefficient

#### 5.3.4 Scanning electron microscopy

### 5.4 Conclusions

### 5.5 References

## Chapter 6: Effect of hydrolysed whey protein on surface composition, water sorption and glass transition temperature of a model infant formula

### Abstract

### 6.1 Introduction

### 6.2 Materials and Methods
6.2.1 Materials ........................................................................................................194
6.2.2 Preparation of IMF powders ........................................................................194
6.2.3 Emulsion fat globule size and powder particle size ........................................195
6.2.4 Viscosity ........................................................................................................195
6.2.5 Emulsion stability ........................................................................................196
6.2.6 Powder characterisation ................................................................................196
6.2.7 Water sorption isotherms ..............................................................................197
6.2.8 Differential scanning calorimetry (DSC) .........................................................198
6.2.9 Scanning electron microscopy (SEM) ..............................................................199
6.3 Statistical analysis ............................................................................................199
6.4 Results and Discussion .....................................................................................199
  6.4.1 Emulsion FGS and Viscosity .......................................................................199
  6.4.2 Powder reconstitution properties ................................................................202
  6.4.3 Powder properties .........................................................................................204
  6.4.4 Differential scanning calorimetry .................................................................206
  6.4.5 Sorption isotherms .......................................................................................209
  6.4.6 Scanning electron microscopy .....................................................................213
6.5 Conclusions .......................................................................................................215
6.6 References ..........................................................................................................216

Chapter 7: Overall Discussion .................................................................................221
  7.1 Influence of protein content on physical and dissolution properties ..............222
  7.2 Effect of formulation and processing conditions on physical properties of powders ..................................................................................................................223
  7.3 Effect of protein type on water diffusivity within a powder formulation ..........224
  7.4 Effect of protein type on surface morphology and physical properties of a model infant formula ........................................................................................................225
Declaration

I hereby declare that I am the sole author of this thesis and it has not been submitted to any other University or higher education institute, for any other academic award. Where the use has been made of other people, it has been fully acknowledged and referenced.

Signature:

__________________

Grace M. Kelly

07/01/2016
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Abstract

The objectives of this thesis were to (i) study the effect of increasing protein concentration in milk protein concentrate (MPC) powders on surface composition and sorption properties; (ii) examine the effect of increasing protein content on the rehydration properties of MPC; (iii) study the physicochemical properties of spray-dried emulsion-containing powders having different water and oil contents; (iv) analyse the effect of protein type on water sorption and diffusivity properties in a protein/lactose dispersion, and; (v) characterise lactose crystallisation and emulsion stability of model infant formula containing intact or hydrolysed whey proteins. Surface composition of MPC powders (protein contents 35 - 86 g / 100 g) indicated that fat and protein were preferentially located on the surface of powders. Low protein powder (35 g / 100 g) exhibited lactose crystallisation, whereas powders with higher protein contents did not, due to their high protein: lactose ratio. Insolubility was evident in high protein MPCs and was primarily related to insolubility of the casein fraction. High temperature (50 °C) was required for dissolution of high protein MPCs (protein content > 60 g / 100 g). The effect of different oil types and spray-drying outlet temperature on the physicochemical properties of the resultant fat-filled powders was investigated and showed that increasing outlet temperature reduced water content, water activity and tapped bulk density, irrespective of oil type, and increased solvent-extractable free fat for all oil types and onset of glass transition (T_g) and crystallisation (T_cr) temperature. Powder dispersions of protein/lactose (0.21:1), containing either intact or hydrolysed whey protein (12 % degree of hydrolysis; DH), were spray-dried at pilot scale. Moisture sorption analysis at 25 °C showed that dispersions containing intact whey protein exhibited lactose crystallisation at a lower relative humidity (RH). Dispersions containing hydrolysed whey protein had significantly higher (P < 0.05) water diffusivity. Finally, a spray-dried model infant formula was produced containing hydrolysed or intact whey as the protein with sunflower oil as the fat source. Reconstituted, hydrolysed formula had a significantly (P < 0.05) higher fat globule size and lower emulsion stability than intact formula. Lactose crystallisation in powders occurred at higher RH for hydrolysed formula. In conclusion, this research has shown the effect of altering the protein type, protein composition,
and oil type on the surface composition and physical properties of different dairy powders, and how these variations greatly affect their rehydration characteristics and storage stability.
### Nomenclature:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
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<tr>
<td>~</td>
<td>Approximately</td>
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<tr>
<td>°</td>
<td>Degree</td>
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<td>=</td>
<td>Equals</td>
</tr>
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<td>&gt;</td>
<td>Greater than</td>
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<td>&lt;</td>
<td>Less than</td>
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<td>-</td>
<td>Minus</td>
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<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>+</td>
<td>Plus</td>
</tr>
<tr>
<td>±</td>
<td>Plus or minus</td>
</tr>
<tr>
<td>2D</td>
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</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>$a_w$</td>
<td>Water activity</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>β-Lg</td>
<td>β-Lactoglobulin</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>C-</td>
<td>Carbon single bond</td>
</tr>
<tr>
<td>C=</td>
<td>Carbon double bond</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal Laser Scanning Microscopy</td>
</tr>
</tbody>
</table>
cm  centimetre
CP  Cream Powder
$\Delta C_{pi}$  Change in heat capacity of component $i$
d  day
D [4, 3]  De Brouckere mean diameter
Da  Dalton
DIC  Differential Interference Contrast
$\Delta C_p$  Change in heat capacity
$D_p$  Diameter of particle
DSC  Differential Scanning Calorimetry
DVS  Dynamic Vapour Sorption
$E_b$  Electron binding energy
$E_k$  Electron kinetic energy
ESCA  Electron Spectroscopy for Chemical Analysis
FCF  For Colouring Food
Fe  Iron
FF  Free Fat
FGS  Fat Globule Size
$g$  gram
$g$  Acceleration due to gravity
$\gamma$  Interfacial tension
$\eta$  Dynamic viscosity
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HP</td>
<td>non-fat powders containing hydrolysed whey protein</td>
</tr>
<tr>
<td>$h\nu$</td>
<td>Photon energy</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Inelastic mean free path</td>
</tr>
<tr>
<td>IDF</td>
<td>International Dairy Federation</td>
</tr>
<tr>
<td>IMF</td>
<td>Infant Milk Formula</td>
</tr>
<tr>
<td>IP</td>
<td>non-fat powders containing intact whey protein</td>
</tr>
<tr>
<td>$I_0$</td>
<td>Intensity of emitted electrons at $z = 0$</td>
</tr>
<tr>
<td>J</td>
<td>Joule</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Kappa</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Viscosity</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>$\mu g$</td>
<td>microgram</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>$\mu L$</td>
<td>microliter</td>
</tr>
<tr>
<td>$\mu mL$</td>
<td>microliter</td>
</tr>
<tr>
<td>$\mu m$</td>
<td>micrometre</td>
</tr>
<tr>
<td>$m_0$</td>
<td>Mass at onset</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>MPa</td>
<td>Mega Pascal</td>
</tr>
<tr>
<td>$m_t$</td>
<td>Mass at time $t$</td>
</tr>
<tr>
<td>$m_{\infty}$</td>
<td>Mass at endpoint</td>
</tr>
<tr>
<td>ME</td>
<td>Microencapsulation Efficiency</td>
</tr>
<tr>
<td>MPC</td>
<td>Milk Protein Concentrate</td>
</tr>
<tr>
<td>MPI</td>
<td>Milk Protein Isolate</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NaCas</td>
<td>Sodium caseinate</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NMC</td>
<td>Native Micellar Casein</td>
</tr>
<tr>
<td>NWI</td>
<td>Native Whey Isolate</td>
</tr>
<tr>
<td>O</td>
<td>Oxygen</td>
</tr>
<tr>
<td>O/W</td>
<td>Oil-in-water</td>
</tr>
<tr>
<td>Pa.s</td>
<td>Pascal seconds</td>
</tr>
<tr>
<td>PLM</td>
<td>Polarised Light Microscopy</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>r</td>
<td>Radius</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density</td>
</tr>
<tr>
<td>$\rho_p$</td>
<td>Density of particle</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>Ra</td>
<td>Surface roughness</td>
</tr>
<tr>
<td>RH</td>
<td>Relative Humidity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RH&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Critical Relative Humidity</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>Φ</td>
<td>Spectrometer work function</td>
</tr>
<tr>
<td>SLS</td>
<td>Static Light Scattering</td>
</tr>
<tr>
<td>SMP</td>
<td>Skim Milk Powder</td>
</tr>
<tr>
<td>SMPG</td>
<td>Agglomerated Skim Milk Powder</td>
</tr>
<tr>
<td>SSA</td>
<td>Specific Surface Area</td>
</tr>
<tr>
<td>SNF</td>
<td>Solids-Non-Fat</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>θ</td>
<td>Theta</td>
</tr>
<tr>
<td>T&lt;sub&gt;cr&lt;/sub&gt;</td>
<td>Crystallisation temperature</td>
</tr>
<tr>
<td>T&lt;sub&gt;g&lt;/sub&gt;</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>T&lt;sub&gt;gi&lt;/sub&gt;</td>
<td>Glass transition temperature of component i</td>
</tr>
<tr>
<td>T&lt;sub&gt;gm&lt;/sub&gt;</td>
<td>Glass transition temperature of mixture</td>
</tr>
<tr>
<td>T&lt;sub&gt;gr&lt;/sub&gt;</td>
<td>Glass –rubber transition</td>
</tr>
<tr>
<td>T&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Freezing/melting temperature</td>
</tr>
<tr>
<td>Torr</td>
<td>Torricelli pressure</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------</td>
</tr>
<tr>
<td>V_{OA}</td>
<td>Volume of occluded air</td>
</tr>
<tr>
<td>V_{IA}</td>
<td>Volume of interstitial air</td>
</tr>
<tr>
<td>V_{Stokes}</td>
<td>Stokes’ creaming/sedimentation velocity</td>
</tr>
<tr>
<td>w_{i}</td>
<td>Mole fraction of component i</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight per weight</td>
</tr>
<tr>
<td>WMP</td>
<td>Whole Milk Powder</td>
</tr>
<tr>
<td>WMPG</td>
<td>Agglomerated Whole Milk Powder</td>
</tr>
<tr>
<td>WPC</td>
<td>Whey Protein Concentrate</td>
</tr>
<tr>
<td>WPC50</td>
<td>Whey Protein Concentrate (50% protein)</td>
</tr>
<tr>
<td>WPC75</td>
<td>Whey Protein Concentrate (75% protein)</td>
</tr>
<tr>
<td>WPI</td>
<td>Whey Protein Isolate</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray Diffraction</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray Photoelectron Spectroscopy</td>
</tr>
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<td>y</td>
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Publications

Peer reviewed publications:


Submitted manuscripts:

Conference and poster presentations

Posters:


Kelly G.M., O’Mahony, J.A., Kelly, A.L., Huppertz, T., O’Callaghan, D.J., “Investigation of the solubility and rehydration properties of a range of spray-dried milk protein concentrate powders” at IUFoST 17th World Congress of Food Science and Technology in Montreal, Canada, August 17th-21st, 2014.
Oral:


Chapter 1: Literature Review
1.1 Introduction

Spray-dried dairy products and infant milk formula (IMF) are important to the Irish economy and the worldwide market, with many milk-derived dry ingredients used in foods such as IMF and other nutritional products. The main ingredients of IMF products on the Irish market are presented in Table 1.1. Apart from oils, ingredients for IMF are generally spray-dried and IMF itself is normally a spray-dried product. IMF contains a source of protein (milk proteins, milk protein hydrolysate or soybean protein) and carbohydrate energy sources (lactose, starch, and/or maltodextrins). Generally, fat sources used in IMF are vegetable oils such as palm oil, rapeseed oil, coconut oil, and sunflower oil with omega-3 and -6 from fish oil. Other ingredients may be added to infant formula and follow-on formulas if their suitability has been clinically evaluated and are complying with Alimentarius (2007).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Demineralised whey, skim milk powder, whey protein concentrate</td>
</tr>
<tr>
<td>Oils</td>
<td>Palm oil, rapeseed oil, coconut oil, sunflower oil, fish oil</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Lactose, starch, maltodextrin, oligosaccharides</td>
</tr>
<tr>
<td>Minerals</td>
<td>Calcium carbonate, potassium chloride, potassium citrate, magnesium</td>
</tr>
<tr>
<td></td>
<td>chloride, choline chloride, sodium citrate, ferrous sulphate, zinc</td>
</tr>
<tr>
<td></td>
<td>sulphate, manganese sulphate, potassium iodide, sodium selenite</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Vitamin C, Vitamin E, biotin, thiamine, vitamin B12, vitamin D3,</td>
</tr>
<tr>
<td></td>
<td>vitamin B6, vitamin K1, niacin, folic acid, pantothenic acid, choline,</td>
</tr>
<tr>
<td></td>
<td>inositol</td>
</tr>
<tr>
<td>Functional</td>
<td>Soy lecithin, carob bean gum</td>
</tr>
<tr>
<td>ingredients</td>
<td></td>
</tr>
</tbody>
</table>

Correct production (formulation and processing), handling and storage of spray-dried dairy ingredients are important for several reasons. These dried foods are extremely sensitive to reactions and interactions which are caused by relative humidity (RH) and temperature changes, leading to modified physical properties (lactose crystallisation, Maillard reaction, oxidation, caking, etc.). Therefore, production, and especially
storage conditions must be controlled to maintain a high quality product, with temperature and RH having a critical role in preserving physico-chemical properties of dairy powders.

Powder properties such as flowability and rehydration are known to be dependent on powder morphology, and on powder surface composition (Kim et al., 2005) with high surface fat generally perceived to have a negative effect on flowability and rehydration properties (Kim et al., 2009c). Powder surface composition can change with RH and temperature changes, another reason RH and temperature conditions of storage are of importance (McCarthy et al., 2013).

In most cases, IMF is formulated from different spray-dried dairy powders to achieve a proportion of proteins (casein: whey protein ratio), carbohydrates and fats that mimic human breast milk. These spray-dried dairy powders are generally rehydrated in water or in an aqueous system; this is the first rehydration step to incorporate the functional ingredients in the food process for IMF. Powders that rehydrate and disperse quickly without forming lumps and sediments are desirable for manufactures and consumers alike. In the IMF manufacturing industry, powdered products with poor rehydration/dissolution properties would result in prolonged processing time leading to increased production costs. Consumers encounter the second rehydration step when preparing IMF bottles and it has been shown that powder dissolution is an important attribute to the consumer. It is undesirable to consumers to have a product that contains sedimentation, flecks and fat pools on the surface due to free fat, as this is organoleptically unappealing. Therefore, storage and rehydration at either manufacture or consumer level is important.

1.2 Proteins

Milk proteins are widely used as food ingredients in either soluble or dispersed forms due to their good surface-active and colloid-stabilising characteristics (Dickinson, 1997). During homogenisation, protein molecules and aggregates rapidly adsorb to the oil surface, resulting in a steric-stabilising layer preventing the oil droplets from
coalescing. This provides stability to emulsions throughout processing and during storage.

There are the two main classes of milk proteins, namely caseins and whey proteins. Four types of bovine casein, $\alpha_s1$-, $\alpha_s2$-, $\beta$- and $\kappa$-casein, represent approximately 38, 10, 36, and 12% of the whole casein, respectively (Fox, 2001; Robson and Dalgleish, 1987). Caseins, particularly $\beta$-casein, contain high levels of proline, which prevents the formation of secondary structures, making them stable to denaturing agents (heat or urea) or processing conditions (homogenisation and pasteurisation). Proline also contributes to their high surface activity, giving them good foaming and emulsifying properties (Fox, 2001). The four main protein fractions in whey protein of bovine milk are: $\beta$-lactoglobulin (50%), $\alpha$-lactalbumin (20%), Bovine Serum Albumin (10%), and Immunoglobulin (10%) (Fox, 2001; Morr and Ha, 1993). Whey proteins are soluble even after coagulation of casein at pH 4.6 at 20 °C. Due to the compact, globular conformation of native whey proteins they remain soluble at, or around, its isoelectric point (4.2 – 5.0) and are completely denatured by heating at 90 °C for 10 min (Fox, 2001).

1.2.1 Caseins and caseinates

Research has been carried out on using sodium caseinate as an encapsulant, with and without the presence of a carbohydrate source. Regarding microencapsulation, caseins and caseinates have better surface-active properties compared to whey proteins, and are more heat-stable. Their excellent surfactant properties make them a useful emulsifier for a variety of applications, including spray drying (Dollo et al., 2003; Hogan et al., 2001; Pedersen et al., 1998; Sliwinski et al., 2003). The efficiency of whey protein, sodium caseinate, and mixtures of sodium caseinate / lactose at different mass ratios, on fat microencapsulation has been reported (Fäldt and Bergenståhl, 1996b, c). The highest encapsulation efficiency was obtained by sodium caseinate with lactose (> 90%), followed by sodium caseinate alone (> 70%), whey protein with lactose (< 45%), whey protein alone (< 45%). Similar results were reported by other studies (Rosenberg and Young, 1993; Young et al., 1993a; Young et al., 1993c).
Vignolles et al. (2009) showed that the presence of the amorphous lactose in a matrix with either micellar casein, whey protein, or heat-denatured whey protein, increases the microencapsulation efficiency of oil.

1.2.2 Whey proteins

The main disadvantage of using whey proteins in dairy powder processing, is their susceptibility to heat-induced denaturation, and subsequent effects on emulsion particle size before spray-drying and after reconstitution (Sliwinski et al., 2003). Aggregation of particles and reduced kinetic stability result from heating whey protein stabilised emulsions to 80 °C (Damodaran, 1997). Denaturation and aggregation of unadsorbed proteins is the main reason for instability; therefore, increasing whey protein concentration accelerates the rate and degree of aggregation (Euston et al., 2000).

Young et al. (1993a) found that WPC50 (whey protein concentrate with 50% protein) gave the best encapsulation efficiency of anhydrous milk fat compared to WPC75 (whey protein concentrate with 75% protein) and WPI. WPC50 had the best microencapsulation efficiency, because it had the highest lactose content (37%). The low encapsulation efficiency of WPI was improved to 95% when lactose was added to WPI in a 1:1 ratio. Other studies have found that whey proteins on their own are poor encapsulating agents; the encapsulation of soybean oil was found to be much lower using whey protein compared to sodium caseinate (Fäldt and Bergensåth, 1996a, b). They found that fat coverage varied from 45 to 60% with increasing whey protein concentration. Poor encapsulation of fat was attributed to less flexible proteins that diffuse at slower rates after denaturation, creating a less stable emulsion that allowed leakage of fat to the surface. Poor encapsulation was likely to be caused by protein aggregation and subsequent fat coalescence.
1.3 Spray-drying

Spray-drying is commonly used for microencapsulation and dehydration of food ingredients (Gharsallaoui et al., 2007; Peighambardoust et al., 2011). The process involves atomising a fully solubilised homogenous (sometimes coarse premix emulsion) liquid feed (producing a spray of droplets) into the spray-drying chamber. Once the atomised droplets are in the chamber, they lose moisture through evaporation at a rate which is controlled by temperature and airflow conditions leading to the formation of dry powder particles. Depending on the spray-drying conditions, particles can be fine (10-50 μm) or larger (200-300 μm) due to agglomeration of powder particles, with spray-drying conditions having implications on the surface composition and morphology of powder particles. Dehydration (through spray-drying) produces powder with low water content and water activity ($a_w$), increasing microbial and reducing storage and transportation costs. Whole milk powder (WMP) is a typical spray-dried product, with homogenised fat protected by the lactose wall, and milk proteins acting as emulsifiers; while the protein matrix provides rigidity and structure, in conjunction with lactose through glass formation.

A commercial spray-dryer consists of a feed pump, atomiser, air heater, drying chamber and conveyor equipment for product discharge, transport and packaging (Devakate et al., 2009). The atomisation process is an important part of the spray-dryer. Atomisation is utilised to create liquid droplets having maximum surface area for heat transfer to the hot air flowing through the chamber. Different types of atomiser can be used to produce the fine spray: centrifugal, pressure, kinetic and sonic (Barbosa-Cánovas and Juliano, 2005), depending on feed viscosity (Bowen, 1938; Masters, 1968). In any type of atomisation, energy is needed to break up liquid bulk to create individual droplets. Rotary or centrifugal atomisers use the energy of a high speed rotating wheel to break up liquid flow into a spray of droplets. They are flexible with regard to product throughput and also easy to operate and maintain. Rotary atomisers generally have no internal blockage problems and can be run for a long time without operator interference, operating under low feed pressure and handling viscous feeds. One drawback is that they cannot be used in horizontal dryers because
the liquid is thrown horizontally. Rotary atomisers produce large quantities of fine particles, which can result in a need for bag filters. They are also expensive to maintain compared to other types of atomisers, and result in powders of low bulk density and high occluded air. Pressure nozzles are commonly used in the dairy industry, and work by using a high pressure pump to feed the liquid to nozzles before entering the dryer chamber. They typically produce powders with high bulk density, a narrow particle size distribution and, in the case of fat containing powders, low free-fat content. High bulk density powders are produced with high flowability. Pressure nozzles also have the advantage of having a low cost. In kinetic energy atomisers, the liquid feed and a supply of compressed air are passed separately to the nozzle head and air turbulence breaks the feed into small droplets. These atomisers are useful for high viscous feeds and require a smaller drying chamber. In sonic atomisation, the nozzle head contains a sonic generator and when the feed passes through the head it breaks up the liquid into droplets. The nature and viscosity of the feed and the desired characteristics of dried product influence the choice of atomiser configuration (Gharsallaoui et al., 2007). The atomised droplets are then dried in the chamber. Two different designs exist, co-current and counter-current drying. Co-current drying is when the liquid is sprayed in the same direction as hot air, whereas counter-current drying the liquid is sprayed in the opposite directions to hot air. Co-current drying uses more moderate outlet temperatures than counter-current drying, and so is used when there are temperature concerns with the product.

In the drying-chamber, removal of moisture from the droplets occurs in two distinct stages. Firstly, evaporation occurs at the droplet surface at a constant water vapour partial pressure and temperature. Then at a critical point, where water cannot migrate to the surface as fast as it evaporates, the droplet forms a dry crust on the particle surface; and, the drying rate decreases dramatically. When evaporation of moisture from the droplet ceases, the particle temperature rises to that of the drying chamber, at which point drying is theoretically finished. Fine powder particles are separated from the humid air via a cyclone and/or bag filter or wet scrubber. Bag filters are the most efficient. Particles which tend to have higher moisture and are heavier, fall to the bottom of the dryer where they are collected for further drying.
Multi-stage dryers with increased residence time and reduced drying temperatures are common as they reduce thermal denaturation. Fluidised beds are commonly used for final drying and cooling. They are situated outside the dryer and can help better control particle size and final water content.

1.3.1 Spray-drying of dairy ingredients

Spray-drying is a common method used for preservation of dairy products. Dehydration minimises the packaging and storage requirements, reducing the storage weight and costs and increasing shelf-life (Landström et al., 2000). The advantages of spray-drying as a dehydration technique include the following:

(i) it is a continuous and easy operation that is fully adaptable to full automatic control,
(ii) a wide range of designs are available depending on the application (such as heat sensitive materials), and
(iii) it leads to increased shelf-life of products and reduced storage and transport costs.

The process has been used commercially for over 100 years. Dairy ingredients such as WMP, skim milk powder (SMP), sodium caseinate (NaCas), milk protein concentrate (MPC), milk protein isolate (MPI), and cream powder (CP), are processed via spray-drying. IMF, composed from a combination of dairy and non-dairy ingredients, in the desired ratio of protein, fat, carbohydrates, vitamins and minerals, are generally reconstituted in a wet mixing process before being spray-dried.

Microencapsulation is achieved in IMF manufacturing when small fat globules and vitamins are embedded inside the walls of powder particles, with the walls generally consisting of lactose. The advantages of microencapsulation are that oxidation of fat is minimised, stickiness of powder is reduced, and the particle size distribution of the original emulsion should be unchanged (Millqvist-Fureby, 2003).
Three crucial steps are required before a liquid product with oil can be spray-dried (Dziezak, 1988). Firstly, the liquid emulsion is prepared by rehydrating dry product and addition of oil, followed by agitation, before the product is homogenised to achieve a fat globule size (FGS) < 1 μm, followed by atomisation of the emulsion in a spray dryer. The first stage involves the formation of a coarse emulsion of the core material (fat) in the wall solution (lactose, protein). The core material (usually hydrophobic) is added to a solution containing the wall material, with which it is immiscible. The coarse emulsion is then heated and homogenised. The emulsion formed after homogenisation should be stable over time with a small FGS (Liu et al., 2001). A highly viscous emulsion can interfere with the atomisation process, leading to large elongated droplets that adversely affect the drying rate (Rosenberg et al., 1990), with a highly viscous feed (> 250 mPa.s) producing larger powder particles. Correct atomisation leads to the evaporation of water and formation of droplets (ideally spherical in shape), with the encapsulated oil and vitamins protected inside the continuous phase (Dziezak, 1988).

1.3.2 Component migration during spray-drying

Different mechanisms relating to component migration have been proposed. In essence, milk and IMF are essentially composed of three main components: lactose, soluble and insoluble proteins and fats. These components have different diffusivity in water, due to differences in molecular size. The difference in relative diffusivity has been investigated by Kim et al. (2003). During drying, the atomised droplets lose moisture from their surfaces, leaving the surface more concentrated with solute, and creating a concentration gradient through the radius of the particle. This concentration gradient causes the suspended material to diffuse inwards inside the droplet. As lactose has the highest diffusivity, it will migrate faster towards the core, followed by protein and then fat molecules (Figure 1.1; Kim et al. (2003)).
Numerous different studies have shown how powder particles are over-represented in fat when spray-dried. Kim et al. (2009a) reported that spray-drying was the most important manufacturing process in determining the surface composition of spray-dried milk powders, with fluidised bed drying having no significant effect on the surface composition of powders. Various theories for powder surface formation have been proposed to explain this:

(i) Due to difference in diffusivity, the powder surface has been shown to accumulate in fat (Kim et al., 2003). Particle formation during drying is influenced by the Peclet number ($Pe$: ratio between the diffusion coefficient of the solute ($D$) and the evaporation rate of solvent ($J$)) and the initial saturation of the concentrate. High fat/protein concentrations should be found only for high $Pe$ values. Droplet viscosity can influence particle shape, size, and surface composition during drying (Huang, 2011);

(ii) Another mechanism of surface composition formation has been proposed; fat globule membranes rupture due to shrinkage caused by evaporation, and as the fat globule itself does not shrink, this allows the fat globule to leak through the ruptured membrane, spreading a thin fat layer (nanometres thick) on the particle surface;
(iii) Fäldt and Bergenståhl (1996b) and Nijdam and Langrish (2006) suggested that, in fat-containing milk powders, milk fat might leak out onto the powder surface during the drying process because the drying temperature is above the melting point of milk fat, and milk fat is therefore in a mobile fluid state;

(iv) Higher inlet temperatures during spray-drying result in a lactose-dominated rather than protein-dominated powder surface. This is due to higher temperatures possibly causing accelerated surface crust formation, decreasing the time for migration of larger molecules, i.e. protein, to the surface. Fat migration to the surface is also minimised by increased viscosity (Nijdam and Langrish, 2006).

1.4 Mechanical and physico-chemical properties of dairy powders

As previously mentioned, powder handling and storage properties are important for a long stable shelf-life and for rehydration purposes. Poor flowability in dairy powders is considered problematic during processing, as that can lead to down-time. Depending on powder composition, storage conditions can alter the powder surface, leading to stickiness, caking, collapse and reduced flowability. Flowability is influenced by numerous factors, including surface composition, particle shape and size distribution, moisture content, compressibility and porosity, etc. (Crowley et al., 2014; Peleg, 1977). High RH, compaction pressure and small particle size are some of the causes of flow difficulties (Peleg et al., 1973). The phenomena of caking, stickiness, collapse and cohesion are very inter-dependent and occur simultaneously during storage. These concepts are discussed in more detail below. The physico-chemical properties of powder being discussed here have some dependence on moisture content, which is determined initially by processing parameters and later by storage conditions. Water content and water availability have a role in physico-chemical stability, which is characterised by water activity ($a_w$) and $T_g$ of the powder. They determine the kinetics of sticking of powders. Water content has a significant influence on the storability of a powder, and increased particle size in spray-drying is associated with an increase in $a_w$. As stated earlier, viscosity of feed entering the spray dryer influences particle size
(Section 1.3). Thus, processing parameters (temperature of the concentrate, pasteurisation conditions, homogenisation, solid contents) have an indirect impact on droplet size and therefore on the final moisture of the powder.

1.4.1 Stickiness, caking and collapse

Caking, stickiness and collapse negatively affect the physical stability of dairy powder, causing free-flowing powders to change into lumps, then into an agglomerated solid, and finally into a sticky material with exposure to higher temperatures and humidity (Adhikari et al., 2001) (Figure 1.2).

It has been shown in the literature that the glass transition temperature ($T_g$) of powders can be correlated with their stickiness and caking potential at given conditions, with stickiness being an early stages of caking, causing a bridge between particles (Hogan and O'Callaghan, 2010). As lactose goes through a glass-rubber transition, there is an increase in molecular mobility on the surface, allowing for interaction of molecules with other surfaces (cohesion and/or adhesion) (Hogan and O'Callaghan, 2010).

Stickiness can be described as a surface property; therefore, surface $T_g$ temperature is more important than the bulk $T_g$. Above the $T_g$, molecular mobility is greatly increased and many amorphous compounds crystallise when viscosity decreases, typically from $10^{12}$ Pa.s to a critical value of $10^6$ - $10^8$ Pa.s, due to an increase in temperature and/or moisture (Bhandari and Howes, 1999; Downton et al., 1982). Generally, the critical viscosity of amorphous solids is reached at temperatures 10-20 °C above its $T_g$, favouring particle cohesion, which can be disrupted with a low shear (Netto et al., 1998; Peleg et al., 1973). Adhikari et al. (2009) showed that adding a small amount of protein (WPI or sodium caseinate, 0.5% w/w) to sucrose reduced powder stickiness and increased the total yield from 18% to 80-85%, with only a minimal increase in $T_g$. 
Cohesiveness, a measure of the attraction between particles, is related to stickiness and is also affected by moisture. Increased cohesiveness at higher moisture contents occurs even when liquid bridges are not present, and is caused by the hydrogen bonds between water molecules in the particles. This leads to decreased flowability of powders (Aguilera et al., 1995).

Caking is a transformation of a free flowing powder to form weak aggregates and potentially a rock-hard mass. Powders will cake during storage if conditions are favourable, as solid particle surfaces change to a rubbery/liquid state (Figure 1.3). Lactose crystallisation releases water and cause stickiness and caking in products. Caking is a common phenomenon in milk powders, with the problem being more pronounced in powders of small particle size and high free fat content. Caking can be accelerated by fat melting, moisture sorption of hygroscopic components and crystallisation of sugar components (mainly lactose in dairy powders). Transportation of powders globally through different climatic zones, and also consolidation pressures,
can accelerate undesirable caking (Özkan et al., 2002). Caking can be measured using the Schulze ring method.

Figure 1.3. Fat sticking of powder particles and powder caking mechanism. Taken from Foster et al. (2005).

Free fat on particle surfaces can contribute to sticking and caking (Foster et al., 2005; Vega and Roos, 2006), and powders with low microencapsulation efficiency (ME), i.e., the percentage of oil content in powder not extractable by solvent, have an increased chance of caking. Nijdam and Langrish (2006) examined the degree of caking in relation to surface composition of milk powders with different fat contents, and indicated that the degree of caking was > 90 % for powders containing 5 - 30% surface fat. When surface fat was reduced to < 3 %, caking was also seen to reduce, and they concluded, that there is a correlation between caking of powders and surface fat.

The term collapse describes the loss of structure in a dried matrix and it is viewed as flow under gravity (Tsourouflis et al., 1976). Amorphous materials collapse under gravity when their viscosity or yield strength gets sufficiently low. Collapse is linked to
a decrease in porosity and an increase in density (Figure 1.2; (Aguilera et al., 1995)).
Collapse is related to the glass transition phenomenon and to viscosity changes.

1.4.2 Flowability

The flowability of a powder refers to the ease with which the particles move with respect to one another, the opposite of resistance to flow (Royal and Carson, 1991), and is a measure of “free-flow” characteristic of a powder. It is an important attribute for the design of manufacturing/ handling machinery, to ensure proper flow of powders and for ease of handling, processing and in the final application (Crowley et al., 2014; Prescott and Barnum, 2000). The flowability of a powder during discharge from a silo depends to a large extent on its composition and physical properties, which are affected by its processing and storage history (Janjatović et al., 2012). Physical properties of a powder, such as particle size and shape, surface structure, particle density, and bulk density, have an impact on flowability, along with surface composition (Schubert, 1987; Teunou et al., 1999). In general, powders with good flow properties are those with large agglomerates and few fines; particles < 200 μm reduce flowability as smaller particles have increased specific surface area (SSA), i.e., area per unit mass of powder, and ideally have a narrow particle size distribution. With the presence of fine particles, more surface area is available for cohesive forces, such as frictional forces, to resist flow (Fitzpatrick et al., 2004; Mathlouthi and Rogé, 2003).

Powder flow, when discharging from a silo is typically classified as core-flow or mass-flow (Crowley et al., 2014). Generally, core-flow tends to be the most problematic of the two, giving rise to a problem known as rat-holing, whereby large quantities of powder remain near the internal walls and do not flow from the hopper (Fitzpatrick et al., 2007). Rat-holing can be prevented by determining the minimum outlet diameter to prevent rat-holing during core flow; however, many flow issues may be resolved by changing the flow pattern from core-flow to mass-flow (Crowley et al. (2014); Figure 1.4).
Figure 1.4 Schematic diagram of flow problems, namely arching and rat-holing, encountered in hoppers during (a) mass-flow and (b) core-flow, respectively, where D = outlet diameter, $D_{\text{arch}}$ = minimum arching diameter, $D_{\text{RH}}$ = minimum rat-hole diameter and $\theta$ = hopper half angle. Taken from Crowley et al. (2014).

For a highly cohesive powder, an effect known as arching may occur, where a stable arch of powder may form at the hopper outlet during mass-flow; this effect creates a no-flow situation and the immobilised powder will require dislodgement for processing to continue (Figure 1.4a). To prevent this, it is important to determine the minimum hopper outlet diameter and the minimum hopper half-angle to prevent arching during mass-flow of a given powder (Iqbal and Fitzpatrick, 2006).

Crowley et al. (2014) examined the flowability of MPC powders increasing in protein content. High protein MPC powders had lower flow index values, requiring larger outlet diameters for optimal discharge from hoppers compared to lower protein MPC powders, suggesting that, as protein content increased, there was a concomitant decrease in flowability. SMP, comparable to the lowest protein powder in Crowley et al. (2014) study, was found to have good flow properties (Sang-Cheon and others 1993). The angle of repose and compressibility were 32.5° and 0.029, respectively, indicative of good free-flowing characteristics.

High moisture levels negatively affect flowability, due to increased liquid bridging and capillary interactions between particles, causing particle-particle and particle-wall sticking, i.e., cohesion and adhesion (discussed in Section 1.4.1). Moisture levels can increase over storage, particularly if lactose is present in the amorphous state, as it is
hygroscopic and readily absorbs moisture (Fitzpatrick et al., 2007). The literature suggests that flowability of powder is a surface-related property (Kim et al., 2005) and that non-fat powders have better flow properties, however, Crowley et al. (2014), who studied non-fat powders, showed that high protein powders are also problematic. Therefore, it is believed that flowability can be influenced by powder surface composition, with surface fat significantly decreasing flowability. Migration and pooling of fat to particle surfaces, which occurs during spray-drying (Nijdam and Langrish, 2006) and storage (Gaiani et al., 2009), reduces flowability by increasing liquid bridging between particles (Kim et al., 2005). The amount of fat on particle surfaces is of importance, with the presence of fat rendering the surface hydrophobic, leading to a decrease not only in flowability but also wettability and dispersibility (Kim et al., 2005). Surface fat gives rise to a sticky powder surface and acts as a bridge between particles, reducing the flowability of some powders. This is more pronounced when the temperature is near or above the fat melting point. Poor flowability of these powders has been overcome by crystallising out the low melting point fraction of fat to obtain powder with medium- or high-melting fat fractions (Ilari and Loisel, 1991). Regarding the effect of elevated storage temperature on WMP, it was concluded that as the fat component reached its melting point it doubled the cohesion of SMP when the temperature was increased from 30 to 65 °C. Other studies have attempted to determine the relationship between free-fat content and flowability of dairy powders, and no correlation was found with approximately the same mean particle size, even when free-fat was expressed per unit of surface area (Buma, 1971).

X-ray photoelectron spectroscopy (XPS) has been used in several studies to determine the surface composition of food powders and will be discussed in detail in Section 1.9. Kim et al. (2005) using XPS, studied the surface composition of industrial spray-dried dairy powders (SMP, WMP, CP and WPC) and examined its influence on flowability. The study determined that non-fat powders, e.g. SMP whose surface is made up of mostly of lactose and proteins, flow well due to the lower percentage of fat coverage on the surface (Kim et al., 2005); however after 6 months of storage, flowability decreased, concomitant to an increase in contact angle from 85° (in fresh powder) to 90 ° (Kim et al., 2009c). Kim et al. (2005) concluded that flowability correlated better
Chapter 1

with surface fat content than with free-fat and total fat content, supporting the importance of surface composition to powder flowability.

1.5 Glass transition temperature

Glass transition temperature, $T_g$, previously mentioned in Section 1.4.1, can be defined as the temperature at which an amorphous system changes from a glassy to a rubbery state, under defined changes of temperature, as the change of state is a function of temperature and time. In some areas $T_g$ has been used as indicator of food stability and to predict the behaviour of foodstuffs. Glass transition theory from polymer science helps to understand textural properties of food systems and explains changes which occur during food processing and storage such as stickiness, caking, and softening (discussed in Section 1.4.1). In choosing conditions for drying of food products, glass transition temperature is a crucial factor that needs to be considered (Labuza et al., 2004). Glass transition temperatures of various dairy ingredients are shown in Table 1.2.

Spray-drying produces powders in an amorphous state. In the course of drying, the material becomes highly viscous as it passes through a glass transition, with typical viscosity above $10^{12}$ Pa.s (Downton et al., 1982). As a consequence, powders are thermoplastic and hygroscopic potentially leading to powder sticking within the chamber during drying or caking during storage. For the above reasons, the state of amorphous lactose in dairy powders is particularly sensitive to moisture and temperature (Bhandari and Howes, 1999). The structural changes that occur in amorphous materials, i.e., lactose, due to high temperature and humidity are related to their $T_g$ (Aguilera et al., 1995; Vega and Roos, 2006). Amorphous materials produced via spray-drying are not as thermodynamically stable as their crystalline form (Flink, 1983; Zhu et al., 2013). Molecular alignment to a lower energy thermodynamically stable crystalline state is favoured, causing alignment of molecules to the crystalline state, and thus, the amorphous material will eventually crystallise over time, at a rate dependent upon the temperature and moisture content (Flink, 1983).
Table 1.2 Calorimetric characteristics of various dairy ingredients (Schuck et al., 2005).

<table>
<thead>
<tr>
<th>Dairy ingredients</th>
<th>Dry glass transition temperature (inflection °C)</th>
<th>Change in heat capacity (J·g⁻¹·°C⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-139</td>
<td>1.94</td>
</tr>
<tr>
<td>Casein</td>
<td>132</td>
<td>0.26</td>
</tr>
<tr>
<td>Whey proteins</td>
<td>127</td>
<td>0.09</td>
</tr>
<tr>
<td>Lactose</td>
<td>98</td>
<td>0.38</td>
</tr>
<tr>
<td>Glucose</td>
<td>31</td>
<td>0.24</td>
</tr>
<tr>
<td>Galactose</td>
<td>30</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Molecular weight is a significant determinant of $T_g$. Low molecular weight polymers (e.g. sucrose) have lower $T_g$ than longer chain molecules (e.g. maltodextrins). $T_g$ of high molecular weight food polymers (e.g., proteins and starches) cannot be determined experimentally, because they decompose at temperatures below their glass transition (Bhandari and Howes, 1999).

1.5.1 Glass transition models in food systems

Carbohydrates, proteins and fat are the main components of multi-component spray-dried food powders, with carbohydrates having the biggest influence on $T_g$. Common sugars (lactose, sucrose, glucose, and fructose) have low glass transition temperatures, so their presence depresses $T_g$ in sugar-rich food. The fat component is not a significant factor in glass transition (Jouppila and Roos, 1994) and the presence of proteins gives a relative increase in $T_g$. Water has a very low $T_g$ (-135 °C), and therefore even a small amount has a significant effect in reducing $T_g$, making it a significant plasticiser in food systems. The $T_g$ of a powder with many components (including water) is a function of the glass transition temperatures of the individual components (Bhandari and Howes, 1999). Mathematical relationships have been developed by Gordon and Taylor (1952) and Couchman and Karasz (1978), to determine the $T_g$ of a binary mixture, or complex mixtures with more than three components, respectively.
The Couchmann-Karasz equation for any number of components (Equation 1.2) is an extension of the two-component Gordon-Taylor equation (Equation 1.1):

\[
T_{gm} = \frac{w_1 T_{g1} + k_{w2} T_{g2}}{w_1 + k_{w2}} \tag{1.1}
\]

\[
T_{gm} = \frac{\sum_{i=1}^{n} w_i \Delta C_{pi} T_{gi}}{\sum_{i=1}^{n} w_i \Delta C_{pi}} \tag{1.2}
\]

where, \( T_{gm} \) is the glass transition temperature of the mixture, \( w_i \) is the mole fraction of component \( i \), \( \Delta C_{pi} \) is the change in heat capacity of component \( i \) between glassy and rubbery states, and \( T_{gi} \) is the glass transition temperature of component \( i \). While the Gordon-Taylor model is for a binary mixture, the Couchmann-Karasz model can be expanded for an \( n \)-component system.

1.5.2 Glass transition and stickiness in spray-drying

Stickiness during spray-drying of sugar-containing products has been related to their low \( T_g \) (Vega and Roos, 2006). The surface of droplets during spray-drying may remain plastic, resulting in sticking inside the dryer wall, depending on product characteristics, composition, and drying conditions. The resulting amorphous product post-spray-drying can be syrup-like, a sticky powder or a relatively free flowing powder at ambient temperatures. Roos and Karel (1991) found that a critical viscosity of \( 10^7 \) Pa.s leading to sticking (Bellows and King, 1973; Downton et al., 1982) is reached at temperatures of circa 10 – 20 °C above \( T_g \), as previously discussed in Section 1.4.1.
1.6 Crystallisation of lactose during storage

Crystallisation of lactose in milk powder during storage is one of many deteriorative phenomena that may occur, along with non-enzymatic browning, protein and lipid oxidation, which influence physical (flow properties due to caking) and functional (solubility, and rehydration) characteristics of powders. Amorphous foods can absorb moisture and change from a metastable glassy state to a rubbery state, followed by crystallisation (Saltmarch and Labuza, 1980; Slade and Levine, 1991). There is a critical RH at a given temperature at which sugars crystallise, which must be taken into account to ensure that storage conditions are optimal for a long shelf life.

Figure 1.5 Amorphous lactose crystallization at 55% RH and 25 °C (Burnett et al., 2016).

Thus, if amorphous powders are not stored under correct conditions (i.e., temperature, RH) relative to their glass transition temperature, then they are susceptible to crystallisation and caking. The rate of crystallisation relates to difference in storage temperature and $T_g$ ($T - T_g$), with increasing temperature accelerating crystallisation rate (Bhandari and Howes, 1999; Roos and Karel, 1991)

The crystalline state is thermodynamically favoured as it has a lower free energy, due to a structured arrangement of molecules in a crystal lattice. The degree of lactose
crystallisation in dairy products has a significant effect on ingredient and food properties, such as texture (e.g., grittiness in ice-cream) and flavour. In milk powders, crystallisation of lactose causes lumping and caking which negatively impacts upon powder reconstitution (Lai and Schmidt, 1990). Listiohadi et al. (2005) concluded that storage at or below 33% RH at 25 °C was sufficient to prevent severe caking and “sandiness” in dairy powders; however, it was not completely sufficient to prevent crystallisation. Amorphous milk powder remains stable if the product temperature remains below the $T_g$ of the product. Nucleation (the beginning of crystallisation) can take place in amorphous products if:

(i) the temperature of the glassy product is raised above its $T_g$; or
(ii) $T_g$ is lowered due to product absorbing moisture from the environment, i.e. increasing RH at the storage temperature.

Lactose crystallisation disrupts powder microstructure, causing internal lipids of fat containing powders to be released (Fäldt and Bergenståhl, 1996a; Shimada et al., 1991), making them more susceptible to oxidation and reducing flowability.

1.6.1 Methods for characterisation of crystalline matter in powders

Different methods are used to quantify crystallisation of amorphous sugars, which can be categorised as microscopy, thermodynamic and spectroscopy techniques. Microscopic techniques have been used to visualise lactose crystals (Maher et al., 2015a; McCarthy et al., 2013). Polarised light is the preferred mode to visualise light crystals against a dark background. Maher et al. (2015a) also utilised confocal scanning light microscopy (CLSM) and scanning electron microscopy (SEM), to visualise lactose crystals (the use of CLSM in dairy products is reviewed in Section 1.8.2). Mahlin et al. (2004) utilised atomic force microscopy (AFM) to quantify the degree of lactose crystallisation on powder surfaces by measuring the surface roughness of lactose powder.

Isothermal differential scanning calorimetry (DSC) has been used to study crystallisation of lactose in the food and pharmaceutical industry (Haque and Roos,
This works on the principle that a difference in respect to heat flow between the sample pan and the reference pan is used to characterise heat capacity changes associated with crystal formation or melting. X-ray diffraction (XRD) has also been used to monitor crystallisation kinetics of lactose, trehalose and lactose/trehalose mixtures (Jouppila et al., 1997; Miao and Roos, 2005). Well-defined peaks are a characteristic of crystallised monohydrate lactose whereas amorphous spray-dried powders, i.e., WPC, are characterised by a broader peak or no defined peaks (Bund and Hartel, 2010). Schuck & Dolivet (2002) have shown how the quantity of α-lactose monohydrate in milk powders can be calculated.

Gravimetric methods can also be used to measure crystallisation by monitoring changes in powder mass upon humidification (Jouppila and Roos, 1994; McCarthy et al., 2013). Dynamic vapour sorption (DVS) is an automated system which measures change in mass over time with increasing RH (Kelly et al., 2014; Maher et al., 2014). Upon crystallisation of an amorphous sugar the molecular structure orientates to a highly ordered crystal lattice, expelling sorbed water, which evaporates and is measured as a decrease in mass in a controlled humidity environment (Burnett et al., 2004; Burnett et al., 2006). Gravimetric methods are generally carried out at ambient temperatures where crystallisation rates are quite low and can be easily monitored in a controlled environment (Kedward et al., 2000).

1.7 Rehydration of dairy powders

Spray-drying can be a means of milk preservation of food for direct consumer user or for later recombination as ingredients used in the food industry (in beverages, ice-cream, energy bars and bread, etc.). For most purposes, spray-dried dairy powders are intended for rehydration directly with water or in some other aqueous medium, for incorporation and expression of functional ingredients, e.g. proteins, fats, lactose, minerals, within a product for the consumer. As discussed in the introduction, instant rehydration properties are desirable, and a rehydrated product should be free from lumps and sedimentation, be it for formulation manufacturers or for consumers.
1.7.1 Mechanism of powder dissolution

Rehydration of food powders occurs according to the following steps (Fang et al., 2008):

(i) Wetting of particles (wettability);
(ii) Sinking of particles (sinkability);
(iii) Dispersing of particles (dispersibility);
(iv) Dissolving of particles into solution (solubility).

Figure 1.6 is a visual representation of what occurs during rehydration.

![Schematic](image)

**Figure 1.6** Schematic representing the rehydration steps for an agglomerated high-protein dairy powder, showing 1. Wetting, 2. Swelling, 3. Sinking, 4. Dispersion and 5. Dissolution. Taken from Crowley et al. (2016)

Powders rehydrate at different rates according to their composition and morphology. For example, in SMP, having very little surface fat, wetting of particles occurs very quickly and agglomerated powders disperse very quickly, due to their size and porosity. Each step of rehydration is discussed in detail below.
1.7.1.1 Wettability

Powder wettability is defined as the ability of powder particles to overcome surface tension at the interface between solid and liquid at a specific temperature. It is measured as the time for a powder to be completely wetted (Niro, 2005).

The rate of wettability has mostly been modelled from the Washburn equation (Equation 1.3), for cylindrical capillary flow (Figure 1.6), ignoring the effect of gravity (Washburn, 1921):

\[
\frac{dl}{dt} = \frac{r \gamma}{\eta 4l} \cos \theta
\]  

(1.3)

where \((dl/dt)\) is the liquid velocity penetrating the capillary, \(r\) is the radius of the capillary, \(\gamma\) is the liquid surface tension, \(\eta\) is the liquid viscosity, \(l\) is the water penetrating distance, and \(\theta\) is the contact angle. Based on this, conditions resulting in fast wettability would be:

- Small contact angle;
- Large particle or agglomerates, forming pores;
- High porosity (as long as critical bulk porosity is not exceeded).

Dairy powders are complex in structure and it is difficult to model the capillary effects and how this impacts the rate of wetting due to the dynamic nature of the wetting process. However, some general principles apply.
Surface tension varies with concentration in solution as different components in dairy powders (lactose, protein, salts, etc.) start to dissolve. At the same time, porosity radius increases as lactose dissolves. The influence of composition on wettability is discussed further in Section 1.7.2. The viscosity of the solution also increases as lactose and protein dissolve, which changes the contact angle. However, as stated above, while cylindrical capillary flow is an over-simplification of wetting dynamics, it provides clues to some of the influencing factors.

1.7.1.2 Sinkability
Sinkability is defined as the ability of particles to sink below the liquid surface, and is dependent on the size and density of particles (Thomas et al., 2004). Particle density accounts for solid density and occluded air content, which causes buoyancy. Generally, larger and denser particles (i.e., low occluded air) result in increased sinking rates (Fang et al., 2008). Composition of powder (protein, carbohydrate, fat, water content) affects solid density. Occluded air in spray-dried powders is affected by foaming.
characteristics of feed liquid, total solids concentration, and atomiser type (Maher et al., 2015b). Keogh et al. (2006) showed that caseins with increasing degree of aggregation (SMP, calcium caseinate, and sodium caseinate) produced powders with decreasing occluded air.

After sinking, powder particles should begin dissolving (Sharma et al., 2012). Particles that have a size > 100 μm and density > 1,500 kg/m³ are able to sink into the solvent (Hogekamp and Schubert, 2003). Other factors influencing sinkability are agglomeration and swelling, agglomerated powders have higher aggregate density and so sink faster. As particles dissolve, it is typically the high density components that dissolve first (lactose and minerals). This reduces the density difference between the solution and the powder particle, which can cause particles to rise after the initial sinking stage.

1.7.1.3 Dispersibility

Dispersibility of a powder in water is defined by its ability to break up into particles that can pass through a sieve with a pre-defined pore diameter. After sinking, agglomerated particles break up and disperse into individual particles in the liquid medium. The rate of dispersion determines whether a food powder can be classified as “instant” or not. “Instant” powders have good wettability and dispersibility. Dispersibility improves with increasing particle size, as small particles (< 90 μm) have poor dispersibility (Neff and Morris, 1968). Large clumps that settle at the bottom of the container are typically the result of powders with poor dispersibility (Písecký, 1997).

1.7.1.4 Solubility

Solubility is considered a key parameter in determining the quality of reconstituted powders. It is critical that powders are completely solubilised, as undissolved material can lead to operational problems during downstream processing (Morr et al., 1985). Fast dissolution is favoured by the presence of small hydrophilic molecules at particle-liquid interfaces (Lillford and Fryer, 1998). Protein solubility depends on soluble, polar
residues interacting with water by hydrogen bonding, while the hydrophobic parts fold inwards to avoid contact with water (Schein, 1990). Fang et al. (2011) showed that the reconstituted particle size of aged (with increasing temperature and time) MPC was greater than that of fresh MPC. This was due to casein molecules forming inter-micellar linkages, making it more difficult for casein micelles to be released into solution, and this was the rate-limiting step of dissolution. Similarly, Anema et al. (2006) showed that aged milk powder had reduced solubility due to the formation of cross-linked protein networks on the powder surface, preventing water penetration.

1.7.2 Factors affecting dissolution

Wetting is a rate-controlling step in the rehydration process of powders (Kim et al., 2002). Surface composition of powders has a significant impact on wettability (Gaiani et al., 2006a). Free fat on the surface of powders reduces wettability due to the hydrophobicity of fat, which prevents water penetration. Therefore, it is important it minimise free fat content of powders to aid rehydration. Factors affecting amount of free fat on powders include:

(i) spray-drying outlet temperatures;
(ii) emulsion particle size;
(iii) type of fat used, and;
(iv) type of emulsifier.

It has been reported that increasing the spray dryer outlet temperature results in increased free fat, suggesting it is due to increased air vacuole expansion, resulting in increased surface crack formation, and thus increased free fat (De Vilder et al., 1976; Kelly et al., 2002; Maher et al., 2014). Maher et al. (2014) reported that the FGS of emulsions pre-spray drying significantly affects powder free fat levels. The authors of that study compared spray dried nano-emulsions (FGS < 200 nm) to spray dried conventional emulsions (FGS < 1200 nm) and found that spray dried nano-emulsions had significantly reduced free fat levels. Millqvist-Fureby (2003) and Jafari et al. (2008) also showed that reducing FGS by increasing the number of homogenisation passes pre-spray-drying reduced free fat levels. The authors suggested that this was due to
the smaller oil droplets being less likely to be ruptured during atomisation, and that the droplets are more embedded in the powder matrix, making them less extractable upon solvent addition. Aguilar and Ziegler (1994) showed that powders with a lactose content > 40% w/w have less solvent extractable free fat. The type of fat also impacts upon free fat levels. Millqvist-Fureby (2003) reported that the fat crystal state had a significant effect on solvent-extractable free fat, with fat that was crystallised prior to spray-drying giving higher free fat. Emulsifier type has a strong influence on free fat, with sodium caseinate being highly surface active, and its inclusion in emulsions being shown in numerous studies to result in very low free fat (Hogan et al., 2003; Maher et al., 2014). The efficiency of whey protein, sodium caseinate, and mixtures of sodium caseinate / lactose at different mass ratios, on fat microencapsulation has been reported (Fäldt and Bergenståhl, 1996b, c), with the highest encapsulation efficiency being obtained by sodium caseinate with lactose (>90%), followed by sodium caseinate alone (>70%), whey protein with lactose (<45%), and whey protein alone (<45%). Similar results were reported by other studies (Rosenberg and Young, 1993; Young et al., 1993b, c). The main challenge in using whey proteins as emulsifiers is their susceptibility to heat–induced denaturation, and subsequent effects on emulsion particle size before spray-drying and after reconstitution (Sliwinski et al., 2003). Denaturation and aggregation of unadsorbed protein is the main reason for instability; increasing whey protein concentration accelerates the rate and degree of aggregation (Euston et al., 2000).

1.7.3 Measurement of powder dissolution

Rapid and complete dissolution of powders in the food manufacturing industry is of foremost importance, with product quality and process efficiency being adversely affected by dissolution time that is either too long or too short. Static light scattering (SLS) is a common method used to quantify dissolution properties of powders in the industry. SLS has been used to quantify the dissolution of MPC powder; by measuring changes in size distribution over time (McCarthy et al., 2014; Mimouni et al., 2009). McCarthy et al. (2014) utilised SLS to investigate the dissolution properties of MPC.
under conventional agitation and ultrasound, while rehydrating at different temperatures and for different durations; particle size was significantly reduced when ultrasound was used. Mimouni et al. (2009) concluded that complete dissolution of powder particles overlapped with agglomerates. A recent review by Crowley et al. (2016) details the mechanism of powder dissolution.

1.8 Use of microscopy for visualisation of micro-structure of dairy powders

1.8.1 Light microscopy

The optical or light microscope, employing visible light, is used widely to examine particle size and shape in the food and pharmaceutical industries. Different modes can be used to examine food samples; bright field, differential interference contrast (DIC), polarised light microscopy (PLM) and phase contrast. Recently, Malvern Instruments launched a new automated light microscope (Morphologi G3), which images a large number of particles simultaneously and collects quantitative results relating to size and shape parameters for each individual particle imaged. Ji et al. (2015) utilised the Morphologi G3 in bright field mode to determine shape parameters (circularity, convexity, and elongation) of MPI which had been modified using a fluidised bed. Both McCarthy et al. (2013) and Maher et al. (2015a) used light microscopy to visualise lactose crystals in dairy powders. McCarthy et al. (2013) observed lactose crystals in model infant formula before and after storage (54.4% RH) using DIC while examining the effect of lowering protein concentration on lactose crystal formation. Maher et al. (2015a) used PLM to visualise lactose crystals within conventional and nano-emulsions. PLM showed clearer images compared to DIC. Light microscopy can be used to observe in-situ dynamics, i.e., such as rehydration and gelatinisation on individual particles (Kett et al., 2012). Phase contrast microscopy was used to visualise individual native phosphate caseinate (NPC) over time, showing swelling in the initial stages of rehydration (Gaiani et al., 2006b). Selomulya and Fang (2013) used time–lapse images of individual powder particles (SMP and MPC) rehydrating over time, showing a comparison in behaviour for a low protein and high protein powder, SMP and MPC,
respectively. SMP dispersed and dissolved within 10 s, whereas MPC particles swelled to twice their size over a 3-hour period. Gelatinisation of maize starch heated on a stage has been also been examined using PLM (Kett et al., 2012).

### 1.8.2 Confocal laser scanning microscopy (CLSM)

CLSM is a non-destructive technique, not requiring sample fixation and/or dehydration, and can image at selective depths, obtaining high-resolution optical images. Image formation is not dependent on transmitting light through the specimen, and, therefore, bulk specimens, i.e. powder particles can be imaged. Optical sectioning (in-focus images from selected depths) is the most valuable feature of confocal scanning. Images are acquired by a laser that scans at high speeds across the specimen and reconstructs an image using software, allowing three-dimensional (3D) reconstructions of the sample. This optical sectioning allows for the interior to be visualised and to expose information typically unobtainable via two-dimensional (2D) micrographs. This operates by gathering information from distant regions by scanning a plane of focus while not blurring the image of the focal plane (Ding and Gunasekaran, 1998). Firstly, a preselected layer of the specimen is obtained and a focused scanning laser is used to illuminate, allowing information from this focal plane to pass back through the specimen and be projected onto a pinhole (confocal aperture); this data is then collected by a detector. This leads to a focal plane image being produced, at the preselected depth within the sample. By moving the specimen up and down relative to the focused laser light, a large number of consecutive optical sections can be obtained with minimum sample preparation. The depth of analysis can be pre-set by software, and when images from each section are obtained, they can be stacked to create a 3D image, as shown in Figure 1.8.
CLSM allows for simultaneous identification of macromolecules, including lipids, protein and polysaccharides, on or within powder particles. This is achieved through specific fluorescence probes emitting light at different wavelengths (Auty et al., 2001; Vignolles et al., 2009a). CLSM has been used to visualise chocolate, emulsions, cereals, yogurts and dried dairy products. Maher et al. (2015a) used CLSM to analyse the distribution of different sized fat droplets within powder particles which were stabilised by sodium caseinate. Dual labelling was achieved using Nile Red/ Fast Green FCF to stain samples for fat and protein, respectively, before and after storage at 0 and 54.4% RH. This allowed for identification of fat and protein within conventional- and nano-emulsions and showed that conventional-emulsions post crystallisation had more obvious needle-shaped crystals (Figure 1.9). CLSM has also been utilised for location of free fat associated with WMP, showing that free fat is not just restricted to the surface but can also be visualised within particles (Auty et al., 2001). Other studies
have shown, when dual labelling is not used and only fat is stained, that $\alpha$-lactose crystals can be seen by negative contrast (Maher et al., 2015a; McKenna, 1997). Kett et al. (2012) used CLSM to follow, *in-situ*, the dynamics of processes such as swelling and gelatinisation of starch, using specially designed stages, allowing heating and cooling of the sample. This gives the possibility of simulating food processes under the microscope and identifying macromolecules.
Figure 1.9. Confocal scanning laser micrographs showing internal microstructures of conventional- (a, c, e) and nano-emulsion (b, d, f) powders after storage for 0 (a, b) and 4 (c, d, e, f) days at 55% RH. Figure 1.8 a–d are fluorescently labelled with Nile red/fast green to show fat (green) and protein (red); Figure 1.8 e and f are labelled with Nile Red to show fat phase. Arrows indicate air vacuoles (a, b), discrete fat droplets (c) and solid lactose crystals (d). (e) inset shows irregularly shaped coalesced fat regions. Scale bars = 100 μm. (taken from Maher et al. (2015a)
1.8.3 Scanning electron microscopy (SEM)

It is important to characterise the morphology of powder particle surface, as it has been shown to provide information on powder flow and handling properties of spray-dried powders. Electron microscopy has been widely employed for the evaluation of surface microstructure (morphology) of food materials, and has been extensively used for detailed analysis of food powders (Danviriyakul et al., 2002). Electron beams rather than light are used to observe the sample; therefore, dairy powder samples need to be prepared before analysis. Sample preparation for dried dairy powders involves coating of powder samples with a thin layer of carbon or gold to make the surfaces conductive. The electron beam allows for gathering of high resolution information, which is much greater than that from light microscopy. Images are formed step by step by scanning a focused electron beam across the specimen. Primary electrons penetrate the solid specimen and are deflected by a large number of elastic scattering processes. Various signals are generated as a result of the incident electron, and it is mainly secondary electrons that are collected to form an image.

Using SEM, low- and high-fat containing powders have been shown to have different surface morphologies. Hogan et al. (2006) found that, at low oil: protein ratio (0.25), powders had higher surface indentation compared to high oil: protein ratio (3). Kim et al. (2002) and Murrieta-Pazos et al. (2011) imaged SMP and WMP and showed significantly different surfaces as fat increased. Hogan et al. (2006) referred to low fat powders having indentations and Murrieta-Pazos et al. (2011) described a similar microstructure, for SMP as having a “brain-type” surface, with WMP surface being characterised by “fat pools” (Kim et al., 2002). Examination of WMP surface after solvent fat extraction revealed powders with dents where these “fat pools” were removed (Kim et al., 2002). SEM images of powders obtained before fat extraction show a smooth surface while after ethanol extraction a dented surface was visible; a less dented surface was obtained when petroleum ether was used as the solvent.

McCarthy et al. (2013) studied model IMF powder and showed a rough surface post lactose crystallisation (storage at 55% RH), whereas a smooth surface was observed for powders using sodium caseinate as the emulsifier post-crystallisation (Hogan et al.,
Sodium caseinate is a very efficient emulsifier (Phillips and Williams, 2000) and has been shown to dominate the surface powder surface even in small concentrations, due to its high surface activity (Fäldt and Bergenståhl, 1994). Kim et al. (2009b) produced milk powders under two spray-drying temperatures (145/85 and 205/105 °C, inlet/outlet), with spherical particles with smoother surfaces being obtained at the higher temperature.

Internal structure of dairy powders have also been analysed by applying appropriate fracturing techniques, namely, manual crushing and cryo-SEM (Kim, 2008; Maher et al., 2015a). Using manual crushing, Kim (2008) examined the internal structure of industrially spray-dried SMP and WMP, before after solvent fat extraction, to understand the distribution of free and encapsulated fat. For SMP, no fat globules could be seen within the powder matrix before and after fat extraction. WMP indicated no difference after free fat extraction, leading Kim (2008) to conclude that encapsulated fat was not removed from the interior and that there were only microscopic cracks and pores on the particle which did not allow for removal of such encapsulated. Cryo-SEM has been used to investigate the internal microstructure of samples, and is a useful technique in analysing nano-sized objects embedded in food matrices and also allowing easy visualisation of air vacuoles (Maher et al., 2015a). Maher et al. (2015a) examined the interior of conventional- and nano-emulsion before and after crystallisation (55 % RH). Oil droplets were seen to be embedded in the matrix wall before lactose crystallisation and were not visible after crystallisation, thus leading to the conclusion that crystallised lactose ruptured the fat globules.

### 1.8.4 Atomic force microscopy

AFM is an extremely high-resolution scanning probe microscopy technique, with demonstrated resolution in the order of nanometres. It is widely used in the medical and pharmaceutical industry to characterise lactose and other powders (Mahlin et al., 2004). While these applications are similar in theory to food powders, the use of AFM on dairy powders is still uncommon.
AFM is based on interaction between a sharp tip and a sample surface. The atomic force microscope uses forces existing between probe and sample to build an image of an object as the tip scans the surface, with the central component of AFM being the tip that “feels” the sample. A nanometre-sharp AFM tip is adhered at the free end of a flexible cantilever that is used as the transducer of the interaction between the tip and sample. The reflection of a laser beam focused at the back side of the cantilever is frequently used by most AFMs to amplify and measure the movement of the cantilever. The reflected beam is directed to a photodiode that provides a voltage depending on the position of the laser beam. For imaging, the tip is scanned over the sample, fine movement of the tip and sample is provided by piezoelectric materials that can move with sub-nanometre precision. At each position, the cantilever deflection is measured, from which a topography map can be constructed. This scanning technique in which the tip is brought into mechanical contact with the sample surface is known as contact mode. Other modes such as non-contact and tapping can be used.

Murrieta-Pazos et al. (2011) published work displaying the surface topography of SMP and WMP by AFM in tapping mode. The 3D surface topography obtained by AFM agreed with SEM images found in the literature, showing fat-pools on the surface of WMP and a “brain type” surface for SMP. Surface roughness (Ra) was also determined, WMP was found to be smoother than SMP, with a Ra of 146 nm and 306 nm, respectively. The rough surface of SMP was due to less fat being found on the surface, corresponding with a wrinkle brain-type surface.

Prime et al. (2011a) examined surface topography and phase images of sodium caseinate stabilised emulsion composed of soya oil and maltodextrin, produced at different inlet/outlet temperatures, i.e., 245/100 °C and 170/80 °C, respectively. Phase imaging is sensitive to viscoelastic changes and hence is sensitive to material composition changes. Images revealed that powder surface was characterised by a uniform distribution of circular features, possibly showing areas of higher oil concentration. However, phase imaging only gives a qualitative analysis of the surface, i.e., indicating differing material properties on the surface. It should be noted that XPS
has shown that, at a lower fat concentration compared to the composition used in the study of Prime et al. (2011a), a powder surface can be dominated by fat.

Prime et al. (2011b) examined the effect of %RH and temperature on a single powder particle. AFM topography images showed significant changes in morphology due to increased temperature and RH%, reflecting a viscous topography. Fyfe et al. (2011) detected an increase in hydrophobicity of MPC powder during storage at 66% RH compared to fresh powder. This was achieved by analysing surface hydrophobic adhesion forces of the powder particles to mica (hydrophobic surface) and graphite (hydrophilic surface) wafer, depending on whether the stored powder was more hydrophobic or hydrophilic in nature.

1.9 Spectroscopic techniques for physico-chemical analysis of dairy powders

1.9.1 Introduction to X-ray photoelectron spectroscopy (XPS)

XPS, also called electron spectroscopy for chemical analysis (ESCA), is an analytical technique widely used in material surface analysis (Briggs, 1998; Rouxhet and Genet, 2011). Since the mid-1960s, it has been used to characterise surfaces of solid materials, i.e., ceramics, fibres, glass, metals, minerals, wood and polymers. In the last 20 years, this analytical technique has progressed to the food science area, allowing for surface analysis of low moisture food products, i.e., dairy powders and flour, and in turn providing compositional data of the surface. The reason for the delay in use of XPS in food powders was due to their complexity, as they contain many different elements and, therefore, the use of XPS was considered to be too complex. However, this was overcome when Fäldt et al. (1993) proposed a matrix which simplified the elemental data obtained from XPS spectra. Fäldt et al. (1993) were the first to use XPS and understand its potential in food science and applied this technique to the surface of spray-dried dairy powder. Since its realisation as an advanced surface elemental mapping spectroscopy technique, it has since been used to determine the surface composition of many different dairy powders (Fyfe et al., 2011; Gaiani et al., 2007; McCarthy et al., 2013; Murrieta-Pazos et al., 2013) and dairy powders containing
different surface-active proteins (Adhikari et al., 2009; Jones et al., 2013). It has since progressed to being used in determination of elemental composition of flours (Rouxhet et al., 2008; Saad et al., 2011; Saad et al., 2009).

It is critical to understand the mechanism behind the formation of the surface composition of industrial spray-dried dairy powders, as it has been shown that the surface has significant implications for powder behaviour, i.e., rehydration and flowability (Kim, 2008). The importance of the technique can be transferred to other dehydrated food products (eggs, cereal, spices) and the pharmaceutical sector (antibiotics and medical ingredients), and other naturally low moisture foods, such as flour. Surface composition by XPS appears to provide the most quantitative information on chemical composition of food powder surface.

1.9.1.1 Principle of XPS

The technique provides elemental and chemical state data from the first ~5nm of the surface of solid samples. The sample is placed under ultra-high vacuum and irradiated with photons from a soft X-ray source with a defined energy; normally a monochromatic Aluminium K\(\alpha\) X-ray (1486.6eV) source is used. However, another common source of radiation is magnesium Mg\(\alpha\) X-rays (1253.6 eV). These photons penetrate from 1 to 10 \(\mu\)m into a solid and interact with atomic and molecular orbital electrons in the surface of the sample, but most of the information acquired pertains to the outer ~5 nm. The method is based on surface irradiation, which causes a complete transfer of photon energy to atomic electrons (Bosquillon et al., 2004). When the electron binding energy (\(E_b\)) is lower than the photon energy (\(h\nu\)), the electron is emitted from the atom with a kinetic energy (\(E_k\)) equal to the difference between the photon energy and the binding energy, minus the spectrometer work function, \(\Phi\), which is the energy needed for the electron to free itself from the surface. i.e., the kinetic energy (\(E_k\)) of the emitted electrons is given by Equation 1.4:

\[
E_k = h\nu - E_b - \Phi
\]  (1.4)
As electron binding energy is unique to each element and orbital. Analysis of the kinetic energy of the emitted photoelectrons allows for identification of the elements and their orbitals. The emitted electrons suffer inelastic collisions with the atoms of the solid as they penetrate the surface. At each inelastic collision, the electron kinetic energy is decreased, as illustrated in Figure 1.9. The electrons originating deeper than ~5 nm lose their energy, and end up as background intensity or do not escape from the surface. Only electrons that originate in the first ~5 nm below the surface do not suffer inelastic collision and therefore contribute to the peaks which are then analysed and fitted to a matrix formulation. Electrons originating far from the surface lose their energy, and end up as background intensity (Figure 1.10; 2 & 3) or do not escape from the surface (Figure 1.10; 4).

**Figure 1.10** Illustration of the loss of kinetic energy (length of arrows), as the photoelectrons (numbers 1-4) penetrate the solid, and their contribution to the XPS spectrum. The electrons photo-ejected near the surface (1) contribute to the peaks, those originating from intermediate depth (2,3), contribute to the background, and those photo-ejected at a greater depth (4) do not escape from the solid. Redrawn from Mozes et al. (1991).
The inelastic process for electrons travelling through a material can be described by:

$$I_z = I_0e^{-z/\lambda \sin \theta} \quad (1.5)$$

where $I_0$ is the intensity of emitted electrons at height $z=0$ and $\theta$ is the analysed take-off angle. The average distance an electron travels before it becomes involved in a collision is a function of its kinetic energy and the density of the solid. The distance is characterised by the inelastic mean free path, $\lambda$. The mean free path varies depending on material being analysed. The signal will weaken with increasing distance, $z$, from the surface of the material, according to Equation 1.5.

An electric kinetic energy analyser detects photoelectrons escaping into the vacuum and counts them as a function of their kinetic energy. The spectrum is obtained as a plot of number of detected electrons per energy interval versus their kinetic energy, which is then converted to binding energy. A unique spectrum is obtained for each element and each covalent bond, e.g., C-, C=, etc.

Peak areas, normalised on the basis of sensitivity factors proposed by the manufacturer, can be used to determine the ratio of the atomic concentrations. It can be deduced that only the surface is determined due to the inelastic scattering of electrons on the outermost molecular layers. Examination of spectra and data collected from XPS make it possible to study functional groups on the surface.

As noted earlier, XPS is performed under high vacuum ($10^{-8}$ torr), which eliminates further scattering but also presents limitations for foods with high moisture content. It can be concluded that this technique is ideal for dehydrated food powders and other low moisture foods, i.e., spray-dried dairy powders, flour and chocolate (James and Smith, 2009).
1.9.1.2 Analysis of spectra

As mentioned in the previous section, the progression of XPS from material surface science to the food industry was first realised on dairy powders and has since advanced to other food materials. In the first application of XPS to the food science sector at Lund University Fäldt et al. (1993), developed an algebraic matrix approach to identify macronutrients on dairy powder surface. Knowing that each component (protein, lactose, fat) in a powder has a specific ratio between elements (C, O and N), it was possible, from analysis of the relative amounts of these different elements in pure components and in the powder samples, to estimate the percentage of each component of interest on the surface. From this mathematical approach, the raw spectrum was further analysed.

To determine the molecular composition a matrix was designed on the basis that the elemental makeup of the surface was a linear combination of the elemental makeup of the different molecular components in the sample (Gaiani et al., 2011; Jayasundera et al., 2009; Kim et al., 2009c). Thus, the data on elemental composition of the surface can be used to estimate the molecular composition of the surface layer by solving the matrix equation (Equation 1.6; Fäldt et al. (1993)):

\[
\begin{pmatrix}
I_{\text{comp.1}}^{n} & \cdots & I_{\text{comp.i}}^{n} \\
\vdots & \ddots & \vdots \\
I_{\text{comp.1}}^{i} & \cdots & I_{\text{comp.i}}^{i}
\end{pmatrix}
\begin{pmatrix}
\gamma_{1} \\
\vdots \\
\gamma_{i}
\end{pmatrix}
= 
\begin{pmatrix}
I_{\text{sample}}^{1} \\
\vdots \\
I_{\text{sample}}^{n}
\end{pmatrix}
\]

(1.6)

In this matrix, the powder particle comprises of \(i\) components, and it is a prerequisite that the sample contain at least \(i\) elements. The relative atomic concentration of element \(n\) in the pure component \(I\) is denoted \(I_{\text{comp.1}}^{n}\), and the relative atomic concentration of element \(n\) in the sample is denoted \(I_{\text{sample}}^{n}\). The unknown relative coverage of component \(I\) is expressed as \(\gamma_{i}\). Thus, from analysis of the relative atomic concentrations of different elements in pure reference components and in the powder sample, it is possible to estimate the relative coverage of components on the powder
surface. The matrix system above can be solved by least squares methods, or exactly if \( i = n \).

Dairy powders are complex systems that contain minerals, some of which are naturally occurring and are associated with the different protein fractions. Previous studies of SMP, WPC, CP and WPC have shown that the surface is free from such minerals (Kim, 2008), or are not present at a sufficiently high concentration for detection. Using the above matrix, only lactose, lipids and proteins were taken into account; other components were neglected (Fyfe et al., 2011; Gaiani et al., 2009). The measurement from XPS was therefore considered to be composed of only the three components (lactose, protein and fat), containing C, O and N, and the relative coverage of different components in the powder surface layer can be expressed as (Equation 1.7):

\[
\begin{pmatrix}
I^C_{\text{lactose}} & I^C_{\text{protein}} & I^C_{\text{fat}} \\
I^O_{\text{lactose}} & I^O_{\text{protein}} & I^O_{\text{fat}} \\
I^N_{\text{lactose}} & I^N_{\text{protein}} & I^N_{\text{fat}}
\end{pmatrix}
\begin{pmatrix}
\gamma_{\text{lactose}} \\
\gamma_{\text{protein}} \\
\gamma_{\text{fat}}
\end{pmatrix}
= \begin{pmatrix}
I^C_{\text{sample}} \\
I^O_{\text{sample}} \\
I^N_{\text{sample}}
\end{pmatrix}
\] (1.7)

The above matrix equation can also be written as:

\[
I^C_{\text{sample}} = I^C_{\text{lactose}} \cdot \gamma_{\text{lactose}} + I^C_{\text{protein}} \cdot \gamma_{\text{protein}} + I^C_{\text{fat}} \cdot \gamma_{\text{fat}}
\]

\[
I^O_{\text{sample}} = I^O_{\text{lactose}} \cdot \gamma_{\text{lactose}} + I^O_{\text{protein}} \cdot \gamma_{\text{protein}} + I^O_{\text{fat}} \cdot \gamma_{\text{fat}}
\]

\[
I^N_{\text{sample}} = I^N_{\text{protein}} \cdot \gamma_{\text{protein}}
\] (1.8)

, and assumes that nitrogen is associated with protein alone.

However, in recent years, Gaiani et al. (2011) developed a technique which detected minerals which are associated with casein and whey protein, and from this were able to differentiate between casein and whey proteins within high protein powders.
1.9.1.3 Use of XPS for analysis of dehydrated dairy products

Numerous authors have shown that some components are highly represented at the powder surface in comparison with the bulk composition (Gaiani et al., 2006a; Kim et al., 2002; Shrestha et al., 2007; Vignolles et al., 2009b). Surface-active components, i.e., fats and proteins have been found to more highly represented at the surface, whereas lactose was less represented. Studies on non-fat powders, for example SMP, MPC and MPI, have demonstrated that the low level of fat present is over-represented at the surface. When a low level of fat is present, protein will migrate to the surface. Nevertheless, fats cover the majority of the surface in the presence of fat and protein.

Nijdam and Langrish (2006), studied the effect of drying temperatures and formulations on surface composition, at laboratory scale and concluded that a small change in bulk fat content resulted in a large change in surface fat coverage, also observing that higher drying temperature resulted in a higher surface coverage of fat and lactose. Nevertheless, there was not concomitant rise of surface lactose with bulk lactose. The authors concluded that at lower drying temperatures (and lower evaporation rates), protein has more time to migrate to the surface of the droplet before sufficient moisture is evaporated to form a skin. Similar results in terms of lactose-protein surface composition were observed by Shrestha et al. (2007b) who studied powders decreasing protein content (34 – 8.5 g / 100 g). Later, Kim et al. (2009b) studied the effect of, (i) increasing total solids of feed pre-spray-drying, (ii) drying temperatures for SMP and WMP and (iii) the use of 2 homogenisation passes for WMP. The authors postulated that higher feed solids content would give rise to more viscous droplets, preventing the migration of components and redistribution at the surface. The authors go on to describe how surface lactose concentration increase with spray-drying temperature, agreeing with Nijdam and Langrish (2006) that the lipid surface concentration is reduced by high temperatures in SMP and does not present an important influence in WMP. Gaiani et al. (2010) observed a high fat surface presence regardless of spray-drying temperature for native micellar casein (NMC) and native whey isolate (NWI). Adding further, that fat was greater in powders containing NWI than NMC and also that higher temperatures increase the protein-lactose surface content and reduce the surface lipid content.
The same authors, also studied the effect of storage on NMC powder at 20 and 50 °C, stored using different types of packaging (standard or watertight bags), over storage times of 15, 30 and 60 days (Gaiani et al., 2007). Significant surface changes were found after 60 days of storage, when powder was stored in watertight bags. When powders were stored in standard bags, changes were found after 30 days of storage.

At industrial scale, the surface composition of WMP, SMP, CP and WPC was studied (Kim et al., 2005; Kim et al., 2002). Later, the same authors (Kim et al., 2009c), determined the effect of storage (6 months) on these powders, and a migration of fat to the surface was observed in all of the powders. WMP and SMP were also collected at different points of the spray-drying and fluidised bed processes (Kim et al., 2009a). Surface composition did not change significantly, suggesting that surface composition is developed during spray-drying and are not modified in subsequent steps. Murrieta-Pazos et al. (2012) compared the surface composition of regular WMP and SMP to that of agglomerated WMP (WMPG) and agglomerated SMP (SMPG). There was no significant difference between regular and agglomerated powders, showing that agglomeration of powders by fines return did not have a significant effect on surface composition. Fyfe et al. (2011) studied the effect of storage on MPC powder surface after 14, 30, 60 and 90 days at 25 and 40 °C at elevated RH levels (44%, 66% and 84%), and reported no significant change to the surface composition.

Finally, different solvents and treatments were applied to fat containing dairy powders (WMP, WMPG, CP) in order to extract the surface free fat (Kim et al., 2002; Murrieta-Pazos et al., 2012). The efficiency of extraction was observed after analysis of surface composition in the “surface free fat” powders, and these powders were then used to evaluate the evolution of functional properties.

Emulsions containing different oil phases with varied melting points were studied before and after crystallisation, and protein was seen to be over represented on the surface relative to the bulk composition. This was consistent with other studies carried out which examined lactose/protein dispersions and emulsions. It was established in this study that lipid type had an influence on surface fat coverage; it was
concluded that higher melting points gave better lipid encapsulation, with hardened rapeseed oil (melting point ~59 °C) and soybean oil representing 3% and 15% of surface fat, respectively. However, the same trend was not seen in other high melting lipid fractions (coconut butter and butter fat, with intermediate melting points of ~40 °C) within the same lactose/protein system. This could be explained by the presence of fat crystals in the oil droplets of the oil/water emulsion, inducing coalescence of emulsion droplets during processing and, therefore, leading to a less stable powder. Above a certain amount of solid fat in an emulsion the rate of coalescence decreases (van Boekel and Walstra, 1981).

1.9.1.4 Functional groups associated with dairy powder surface

Using XPS, Fyfe et al. (2011) determined the surface elemental bonding state of MPC80 powder after storage, with a particular focus on the bonding state of carbon (C). It was hypothesised that, as particle surface hydrophobicity increased during storage, there would be an increase in non-polar bonds (C–C) at the surface. A small increase in C-C bonds was observed during storage, with the significant ($P \leq 0.05$) factors contributing to an increase in C-C bonds being temperature, followed by time and then humidity. The most significant changes occurred between 14 and 30 days of storage, after which the number of C-C bonds plateaued. As previously mentioned, temperature had the greatest effect on C-C bonds, with the largest number of C-C bonds occurring after 14 days at 40 °C and 30 days at 25 °C for all RH values examined. The authors also detected a decrease in surface fat after storage; however, powder hydrophobicity increased over time, which possibly could be related to increased cross-linking of proteins in MPC during storage, as suggested by others (Anema et al., 2006; Havea, 2006).
1.10 Conclusions

There is significant knowledge and understanding of dairy powders in terms of their individual ingredients, physical properties, and their many potential uses in the food industry. However, there is a lack of knowledge regarding the newer, more novel ingredients, such as MPC and hydrolysed whey protein powders being increasingly used commercially. The utilisation of microscopic techniques, such as XPS, is a relatively new concept in dairy science with a limited number of publications in the area. Further research is required in this area so that a better understanding of powder surfaces can be achieved to facilitate production of dairy products with desired functional characteristics and improved storage stability. The research in this thesis details the effect of surface composition on powder properties, functionality and stability by formulating, preparing at pilot scale and analysing powders of varied oil type, protein type, and protein composition.
1.11 References


Objectives

This thesis describes a series of studies, on the effects of changing powder composition and processing conditions on the surface characteristics, physical properties, storage stability and rehydration properties of dairy powders. In the studies described, different powders were produced, reflecting fat-free commonly-used dairy powdered ingredients and fat-containing model infant formulae. Dairy powder ingredients and dairy powders for end use are a diverse range of products designed to meet consumer and company demand, which are produced worldwide by different companies. Consequently, different manufacturing processes can be employed. Defatted dairy powders were produced via two processes, either from skim milk or by recombination of lactose and protein ingredients. Firstly, the influence of protein level in a defatted powder was studied using membrane process filtration. Secondly, in two of the studies, protein source differed by inclusion of either intact or hydrolysed whey protein, with hydrolysed whey protein having uses in specialised IMF products, to reduce allergenicity or increase digestibility (Maldonado, Gil, Narbona, & Molina, 1998). Also, another two studies examined fat containing powders, which were produced via recombination of ingredients and studied the effect of different processing condition and ingredients on powder surface characteristics, functional properties and storage stability.
Chapter 2: Influence of protein concentration on surface composition and physico-chemical properties of spray-dried milk protein concentrate powders


Declaration: Production of MPC powders and chemical composition was carried out in NIZO, Ede, The Netherlands, except for particle size and fat analysis, which were carried out by Grace Kelly. Glass-rubber transition and sorption isotherm analysis was also carried out by Grace Kelly. Dr. Deirdre Kennedy took images using SEM. XPS was carried out by Dr. Fathima Laffir, University of Limerick. Experimental results/ data were analysed and the chapter was written by Grace Kelly.
Abstract

Surface composition, moisture sorption behaviour and glass-rubber transition temperature ($T_{gr}$) were determined for spray-dried milk protein concentrate (MPC) powders over a range of protein contents (35 – 86 g / 100 g). Surface characterisation of MPC powders, indicated that fat and protein were preferentially located on the surface of the powder particles, whereas lactose was located predominantly in the bulk. Moisture sorption analysis at 25 °C showed that MPC35 exhibited lactose crystallisation, whereas powders with higher protein contents did not and continually absorbed moisture upon humidification up to 90% RH. The GAB equation, fitted to sorption isotherms of MPCs, gave increases in monolayer moisture value ($m_m$) with protein content. $T_{gr}$, measured with a rheometer, decreased significantly ($P < 0.05$) with increasing water content and increased with increasing protein content ($P < 0.05$). In conclusion, increasing protein concentration of MPCs resulted in altered surface composition and increased $m_m$ value and $T_{gr}$ values.
2.1 Introduction

Milk protein concentrates (MPCs) are high-protein spray-dried powders derived from membrane-separated skimmed milk (Chandan & Kilara, 2010). Ultrafiltration (UF) and diafiltration (DF) are applied to skimmed milk, followed by evaporation and spray-drying (Mistry & Hassan, 1991), resulting in powders with protein concentrations ranging from 35 to 86 g / 100 g, with a concomitant reduction in lactose and non-protein soluble component levels as protein content increases (Havea, 2006).

In recent years, MPCs of different protein contents have been produced and incorporated into a wide range of products. For example, MPC powders are often used to standardise the protein content of milk for cheese-making, and are also used in recombined cheese, infant milk formula (IMF), dairy-based beverages and sports and nutritional foods.

There are issues in the handling and storage of dried milk protein ingredients, e.g., powder blockage in spray dryers, powder silos and hoppers, and shelf-life issues involving caking. These issues can be related to surface composition and moisture levels, which affect key functional properties, e.g., stickiness, wettability, bulk density and flowability of powders (Kim, Chen, & Pearce, 2002; Nijdam & Langrish, 2006). Non-uniform distribution of components, e.g., on particle surfaces versus their interior, can occur through various mechanisms. Spray-drying involves the rapid removal of water from a concentrated dispersion, during which milk components are concentrated, as moisture evaporates from the droplet surface (Kim, Chen, & Pearce, 2009), causing concentration gradients of solutes. Osmotic forces cause dissolved milk components to migrate toward the core of the particle to balance concentration gradients (Birchal, Huang, Mujumdar, & Passos, 2006). Another influence is hydrophobicity, whereby hydrophobic molecules, such as fat, will preferentially take up position on the surface. One effect of this migration is that concentrations of lactose, fat, and protein at the surface of spray-dried dairy powders have been found to be different from the bulk composition (Fäldt & Bergenståhl, 1996; Kim, et al., 2002; Nijdam & Langrish, 2006). Powders with high levels of surface fat are less wettable, less flowable, and more...
prone to lipid oxidation compared to those with lower levels of free fat (Vignolles, Jeantet, Lopez, & Schuck, 2007).

Moisture sorption behaviour and glass-rubber transition data provide information which is useful in selecting processing conditions for spray-dried milk powders, and which influence physical characteristics (such as stickiness, hygroscopicity and caking behaviour) and stability (storing and handling) of the final product. Moisture sorption isotherms show the quantity of water absorbed by powders and the relative humidity (RH) at which lactose crystallises in powders. Crystallisation of non-fat powders is undesirable as it causes sticking, caking and cohesion (Jouppila & Roos, 1994). Sorption isotherms can be modelled using equations such as Guggenheim-Anderson-de Boer (GAB) to determine factors such as the monolayer value of powders (Foster, Bronlund, & Paterson, 2005). Glass-rubber transition temperatures ($T_{gr}$) of powders influences their storage stability, as, at higher $T_{gr}$ values, powder is likely to crystallise at a slower rate at a given temperature during storage.

X-ray photoelectron spectroscopy (XPS) is an established method for elemental analysis of a variety of materials, including dairy powders (Kim et al., 2009; Nijdam & Langrish, 2006). The analysis provides relative atomic percentages of elements present at the surface of milk powder particles using measurements within ~ 5 nm of the surface (Gaiani, et al., 2006). Surface composition of powder is of importance in understanding functional properties.

The physico-chemical properties of spray-dried dairy powders of different composition, such as milk powders with various fat levels, skimmed milk powder (SMP) with added lactose, and SMP with hydrolysed lactose or whey protein/lactose blends have been studied (Hogan & O’Callaghan, 2013; Nijdam & Langrish, 2006; Shrestha, Howes, Adhikari, & Bhandari, 2007a; Shrestha, Howes, Adhikari, Wood, & Bhandari, 2007b). However, the effect of increasing protein content of MPCs on their physico-chemical properties, such as surface composition, moisture sorption behaviour and $T_{gr}$, has not yet been studied.
The aim of the present study was to investigate the effect of protein concentration (35-86 g / 100 g) on physico-chemical properties of a series of MPC powders made from the same milk source. Hence, differences in milk composition were not a factor, as would be the case if powders were collected from industrial sources. Powder particle surface composition of MPC powders was analysed using XPS, and moisture sorption isotherms and \( T_g \) of the powders were also measured.

2.2 Materials and methods

2.2.1 Sample preparation

Seven non-agglomerated MPC powders were produced from skimmed milk by ultrafiltration, diafiltration and spray-drying, as reported by Crowley, Megemont, Gazi, Kelly, Huppertz, and O'Mahony (2014a). The powders, referred to as MPC35, MPC50, MPC60, MPC70, MPC80, MPC85, MPC90, in order of increasing protein content, had measured protein levels in the range 35 to 86 g / 100 g (Table 2.1). Chemical composition of the powders was determined by NIZO food research (Ede, The Netherlands), using the Kjeldahl method for protein, furnace method for ash and HPLC for lactose content, as detailed by Crowley et al. (2014a).

2.2.2 Powder particle size

Powder particle size was determined by laser-light-scattering using a Malvern Mastersizer 3000 with an Aero S unit (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Powder sample was added to the standard venturi disperser with a hopper gap of 4 mm and then fed into the dispersion system. The feed rate was 18 - 25% to keep the laser obscuration level at 1 - 6%. Compressed air at 0.5 bar was used to transport and suspend the powder particles through the optical cell, a measurement time of 10 s was used, and background measurements were made using air for 20 s. Volume mean diameter, \( D[4,3] \), was used to characterise the particle size of powders.
2.2.3 Surface structural analysis by scanning electron microscopy

Surface structure was observed using scanning electron microscopy (SEM). Samples were imaged using a field emission scanning electron microscope (FE-SEM; Zeiss Supra Gemini, Darmstadt, Germany) at 2.00 kV. Samples were mounted on double-sided carbon tape, attached to SEM stubs, and then sputter-coated with chromium (Emitech K550X, Ashford, UK). Representative micrographs were taken at 1000× and 5000× magnification in order to visualise surface characteristics.

2.2.4 Surface composition of powders

Surface composition of powders was determined as described by McCarthy, Gee, Hickey, Kelly, O’Mahony and Fenelon (2013). In brief, a Kratos Axis 165 X-ray photoelectron spectrophotometer (XPS; Kratos Analytical, Manchester, UK), using a monochromatic Al Kα X-ray source (1486.58 eV) at 150W (15 kV, 10 mA) was used. The powder samples were attached to the sample holder prior to analysis using double-sided conductive tape. Using theoretical elemental compositions, a matrix formula was used to determine relative amounts of protein, fat and lactose on the powder surface, as described by Fäldt, Bergenståhl and Carlsson (1993).

2.2.5 Glass-rubber transition temperature

Powders were dried overnight at 70°C in a vacuum oven, followed by further drying in a desiccator over P₂O₅ for two days. Samples were placed in vacuum desiccators over saturated salt solutions of LiCl, CH₃COOH, MgCl₂ or K₂CO₃, to give respective RH of 11.4%, 23.1%, 33.2%, and 44.1%, at room temperature, giving a_water values of 0.01 x RH (Labuza, Kaanane, & Chen, 1985). The samples were stored under these conditions for up to 15 d before analysis of $T_{gr}$ values of powders by a thermo-mechanical technique, as described by Hogan, Famelart, O’Callaghan, and Schuck (2010), using a standard laboratory rheometer (AR2000, TA Instruments Ltd., Crawley, UK). Powder samples (~ 1 g) were compressed (30 N) between a Peltier base plate 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). The T_{gr} was identified as the point of inflection on the normal force versus temperature curve. All T_{gr} measurements were carried out in duplicate.

### 2.2.6 Sorption isotherm studies

Water sorption isotherms were determined gravimetrically using a dynamic vapour sorption (DVS) technique (DVS Advantage 1 Surface Measurement Systems Ltd., London, UK). The DVS apparatus monitors the moisture sorption capacities of powders during storage by recording changes in sample weight over time at a constant temperature (25 °C) and varying RH (between 0 and 90%). Samples (30 mg) dried as described in Section 2.2.5 were loaded into the sample pan. Accurate RH settings were obtained by mixing dry nitrogen gas with saturated water vapour in defined proportions. Samples were humidified from 0 to 90% RH in increments of 10% RH. For each step, equilibrium was considered to be reached when change in mass with time (dm/dt) was < 0.001 mg / min for at least 10 min. Graphs of water uptake over time (isotherms) for each powder sample were obtained using the DVS Data Analysis Suite which runs from within Microsoft Excel (Microsoft Office Excel 2003).

The GAB equation (Van den Berg, 1984) was used to model water sorption isotherms and to determine the monolayer value (m_{m}). The GAB model isotherm, is given in Equation 2.1:

\[
\frac{m}{m_m} = \frac{C K a_w}{(1-K a_w)(1+K a_w(C-1))}
\]  

(2.1)

where m is the moisture content (g / 100 g dry solids), m_{m} is the monolayer value (g / 100 g), a_{w} is water activity and C and K are dimensionless constants.

Bizot (1983) showed that this equation could be converted to a second-order polynomial giving a quadratic equation (Equation 2.2):
\[
\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma
\]  

(2.2)

Values for the parameters, \(\alpha, \beta, \gamma\) were determined by quadratic regression analysis of \(\frac{a_w}{m}\) as a function of \(a_w\) using DVS data, over a range of \(a_w\), e.g., 0 to 0.4. The solution to equations (2.1) and (2.2) give the values for \(m, C\) and \(K\) as:

\[
m = \frac{1}{\sqrt{\beta^2 - 4\alpha\gamma}}
\]

(2.3)

\[
K = \frac{\beta - \frac{1}{m}}{-2\gamma}
\]

(2.4)

\[
C = \frac{1}{m K \gamma}
\]

(2.5)

The point of inflection on sorption isotherms was determined as the point where the second derivative of the GAB equation equals 0. This was calculated using the goal-seek procedure in Excel 2010.

2.2.7 Statistical analysis

Analysis of variance (ANOVA) was carried out using Minitab 15 statistical package (Minitab Ltd., Coventry, UK). A single factor ANOVA test was used to determine significant results and Fisher’s one-way multiple comparison tests were used to compare different levels. Results were deemed statistically significant if \(P < 0.05\). Second order polynomial regression was carried out to determine the GAB model and to analyse the fit. Root mean square error (\(E_{rms}\)) was used as an index of quality of fit, where
\[ E_{\text{rms}} = \sqrt{\frac{\sum_{i=1}^{4} (\frac{m-m_i}{m_i})^2}{n}} \] (2.6)

and \( m_1, m_2, m_3 \) and \( m_4 \) were the moisture contents at \( a_w = 0.1, 0.2, 0.3 \) and 0.4, respectively.

2.3 Results and discussion

2.3.1 Scanning electron microscopy

Representative SEM micrographs of the MPC powders are shown in Figure 2.1. In general, powder particles were small, \( \sim 10-90 \mu m \), as confirmed by particle size analysis (Table 2.1). The low protein powders (e.g., MPC35) had a wrinkled surface consistent with that of SMP observed in other studies, which is due to minimal fat level (Fyfe, Kravchuk, Nguyen, Deeth, & Bhandari, 2011; Gaiani, et al., 2006; Kim, et al., 2002; Murrieta-Pazos, Gaiani, Galet, & Scher, 2012; Nijdam & Langrish, 2006). Crowley, Gazi, Kelly, Huppertz and O’Mahony (2014b) reported a low volume of occluded air \( (V_{OA}) \) for low protein MPC powders, which is consistent with the SEM images of low-protein powders displaying deflated powder particles. As protein content increased, powder surfaces became smoother, with smooth dimples being evident for MPC70 to MPC90. These observations were similar to those reported by Fyfe et al. (2011) for spray-dried low and high protein content MPCs.
Figure 2.1 SEM images showing microstructure of MPC powders (1) MPC35, (2) MPC70, and (3) MPC90 at a magnification of (A) 1,000x and (B) 5,000x.
2.3.2 Surface composition

The application of XPS to analysis of surface composition of food powders has been well documented (Drusch, et al., 2012; Gaiani, et al., 2011; McCarthy, et al., 2013). Elemental analysis of the surface of MPC samples was based on binding energies of C₁s, N₁s and O₁s, which were in the ranges 281-293, 397-408, and 528-533 eV, respectively (Fadley, 1976; Shrestha, et al., 2007b). These peaks can be decomposed into components that relate to well-identified chemical functions, typical of classes of biochemical compounds found in food, i.e., proteins, fats and carbohydrates.

The surface and bulk composition of MPCs in terms of protein, lactose and fat is presented in Table 2.1. In this study, as ca. 85-86% of bulk composition is made up of protein and lactose, it is implicit that any increase in protein concentration (by UF and/or DF) is accompanied by a corresponding decrease in lactose. Fat content in the bulk composition was low and increased from 0.6 to 1.4 g / 100 g with increasing protein level.
Table 2.1 Powder particle size (mean ± standard deviation; n=2), surface\(^a\) and bulk\(^b\) compositions of MPCs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MPC35</th>
<th>MPC50</th>
<th>MPC60</th>
<th>MPC70</th>
<th>MPC80</th>
<th>MPC85</th>
<th>MPC90</th>
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<tbody>
<tr>
<td>D([4,3]) ((\mu)m)</td>
<td>40.3 ± 0.4</td>
<td>46.8 ± 0.3</td>
<td>58.8 ± 1.1</td>
<td>46.4 ± 0.3</td>
<td>31.4 ± 0.2</td>
<td>30.6 ± 1.2</td>
<td>32.0 ± 0.8</td>
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<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Surface</td>
<td>62.7</td>
<td>63.4</td>
<td>73.9</td>
<td>78.9</td>
<td>90.8</td>
<td>97.2</td>
<td>93.0</td>
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<tr>
<td>Bulk</td>
<td>35.4</td>
<td>49.9</td>
<td>60.8</td>
<td>68.2</td>
<td>79.1</td>
<td>84.0</td>
<td>85.8</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Surface</td>
<td>31.3</td>
<td>26.5</td>
<td>18.6</td>
<td>12.4</td>
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<td>&lt;1</td>
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<tr>
<td>Bulk</td>
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<td>35.8</td>
<td>24.5</td>
<td>18.0</td>
<td>6.4</td>
<td>1.8</td>
<td>0.4</td>
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<tr>
<td>Fat</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Surface</td>
<td>5.9</td>
<td>10.2</td>
<td>7.5</td>
<td>8.8</td>
<td>8.2</td>
<td>3.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Bulk(^b)</td>
<td>0.6</td>
<td>0.8</td>
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<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>1.4</td>
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<tr>
<td>Elemental(^c)</td>
<td>2.1</td>
<td>1.9</td>
<td>1.5</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
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<tr>
<td>Bulk</td>
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<tr>
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<td>3.6</td>
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</tbody>
</table>

\(^a\) Bulk powder composition values for protein, lactose, ash and moisture of MPCs were taken from Crowley et al. (2014a) (g / 100 g)

\(^b\) Fat was determined by the Rose Gottlieb method

\(^c\) Total surface elemental mineral composition was measured by XPS (% atomic concentration)
Surface analysis showed that protein and fat levels were markedly higher on the surface than in the bulk, with protein contents on the surface > 60 % for all powders. There was a trend towards increasing protein and decreasing lactose on the surface with increasing concentration of protein in the bulk. There was also a large decrease in the level of lactose on the surface for MPC80 compared to MPC70, coinciding with a considerable decrease in total solids in the feed (Crowley et al., 2014b). XPS indicated that fat was present on powder surfaces, typically at levels of 6-10% of surface area, and this level was not correlated with fat level in the bulk composition. In general, the broad trends in these results are consistent with other studies of spray-dried powders containing milk proteins and lactose (Fäldt & Bergenståhl, 1994; Kim et al., 2009).

Due to the association of Ca and P with the casein micelle, it can be inferred from the high levels of these minerals on the surface (Table 2.2) that casein is the dominant protein on the surface (Gaiani, et al., 2011). After protein, the next principal constituent on the surface is lactose, which occupies space between the proteins. The increased fat on the surface is probably due to its hydrophobicity, i.e., a small amount of fat near the surface migrates to the droplet/powder particle surface during drying.
Table 2.2 Elemental surface composition of MPC powders as measured by XPS

<table>
<thead>
<tr>
<th>Elements</th>
<th>% Atomic concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPC-35</td>
</tr>
<tr>
<td>P</td>
<td>0.5</td>
</tr>
<tr>
<td>S</td>
<td>0.2</td>
</tr>
<tr>
<td>Cl</td>
<td>0.4</td>
</tr>
<tr>
<td>Ca</td>
<td>0.3</td>
</tr>
<tr>
<td>K</td>
<td>0.6</td>
</tr>
<tr>
<td>Na</td>
<td>0.1</td>
</tr>
<tr>
<td>Total Minerals</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*nd = undetectable
2.3.3 Glass-rubber transition temperature of powders

$T_{gr}$ values provide information on state transition temperatures of powders and can be used as an indicator of their overall stability to moisture- and temperature-induced changes (Roos, 2010). $T_{gr}$ values of powders at a range of water activities are given in Figure 2.2. As expected, the $T_{gr}$ increased significantly ($P < 0.05$) as protein content increased. Casein and whey proteins have high $T_{gr}$ (132 °C and 127 °C, respectively), (Schuck et al., 2005). In keeping with results of previous studies for dairy ingredients, $T_{gr}$ decreased with increasing water activity, due to the plasticisation effect of water, and increased with increasing protein content (Maher, Fenelon, Zhou, Haque, & Roos, 2011). $T_{gr}$ of MPC35, which is comparable to SMP in terms of lactose and total protein, had similar results to SMP as reported by Shrestha et al. (2007b). Powders with low $T_{gr}$ are more likely to present problems in drying or storage, i.e., sticking of particles during spray-drying or crystallisation during storage (Hogan & O'Callaghan, 2010). As expected, increasing protein content in the bulk from 35 to 86 g / 100 g resulted in delayed $T_{gr}$, possibly due to interactions between large protein molecules and lactose. It has been documented in the literature that lactose has the greatest effect on the glass transition temperature of an amorphous dried food material (Bhandari & Howes, 1999).
Figure 2.2 Glass-rubber transition temperatures ($T_{gr}$) of MPC powders at different water activities. Solid fill represents $T_{gr}$ from one replicate and empty fill the difference from duplicate.

- Data for lactose was taken from (Jouppila and Roos (1994))
- Pooled variance of data was calculated to be 3.37 from $\sum_i (n - 1)(\text{var}_i)/\sum_i (n - 1)$
2.3.4 Sorption isotherms

Moisture is an important component of many dehydrated food products, the functional properties of which (i.e., glass transition, crystallisation behaviour, particle porosity and stickiness) are closely dependent on modes of water-binding to other constituents (in particular protein and lactose) (Sharma, Jana, & Chavan, 2012). The moisture sorption isotherms of the MPCs are shown in Figure 2.3; because fat does not absorb water, the moisture content is presented on a solids-non-fat (SNF) basis.

Crystallisation is apparent when there is a sudden decrease in mass at a certain RH (Burnett, Thielmann, & Booth, 2004; Burnett, Thielmann, Sokoloski, & Brum, 2006). MPC35 was the only powder that showed lactose crystallisation upon humidification (0 – 90% RH), with crystallisation occurring near 70% RH. Crystallisation is due to an increase in molecular mobility, allowing lactose molecules to orientate in a more ordered crystalline structure. As water can only be adsorbed at the surface of a crystal, the internal water in the amorphous phase is released upon crystallisation and evaporates. Similar results were found by Jouppila and Roos (1994), who reported that lactose in SMP crystallised when the powder was stored at 66.2% RH. Pure amorphous lactose crystallises at ~ 40% RH at 24 °C (Jouppila & Roos, 1994). Thus, it can be inferred that proteins significantly delay crystallisation of lactose in powders, as has been shown by numerous other studies (Foster, et al., 2005; Thomas, Scher, Desobry-Banon, & Desobry, 2004). Foster et al. (2005) indicated that whey protein delays the crystallisation of amorphous lactose, as both protein and lactose compete for water.
Figure 2.3 Moisture sorption isotherms for powders, MPC35 ( ), MPC50 ( □ ), MPC60 ( △ ), MPC70 ( ✗ ), MPC80 ( ✖ ), MPC85 ( ○ ), MPC90 ( ✦ ). Powder moisture is expressed on a solids-non-fat basis. Inset shows the GAB equation fitted to sorption isotherms.
Between 40–60% RH, MPC35 goes through a glass transition, as indicated by increasing amounts of water uptake in this RH range, just prior to lactose crystallisation (Berlin et al., 1968) at 70% RH. The glass transition is confirmed by the data in Figure 2.2, showing $T_{gr}$ approaching 25 °C as RH exceeds 44%. The other powders did not crystallise due to their higher protein: lactose ratios; $T_{gr}$ was delayed as protein content increased, indicating delayed onset of crystallisation. This is consistent with reports that increasing the lactose content in milk powders decreases the RH at which crystallisation begins (Hogan & O’Callaghan, 2010), and that the presence of high molecular weight compounds such as proteins delays the onset of crystallisation in milk powders (Jouppila & Roos, 1994). Results in Figure 2.2 show that powders with higher protein content had higher $T_{gr}$, and so higher RH would be required at 25 °C for these powders to go through a glass transition.

In the initial stages of moisture sorption (0-20% RH), high protein powders (MPC80 to MPC90) adsorbed more moisture (4.62 – 5.06 g water / 100 g) compared to lower protein powders (MPC35 to MPC60; 2.16 – 2.39 g water / 100 g), (Figure 2.3 inset). This is consistent with the findings of Berlin, Anderson and Pallansch (1968), who studied moisture sorption characteristics of milk powder components and concluded that macromolecular materials (proteins) dominate sorption behaviour at low RH (ca. 0–20% RH). In contrast, at ~ 20–60% RH, soluble components (lactose) exert their effects as the powder goes through a glass transition and, above 60% RH, the predominant contribution to moisture sorption comes from salts. At high humidity levels (i.e., in going from 80 to 90% RH), high protein powders, MPC85 and MPC90, with less minerals on the surface, sorbed less water (~5.6 g water / 100 g) compared to lower protein powders (e.g., up to 9.3 g water / 100 g) (Table 2.1 and Figure 2.3). Over a full sorption cycle (0-90% RH), MPC85 and MPC90 sorbed less water, i.e., ~24 g water / 100 g, compared to lower protein powders, which sorbed up to 30 g water / 100 g (Figure 2.3); this shows the effect of protein, as the bulk mineral content was relatively similar for all powders (7.5-8.1 g / 100 g) and bulk fat content was not a factor in moisture sorption, as milk fat does not significantly affect water sorption isotherms (Foster, et al., 2005; Kelly, O’Mahony, Kelly, & O’Callaghan, 2014) and, in any case, was present at low levels, i.e., in the range 0.6-1.34 g / 100 g (Table 2.1).
The GAB model was fitted to the experimental sorption data of MPCs (Table 2.3). As MPC35 went through a glass transition at > 40% RH, sorption data was modelled up to 40% RH for all powders (Figure 2.3 inset). In this range of RH, isotherms demonstrated a concurrent increase in equilibrium moisture content with increasing equilibrium RH. All MPCs had a sigmoidal curve, namely a Type II isotherm according to Brunauer’s classification (Brunauer, Emmett, & Teller, 1938). Models showed good agreement with experimental data ($R^2 \geq 0.93$) for all powders (Table 2.3).

The calculated GAB isotherm constants, $m_m$, K and C values, are presented, along with the $\alpha$, $\beta$, and $\gamma$ coefficients of equation 2.2, in Table 2.3. The initial rate of moisture sorption was higher for powders MPC70, -80, -85 and -90, than for MPC35 and -50. The monolayer value, $m_m$, originally developed in the context of the BET model, but physically more applicable to the GAB model, theoretically represents a level of moisture at which each polar group in a molecule binds one molecule of water (Timmermann, Chirife, & Iglesias, 2001; Timmermann, 2003; Zayas, 1997). Below this moisture level, the rate of chemical reactions in food is very low, since the water molecules are bound strongly to the surface of the monolayer (Arslan & Togrul, 2006). As protein content of MPC decreased, a decrease in $m_m$ capacity (7.8 to 1.7 g / 100 g dry basis) was observed. However, it must be noted that powder MPC-60 had the largest particle size (Table 2.1), giving rise to lower specific surface area (SSA); as SSA decreases, it would be expected that there would be a decrease in available area for moisture uptake. As protein content increased, the C values, which are related to the water-binding energy of the monolayer, decreased (from 9.5 to 6.1), as did the K values (from 1.9 to 0.8). McCarthy et al. (2013) reported similar trends for model infant formula powders with increasing protein content. These trends also confirm observations carried out on micellar casein powders (Foster, et al., 2005; Gaiani, et al., 2009) which reported $m_m$ values of approximately 7 g water / 100 g dry basis, C values around 10, and K values around 0.7, which are consistent with results reported for high protein powders in this study. The point of inflection on the sorption isotherms ($a_{\text{winfl}}$) increased with protein level, indicative of a more extended influence of preferential sorption by protein, which was further confirmed by the increasing moisture levels ($m_{\text{infl}}$) at the point of inflection (Table 2.3).
Table 2.3 Guggenheim-Anderson-de Boer (GAB) isotherm constants C, K, and monolayer value (m_m) of powders

<table>
<thead>
<tr>
<th>Powder</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>C</th>
<th>K</th>
<th>m_m</th>
<th>R²</th>
<th>E_{rms}^a</th>
<th>a_{winf}^b</th>
<th>m_{infl}^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC35</td>
<td>-0.99</td>
<td>0.47</td>
<td>0.033</td>
<td>9.54</td>
<td>1.89</td>
<td>1.70</td>
<td>1.00</td>
<td>0.56</td>
<td>0.13</td>
<td>1.72</td>
</tr>
<tr>
<td>MPC50</td>
<td>-0.74</td>
<td>0.36</td>
<td>0.053</td>
<td>6.11</td>
<td>1.66</td>
<td>1.87</td>
<td>0.93</td>
<td>2.28</td>
<td>0.15</td>
<td>1.63</td>
</tr>
<tr>
<td>MPC60</td>
<td>-0.54</td>
<td>0.33</td>
<td>0.038</td>
<td>8.41</td>
<td>1.39</td>
<td>2.28</td>
<td>0.99</td>
<td>0.93</td>
<td>0.18</td>
<td>2.24</td>
</tr>
<tr>
<td>MPC70</td>
<td>-0.22</td>
<td>0.17</td>
<td>0.024</td>
<td>8.49</td>
<td>1.10</td>
<td>4.50</td>
<td>1.00</td>
<td>0.31</td>
<td>0.23</td>
<td>4.44</td>
</tr>
<tr>
<td>MPC80</td>
<td>-0.15</td>
<td>0.13</td>
<td>0.024</td>
<td>7.22</td>
<td>1.01</td>
<td>5.77</td>
<td>1.00</td>
<td>0.83</td>
<td>0.25</td>
<td>5.41</td>
</tr>
<tr>
<td>MPC85</td>
<td>-0.12</td>
<td>0.11</td>
<td>0.023</td>
<td>6.87</td>
<td>0.95</td>
<td>6.58</td>
<td>1.00</td>
<td>0.56</td>
<td>0.26</td>
<td>6.06</td>
</tr>
<tr>
<td>MPC90</td>
<td>-0.09</td>
<td>0.09</td>
<td>0.025</td>
<td>6.14</td>
<td>0.83</td>
<td>7.78</td>
<td>1.00</td>
<td>0.75</td>
<td>0.29</td>
<td>6.83</td>
</tr>
</tbody>
</table>

α_w range of all powders was 0.1-0.4 for all experimental data points; four data points were taken for all powders. The terms C, K, and m_m (g water / 100 g dry weight) are derived from constants α, β, and γ.

^a Root mean square error.

^b a_w and moisture at point of inflection on GAB fit to sorption isotherm.
2.4. Conclusions

XPS analysis indicated that the outer surface of MPC powders of different protein content differed in composition in comparison with the interior bulk, with pronounced increases in concentration of fat and protein and decreases in lactose and mineral content on the surface. It is hypothesised that the shrinkage of the soluble phase during drying is a major factor in the increase in protein concentration, and decrease in soluble components, on the surface. The relatively high fat concentration on the surface is thought to be mainly due to hydrophobic effects. Surface morphology was more wrinkled for the highest lactose powder (MPC35) and smoother and dimpled for the higher protein powders. The powder with the highest lactose content (MPC35) was the only powder to show lactose crystallisation during water sorption, consistent with higher protein levels interacting with lactose in a way that reduces the tendency to crystallise. Increasing protein level increased the $m_m$ value and $T_{gr}$, indicating a higher threshold to humidity and decreased tendency to crystallise, which is advantageous for processing and storage stability.
2.5 References


Chapter 3: Effect of increasing protein concentration on the dissolution characteristics of dairy ingredients

Declaration: Production of MPC powders and chemical composition was carried out at NIZO, Ede, The Netherlands, except for particle size and fat analysis, which were carried out by Grace Kelly. Rehydration work and experimental results/data were analysed and the chapter was written by Grace Kelly.
Abstract

Milk protein concentrate (MPC) powders with protein content ranging from 35 (MPC35) to 87 g / 100 g (MPC90) (w/w) protein in dry matter were monitored during the course of rehydration. Particle size distribution data was gathered during rehydration using static light scattering (SLS) and classified into three categories; fine particles, < 1 μm; medium particles, 1-100 μm, and large/agglomerate particles > 100 μm, to follow dissolution patterns. Solubility of the MPC powders and their individual caseins and whey fractions were then determined by RP-HPLC. A novel methodology to characterise the solubility of MPC using SLS is presented, monitoring the changes in particle size with time. Faster decrease in particle size implied better solubility.
3.1 Introduction

Complete, ideally rapid, rehydration is an essential attribute for expression of powder functionality, knowing that high protein concentrate powders can be difficult to solubilise. It is thought that casein micelles dominate at MPC powder surfaces (Kelly et al., 2015), forming a skin-layer and giving rise to rehydration difficulties due to the slow transfer of water into particles (Schuck et al., 2007). Solubility rate of MPC powders decreases when they are stored at elevated temperatures and for prolonged periods of time (Anema et al., 2006). The loss of solubility rate on storage has been attributed to cross-linking of casein (αs- and β-casein) at the surface of powder particles (Havea, 2006), and preferential migration of fat to the surface of the powder particle (Gaiani et al., 2007a; Gaiani et al., 2009; McCarthy et al., 2013).

Increasing the reconstitution temperature and rehydration time improves the solubility rate of MPC powders (Mimouni et al., 2009; Mimouni et al., 2010a). However, poor solubility of the casein fraction can occur at high reconstitution temperatures after extended storage of powder (50 °C for 120 d) (Gazi and Huppertz, 2015). The loss of solubility rate has been attributed to the slow release of casein micelles from the dispersed powder particle due to the increased crosslinking of micelles (αs- and β-casein) (Anema et al., 2006) rather than to the formation of insoluble material (Mimouni et al., 2010b; Mimouni et al., 2010a).

Focused beam reflectance measurement (FBRM) has been used to monitor powder rehydration of MPC online by mapping chord length over time (Fang et al., 2010) and classifying these into three categories: fine particles (1–10 μm), medium particles (10–150 μm), and the big/ agglomerate particles (150–300 μm). With chord length being defined as the straight-line distance from one edge of a particle to another (Fang et al., 2010). Fang et al. (2011) observed a dynamic change in chord length as powder dissolved, with a movement from larger to smaller chord lengths and concluded that, by monitoring the change in chord length of the sample, the solubility of MPC can be quantitatively represented and reported on a size classification basis.

Effect of storage temperature and rehydration temperature on MPC has been previously investigated (Gazi and Huppertz, 2015; Crowley et al., 2015). Gazi and
Huppertz (2015) focused on the effect of increasing protein content on the solubility of casein and whey fractions at different storage temperatures (20, 37 and 50 °C), for up to 4 hrs. The influence of pre-heat treatment (low- and medium-) on MPC solubility was also investigated, finding that the insolubility of whey proteins was primarily found in MPC containing high levels of denatured whey protein. Crowley et al. (2015) investigated the rehydration properties of MPC, with an emphasis on the formation of powder sedimentation after rehydration for 90 min, concluding that high protein powders had a significantly higher sedimentation height in comparison to lower MPC, also noting that increased rehydration temperature (50 °C) decreased sedimentation height in high protein MPC powders.

The objectives of this study were (i) to demonstrate the potential of particle size measurement as a technique for monitoring MPC powder dissolution and (ii) to characterise the solubility of MPC powders as a function of time and temperature using a static light scattering (SLS) particle size analyser. High-performance liquid chromatography was then employed to identify each soluble protein fraction.

3.2 Materials and Methods

3.2.1 Materials

The MPC powders used were as reported by Gazi and Huppertz (2015). Seven MPC powders were produced under controlled conditions by NIZO, Ede, The Netherlands from skim milk. The protein levels in the prepared powders were 35.4, 49.9, 60.8, 68.25, 79.06, 83.96 and 85.85 g / 100 g which will be referred to as MPC35, MPC50, MPC60, MPC70, MPC80, MPC85, MPC90, in order of increasing protein content. Processing conditions for manufacturing of all powders are given by Crowley et al. (2014) and their chemical composition is provided in Table 3.1.
3.2.2 Reconstitution of powders

MPC powders were dispersed (200 g sample; 5%, w/w) in pre-tempered distilled water (20 or 50 °C), using an overhead stirrer (Euro-st digital, IKA®-Werke GmbH & Co. KG, Janke & Kunkel-Str. 10, 79219 Staufen, Germany) equipped with a propeller (3-bladed, R 1381 propeller, IKA®-Werke GmbH & Co. KG, Janke & Kunkel-Str. 10, 79219 Staufen, Germany) at a rotating speed of 600 rpm. The pH was adjusted to 6.9 using 0.1M NaOH or HCl. After powder incorporation, samples (~ 2 mL) were taken at defined time points (10, 20, 30, 60, 90, and 150 min) with a Pasteur pipette for analysis.

3.2.3 Dissolution profile over time

Particle size distribution (PSD) of dispersions/solutions were determined by static light-scattering (SLS) using a Malvern MasterSizer 3000 apparatus (Malvern Instruments Ltd., Malvern, UK) operating with two laser sources (466 and 633 nm). Measurements were recorded when the laser obscuration reached 7%. Samples were introduced into 800 mL of distilled water re-circulating at 20 °C in the dispersion unit (Hydro MU) at 1800 rpm and the measurement started 10 s after the introduction of the suspension into the dispersing unit. Size distribution of particles was calculated using software provided with the Malvern MasterSizer (V 2.2) using the general polydisperse analysis mode. Refractive index values of 1.57 and 1.33 were used for particle and dispersant, respectively (Mimouni et al., 2009). PSDs were obtained during rehydration under controlled conditions of temperature, time and agitation rate as described in Section 3.2.2. All particle size measurements were performed in triplicate. In this study, the general dissolution behaviour of the powders was investigated with particle populations classified into 3 categories: fine particles (< 1 μm), medium particles (1-100 μm), and the large/agglomerate particles (> 100 μm). It is expected that during dissolution, the fine particle fraction (< 1 μm) increases with time while the large particle fraction (> 100 μm) decreases with time. A faster rate of size reduction implies a better solubility, as more particles break down and dissolve.
3.2.4 Solubility

The procedure of McCarthy et al. (2014) was used to measure solubility of the powders with modifications. Rehydration was carried out for 30 min in duplicate under controlled conditions of temperature and agitation rate as described in Section 3.2.2. Aliquots of MPC dispersions (50 mL) were centrifuged at 700 g for 10 min (20 °C). Soluble protein fractions in the supernatant were measured by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC), using a method previously reported by Mounsey and O’Kennedy (2009).

3.2.5 Scanning electron microscopy

Morphological properties of the powders were observed using scanning electron microscopy (SEM). Samples were visualised using a field emission scanning electron microscope (FE-SEM; Zeiss Supra Gemini, Darmstadt, Germany) at 2.00 kV. Samples were mounted on double-sided carbon tape, attached to SEM stubs, and then sputter-coated with chromium (Emitech K550X, Ashford, Kent, UK). Representative micrographs were taken at 1000×, 5000× and 10000× magnification in order to visualise surface characteristics.

3.2.6 Statistical analysis

Analysis of variance (ANOVA) was carried out using Minitab 15 (Minitab Ltd., Coventry, UK) statistical package. Fisher’s one-way multiple comparison tests were used to compare different levels. Results are deemed statistically significant if $P < 0.05$. 
3.3 Results and discussion

3.3.1 Composition and morphology

Protein and lactose contents ranged from 35.4 to 85.9 g / 100 g and 49.6 – 0.37 g / 100 g for MPC powders, respectively. As for particle size, the particles were larger with broader size variation for lower MPC powders (Table 3.1).

Representative SEM micrographs of the MPC powders are shown in Figure 3.1. The powder surface of lower protein powders had a wrinkled surface consistent with that of SMP observed in other studies which is due to minimal fat content (Murrieta-Pazos et al., 2012; Kim et al., 2002; Gaiani et al., 2006; Nijdam and Langrish, 2006). As protein content increased, powder surface changed, with a smoother surface being evident. It is clear from SEM images that powder particles were not agglomerated, which was also reconfirmed by particle size data, with agglomeration known to have positive effects on rehydration properties (Gaiani et al., 2007b).
Table 3.1 Composition and particle size distribution of milk protein concentrate (MPC) powders

<table>
<thead>
<tr>
<th>Powder</th>
<th>Moisture $^1$ (g / 100 g)</th>
<th>Protein (g / 100 g)</th>
<th>Lactose (g / 100 g)</th>
<th>Fat $^2$ (g / 100 g)</th>
<th>Ash (mg / g)</th>
<th>D(v, 0.1) (μm)</th>
<th>D(v, 0.5) (μm)</th>
<th>D(v, 0.9) (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC35</td>
<td>3.4</td>
<td>35.4</td>
<td>49.6</td>
<td>0.6 ± 0.0</td>
<td>8.1</td>
<td>14.6 ± 0.2</td>
<td>37.3 ± 0.3</td>
<td>70.2 ± 0.3</td>
</tr>
<tr>
<td>MPC50</td>
<td>3.8</td>
<td>49.9</td>
<td>35.8</td>
<td>0.8 ± 0.1</td>
<td>7.8</td>
<td>16.6 ± 0.2</td>
<td>43.1 ± 0.4</td>
<td>82.2 ± 0.6</td>
</tr>
<tr>
<td>MPC60</td>
<td>4.0</td>
<td>60.8</td>
<td>24.5</td>
<td>1.0 ± 0.0</td>
<td>7.7</td>
<td>21.4 ± 0.1</td>
<td>53.6 ± 0.1</td>
<td>104 ± 0.6</td>
</tr>
<tr>
<td>MPC70</td>
<td>3.6</td>
<td>68.3</td>
<td>18.0</td>
<td>1.1 ± 0.1</td>
<td>8.0</td>
<td>16.4 ± 0.2</td>
<td>42.7 ± 0.6</td>
<td>81.4 ± 0.2</td>
</tr>
<tr>
<td>MPC80</td>
<td>4.6</td>
<td>79.1</td>
<td>6.4</td>
<td>1.3 ± 0.0</td>
<td>7.7</td>
<td>9.39 ± 0.2</td>
<td>28.3 ± 0.5</td>
<td>57.7 ± 2.2</td>
</tr>
<tr>
<td>MPC85</td>
<td>4.8</td>
<td>84.0</td>
<td>1.8</td>
<td>1.2 ± 0.1</td>
<td>7.5</td>
<td>9.11 ± 0.2</td>
<td>27.5 ± 0.5</td>
<td>56.8 ± 0.6</td>
</tr>
<tr>
<td>MPC90</td>
<td>4.2</td>
<td>85.9</td>
<td>0.4</td>
<td>1.4 ± 0.0</td>
<td>7.6</td>
<td>10.0 ± 0.2</td>
<td>28.3 ± 0.3</td>
<td>59.5 ± 0.2</td>
</tr>
</tbody>
</table>

$^1$ Powder compositional values for protein, lactose, ash and moisture of MPC powders were taken from Crowley et al. (2014) (g / 100 g)

$^2$ Fat was measured by Rose Gottlieb as described in Kelly et al. (2015)
Figure 3.1 SEM micrographs showing morphology of MPC powders in increasing protein content; MPC35 (A), MPC50 (B), MPC60 (C), MPC70 (D), MPC80 (E), MPC85 (F) and MPC90 (G).
3.3.2 MPC dissolution profiles

The dissolution profile of MPC powders was monitored as described in Section 3.2.3. Before dissolution, most powder particles were in the range 1–100 μm (Table 3.1). Figure 3.2 and Figure 3.3 show the particle size fractions (< 1 μm, 1-100 μm, and > 100 μm) of powders during the course of dissolution at 20 and 50 °C over 150 min for MPC35 – MPC60 and MPC70 - MPC90, respectively. Decreased volume fraction in the range 1-100 μm, along with increases in small particle fraction (< 1 μm) indicated dissolution of the powder (Fang et al., 2010; Fang et al., 2011). Powder particles of size < 1 μm were assumed to be fully solubilised.

In Figure 3.2 A and D, the rehydration of MPC35 is shown at 20 and 50 °C, respectively. Little observable difference was found for MPC35 as at both temperatures there are very similar rehydration profiles. At both temperatures there was a high proportion of particles < 1 μm, showing that this powder is readily dissolved even at room temperature. The rehydration of MPC50 in Figure 3.2 B and E was similar to that of MPC35, with a high degree of dissolution at both temperatures indicated by a high volume of particles < 1 μm and low volume (~ 7% of total particle volume) of particles > 1 μm. Both of these powders were readily soluble due to their relatively high quantity of lactose, as lactose is more rapidly solubilised in water than protein (Lowe and Paterson, 1998). In contrast, there was a noticeable difference in solubility at 20 °C for MPC60 (Figure 3.2C). In the early stages of hydration (0 – 60 min), there was a large amount of particles in the 1 – 100 μm range, with minimal particles < 1 μm. After 90 min hydration, there was a cross-over of particle fractions, with the 1 – 100 μm range disappearing and being replaced by smaller particles, which is indicative of increasing solubility. Dissolution of MPC60 was dramatically improved at 50 °C (Figure 3.2F), with > 80 % by volume fraction of particles being < 1 μm after 10 min.
The high protein powders (MPC70 – MPC90) all exhibited very poor dissolution properties at 20 °C (Figure 3.3). At 50 °C, dissolution was initially low (at t = 10 min) for MPC70 but increased steadily up to 60 min, at which time the powder was fully solubilised, with nearly 100% of particles < 1 μm. At 50 °C, MPC80 dissolves steadily over 150 min and the amount of particles < 1 μm increases from ~ 0 to 60% (Figure 3.3F). This trend suggests that dissolution would continue given further time and that solubility would increase further towards 100% solubilisation. For MPC85 and MPC90 at 50 °C, the rate of dissolution was slower compared to MPC70 and MPC80, with < 50% by volume in the range 1 – 100 μm (Figure 3.3G and H) being dissolved after 150 min.
More time is required to fully dissolve high protein powders. Overall, protein content of powders and the temperature of water used to rehydrate have large effects on solubilisation, with decreasing protein content and increasing temperature and time increasing solubilisation. In general, undissolved powder particles were mostly in the size range 1 – 100 µm, with very few cases where particles > 100 µm were measured. Results show that relatively low protein powders (MPC35 and MPC50) can be fully hydrated given a short amount of time without the need for high temperature (50 °C). MPC60 will solubilise over time at 20 °C but will take longer than MPC35 or MPC50. For
high protein powders (MPC70, MPC80, MPC85, and MPC90), a rehydration temperature of 20 °C is not sufficient and a higher temperature, e.g., 50 °C, with longer rehydration time is required to dissolve these powders. These results are in agreement with previous studies (Fang et al., 2011), where MPC85 showed the lowest particle size at 50 °C compared to a rehydration temperature of 20 °C.

This information is of importance to ensure complete dissolution of MPC powders is achieved prior to their use in food formulations, as dissolution is considered as the key determinant of overall reconstitution quality of MPC powders.

3.3.3 Solubility of individual protein fractions

The amount of each individual protein fraction, caseins (αs1-, αs2-, β- and κ-casein) and whey proteins (α-lactalbumin, β-lactoglobulin A, and β-lactoglobulin B), solubilised in each MPC powder was determined by HPLC analysis of the supernatant after solubilisation at 20 or 50 °C for 30 min. Table 3.2 shows the percentage of each protein fraction that solubilised, i.e., quantity of protein fraction measured in supernatant (after centrifugation) by HPLC divided by the quantity of each protein fraction in the dispersion. Statistical significance is shown independently with respect to hydration temperature and protein fraction.

Figure 3.4 gives a visual representation of this data for MPC35 and MPC90. In brief, for caseins, high temperature and longer reconstitution time had a significant ($P < 0.05$) effect on solubilisation only for MPC90. For whey proteins, increasing temperature had no significant ($P > 0.05$) effect on solubility for MPC35 and MPC90. Solubility of the caseins was found to increase strongly with increasing reconstitution time, and in particular with increasing reconstitution temperature.
In agreement with the study of Gazi and Huppertz (2015), the whey proteins were shown to be more soluble than the caseins, which was observed at temperatures as low as 20 °C (Table 3.2). The solubility of κ-casein at 20 °C generally decreased with increasing protein content (on a solids basis), with MPC35 and MPC50 having significantly ($P < 0.05$) higher solubility than powders of higher protein content (MPC60 – MPC90). The low solubility of κ-casein at 20 °C for MPC70 – MPC90 was improved at 50 °C. At 50 °C, there was no significant ($P > 0.05$) difference between the solubility of low protein and high protein MPC powders, showing the beneficial effect of high temperature for solubilisation of this particular protein fraction in MPC powder. The solubility of $\alpha_s2$-, $\alpha_s1$- and $\beta$-casein displayed similar trends. At 20 °C, their solubility was significantly ($P < 0.05$) lower for high protein MPC powders than for lower protein MPC powders, but raising the hydration temperature to 50 °C increased their solubility to levels similar to those of the lower protein MPC powders.
Table 3.2 Soluble protein fractions in MPC powder after 30 min rehydration at 20 and 50 °C, respectively.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Powder</th>
<th>(\kappa)-casein</th>
<th>(\alpha_2)-casein</th>
<th>(\alpha_s)-casein</th>
<th>(\beta)-casein</th>
<th>(\alpha)-lactalbumin</th>
<th>(\beta)-lactoglobulin-A</th>
<th>(\beta)-lactoglobulin-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>MPC35</td>
<td>31.6\textsuperscript{aA}</td>
<td>30.8\textsuperscript{aA}</td>
<td>50.6\textsuperscript{aA}</td>
<td>45.7\textsuperscript{aA}</td>
<td>48.5\textsuperscript{aA}</td>
<td>82.7\textsuperscript{aA}</td>
<td>49.5\textsuperscript{aA}</td>
</tr>
<tr>
<td></td>
<td>MPC50</td>
<td>28.7\textsuperscript{aA}</td>
<td>22.0\textsuperscript{aB}</td>
<td>44.4\textsuperscript{aA}</td>
<td>39.9\textsuperscript{aA}</td>
<td>34.5\textsuperscript{aB}</td>
<td>62.4\textsuperscript{aA}</td>
<td>39.5\textsuperscript{aA}</td>
</tr>
<tr>
<td></td>
<td>MPC60</td>
<td>17.1\textsuperscript{bA}</td>
<td>29.1\textsuperscript{bA}</td>
<td>39.3\textsuperscript{aA}</td>
<td>37.8\textsuperscript{aA}</td>
<td>43.0\textsuperscript{aB}</td>
<td>46.5\textsuperscript{aB}</td>
<td>41.1\textsuperscript{aA}</td>
</tr>
<tr>
<td></td>
<td>MPC70</td>
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<td>13.8\textsuperscript{bC}</td>
<td>20.6\textsuperscript{bA}</td>
<td>18.6\textsuperscript{bA}</td>
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<td>6.1\textsuperscript{cA}</td>
<td>9.9\textsuperscript{bA}</td>
<td>12.7\textsuperscript{bA}</td>
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<td>MPC85</td>
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<td>22.4\textsuperscript{bA}</td>
<td>21.2\textsuperscript{bA}</td>
<td>35.7\textsuperscript{aB}</td>
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<td>MPC90</td>
<td>15.6\textsuperscript{bA}</td>
<td>10.6\textsuperscript{bC}</td>
<td>17.8\textsuperscript{bA}</td>
<td>17.6\textsuperscript{bA}</td>
<td>35.3\textsuperscript{aB}</td>
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<tr>
<td>50</td>
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<tr>
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<td>23.9\textsuperscript{aA}</td>
<td>40.1\textsuperscript{aA}</td>
<td>40.7\textsuperscript{aA}</td>
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<td>31.8\textsuperscript{dA}</td>
<td>27.1\textsuperscript{cB}</td>
</tr>
<tr>
<td></td>
<td>MPC70</td>
<td>31.1\textsuperscript{bC}</td>
<td>32.3\textsuperscript{ab}</td>
<td>53.9\textsuperscript{ab}</td>
<td>47.2\textsuperscript{ab}</td>
<td>33.9\textsuperscript{bA}</td>
<td>69.0\textsuperscript{aA}</td>
<td>40.6\textsuperscript{aA}</td>
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<tr>
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<td>MPC80</td>
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<td>36.0\textsuperscript{ab}</td>
<td>39.9\textsuperscript{ab}</td>
<td>44.1\textsuperscript{ab}</td>
<td>54.2\textsuperscript{bB}</td>
<td>53.3\textsuperscript{cB}</td>
<td>32.8\textsuperscript{abC}</td>
</tr>
<tr>
<td></td>
<td>MPC85</td>
<td>52.0\textsuperscript{ab}</td>
<td>35.3\textsuperscript{ab}</td>
<td>48.2\textsuperscript{ab}</td>
<td>44.7\textsuperscript{ab}</td>
<td>42.6\textsuperscript{bb}</td>
<td>52.4\textsuperscript{cA}</td>
<td>30.9\textsuperscript{bcB}</td>
</tr>
<tr>
<td></td>
<td>MPC90</td>
<td>37.2\textsuperscript{abB}</td>
<td>28.5\textsuperscript{ab}</td>
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<td>34.4\textsuperscript{ab}</td>
<td>41.2\textsuperscript{ba}</td>
<td>55.1\textsuperscript{bcA}</td>
<td>36.9\textsuperscript{abA}</td>
</tr>
</tbody>
</table>

\(a\)Within a column, small letters represent significant difference between different MPC powders at one temperature. Capital letters represent significant differences with regard to 20 and 50 °C for each MPC.
Generally, whey protein fractions showed increased dissolution compared to the casein fractions at 20 °C; however, increasing the hydration temperature to 50 °C had less significant effect, when comparing these two temperatures (Figure 3.4).

β-lactoglobulin-A was the most soluble protein fraction, with κ-casein and αs2-casein having the lowest solubility. High protein MPC powders are prone to skin formation on rehydration, which is generally regarded as aggregated casein micelles on the surface of the powder (McKenna, 2000; Mimouni et al., 2010a); this layer is poorly dispersible and acts as a barrier that prevents the solubilisation and dissolution of other constituents from the core of the powder.

While whey proteins are more soluble than casein proteins, they are heat- and pH-sensitive, with thermal denaturation occurring for whey proteins (β-lactoglobulin) at > 70 °C (Tobin et al., 2010). At a rehydration temperature of 50 °C, the protein structure is not affected by heat (Pelegrine and Gasparetto, 2005); however, pH could negatively affect solubilisation if it was near the isoelectric point (pH 4.5). Values below or above the isoelectric point leads to increased solubility, due to proteins having positive or negative net charges, such that more water can interact with the protein molecules (Pelegrine and Gasparetto, 2005).

The high level of lactose in the low protein powders can provide a hygroscopic matrix in which proteins (casein micelle and whey proteins) are dispersed (Thomas et al., 2004), thus inhibiting their interactions. High protein MPC powders lack sufficient lactose to form a hygroscopic matrix and interactions of casein micelles are thus facilitated. Throughout storage at low humidity a potential dehydration process can occur due to migration of moisture from the core to the surface of the particle, leading to further “jamming” and compaction of casein micelles in high protein MPC.
3.4 Conclusions

Overall, > 30 min is required to solubilise and to fully express the protein fractions of MPC, while a higher reconstitution temperature gave a significant increase in protein solubilisation. Results indicate that rehydration temperature and time are key factors in rehydration of MPC powders in particular with high-protein MPC powders (e.g., MPC70, MPC80, MPC85, MPC90), which have the poorest rehydration properties. In particular, static light scattering has been demonstrated as a potential technique to monitor powder dissolution. This shows potential for online techniques for monitoring dissolution of powder particle size and for size classification.
3.5 References


Chapter 3


Chapter 4: Effect of type of oil and spray drying conditions on physical characteristics of a spray-dried dairy emulsion


Declaration: Experimental design, powder production and all analysis were carried out by Grace Kelly. Experimental results/ data were analysed and the chapter was written by Grace Kelly.
Abstract

The objective of this study was to investigate the physical characteristics of spray-dried dairy powders formulated with different oil types, spray-dried at different outlet temperatures. A model fat-filled dairy formulation (target 40% w/w total solids, comprising protein, oil and lactose) containing lactose (23.9 g / 100 g), sodium caseinate (5.11 g / 100 g) and sunflower (SO) or palm (PO) oil or a 50:50 mixture of SO/PO (in all cases 11.5% total oil) were heat-treated, homogenised and spray-dried at an outlet temperature of 80 or 90 °C. Increasing outlet temperature reduced water content, water activity and tapped bulk density, irrespective of oil type, and increased solvent-extractable free fat for all oil types. Onset of glass transition ($T_g$) and crystallisation ($T_c$) decreased at the lower outlet temperature. Oil type had no effect on powder moisture, water activity ($a_w$), powder bulk density, particle size, fat globule size of emulsion or fat globule size of reconstituted fat-filled dairy powders.
4.1 Introduction

The shelf-life of dairy products can be increased by transforming them into a dry product by spray-drying. In a powder form, storage, handling and transport are also easier. The oil component in dairy powders influences flowability and rehydration (Fälldt, 1995). Oils of non-dairy origin, from a wide range of sources, are frequently utilised in the formulation of dairy-based powders, such as infant formulae, to achieve fatty acid profiles. In the manufacturing process, proteins act as surfactants to produce stable oil-in-water emulsions, stabilising the system to separation/creaming, protecting the fat from oxidation and reducing powder handling problems (Matsuno & Shuji, 1993).

Various forms of casein and whey proteins (WP) are used as emulsifiers in dairy-based formulations and powders. Non-micellar casein as found in sodium caseinate (NaCas) may be considered a flexible protein that can readily unfold to form an interfacial layer. Micellar casein behaves differently to non-micellar casein, as calcium bridges restrict the extent to which casein micelles unfold and adsorb at the interface. NaCas depresses interfacial tension more effectively than whey proteins, as it diffuses more quickly to an interface and, on reaching the interface, absorbs more quickly than the other proteins (Phillips & Williams, 2000). WP denaturation occurs during concentration, evaporation and drying, which leads to a less stable emulsion and increased surface fat and larger droplets after reconstitution, whereas NaCas-stabilised emulsions are considerably more heat-stable (Vega & Roos, 2006).

Free fat, i.e. fat that is no longer emulsified, is located at the powder particle surface and in pores and capillaries created during the drying process. In spray-dried powders free fat is of concern because it has a tendency to oxidise, and the reconstituted product loses its organoleptic appeal due to the formation of free fat pools on the surface of the liquid. Free fat in dairy powders is influenced by product composition, homogenisation pressure, spray-drying inlet/outlet temperatures and storage conditions of powders (Vignolles et al., 2007).

Other studies have examined the effect of spray-drying temperature on the surface fat of dairy powders but have varied both inlet and outlet temperatures simultaneously.
rather than independently (Gaiani et al., 2010; Kim et al., 2009). Prolonged storage of dairy powders at a high relative humidity (RH) can lead to lactose crystallisation which reduces the integrity of the protein-based interfacial layer and results in increased free fat levels (McCarthy et al., 2013). One study found that the use of a fat in its native state compared to a blend of native and hardened fats reduced free fat level in a NaCas-stabilised fat-filled emulsion containing lactose (Millqvist-Fureby, 2003).

Confocal laser scanning microscopy (CLSM) has been used to visualise fat distribution on particles of whole milk powder (WMP) and spray-dried cream powder (Auty et al., 2001; Vignolles et al., 2010). In the last 15 years, x-ray photoelectron spectroscopy (XPS) has been applied to investigate the surface composition of dairy powders. From carbon, oxygen and nitrogen percentages, surface composition can be calculated by empirical calculations. During spray-drying, rapid evaporation of water occurs, which leads to the migration of milk components to or from the surface of the drying droplet, due to concentration gradients. Studies using XPS have shown that fat can be overly-represented on the surface of powders compared to bulk phase composition, e.g., powder surface being composed of 98% fat of WMP compared to 29% in the bulk (Kim et al., 2003). Fat type influences the level of surface fat, with fats having intermediate melting points showing the highest surface fat values, while increased homogenisation pressure creates smaller fat globules, resulting in less free fat (Millqvist-Fureby, 2003). During spray-drying, rapid evaporation of water occurs, which leads to the migration of milk components to or from the surface of the drying droplet, due to concentration gradients.

The aim of this study was to investigate the physical characteristics of a spray-dried dairy powder made with different blends of vegetable oils, namely palm oil, sunflower oil or a 50:50 blend of the two oils in order to determine if oil type affects physical properties in a model dairy-based emulsion, produced at two different spray-dryer outlet temperatures while keeping inlet temperature constant.
4.2 Materials and Methods

4.2.1 Materials

Sunflower oil and palm oil were purchased from Trilby Trading (Drogheda, Ireland). Sodium caseinate was purchased from Kerry Ingredients (Listowel, Co. Kerry Ireland) with a protein content of 89.8% and lactose from Glanbia Ingredients (Ballyraggett, Co. Kilkenny, Ireland). Petroleum ether (40-60 °C boiling point) of analytical grade, Nile Red, Fast green and PEG 400 were obtained from Sigma-Aldrich (Wicklow, Ireland).

4.2.2 Experimental Design

The experimental design consisted of six unique trials (Table 4.1) carried out in triplicate, where the trials for each replicate were carried out in random order. The emulsion consisted of 5.1% NaCas, 11.5% fat, and 23.9% lactose. The experimental design included 3 compositional variations, where the oil fraction was made up of sunflower (SO) and palm (PO) at different ratios as presented in Table 4.1.

Table 4.1 Experimental design for formulation and spray drying conditions.

<table>
<thead>
<tr>
<th>Code</th>
<th>Fat type or blend</th>
<th>Outlet temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO-L</td>
<td>SO</td>
<td>80</td>
</tr>
<tr>
<td>SO-H</td>
<td>SO</td>
<td>90</td>
</tr>
<tr>
<td>SOPO-L</td>
<td>SOPO</td>
<td>80</td>
</tr>
<tr>
<td>SOPO-H</td>
<td>SOPO</td>
<td>90</td>
</tr>
<tr>
<td>PO-L</td>
<td>PO</td>
<td>80</td>
</tr>
<tr>
<td>PO-H</td>
<td>PO</td>
<td>90</td>
</tr>
</tbody>
</table>

Note: Suffix L and H correspond to lower (80 °C) and higher (90 °C) outlet temperatures, respectively.

Sunflower oil, SOPO-Sunflower oil: Palm oil at 50:50 and PO-Palm oil

Spray dryer inlet temperature was 185 °C in all cases.
4.2.3 Preparation of model emulsions

Emulsions (15 kg batch size) were prepared as follows. Lactose powder was dissolved in hot water (~65 °C), using a Silverson L4RT (Silverson Machines Ltd., Waterside, Chesham, Bucks, England) mixer to aid reconstitution. Some fat (~10% of total fat) was added during mixing to reduce foaming. When palm oil was used, it was melted in a separate vessel before addition. Sodium caseinate (NaCas) was then added slowly before addition of the remaining fat component. The mix was tempered to 60 °C and adjusted to pH 6.8 by adding 1M KOH and kept under high shear for 120 mins to ensure complete hydration of the NaCas. The feed was agitated prior to high-temperature short-time heat treatment (100 °C x 30 s) using a Microthermics (Model 25HV; North Carolina, USA) tubular heat exchanger. The mix was homogenised using an in-line 2-stage homogeniser (Model NS20006H, GEA Niro, Soavi, Parma, Italy) using a first-stage pressure of 13.8 MPa and a second-stage pressure of 3.45 MPa, and spray-dried in a pilot-scale Anhydro Spray dryer (Model Plant No. 3 type I KA, Copenhagen, Denmark), equipped with a two-fluid nozzle atomization system (Type 1/8 JAC 316ss) and counter-current drying, with a typical water evaporation rate of 20 L/hr. Dryer inlet temperature was held constant at 185 °C and outlet temperature was either 80 or 90 °C to vary the moisture level in the powder. Samples of the emulsion were taken post-heat treatment and post-homogenisation for physico-chemical measurements. Samples of powder were stored at 10 °C in sealed foil bags until analyses were carried out.

4.2.4 Measurement of fat globule size and powder particle size

Mean fat globule size of emulsions was determined post-homogenisation and in reconstituted emulsions by laser light scattering with a MasterSizer S laser diffraction instrument (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 300 RF lens. Distilled water was used as the dispersing medium and refractive index values of 1.46 and 1.33 were used for particles and dispersant, respectively (Gompone, 2011). Mean powder particle size analysis was carried out by laser diffraction using the dry-feeding unit for the MasterSizer S, with a pressure setting of 1 bar.
4.2.5 Rheological measurements

Viscosity was measured at 55 °C using an AR-G2 controlled stress rheometer (TA Instruments, UK) equipped with concentric cylinder geometry in shear rate mode. Samples were pre-sheared at 500 s\(^{-1}\) for 1 min, followed by equilibration for 2 min. An upward shear rate sweep was then applied from 5 to 500 s\(^{-1}\) over 3 min, followed by holding at 500 s\(^{-1}\) for 1 min. The average apparent viscosity measured at 500 s\(^{-1}\) was used for comparison of the model formulations. Rheological behaviour was modelled using the power law equation, (Equation 4.1) with a logarithmic transformation and fitted using least squares regression in Microsoft Excel.

\[
\log \sigma = \log K + n \log \dot{\gamma}
\] (4.1)

where \(\sigma\) is the shear stress (Pa), \(K\) is the consistency index (Pa·s\(^{n}\)), \(\dot{\gamma}\) is the shear rate (s\(^{-1}\)) and \(n\) is the flow index, with \(n < 1\) for a shear-thinning fluid and \(n = 1\) for a Newtonian fluid.

4.2.6 Determination of physico-chemical properties

Sample pH was monitored using a pH 330i meter (WTW, Weilheim, Germany). The powder water content was determined using a halogen rapid moisture analyser (HR-83 Halogen, Mettler Toledo, Switzerland). The samples were dried at a temperature of 105 °C until a constant weight was attained (< 1 mg change over 140 s, equivalent to ± 0.025%). Water activity (\(a_w\)) was measured with a Novasina LabMaster-\(a_w\) water activity meter (Novatron Scientific Ltd, West Sussex, UK). Powder bulk density was determined according to the IDF Standard 134A:1995.
4.2.7 Free fat content of powders

Free fat content of powders was determined by the GEA Niro analytical method (GEA Niro, 2005). Powder (10 g) was mixed with petroleum ether (50 mL), agitated for 15 min using a laboratory shaker, and filtered (No. 4, Whatman, Maidstone, Kent, UK). Filtrate (25 mL) was placed in a pre-weighed aluminium dish and placed in a fume hood for the evaporation of petroleum ether. Once most of the petroleum had evaporated, the aluminium dish was placed in an oven at 105 °C for 1 h, and samples were cooled in a desiccator before weighing. Free fat was expressed as a percentage of powder weight.

4.2.8 Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC Q2000, TA Instruments, Crawley, UK) was used to determine the onset temperature of glass transition ($T_g$), onset temperature of lactose crystallisation ($T_{cr}$) and melting temperature ($T_m$) of the oils in the powders. Samples (8-12 mg) were scanned in hermetically sealed aluminium pans while subjected to heating from 0 to 100 °C at 5 °C / min, followed by cooling to 0 °C at 10 °C / min, and then heated at 5 °C / min from 0 to 160 °C. The $T_g$ and $T_{cr}$ of the samples were determined on the second heating scan. Using the TA Universal analysis software, the onset and midpoint transitions were calculated. Start point and end points of a transition were selected visually and the software generates tangent lines to calculate both the onset and midpoint of glass transition. An empty aluminium pan was used as a reference. The DSC was calibrated by means of indium standards and dry nitrogen (50 mL / min) as the purge gas was used.

In the literature, $T_g$ has been extrapolated from equilibrium powder moisture contents, generated from moisture sorption isotherms, using established mathematical relationships such as the Couchmann-Karasz equation (Couchman & Karasz, 1978) (Equation 4.2),
where $T_g$ is the glass transition temperature of each component (i.e., water, lactose, and casein protein), $\Delta C_{pi}$ is the change in heat capacity at $T_g$ (J/kg°C) and $W_i$ is the weight fraction of each component. In this study, we include predicted values of $T_g$ by this technique, using parameter values from Schuck et al. (2005), for comparison with $T_g$ values determined by DSC curves.

4.2.9 Dynamic Vapour Sorption (DVS)

A DVS was used to monitor the water sorption capacities of the powders during storage. Water sorption isotherms were determined gravimetrically using a DVS system ((DVS-1) Surface Measurement Systems, Ltd., London, UK) equipped with a Cahn microbalance. A sample (~30 mg) of powder was loaded into the sample pan. The changes in sample weight over time at 25 °C and at varying RH (between 0% and 90%) were recorded. Samples were humidified from 0% to 90% RH and back to 0% RH in increments of 10% RH in a 2-cycle procedure. For each step, the changes in mass ($m$) were plotted against time. Equilibrium was considered to be reached when change in mass with time (dm/dt) was lower than 0.001 mg/min for at least 10 min. Accurate RH settings were obtained by mixing dry nitrogen gas with saturated water vapour in correct proportions using mass flow controllers. Graphs of water uptake over time for each powder sample were obtained using the DVS Data Analysis Suite. The Guggenheim-Anderson-de Boer (GAB) equation was used to model water sorption isotherms and to determine the critical water content and water activity ($a_w$). The GAB model isotherm, with constants $C$ and $K'$, is given in Equation 4.3 and was converted to a second-order polynomial (Bizot, 1983), giving a quadratic equation (Equation 4.4):

$$\frac{m}{m_m} = \frac{CK' a_w}{(1-K'a_w)(1-K'a_w + CK'a_w)}$$

(4.3)
\[
\frac{a_w}{m} = \alpha a_{w}^2 + \beta a_w + \gamma
\]  

(4.4)

where \( m \) is the moisture content (g / 100 g dry solids), \( m_m \) is the monolayer value, and \( \alpha, \beta \) and \( \gamma \) are constants determined by quadratic regression analysis. Accuracy of fit was estimated by mean square error.

### 4.2.10 Confocal laser scanning microscopy

All observations were performed using a Leica TCS SP5® Confocal Laser Scanning Microscope (CLSM) (Leica Microsystems, Baden–Württemberg, Germany). Dual labelling of the powders was carried out to visualize the fat and protein phases. The fluorescent probes Nile Red (0.1% w/v, in polyethylene glycol, PEG 400) and Fast Green FCF (0.1% w/v, in distilled water) (Sigma Aldrich, Ireland) label the fat and protein phases, respectively. A small sample of powder was transferred onto a glass slide and stained with a mixture of Nile Red: Fast Green (100:1, v/v). Images of representative areas of each sample were taken using a 63x oil immersion objective (numerical aperture = 1.4) at excitation wavelengths of 488 nm and 633 nm, provided by Ar and He/Ne lasers, and emission was measured in the wavelength range of 500-580 nm and 550-700 nm, respectively. Red, Green, Blue (RGB) colour images (24 bits), 1024 x 1024 pixels in size, were acquired using 1x, 3x and 5x zoom factors. At least three images were obtained per sample for each replicate.

### 4.2.11 Statistical Analysis

Analysis of Variance ANOVA was carried out using the general linear model (GLM) in Minitab 15 (Minitab Ltd., Coventry, UK). All 2-way interaction terms were included initially for screening. The non-significant interaction terms were removed in the final models. Effects were deemed statistically significant if \( P < 0.05 \).
4.3 Results and Discussion

4.3.1 Physical properties of powders

Moisture and water activity levels decreased from 2.2 to 1.75 g / 100 g and 0.17 to 0.13 respectively, with increase in outlet temperature (from 80 to 90 °C) (Table 4.2). Powder particle size, (D[4,3]) decreased with increasing outlet temperature but was not affected by oil type (Table 4.2). The effect of outlet temperature may have been due to greater particle shrinkage due to undergoing more rapid drying. The powders produced at a higher outlet temperature had a lower bulk density than powders produced at the lower outlet temperature for all powders, regardless of oil type used (Table 4.2).

The powders produced at a lower outlet temperature had a lower level of free fat, implying greater fat encapsulation, compared with powders produced at higher outlet temperature (Table 4.2). Kelly et al. (2002) observed a similar effect for fat-based dairy powders produced at higher outlet temperature. Two studies reported decreasing amounts of free fat with higher spray-drying outlet temperatures (Gaiani et al., 2010; Kim et al., 2009). However, it needs to be pointed out, that, in the study of Kim et al., (2009), (i) inlet and outlet temperature were varied simultaneously, rather than independently, making it impossible to isolate the effect of outlet temperature, (ii) extreme inlet temperatures were used, i.e., 145 and 205 °C, (iii) the feed had low total solids contents, i.e., 10, 20 or 30 %, and (iv) it was not stated whether the differences in free fat with respect to drying temperatures were significant. It is acknowledged that inlet temperatures as high as 205 °C cause surface crust formation on powder particles, which can limit the diffusion of components, including fat, to the surface (Kim et al., 2009). In the present study, inlet temperature was not varied and free fat was expected to increase with exposure to higher outlet temperatures. Free fat level was not significantly affected by oil type in this study (Table 4.2). Thus, fat encapsulation efficiency was not significantly affected by oil type. The effect of outlet temperature on bulk density may be due to the more free-flowing nature of the powder with lower free fat, resulting in more dense packing.
Table 4.2 Physical properties of powders and reconstituted emulsions as affected by oil type and outlet temperature.

<table>
<thead>
<tr>
<th>Code</th>
<th>Moisture content&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Water activity</th>
<th>Powder particle size</th>
<th>Bulk density</th>
<th>Free fat</th>
<th>Micro-encapsulation Efficiency (ME)</th>
<th>Reconstituted fat globule size, D&lt;sub&gt;4,3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g / 100 g)</td>
<td>(%)</td>
<td>D(v,0.1) (μm)</td>
<td>D(v,0.5) (μm)</td>
<td>D(v,0.9) (μm)</td>
<td>D[4,3] (g/mL)</td>
<td>(g / 100 g of powder)</td>
</tr>
<tr>
<td>SO-L</td>
<td>2.27</td>
<td>0.19</td>
<td>32.4 ± 2.8</td>
<td>80.1 ± 9.1</td>
<td>151 ± 20.5</td>
<td>86.2 ± 10.3</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td>SO-H</td>
<td>1.77</td>
<td>0.15</td>
<td>31.1 ± 2.9</td>
<td>75.0 ± 5.3</td>
<td>139 ± 10.2</td>
<td>80.4 ± 5.8</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>SOPO-L</td>
<td>2.04</td>
<td>0.17</td>
<td>33.2 ± 5.5</td>
<td>80.2 ± 14.1</td>
<td>168 ± 96.3</td>
<td>87.2 ± 15.2</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>SOPO-H</td>
<td>1.68</td>
<td>0.12</td>
<td>28.5 ± 0.9</td>
<td>70.1 ± 1.9</td>
<td>137 ± 10.3</td>
<td>77.2 ± 4.0</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>PO-L</td>
<td>2.29</td>
<td>0.15</td>
<td>31.7 ± 5.6</td>
<td>76.9 ± 11.6</td>
<td>157 ± 0.5</td>
<td>89.4 ± 18.4</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>PO-H</td>
<td>1.81</td>
<td>0.13</td>
<td>26.6 ± 0.9</td>
<td>67.5 ± 4.4</td>
<td>139 ± 21.1</td>
<td>74.0 ± 5.4</td>
<td>0.34 ± 0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean or mean ± standard deviation as appropriate (n = 3).

<sup>a</sup> Code as per Table 4.1

<sup>b</sup> Moisture content is expressed on a wet basis
4.3.2 Fat globule size in emulsions and in reconstituted powders

Emulsification is one of the key steps in the preparation of fat filled milk powders and milk protein-based nutritional formulae (Liu et al., 2001). The size distribution of oil droplets in emulsions post-homogenisation, containing, SO, blends of SOPO and PO, were monomodal with a normal distribution. The volume average diameter ($D_{[4,3]}$) ranged from 0.95 to 1.25 µm. The different oil types had no significant effect at the 5% level on emulsion droplet size (Table 4.3). Fat globule size in the reconstituted formula was not affected by oil type or spray-dryer outlet temperature (Table 4.2). Controlling fat globule size (< 1 µm) is important in ensuring emulsion stability and reducing free fat and loss of any volatiles (Hogan et al., 2001; Sheu & Rosenberg, 1995). This can be achieved by control of homogenisation conditions, as increasing homogenisation pressure reduces fat globule size (Floury et al., 2000; Millqvist-Fureby, 2003).

Table 4.3 Influence of oil type on mean fat globule size and viscosity of the emulsions prior to spray drying.

<table>
<thead>
<tr>
<th>Oil type</th>
<th>D[4,3]</th>
<th>Viscosity $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µm)</td>
<td>Pre $^2$</td>
</tr>
<tr>
<td>SO</td>
<td>1.25 ± 0.36$^a$</td>
<td>8.93 ± 0.49$^b$</td>
</tr>
<tr>
<td>SOPO</td>
<td>0.99 ± 0.17$^a$</td>
<td>7.89 ± 0.51$^{bc}$</td>
</tr>
<tr>
<td>PO</td>
<td>0.95 ± 0.14$^a$</td>
<td>7.54 ± 0.43$^c$</td>
</tr>
</tbody>
</table>

Mean values in the same column with common superscript are not significantly different. Values represent mean ± standard deviation (n = 3).

$^1$ Shear rate 500 s$^{-1}$ at 55 °C

$^2$ Pre-homogenisation (coarse emulsion)

$^3$ Post-homogenisation
4.3.3 Viscosity

Viscosity of concentrated emulsions before drying is industrially important as a highly viscous feed gives rise to large droplets on atomization, which in turn leads to larger particle size and an increase in free fat level in the powder (Rosenberg et al., 1990). In addition, small droplets, and a low viscosity, are required to reduce the likelihood of air inclusion in the powder particle and produce powders with a high bulk density (Drusch, 2007). All the pre-homogenised formulations were found to be Newtonian in their flow behaviour ($n = 1.02 \pm 0.01$), while the homogenised emulsions were shear-thinning ($n = 0.89 \pm 0.01$) (Figure 4.1).

While there were differences in pre-homogenisation viscosity for samples containing PO and SO, viscosity was not significantly different for any emulsion post-homogenisation (Table 4.3). The viscosity of the various vegetable oils depends on their fatty acid composition; viscosity increases as chain length and degree of saturation of fatty acids increase (Yalcin et al., 2012). PO contains a high level of saturated fatty acids, at 44%, compared to SO which contains 6% (O'Dwyer et al., 2013). PO has a higher $T_m$ compared to SO (Table 4.4). While emulsion viscosity decreased after homogenisation, this was not significantly influenced by oil type, consistent with the oil being in a liquid state at the processing temperature used (Table 4.3). This is also consistent with the lack of effect of oil type on powder particle size.
Figure 4.1 Viscosity profiles of the emulsions pre (---) and post (——) homogenisation. Error bars represent ± standard error, n = 9.
4.3.4 Differential scanning calorimetry

Glass transition temperature, $T_g$, as determined by DSC decreased with increasing moisture content for all three powder types (Table 4.4). This is because water acts as a plasticiser and thus causes the $T_g$ to decrease. While predicted $T_g$, determined using the Couchmann-Karasz formula, were higher than those determined by DSC in this study (4-10 °C), the trend with regards to water content was similar. Zhu et al. (2011) also reported that predicted $T_g$ values for infant formula concentrates containing fat were higher than those measured by DSC. A difference of ~ 4 °C between the high and low moisture powders was observed for the three types of oil used for $T_g$-onset and $T_g$-mid. Less than 1 ºC (not significant) difference was observed between the $T_g$ values for powders of different oil type at the same moisture, showing that the type of oil used had no effect on $T_g$. ANOVA confirmed an interactive effect between replicate and oil type for glass transition temperatures and crystallisation temperature (Table 4.5). Hence, even where oil type gave a high F value, there was not a significant effect and, considering the relatively small differences in glass transition temperature for different experimental treatments, it can be concluded that the type of oil does not affect glass transition temperature. Increasing moisture level also lowered the onset temperature of lactose crystallization ($T_{cr}$) (Table 4.4). This can be attributed to increasing molecular mobility and thus an accelerating rate of lactose crystallisation (Roos & Karel, 1991).

The contrasting melting characteristics of the different oils were confirmed by DSC analysis. The melting points of the different oils were determined by scanning the oils individually. The melting endotherms for sunflower oil and palm oil were confirmed as being at ~ -17 °C and ~ 35 °C, respectively. The SOPO oil mixture exhibited the melting endotherms of the constituent oils, but they were not as pronounced as those for the individual oils. Each of the DSC graphs equilibrated to the same heat flow value at ~ 50 °C (after melting), i.e., the oil types had no effect on the $T_g$ values, which were all greater than 50 °C. DSC analysis of the powders showed melting endotherms between 35 and 37 °C for powders containing palm oil; the oil melting points could be observed in the powder using DSC, because the glass transition was not masked by melting endotherm as $T_m$ was well below $T_g$. 


Table 4.4 Onset glass transition temperature ($T_{g}$), onset lactose crystallisation temperature ($T_{cr}$), melting temperature of constituent oil ($T_{m}$) and $T_{g}$ using Couchmann-Karasz equation (predicted $T_{g}$) for powders of differing fat type and outlet temperature.

<table>
<thead>
<tr>
<th>Powder</th>
<th>$T_{g}$ onset ($^\circ C$)</th>
<th>$T_{g}$ mid ($^\circ C$)</th>
<th>Predicted $T_{g}$ $^a$ ($^\circ C$)</th>
<th>$T_{cr}$ ($^\circ C$)</th>
<th>$T_{m}$ ($^\circ C$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO-L</td>
<td>56.5 ± 2.0</td>
<td>60.0 ± 1.4</td>
<td>66.0 ± 2.7</td>
<td>127.1</td>
<td>-17.0$^b$</td>
</tr>
<tr>
<td>SO-H</td>
<td>59.6 ± 1.5</td>
<td>63.7 ± 1.7</td>
<td>73.1 ± 2.4</td>
<td>131.4</td>
<td>-17.0$^b$</td>
</tr>
<tr>
<td>SOPO-L</td>
<td>57.6 ± 1.3</td>
<td>61.5 ± 1.7</td>
<td>69.1 ± 0.8</td>
<td>117.4</td>
<td>36.0</td>
</tr>
<tr>
<td>SOPO-H</td>
<td>59.2 ± 2.0</td>
<td>64.4 ± 1.6</td>
<td>74.5 ± 1.5</td>
<td>122.7</td>
<td>35.3</td>
</tr>
<tr>
<td>PO-L</td>
<td>57.6 ± 1.1</td>
<td>61.5 ± 1.0</td>
<td>65.6 ± 0.9</td>
<td>121.4</td>
<td>36.6</td>
</tr>
<tr>
<td>PO-H</td>
<td>59.2 ± 1.8</td>
<td>63.9 ± 1.5</td>
<td>72.5 ± 1.5</td>
<td>128.1</td>
<td>36.9</td>
</tr>
</tbody>
</table>

$^a$ Parameter values used in Couchmann-Karasz equation for $T_{g}$ and $\Delta C_p$ were taken from Schuck et al. (2005).

$^b$ Melting temperature of SO measured independently.
Values represent mean ± standard deviation (n = 3).
### Table 4.5 General linear model (GLM) ANOVA table showing effect of outlet temperature, oil type and replicate on glass transition and crystallisation temperature.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>$T_g$ onset Adj MS</th>
<th>$T_g$ onset F</th>
<th>$T_g$ onset P</th>
<th>$T_g$ mid Adj MS</th>
<th>$T_g$ mid F</th>
<th>$T_g$ mid P</th>
<th>$T_g$ pred Adj MS</th>
<th>$T_g$ pred F</th>
<th>$T_g$ pred P</th>
<th>$T_{cr}$ Adj MS</th>
<th>$T_{cr}$ F</th>
<th>$T_{cr}$ P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>2</td>
<td>2.26</td>
<td>2.87</td>
<td>0.12</td>
<td>4.08</td>
<td>2.93</td>
<td>0.11</td>
<td>4.46</td>
<td>6.42</td>
<td>0.02</td>
<td>37.0</td>
<td>9.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Oil</td>
<td>2</td>
<td>0.26</td>
<td>0.33</td>
<td>0.73</td>
<td>1.19</td>
<td>0.35</td>
<td>0.46</td>
<td>13.1</td>
<td>18.8</td>
<td>0.00</td>
<td>132</td>
<td>32.9</td>
<td>0.00</td>
</tr>
<tr>
<td>OT</td>
<td>1</td>
<td>20.5</td>
<td>26.0</td>
<td>0.00</td>
<td>44.1</td>
<td>31.6</td>
<td>0.00</td>
<td>188</td>
<td>270</td>
<td>0.00</td>
<td>120</td>
<td>29.9</td>
<td>0.00</td>
</tr>
<tr>
<td>Rep*Oil</td>
<td>4</td>
<td>8.15</td>
<td>10.3</td>
<td>0.00</td>
<td>10.7</td>
<td>7.67</td>
<td>0.01</td>
<td>6.42</td>
<td>9.24</td>
<td>0.00</td>
<td>44.2</td>
<td>11.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.79</td>
<td>1.39</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>4.01</td>
<td>4.01</td>
<td>0.00</td>
<td>4.01</td>
<td>4.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The GLM was run with interaction terms and terms giving non-significant interactions were removed.

- $T_g$ refers to onset of glass transition
- $T_g$ mid refers to mid-point of glass transition
- $T_g$ pred refers to predicted glass transition temperature using the Couchmann-Karasz equation.
- $R^2$ (adj) refers to adjusted $R^2$
4.3.5 Dynamic Vapour Sorption

Lactose crystallisation in dairy powders can be inferred from moisture sorption characteristics (Vollenbroek et al., 2010). Different quantities of proteins within a dairy powder have been shown to impact lactose crystallisation due to protein having different hydration properties (McCarthy et al., 2013). Two cycles of sorption/desorption were carried out; first and second sorption cycles for an outlet temperature of 90 °C are shown in Figures 4.2 and 4.3. The sorption models were fitted with GAB models (Table 4.6); the crystallisation peak observed in the first sorption cycle did not appear on the subsequent sorption cycle, showing the completeness and irreversibility of lactose crystallisation for all powders (Figures. 4.2 and 4.3). Water sorption capacity of crystalline solids decreases compared to amorphous solids, due to decreased void space, free energy and surface area, resulting in release of water upon crystallisation (Burnett et al., 2004). This released water evaporates as equilibrium of vapour pressure with the controlled humidity environment is reached, leading to an observed decrease in mass. When no decrease in mass is observed upon further humidification, then the powder is fully crystallised (Burnett et al., 2004; Burnett et al., 2006).

The results show very little difference in water sorption properties for the six powders at either outlet temperature (data not shown for outlet temperature of 80 °C). All powders sorbed and desorbed water at similar rates for powder produced at different outlet temperatures, i.e., the two-cycle process took approximately the same amount of time (~1400 min) (data not shown). With regard to outlet temperature, free fat and moisture, the outlet temperature directly affects moisture and free fat. However, it would not be expected that free fat would directly affect the sorption characteristics as it is mainly protein, lactose and salts that are involved in sorption (Berlin et al., 1968).
Table 4.6 Guggenheim-Anderson-de Boer (GAB) isotherm constants $\alpha$, $\beta$, $\gamma$, $C$, $K'$ and monolayer value ($m_m$) of powders for sorption cycles 1 and 2.

<table>
<thead>
<tr>
<th>Powder</th>
<th>$a_w$ range</th>
<th>$n$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$K'$</th>
<th>$C$</th>
<th>$m_m$</th>
<th>%RMS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO-H</td>
<td>0.1-0.4</td>
<td>4</td>
<td>-0.37</td>
<td>0.57</td>
<td>0.07</td>
<td>15.45</td>
<td>0.60</td>
<td>1.52</td>
<td>0.57</td>
<td>0.0001</td>
</tr>
<tr>
<td>SOPO-H</td>
<td>0.1-0.4</td>
<td>4</td>
<td>-0.38</td>
<td>0.64</td>
<td>0.09</td>
<td>14.98</td>
<td>0.56</td>
<td>1.36</td>
<td>0.48</td>
<td>0.0001</td>
</tr>
<tr>
<td>PO-H</td>
<td>0.1-0.4</td>
<td>4</td>
<td>-0.70</td>
<td>0.72</td>
<td>0.07</td>
<td>12.71</td>
<td>0.89</td>
<td>1.18</td>
<td>0.99</td>
<td>0.0004</td>
</tr>
<tr>
<td>SO-H</td>
<td>0.1-0.9</td>
<td>9</td>
<td>-0.36</td>
<td>0.46</td>
<td>-0.01</td>
<td>-101.41</td>
<td>0.78</td>
<td>2.21</td>
<td>32.03</td>
<td>0.0043</td>
</tr>
<tr>
<td>SOPO-H</td>
<td>0.1-0.9</td>
<td>9</td>
<td>-0.61</td>
<td>0.68</td>
<td>0.01</td>
<td>145.89</td>
<td>0.89</td>
<td>1.45</td>
<td>31.91</td>
<td>0.0030</td>
</tr>
<tr>
<td>PO-H</td>
<td>0.1-0.9</td>
<td>9</td>
<td>-0.66</td>
<td>0.72</td>
<td>0.01</td>
<td>88.76</td>
<td>0.91</td>
<td>1.35</td>
<td>33.60</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

*a$_w$ range of experimental sorption data.

*b Number of experimental data points.

The terms $C$, $K'$, and $m_m$ (g water / 100 g dry weight) are derived from quadratic constants $\alpha$, $\beta$, and $\gamma$ cf. equations (4.3) and (4.4).
Powders sorbed water with humidification up to ~60% RH, after which mass decreased until ~ 70% RH corresponding to lactose crystallisation. Thomas et al. (2004) reported that crystallisation in pure lactose and non-fat milk powders started at ~ 40% and ~ 50% RH, respectively. In the case of WMP, they found that lactose crystallisation did not occur until ≥ 66% RH, due to the presence of milk fat, which is believed to act as a hydrophobic barrier and to limit the diffusion of hydrophilic molecules and the growth of lactose crystals (McSweeney & Fox, 2009). When lactose crystallises, water is released as the crystalline structure is formed, and this water evaporates. The second sorption cycle showed no decrease in mass upon humidification, indicating that all the lactose had crystallised (Figure 4.3). At the end of the 2nd cycle, the mass had increased by ~ 1.5% at 0% RH (data not shown); this water is permanently bound to the crystalline lactose structure. For the first sorption cycle, the GAB monolayer \( (m_m) \) values ranged from 1.18 to 1.52 g water / 100 g solids for powders produced at the higher outlet temperature (Table 4.6). Water sorption behaviour did not vary significantly with oil type for \( a_w \) values in the range 0.0 - 0.6. Net moisture loss after crystallisation (i.e. from the moisture peak to the moisture trough for \( a_w > 0.7 \)) was less for powder containing SO than for powders containing SOPO or PO (Figure 4.2). As \( a_w \) increased from 0.6 to 0.9, the differences observed between powders became more apparent, which may be related to the state of lactose, i.e., relative amounts of \( \alpha \) monohydrate and \( \beta \) anhydrous (Figure 4.2). Powders showed typical water sorption isotherms for protein/lactose mixtures, with good agreement between predicted and experimental data over the \( a_w \) range 0.00 - 0.44, until lactose crystallisation occurred, between \( a_w \) values of 0.6 and 0.7. After lactose crystallisation, SO powder had a higher weight, which was carried through to the desorption cycle and was maintained in the subsequent sorption cycle (Figure 4.3). It is postulated that, during crystallisation of lactose, the more fluid SO can interact with the lactose molecules and influence isomer formation.
Figure 4.2 Moisture sorption isotherms for first vapour sorption cycle for powders made with sunflower oil (SO, - ◊ -), 50:50 blends of sunflower and palm oil (SOPO, - ◊ -) and palm oil (PO, - △ -), illustrating crystallisation of lactose. GAB model fits are superimposed as continuous lines from 0 – 40% RH. Powder moisture is expressed on a dry basis. Drying outlet temperature was 90 °C.
Figure 4.3 Moisture sorption isotherms for second vapour sorption cycle for powders made with sunflower oil (SO, ⬤), 50:50 blends of sunflower and palm oil (SOPO, ◇) and palm oil (PO, Δ) illustrating absence of further crystallisation of lactose. GAB model fits are superimposed as continuous lines from 10 – 90% RH. Powder moisture is expressed on a dry basis. Dryer outlet temperature was 90 °C.
4.3.6 Confocal Microscopy

Distribution of fat in dairy powders has a significant effect on functional properties (Píseký, 1997). The CLSM of spray dried powders which were dual labelled with Nile Red/Fast Green FCF showed that fat was not restricted to the surface of powder particles, but was present within particles, confirming previous electron microscopy studies (Buchheim, 1982; Buma, 1971). Spray-dried powders produced at the higher outlet temperature of 90 °C (Figure 4.4, Image 1b, 2b and 3b) showed more surface fat compared to the powders produced at 80 °C (Figure 4.4, Image 1a, 2a and 3a). This may be attributed to the greater mobility of oil when particle surfaces are exposed to temperatures approaching the outlet temperature, as occurs after the critical stage of spray drying. It could also be due to rupturing of the fat globule membrane due to shrinkage caused by evaporation, resulting in fat leaking through the ruptured membrane and going to powder surface.
Figure 4.4 Confocal images of powder particles spray-dried at (A) 80 °C or (B) 90 °C at a magnification of 63x (3 zoom factor) from emulsions with palm oil (Image 1a, 1b), a 50:50 mix of palm oil: sunflower oil (Image 2a, 2b) and sunflower oil (Image 3a, 3b). Scale bar 25 µm. Protein phase appears red while the oil phase appears green.
4.4 Conclusions

Oil type had no significant effect on the principal physical characteristics of fat-filled dairy-based powders, i.e., bulk density, particle size, water sorption isotherm characteristics, moisture and $a_w$. Likewise, oil type did not affect fat globule size of the emulsion or of the reconstituted formula. Thus, while the oil type, PO or SO, affected viscosity of the emulsion, this effect did not impact on physical characteristics of the powders. Oil type also did not influence glass transition or water sorption characteristics. Conversely, outlet temperature influenced each of the parameters tested, except for fat globule size of the reconstituted product. Free fat level increased as expected with increasing outlet temperature, but was not influenced significantly by oil type used in the emulsion. Fat substitution did not impact significantly on physical properties of dairy-based formulations.
4.5 References


Chapter 5: Water sorption and diffusion properties of spray-dried dairy powders containing intact and hydrolysed whey protein


Declaration: Experimental design, powder production and all analysis except SEM imaging were carried out by Grace Kelly. Experimental results/ data were analysed and the chapter was written by Grace Kelly. Dr. Deirdre Kennedy from National Food Imaging Centre, Teagasc Food Research Centre, Moorepark, Co. Cork, Ireland analysed powders using SEM.
Abstract

The aim was to compare the effect of intact or hydrolysed whey protein in spray-dried lactose/protein powders on water diffusion properties and microstructure. Dispersions of protein/lactose (0.21:1) containing either intact or hydrolysed whey protein were spray-dried at pilot scale, and physical properties were determined. Lactose/hydrolysed whey protein powders had significantly increased ($P < 0.05$) particle density, resulting in lower bulk density and occluded air, and higher interstitial air. Moisture sorption analysis at 25 °C showed that dispersions containing intact whey protein exhibited lactose crystallisation at a lower relative humidity (RH) compared to the dispersions containing hydrolysed whey protein. Hydrolysed whey protein dispersions had a lower monolayer moisture value ($m_m$) than intact whey protein dispersions, as calculated using the Guggenheim-Anderson-de Boer (GAB) equation. Water diffusivity, determined at 25 °C from water sorption kinetics and the application of a mathematical model based on Fick’s 2nd law, was significantly different ($P < 0.05$) with respect to the presence of intact or hydrolysed whey protein over the RH range examined (0-60% RH), except at 40% RH. The presence of hydrolysed whey protein resulted in a significantly higher ($P < 0.05$) water diffusivity in powders, with potential implications for hygroscopicity, caking, stickiness and flowability in humid environments.
5.1 Introduction

Carbohydrates and proteins are major non-fat solid components in food products, and their interaction with water and with each other influence their physical properties (Haque & Roos, 2005; Roos, 2002). These macronutrients are commonly derived from dairy sources. The proteins can be intact or hydrolysed, with the latter being composed of peptides produced from hydrolysis of intact proteins, giving lower average molecular mass and less secondary structure than intact proteins (Flanagan & FitzGerald, 2002). Hydrolysed proteins have reduced immunological reactivity and are commonly used in infant formulas for hypoallergenic infants and in the nutritional management of individuals who cannot digest whole or intact protein (Hochwallner, Schulmeister, Herz, Focke-Tejkl, Swoboda, Reininger et al., 2015). Peptides generated by hydrolysis are readily absorbed, and are therefore an attractive source of nitrogen in sports nutrition. Hydrolysis of proteins alters functional characteristics such as solubility, emulsification and foaming properties in food products.

Lactose, a low molecular weight carbohydrate in milk, commonly exists in the amorphous state in a number of low-moisture foods, i.e., dairy powders. The state of lactose in dairy powders may change during storage to a glassy or crystalline state, depending on composition and storage conditions, e.g., relative humidity, temperature and duration of storage (Jouppila, Kansikas & Roos, 1997; Kelly, O'Mahony, Kelly, Huppertz, Kennedy & O'Callaghan, 2015; McCarthy, Kelly, O'Mahony, Hickey, Chaurin & Fenelon, 2012).

Water transfer in porous food products is complex, with different mechanisms occurring; vapour diffusion in air-filled pores as a result of vapour pressure gradients and movement of liquid due to capillary action (Gekas, 1992). The relative impact of these mechanisms to overall water diffusivity is difficult to determine, and therefore apparent water diffusivity is obtained using Fick’s second law. Water diffusion of flour has been studied using sorption isotherms (Lomauro, Bakshi & Labuza, 1985), with limited studies on water diffusion within dairy powders. Murrieta-Pazos, Gaiani, Galet, Cuq, Desobry and Scher (2011) examined water transfer in skimmed milk powder (SMP) and whole milk powder (WMP), and showed that WMP had a lower diffusion coefficient than SMP, suggesting that the presence of fat retards the diffusion of
moisture. Diffusion of water is affected by the physical structure of the food material (Murrieta-Pazos, Galet, Gaiani & Scher, 2014); an increase in moisture gives rise to a change in porosity, which can lead to collapse of the food structure.

 Powders with higher protein: lactose ratios are less susceptible to sticking (Kelly, O'Mahony, Kelly, Huppertz, Kennedy & O'Callaghan, 2015). Hydrolysed protein powders also tend to have higher hygroscopicity and thermoplasticity compared with powders containing intact proteins.

 The aim of this work was to compare the effect of inclusion of intact versus hydrolysed whey protein in spray-dried lactose / protein dairy dispersions on water diffusion properties and the effects of storage under different RH levels on microstructure.

 5.2 Materials and Methods

 5.2.1 Materials

 Intact whey protein concentrate powder (80 g protein/ 100 g powder) and hydrolysed whey protein concentrate powder (80 g protein/ 100 g powder) with a degree of hydrolysis (DH) value of 12 were obtained from Carbery Ingredients Ltd. (Ballineen, Co. Cork, Ireland). According to the supplier, the hydrolysed whey protein had an average molecular weight of 5.84 kDa, with more than 70% > 5 kDa. Edible-grade α-lactose monohydrate was obtained from Glanbia Ingredients (Ballyraggett, Co. Kilkenny, Ireland), and SMP was purchased from Dairygold Food Ingredients (Mitchelstown, Co. Cork, Ireland).

 5.2.2 Preparation of spray-dried powders and experimental design

 The experiment was carried out in triplicate, where the trials for each replicate were carried out in random order using Design Expert Version 7.1.6 (Stat-Ease, USA). The dispersion (20% total solids) consisted of 3.34 g protein / 100 g (whey protein: casein, 60:40; either intact or hydrolysed whey protein), and 16.04 g lactose / 100 g. Batches (15 kg) were prepared as follows; lactose powder was dissolved in water at ~70 °C, using a Silverson L4RT (Silverson Machines Ltd., Waterside, Chesham, Bucks, England).
mixer to aid reconstitution. SMP was then added slowly, followed by the whey protein. The batches were tempered to 60 °C and adjusted to pH 6.9 by adding 4 mol/L KOH and maintained under high shear for 30 min to ensure complete hydration of the whey protein. The blend was agitated prior to heat treatment at 100 °C for 30 s using a pilot scale tubular heat exchanger (Microthermics Model 25HV; North Carolina, USA). The product was then spray-dried in a pilot-scale Anhydro Spray dryer (Model Plant No. 3 type I KA, Copenhagen, Denmark), equipped with a two-fluid nozzle atomisation system (Type 1/8 JAC 316ss) and counter-current drying. Dryer inlet temperature was maintained constant at 185 °C and outlet temperature was maintained at 80 °C. Powders were labelled IP and HP, for powders containing intact and hydrolysed whey protein, respectively. Samples of powder were stored at 10 °C in sealed foil bags until analysis was completed.

5.2.3 Viscosity

Viscosity for each formulation post heat-treatment was measured at 55 °C and reconstituted product at 12.5 g / 100 g was measured at 20 °C using the method described by Kelly, O’Mahony, Kelly and O’Callaghan (2014). The apparent viscosity, measured at 500 s⁻¹, was used for comparison of formulations.

5.2.4 Powder characterisation

Water content of powders was determined using a halogen rapid moisture analyser (HR-83 Halogen, Mettler Toledo, Switzerland). The samples were dried at a temperature of 105 °C until a constant weight was attained (< 1 mg change over 140 s, equivalent to ± 0.025%). Water activity (aw) was measured with a Novasina LabMaster aw water activity meter (Novatron Scientific Ltd., West Sussex, UK). Protein (N x 6.38) was determined by macro-Kjeldahl (IDF 2001). Ash content was determined after overnight incineration at 550 °C. Lactose content was estimated by difference in weight.
Particle density ($\rho_p$) was measured with a pycnometer (AcuPyc 1340, Micromeritics, Norcross, GA, USA). The method uses helium to measure the particle density of an air-free volume of a known weight of powder (GEA Niro, 2006b). The occluded air ($V_{OA}$), measured in mL/100g, is defined as the difference between the volume of a given mass of particles and the volume of the same mass of air-free solids. It is calculated from the equation:

$$V_{OA} = \frac{100}{D_{\text{particle}}} - \frac{100}{D_{\text{solids}}}$$

(5.1)

Here, $D_{\text{particle}}$ is the measured particle density and $D_{\text{solids}}$ is the calculated density of powder solids (g/mL). The interstitial air ($V_{IA}$), measured in mL/100 g, is defined as difference between the volume of a given mass of particles and the volume of the same mass of 100 x tapped powder. It is calculated from the equation:

$$V_{IA} = \frac{100}{D_{\text{powder}}} - \frac{100}{D_{\text{particle}}}$$

(5.2)

Here, $D_{\text{powder}}$ is the powder bulk density (g/mL) measured after 100 taps by the GEA Niro method (GEA Niro, 2006a). Samples were measured in duplicate.

5.2.5 Powder particle size distribution

Powder particle size was determined by laser light scattering using a Malvern Mastersizer 3000 with the Aero S unit (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Powder sample was added to the standard venturi disperser with a hopper gap of 4 mm and then fed into the dispersion system. Compressed air at 50,000 Pa was used to transport and suspend the powder particles through the optical cell, with a measurement duration of 10 s. Background measurements were made using air for 20 s.
5.2.6 Water sorption isotherms

Sorption isotherms were determined gravimetrically by DVS technique (DVS Advantage 1, Surface Measurement Systems Ltd., London, UK) as described in Section 2.2.6. Dried samples (~30 mg) were loaded into the sample pan and humidified from 0 to 90% RH in 10% RH increments. Equilibrium was considered to be reached when change in mass with time (dm/dt) was < 0.001 mg/min for at least 10 min for each step. DVS Data Analysis Suite, which runs with a Microsoft Excel add-on (Microsoft Office Excel, 2003), was used to graph and analyse data.

The Guggenheim-Anderson de Boer (GAB) equation (Equation 5.3) (Van den Berg, 1984) was used to model water sorption isotherms and to determine the critical water content and water activity (a_w).

\[
\frac{m}{m_m} = \frac{CKa_w}{(1-Ka_w)(1+Ka_w(C-1))}
\]  

(5.3)

where \(m\) is the moisture content (g / 100 g dry solids), \(m_m\) is the monolayer value (g / 100 g), \(a_w\) is water activity, and \(C\) and \(K\) are constants, \(K\) having units inverse to \(a_w\) and \(C\) being dimensionless.

Bizot (1983) showed that this equation could be converted to a second-order polynomial giving a quadratic equation (Equation 5.4):

\[
\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma
\]  

(5.4)
Values for the parameters, $\alpha$, $\beta$, $\gamma$ were determined by quadratic regression analysis of $\frac{a_w}{m}$ as a function of $a_w$ using DVS data, e.g., over a range of $a_w$, from 0 to 0.4. The solution to equations (5.1) and (5.2) give the values for $m_m$, $K$ and $C$ as:

$$m_m = \frac{1}{\sqrt{\beta^2 - 4\alpha\gamma}}$$  \hspace{1cm} (5.5)

$$K = \frac{\beta - \frac{1}{m_m}}{-2\gamma}$$  \hspace{1cm} (5.6)

$$C = \frac{1}{m_m K \gamma}$$  \hspace{1cm} (5.7)

5.2.7 Initial rate of water sorption

Change in sample mass as a function of time during hydration, i.e., during each discrete increment of RH, was recorded by the DVS microbalance and modelled using an exponential function (Roman-Gutierrez, Mabille, Guilbert & Cuq, 2003):

$$q(t) = Q \left[1 - \exp \left(-\frac{t}{B}\right)\right]$$  \hspace{1cm} (5.8)

where $q(t)$ is the change in sample mass (g of water/100 g of dry matter) as a function of time; $t$ is the time (min); $Q$ is the equilibrium water adsorption capacity (g of water/100 g of dry matter) between two relative humidities; and $B$ is a time constant for equilibration of water adsorption (min). Model parameters $Q$ and $B$ of Equation 5.8
were determined by nonlinear least squares optimisation, i.e., by minimizing the sum of squares error (SSE) between the experimental and the predicted moisture content (q(t)) values, using the Solver add-on in Microsoft Excel (2010). The standard error of estimate (SEy) was calculated from SSE (Equation 5.9):

\[ SEy = \sqrt{\frac{SSE}{n}} \]  

(5.9)

The initial rate of water vapour adsorption, \( R_0 \), was calculated from the values of Q and B (Equation 5.10):

\[ R_0 = \frac{Q}{B} \]  

(5.10)

where \( R_0 \) has units: g of water/100 g of dry matter/ min.

5.2.8 Water diffusion coefficient by Fick’s law

Diffusion coefficient of water vapour through particles is defined from Fick’s first law (Roman-Gutierrez, Mabille, Guilbert & Cuq, 2003), with Fick’s second law being used to model water diffusion coefficient for spherical particles (Roman-Gutierrez, Mabille, Guilbert & Cuq, 2003). Calculation of apparent water diffusion coefficient, D, for dairy powders requires consideration of the size distribution of particle diameters. The total water gain at any time \( t \) in powders formed by spherical particles with a range of diameters is then defined by the sum of the individual contributions of each class of particle diameters. Spherical powder particles with a dispersed diameter can be expressed in the following equation (Equation 5.11) which assumed conditions proposed by Roman-Gutierrez, Mabille, Guilbert and Cuq (2003):
\[ M_t = (M_i - M_{eq}) - \frac{6}{\pi^2} \sum_i w(a_i) \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( \frac{-D \cdot n^2 \cdot \pi^2 \cdot t}{a_i^2} \right) + M_{eq} \quad (5.11) \]

where \( M_t \) is the water content (kg of water/kg dm) at time \( t \) (min), \( M_i \) and \( M_{eq} \) is the water content (kg of water/kg dm) at initial (\( t=0 \)) and equilibrium (\( t=\infty \)), \( a_i \) is the radius (m) of the spherical particle \( i \); \( w(a_i) \) is the weight fraction of particles that are characterised by the radius; \( n \) is the calculation increment. \( D \) was calculated by minimizing the sum of squares error (SSE) between the experimental and the predicted data values using the Solver add-on in Microsoft Excel (2010).

5.2.9 Scanning electron microscopy (SEM)

Powders were stored for 2 weeks at room temperature at 0% and 54.4% RH, respectively, prior to SEM observation. Samples were imaged using a field emission scanning electron microscope (FE-SEM; Zeiss Supra Gemini, Darmstadt, Germany) at 2 kV beam energy. For analysis, samples were mounted on double-sided carbon tape, attached to scanning electron microscope (SEM) stubs, and sputter-coated with chromium (Emitech K550X, Ashford, UK). Representative micrographs were taken at 1000x and 5000x magnification in order to visualize surface morphology.

5.3 Results

5.3.1 Viscosity and powder properties

In comparing apparent viscosity of formulations post heat treatment, no significant difference \( (P > 0.05) \) was observed between mixes (Table 5.1). However, on reconstitution, IP had a significantly higher \( (P < 0.05) \) viscosity compared to HP. Hogan and O’Callaghan (2013) demonstrated a decrease in viscosity as degree of hydrolysis of whey protein increased. Spray-dried dispersions containing the different types of protein were similar in their contents of protein, fat, lactose and moisture (Table 5.2).
The mineral content of HP powder was significantly higher ($P < 0.05$) compared to IP powder, possibly due to addition of alkali to neutralize hydrolysis-induced reduction in pH during manufacture of hydrolysed whey protein ingredient (Séverin & Wen-shui, 2006). Hogan and O’Callaghan (2013) also reported an observed increase in mineral composition in powders that were produced using extensively hydrolysed whey protein.

**Table 5.1** Viscosity of dispersions post heat treatment and upon reconstitution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Post heat treatment</th>
<th>Reconstituted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viscosity (mPa s)$^1$</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>3.13 ± 0.04$^a$</td>
<td>3.71 ± 0.08$^a$</td>
</tr>
<tr>
<td>HP</td>
<td>3.23 ± 0.12$^a$</td>
<td>3.60 ± 0.01$^b$</td>
</tr>
</tbody>
</table>

$^1$ Results are shown as means ± standard deviation (n=3). Viscosity was measured at 20% total solids / 55 °C post heat treatment and 12.5 g / 100 g total solids / 20 °C upon reconstitution.

(a–b) Values within a column not sharing a common lower-case letter differ significantly ($P < 0.05$)
Table 5.2 Composition of protein/lactose dispersions containing intact or hydrolysed whey protein.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Lactose (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Moisture (g/100 g)</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>16.4 ± 0.02a</td>
<td>0.80 ± 0.0a</td>
<td>78.1 ± 0.1a</td>
<td>3.6 ± 0.03b</td>
<td>2.5 ± 0.1a</td>
<td>0.19 ± 0.01b</td>
</tr>
<tr>
<td>IP</td>
<td>16.3 ± 0.05a</td>
<td>0.83 ± 0.0a</td>
<td>78.3 ± 0.2a</td>
<td>2.6 ± 0.05a</td>
<td>2.4 ± 0.27a</td>
<td>0.14 ± 0.00a</td>
</tr>
</tbody>
</table>

Results are shown as means ± standard deviation (n=3).

(a-b) Values within a column not sharing a common lower-case letter differ significantly (P < 0.05)

There were no significant (P > 0.05) differences in powder particle size D[4,3], which may be due to similar viscosity pre spray-drying (Table 5.3). Crowley, Gazi, Kelly, Huppertz and O’Mahony (2014) reported that as feed viscosity (milk protein concentrate) increased there was a concomitant increase in powder particle size. Although the fraction of fine particles, D(v,0.1), was not significantly different, HP showed a bimodal size distribution (Figure 5.1). IP had a significantly lower (P < 0.05) particle density (ρp) than HP powder, due to the higher volume of occluded air (Voa) in IP powders (Table 5.3); lower (P < 0.05) tapped bulk density values for HP compared to IP powder were also due to higher volume of interstitial air (Via).

Occluded air is affected by formulation and concentrates with high foamability allow incorporation of air before drying, resulting in high occluded air (Keogh, O’Kennedy, Kelly, Auty, Kelly, Fureby et al., 2001; Sánchez & Patino, 2005), while the addition of oil reduces foaming of concentrates. Jambrak, Mason, Lelas, Herceg and Herceg (2008) examined the foamability of hydrolysed and intact whey protein and concluded that hydrolysed whey protein had increased foaming properties in comparison to intact whey protein.
Figure 5.1 Particle size distributions of powders, containing intact (---) and hydrolysed (-----) whey protein.
Table 5.3 Powder particle size, density and pycnometer parameters of protein/lactose dispersions containing intact or hydrolysed whey protein.

<table>
<thead>
<tr>
<th>Sample</th>
<th>D(v0.1)</th>
<th>D(v0.5)</th>
<th>D(v0.9)</th>
<th>D[4,3]</th>
<th>ρ_p</th>
<th>ρ_tapped</th>
<th>V_{OA}</th>
<th>V_{IA}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μm</td>
<td></td>
<td></td>
<td></td>
<td>g/mL</td>
<td>mL/100g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>10.8 ± 2.86^a</td>
<td>40.8 ± 13.3^a</td>
<td>96.8 ± 21.8^a</td>
<td>48.8 ± 13.7^a</td>
<td>1.01 ± 0.11^b</td>
<td>0.28 ± 0.01^a</td>
<td>30.1 ± 0.11^a</td>
<td>251 ± 8.49^b</td>
</tr>
<tr>
<td>HP</td>
<td>8.17 ± 1.26^a</td>
<td>46.2 ± 12.5^a</td>
<td>146 ± 34.9^a</td>
<td>64.3 ± 15.0^a</td>
<td>1.35 ± 0.00^b</td>
<td>0.21 ± 0.02^b</td>
<td>6.07 ± 0.18^b</td>
<td>407 ± 37.1^a</td>
</tr>
</tbody>
</table>

Results are shown as means ± standard deviation (n=3).

[^a-b]: Values within a column not sharing a common lower-case letter differ significantly (P < 0.05)
5.3.2 Sorption isotherms

Sorption isotherms (0-90 % RH) of dispersions, containing intact and hydrolysed whey protein are shown in Figure 5.2. Moisture sorption isotherms could be classified as a type II isotherm according to Brunauer classification (Brunauer, Deming, Deming & Teller, 1940). Isotherms displayed three distinct regions, namely 0-30 % RH, 30-40 % RH and > 40% RH (Figure 5.2).

In the first distinct region, moisture sorption occurred at a rate of 1.21 and 1.14 g / 100 g per 10% increase in RH for IP and HP, respectively, without glass transition. The literature suggests that this occurs without change in structure and is mostly due to surface adsorption (Burnett, Thielmann, Sokoloski & Brum, 2006; Maher, Auty, Roos, Zychowski & Fenelon, 2015). This was not expected, as the literature shows that as DH% increases, there is also a concomitant increase in moisture sorption in the lower RH range, due to there being more available binding sites associated with lower molecular mass fractions (Hogan & O’Callaghan, 2013; Mounsey, Hogan, Murray & O’Callaghan, 2012).
Figure 5.2 Moisture sorption isotherms for dispersions containing intact (—) and hydrolysed (—) whey protein. Powder moisture is expressed on a solid-non-fat (SNF) basis. Inset shows the GAB equation fitted to sorption isotherms.
In the next region (30-40% RH), there was a rapid increase in the rate of moisture sorption, 2.96 and 3.40 g /100 g, for IP and HP, respectively, indicative of water migrating into the bulk of the powder as it approaches a glass transition region, prior to crystallisation.

As the humidity rapidly increased to > 40% RH, the sample mass increased dramatically due to water sorption. After a further period of time, the mass decreased at a particular RH, referred to as crystallisation relative humidity (RHc). This characteristic mass loss feature is associated with the crystallisation of amorphous lactose, where crystallisation of lactose is evidenced by a decrease in moisture, or discontinuity in the isotherm, due to the release of moisture previously bound by amorphous lactose (Burnett, Thielmann & Booth, 2004; Kelly, O’Mahony, Kelly & O’Callaghan, 2014); this was apparent for both powders, with RHc of IP and HP powder occurring at 50% and 60% RH, respectively (Figure 5.2). The amount of moisture sorbed by powders prior to lactose crystallisation was higher for HP powder. Crystallisation of lactose was significantly delayed with respect to RH in the HP powder.

When powder equilibration was examined with respect to time over successive increments of RH, HP had a significantly shorter equilibration cycle compared to IP (data not shown), crystallisation occurred earlier for HP (480 min) than IP (1373 min) corresponding to moisture contents of 14.5 and 9.77 g / 100 g, respectively. Thus, it appears that hydrolysed whey proteins absorb water more quickly, reaching glass transition more quickly, but because crystallisation is a process that takes place over a period of time, the crystallisation phenomenon is observed at a higher water content than for intact proteins.

A reduction in mass of sorbed water from ~9.77 to ~4.82 g / 100 g and 14.5 to 7.26 g / 100 g due to crystallisation of amorphous lactose was observed for IP and HP, respectively (Figure 5.2). Post crystallisation, the uptake in water increased to ~12.4 and ~18.4 g / 100 g at 90% RH for IP and HP. Maximum water sorption occurred more slowly for IP than for HP (~1843 min and ~772 min); this significant difference in equilibration time may be explained by the increase in hydrophilic charged peptides and amino acids due to hydrolysis, leading to rapid sorption of water molecules in HP.
powder (Mahmoud, Malone & Cordle, 1992). Water diffusion increases in polar hydrophilic matrices (Palzer, 2010) and has been shown to increase with increasing water activity up to the point of lactose crystallisation in SMP and WMP powders (Murrieta-Pazos, Gaiani, Galet, Cuq, Desobry & Scher, 2011) and hydrolysed protein/lactose dispersions (Hogan & O’Callaghan, 2013; Mounsey, Hogan, Murray & O’Callaghan, 2012).

GAB monolayer ($m_m$) values were 1.6 and 2.19 g water / 100 g solids for HP and IP powders, respectively (Table 5.4). Although HP powders have more active sites for water adsorption, the calculated $m_m$ value was lower, possibly due to HP having a larger powder particle size than IP and hence, lower surface area. The $m_m$ value can indicate the optimum quantity of moisture in dried foods associated with negligible loss in product quality in terms of aroma retention, colour and biological value (Andrade, Lemus & Pérez, 2011). Above the $m_m$ value (RH > 30%), food powders may have enough water to go through a glass transition, which can lead to powder deterioration (caking, crystallisation etc.).

**Table 5.4** Guggenheim Anderson de Boer (GAB) isotherm constants $\alpha$, $\beta$, $\gamma$, $C$, $K$, and monolayer value ($m_m$) of protein/lactose dispersions containing intact or hydrolysed whey protein for 0-40% RH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$K$</th>
<th>$C$</th>
<th>$m_m$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>-0.49</td>
<td>0.09</td>
<td>0.10</td>
<td>1.80</td>
<td>2.46</td>
<td>2.19</td>
<td>0.98</td>
</tr>
<tr>
<td>HP</td>
<td>-0.84</td>
<td>0.23</td>
<td>0.10</td>
<td>1.97</td>
<td>3.66</td>
<td>1.61</td>
<td>0.97</td>
</tr>
</tbody>
</table>
5.3.3  Sorption kinetics and water diffusion coefficient

Water vapour sorption kinetics for powders were evaluated by fitting the exponential model (Equation 5.8) to DVS data. The exponential model fitted well ($R^2 \geq 0.99$ and $SEy \leq 0.088 \text{ g / 100 g}$) for each equilibration step for powders (Table 5.5).

The time taken for powders to equilibrate at each RH ($B$, min; Table 5.5) was generally lower in HP powder, suggesting that equilibration was achieved due to more hydrophilic sites associated with hydrolysed whey proteins. The equilibrium water sorption capacity, $Q$, was not significantly different ($P > 0.05$) between the two powders examined at the same RH values ($0$ – $60 \%$ RH). However, there was a concomitant increase in $Q$ with RH, mirroring the increments observed in sorption isotherms (Figure 5.2), with a significant difference ($P < 0.05$) between RH levels (Table 5.5). The rate of water sorption ($R_0$ in Equation 5.10; Figure 5.3) was not significantly different ($P > 0.05$) between the two powders, when examined over the RH range $0$ – $50 \%$ (Table 5.5). $R_0$ did not show an overall increase over the RH range $0$ to $40\%$, but increased by a factor of 2 or more above $40\%$ RH.

Water diffusion coefficient ($D$) (Figure 5.4) was calculated for each 10\% RH increment (for initial RH values of 0-50\% RH) from water sorption kinetic curves obtained from DVS and powder particle size distribution data, by applying Fick’s second law to spherical particles (Equation 5.11), taking powder particle size distribution into account. Other studies have used Fick’s second law to model water diffusivity in food powders but assumed a constant particle size ($D_{v50}$) for the calculation, and did not account for size distribution (Murrieta-Pazos, Galet, Gaiani & Scher, 2014). Diffusion coefficients were modelled below the onset of crystallisation at $25 ^\circ C$, i.e., up to 50 and 60\% RH for IP and HP, respectively. $D$ varied for IP and HP powder, from 1.93 to 0.41 and 4.15 to 0.57, ($\text{units of } 10^{-12} \text{ m}^2/\text{s}$), respectively, depending on the RH conditions (Figure 5.4). These values are lower than those reported in the literature for other food products (e.g., sponge cake, bread biscuit and muffin) (Guillard, Broyart, Bonazzi, Guilbert & Gontard, 2003), with the difference being attributed to differences in food structure and composition.
Table 5.5 Dynamic water vapour sorption properties of dairy powder dispersions containing intact and hydrolysed whey protein exposed to RH increments of 10% (over 0 % RH and increasing to 60 % RH).

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>B (min)</th>
<th>Q (g/100 g of dry matter)</th>
<th>R₀ (g/100 g/min)</th>
<th>SEy (g/100 g)</th>
<th>R² (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.66 ± 2.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.78 ± 0.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.91 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.04 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>18.1 ± 4.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.7 ± 1.13&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.01 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.06 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>39.9 ± 4.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.1 ± 4.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.81 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.58 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>45.5 ± 11.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.3 ± 11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.71 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.05 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>17.7 ± 3.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2 ± 0.19b&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.44 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.34 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>-</td>
<td>20.9 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>4.21 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

SEy and R² refers to standard error of estimate and coefficient of determination, respectively, in fitting Equation 5.8 to DVS data.

<sup>a-d</sup> Values within a column not sharing a common lower-case letter differ significantly (P < 0.05)

<sup>A-B</sup> Values within a row not sharing a common lower-case letter differ significantly (P < 0.05)
Figure 5.3 Influence of initial relative humidity conditions on water vapour adsorption rates at 25 °C for dispersion powders containing intact (–○–) and hydrolysed (←●→) whey protein exposed to RH increments of 10%, error bars represent standard deviation, n=2.
Figure 5.4 Influence of initial relative humidity conditions on the apparent water diffusion coefficient at 25 °C for dispersion powders containing intact (---) and hydrolysed (----) whey protein exposed to a RH increments of 10%, error bars represent standard deviation, n=2.
The maximum values for $D$ were observed for an initial RH of 0% (equilibrium RH of 10%), at 1.93 and 4.15, (units of $10^{-12} \text{ m}^2/\text{s}$), for IP and HP, respectively, corresponding to the formation of a monolayer (Murrieta-Pazos, Galet, Gaiani & Scher, 2014). $D$ decreased gradually for both powders as moisture content increased to initial RH of 30% (where the curves converged; Figure 5.4). A similar trend of decreasing $D$ value with increasing RH above 10 % was observed in durum wheat (Murrieta-Pazos, Galet, Gaiani & Scher, 2014). HP powder generally had a significantly higher ($P < 0.05$) diffusivity in comparison to IP powder. The difference in $D$ value between powders possibly reflects the number of available water binding sites associated with lower average molecular weight peptides in hydrolysed proteins. Hogan & O’Callaghan (2013) examined lactose/protein dispersions of varying DH and observed an increase in moisture uptake with increasing DH after equilibration at all RH, noting this was done under static rather than dynamic conditions, (i.e., the rate of moisture uptake was not monitored).

At an initial RH of 40% (equilibrium 50% RH; Figure 5.4), there was a significant ($P < 0.05$) acceleration of water diffusivity in IP and HP, corresponding to the glass transition of powders, when there is a rapid increase in moisture, with the increase for HP powder being significantly higher than that of IP powder, which possibly corresponds to a lower $T_g$ in HP in comparison to IP powder.
Figure 5.5 Scanning electron microscope (SEM) images of IP (A and B, pre-crystallisation, low and high magnification; C and D, post-crystallisation, low and high magnification) and HP (E and F, pre-crystallisation, low and high magnification; G and H, post-crystallisation (low and high magnification). Scale bar indicates 10 μm for low magnification and 2 μm for high magnification.
5.3.4 Scanning electron microscopy

Images of powders were obtained at two levels of RH (0 and 54.4 % RH; Figure 5.5), to determine the effect of RH on powder surface morphology, in intact and hydrolysed whey protein dispersion systems. Powder surfaces at different RH can be observed in Figure 5.5 A, C, E and G, and interior of particles can be observed, at higher resolution, in Figure 5.5 B, D, F and H. At 0% RH (before lactose crystallisation), IP and HP powders appear similar in shape and structure with a smooth surface (Figure 5.2; A and E), while at higher magnification similarities in wall structure can be seen (Figure 5.2; B and F). In contrast, both powders stored at 54.4% RH (post lactose crystallisation) were noticeably rougher, possibly due to protruding lactose crystals (Figure 5.2; C and G), and an agglomerated structure of powder particles was apparent in HP.

5.4 Conclusions

This study investigated the effects of whey protein hydrolysis on viscosity, moisture sorption, water diffusion and morphology of whey protein/lactose powders. Hydrolysed whey protein-based powders demonstrated a delay in lactose crystallisation with respect to % RH. During hydration, the dispersion containing hydrolysed whey protein had higher water diffusivity, possibly reflecting the number of available water binding sites associated with lower molecular weight peptides. From SEM images of powders before and after lactose crystallisation, HP displayed a more agglomerated structure in comparison to IP dispersion after crystallisation. The higher water diffusivity of hydrolysed whey-protein-based powders can lead to powder handling and quality issues related to caking, stickiness and flowability in humid environments. Consequently, these issues need to be considered in the design of packaging and conditions of storage and powder handling.
5.5 References


Chapter 6: Effect of hydrolysed whey protein on surface composition, water sorption and glass transition temperature of a model infant formula

**Declaration:** Experimental design, powder production and all analysis except SEM imaging were carried out by Grace Kelly. Experimental results/data were analysed and the chapter was written by Grace Kelly. Dr. Deirdre Kennedy from National Food Imaging Centre, Teagasc Food Research Centre, Moorepark, Co. Cork, Ireland analysed powders using SEM.
Abstract

Physical properties of spray-dried dairy powders depend on their composition and physical characteristics. Model infant formulae were produced containing either intact (Degree of Hydrolysis, DH, = 0%) or hydrolysed (DH = 12%) whey protein. This study investigated the effect of hydrolysed whey protein on the microstructure and physical stability of dried model infant formula. Prior to spray drying, apparent viscosities of liquid feeds (at 55 °C) at a shear rate of 500 s$^{-1}$ were 3.02 and 3.85 mPa.s, for intact and hydrolysed infant formula, respectively. On reconstitution, powders with hydrolysed whey had a significantly ($P < 0.05$) higher fat globule size and lower emulsion stability than intact whey protein formula. Lactose crystallization in powders occurred at higher RH for hydrolysed formula. The GAB equation, fitted to sorption isotherms, gave increases in monolayer moisture value ($m_m$) when intact protein was present. As expected, glass transition decreased significantly ($P < 0.05$) with increasing water content. Partial hydrolysis of whey protein in model infant formula resulted in altered powder particle surface morphology, emulsion stability, lactose crystallization properties and storage stability.
6.1 Introduction

Whey protein hydrolysates (WPH) and whey protein isolates (WPI) are widely used sources of protein in the food industry, e.g., in performance foods and infant milk formula (IMF). The IMF industry use WPI and WPH, the latter being used for ease of digestion in infant comfort foods. WPH are used in the nutritional management of individuals unable to digest intact protein, providing complete nutritional requirements with positive health benefits. WPH have lower molecular mass and less secondary structure than WPI (Chobert et al., 1988).

Proteins play an important role in the stabilization of oil-in-water emulsions in IMF (Damodaran, 2005, McCarthy et al., 2012). Because proteins are incorporated into spray-dried food systems, it is of interest to study the effects of processing on the physical properties of resulting powders, especially in relation to storage stability and rehydration. Physico-chemical changes in food powders have been related to glass transition temperature (T_g) (McCarthy et al., 2013, Roos, 1995). For example, powders with low T_g, caused by increased moisture content, may exhibit accelerated deteriorative changes such as stickiness, caking, cohesion and sugar crystallisation.

Lactose, due to its hygroscopic nature, can readily absorb moisture, which may lead to deteriorative reactions (e.g., caking, cohesion and crystallization) in milk powders. Previous work has explored the effects of milk proteins on the physical behaviour of lactose in dairy powders (Haque and Roos, 2004, Hogan and O'Callaghan, 2010, Murphy et al., 2015). It has been shown that as protein: lactose increases, there is a concomitant increase in T_g, making high protein: lactose powders more resistant to stickiness and crystallisation (McCarthy et al., 2013, Thomas et al., 2004, Kelly et al., 2015). Netto et al. (1998) reported that the T_g of pure protein hydrolysates is dependent on the source of protein, e.g., casein or whey protein, as well as the degree of hydrolysis (DH), and suggested that proteins may be equally as important as sugars in altering T_g. Mounsey et al. (2012) reported that stickiness of hydrolysed sodium caseinate/lactose mixtures was affected by protein hydrolysis, intact sodium caseinate/lactose mixture was less susceptible to sticking compared with powders with
hydrolysed sodium caseinate/lactose, with the extent of protein hydrolysis having no significant effect on the stickiness behaviour.

Hogan and O’Callaghan (2013) studied the effect of varying the DH of whey in protein/lactose dispersions, and concluded as DH % increased there was an increase in hygroscopicity and a delay in crystallisation. Recently, Murphy et al. (2015) examined the effect of partially- and selectively-hydrolysed casein and whey protein within a model infant formula, concluding that selectively-hydrolysed (in which β-Lg was selectively hydrolysed) milk proteins may be successfully used to produce IMF powders with good physical characteristics. Protein ingredients require good solubility, emulsification capacity and thermal stability when used in IMF. Stable emulsions are required during manufacture of IMF to minimize surface free fat during manufacture of IMF and protect against creaming in reconstituted IMF products.

The majority of studies on hydrolysed proteins in IMF have focused on nutritional and allergenic aspects, rather than functional characteristics such as emulsification and viscosity. The significance of protein content on IMF emulsion stability has recently been reported (McCarthy et al., 2013, McCarthy et al., 2012, Murphy et al., 2015).

The objective of the present study was to compare the effects of hydrolysed whey (12% DH) compared to intact whey on the stability of model infant milk formula emulsions during processing, and also on physico-chemical properties in the resultant powders. To determine emulsion stability, emulsion FGS and viscosity were evaluated systematically throughout processing and reconstitution (i.e., post-homogenisation, spray-drying and reconstitution). Stability of spray-dried powders was examined in relation to RH.
6.2 Materials and Methods

6.2.1 Materials

Intact whey protein concentrate (80% protein w/w) and hydrolysed whey protein concentrate (80% protein w/w) with a DH value of 12 % were purchased from Carbery Ingredients Ltd. (Ballineen, Co. Cork, Ireland). According to the supplier, molecular weight profile of WPH had an average molecular weight of 5.84 kDa, with more than 70% being > 5 kDa. Edible-grade α-lactose monohydrate was obtained from Glanbia Ingredients (Ballyragget, Co. Kilkenny, Ireland), sunflower oil was purchased from Trilby Trading (Drogheda, Co. Louth, Ireland) and SMP (consisting of intact casein and whey, 80:20 ratio) was purchased from Dairygold Food Ingredients (Mitchelstown, Co. Cork, Ireland).

6.2.2 Preparation of IMF powders

Emulsions consisting of 11.8 g / 100 g lactose, 2.5 g / 100 g protein (whey: casein 60:40) and 5.7 g / 100 g fat were prepared (20 g / 100 g total solids). Batches (15 kg) were produced as follows; lactose powder was dissolved in preheated water (~70 °C), using a Silverson L4RT (Silverson Machines Ltd., Waterside, Chesham, Bucks, England) mixer to aid reconstitution. Approximately 10% of the total fat was added to the batch to reduce foaming prior to addition of protein. SMP was then added slowly, followed by whey protein, before addition of the remaining fat. The batches were tempered at 60 °C and adjusted to pH 6.9 by adding 2M KOH and kept under high shear for 30 min to ensure complete hydration of the protein. The feed was subjected to heat treatment (100 °C x 30 s) using a Microthermics (Model 25HV; North Carolina, USA) tubular heat-exchanger. The coarse emulsion was then homogenised using a LAB 60 homogeniser (APV, Lübeck, Germany) using a first-stage pressure of 13.6 MPa and a second-stage pressure of 3.4 MPa. It was subsequently spray-dried in a pilot-scale Anhydro spray dryer (Model Plant No. 3 type I KA, Copenhagen, Denmark), equipped with a two-fluid nozzle atomization system (Type 1/8 JAC 316ss) and counter-flow
drying. Dryer inlet temperature was held constant at 185 °C and outlet temperature was 80 °C.

6.2.3 Emulsion fat globule size and powder particle size

Emulsion FGS was measured post-homogenisation and after reconstitution of powder using dynamic light scattering (Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The optical parameters used were refractive indices of 1.46 and 1.33 for sample and dispersant, respectively, and particle absorbance of 0.001. Product was reconstituted (12.5g/100g) at ~40 °C.

Powder particle size was determined by laser light scattering using a Malvern Mastersizer 3000 with the Aero S dry powder feeder unit. The powder sample was added to the standard venturi disperser with a hopper gap of 4 mm and then fed into the dispersion system at a feed rate of 18 - 25% to keep the laser obscuration level at 1 - 6%. Compressed air at 0.5 bar was used to transport and suspend the powder particles through the optical cell and a measurement time of 10 s was used. Background measurements were made using air for 20 s.

6.2.4 Viscosity

Viscosity for each formulation, pre- and post-homogenisation, was measured at 55 °C. Reconstituted product at 12.5 g / 100 g was measured at 20 °C using an AR-G2 controlled stress rheometer (TA Instruments, Crawley, UK), equipped with concentric cylinder geometry in shear rate sweep mode. Samples were pre-sheared at 500 s\(^{-1}\) for 1 min, followed by equilibration for 2 min. An ascending shear rate sweep was then applied from 5 to 500 s\(^{-1}\) over 3 min, followed by holding at 500 s\(^{-1}\) for 1 min. The average apparent viscosity measured at 500 s\(^{-1}\) was used for comparison of the formulations.
6.2.5 Emulsion stability

A LUMiSizer® stability analyser (L.U.M. GmbH, Berlin, Germany) was used to measure the separation rate (creaming rate) of hydrolysed and intact formula at 25 °C. Polycarbonate sample cells were loaded with the reconstituted product (0.4 mL aliquots at 12.5 g / 100 g) and centrifuged (285 x g) for approximately 7.2 h, simulating approximately 3 months of separation under normal gravity. Separation rates were determined using the software package SepView 4.1 (L.U.M GmbH). The velocity of separation of individual particles under normal gravity conditions (mm/day) was calculated from the measurement results.

6.2.6 Powder characterisation

Water content of powders was determined using a halogen rapid moisture analyser (HR-83 Halogen, Mettler Toledo, Switzerland). The samples were dried at a temperature of 105 °C until a constant weight was attained (< 1 mg change over 140 s, equivalent to ± 0.025%). Water activity (aw) was measured with a Novasina LabMaster aw water activity meter (Novatron Scientific Ltd., West Sussex, UK). Protein content (N x 6.38) was determined by macro-Kjeldahl (IDF, 2001). Fat and lactose contents were estimated from composition of ingredients. Ash content was determined after overnight incineration at 550 °C. Tapped bulk density was measured by GEA Niro (2006a) method, and surface free fat level of powders was determined by GEA Niro (2005) method, as described in detail in by Kelly et al. (2014). Microencapsulation efficiency (ME) was calculated by Equation 6.1:

\[
ME = \frac{Total\ oil - Extractable\ oil}{Total\ oil} \times 100
\]

(6.1)

Chemical analysis of powders was carried out in duplicate immediately after manufacture, in duplicate.
6.2.7 Water sorption isotherms

Water sorption isotherms were determined gravimetrically as described by (Kelly et al., 2015) using a dynamic vapour sorption (DVS) technique. Dried samples (~ 30 mg) were loaded into the sample pan and were humidified from 0 to 90% RH in 10% RH increments. Equilibrium was considered to be reached when change in mass with time \( (\frac{dm}{dt}) \) was < 0.001 mg/min for at least 10 min for each step. DVS Data Analysis Suite, which runs with a Microsoft Excel add-on (Microsoft Office Excel, 2003), was used to graph and analyse data.

The Guggenheim-Anderson de Boer (GAB) equation (Van den Berg, 1984) was used to model water sorption isotherms and to determine the critical water content and water activity \( (a_w) \). The GAB model isotherm, with constants \( C \) and \( K \), Equation 6.2, was converted to a second-order polynomial (Bizot, 1983), giving a quadratic equation (Equation 6.3).

\[
\frac{m}{m_m} = \frac{CKa_w}{(1-Ka_w)(1+Ka_w(C-1))} \tag{6.2}
\]

where \( m \) is the moisture content \( (g / 100 \text{ g dry solids}) \), \( m_m \) is the monolayer value \( (g / 100 \text{ g}) \), \( a_w \) is water activity, and \( C \) and \( K \) are constants, \( K \) having units inverse to \( a_w \) and \( C \) being dimensionless.

\[
\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma \tag{6.3}
\]
Values for the parameters, $\alpha$, $\beta$, $\gamma$ were determined by quadratic regression analysis of $\frac{a_{w}}{m}$ as a function of $a_{w}$ using DVS data, e.g., over a range of $a_{w}$ from 0 to 0.4. The solution to equations 6.2 and 6.3 give the values for $m_{m}$, $K$ and $C$ as;

$$m_{m} = \frac{1}{\sqrt{\beta^2 - 4\alpha \gamma}}$$  \hspace{1cm} (6.4)

$$K = \frac{\beta - \left(\frac{1}{m_{m}}\right)}{-2\gamma}$$  \hspace{1cm} (6.5)

$$C = \frac{1}{m_{m} K \gamma}$$  \hspace{1cm} (6.6)

6.2.8 Differential scanning calorimetry (DSC)

Powders were stored at ~ 22 °C in vacuum desiccators containing $P_{2}O_{5}$ (0% RH) for 14 days. Samples were then placed in vacuum desiccators containing LiCl, CH$_3$COOK, MgCl$_2$, K$_2$CO$_3$, Mg(NO$_3$)$_2$, giving RH values of 11.4%, 23.1%, 33.2%, 44.1%, and 54.4%, respectively, with $a_{w}$ as 0.01 x %RH. A differential scanning calorimeter (DSC Q2000, TA Instruments, Crawley, UK) was used to determine the glass transition temperature ($T_g$). Samples (8 – 12 mg) were scanned in hermetically sealed aluminium pans while subjected to heating from 0 to 100 °C at 5 °C / min, followed by cooling to 0 °C at 10 °C / min, and then heated at 5 °C / min from 0 to 160 °C. Using the TA Universal Analysis software, the onset ($T_{g \text{ onset}}$) and midpoint ($T_{g \text{ mid}}$) glass transition temperatures were determined. An empty aluminium pan was used as a reference. The DSC was calibrated by means of indium standards and dry nitrogen (50 mL / min) was the purge gas.
6.2.9 Scanning electron microscopy (SEM)

Powders were stored at 0% and 54.4% RH prior to SEM observation. Samples were imaged using a field emission scanning electron microscope (FE-SEM; Zeiss Supra Gemini, Darmstadt, Germany) at 2.00 kV. They were mounted on double-sided carbon tape, attached to scanning electron microscope (SEM) stubs, and then sputter-coated with chromium (Emitech K550X, Ashford, UK). Representative micrographs were taken at 1000x and 5000x magnification in order to visualize surface morphology.

6.3 Statistical analysis

Formulation manufacture and spray drying trials were carried out in triplicate, with trials for each replicate being carried out in random order, as generated by Design Expert Version 7.1.6 (Stat Ease, USA). One-way analysis of variance (ANOVA) was used with Minitab 15 (Minitab Ltd., Coventry, UK) to determine significant differences between both powders by Fisher’s one-way multiple comparison test. Results were deemed statistically significant if \( P < 0.05 \).

6.4 Results and Discussion

6.4.1 Emulsion FGS and Viscosity

During processing, emulsion FGS was monitored post-homogenisation (Table 6.1). HF had significantly (\( P < 0.05 \)) higher FGS (D\([4,3]\), and D\([v,0.9]\)) compared to IF, reflecting an observed bimodal size distribution (Figure 6.1). For HF, homogenisation did not reduce particle size to below the desired mean FGS of < 1 µm (Sheu and Rosenberg, 1995), which has negative implications for emulsion stability. FGS for HF was higher because hydrolysed proteins, due to their numerous short peptides, are not as efficient as emulsifiers and have reduced stabilising properties compared to intact proteins (Agboola and Dalgleish, 1996). The main difficulties arising from using hydrolysed proteins are; (i) they are less likely to adsorb to the fat globule surface due to their poor hydrophobicity; (ii) the least hydrophobic peptides are likely to be the most charged in solution, resulting in reduced charge of oil droplets; and (iii) if the
peptides are short, then steric stabilisation is less likely (Singh and Dalgleish, 1998). However, Drapala et al (2016) showed that WPH emulsions can be produced with improved thermal stability, without changes in FGS, by conjugation of WPH with carbohydrate (maltodextrin), due to increased steric and electrostatic repulsion. Emulsifiers such as lecithin and mono-glycerides are commonly added to formulae with hydrolysed protein to aid emulsification and hence reduce FGS during homogenisation (Drapala et al., 2015).

In comparing apparent viscosity of formulations pre- and post-homogenisation, no significant difference ($P > 0.05$) was observed for IF, a significant ($P < 0.05$) increase in viscosity was found in HF post-homogenisation (Table 6.1). In this study, HF post-homogenisation emulsions, which have higher FGS compared to IF, have higher viscosity than IF emulsions. This is consistent other studies in which emulsions with reduced FGS have been shown to have reduced viscosity (Floury et al., 2000, Maher et al., 2014). Another possible reason for increased FGS of HF is that it has a higher mineral content, which has been shown to cause increased protein aggregation (Figure 6.1) and, thus, increased viscosity (Barbut, 1995, Murphy et al., 2015). Singh and Dalgleish (1998) showed that increasing DH (8 – 45%) in hydrolysed whey proteins resulted in increased aggregation, with the most stable emulsions being similar to the DH content of the hydrolysed protein used in the current study.
Table 6.1 Fat globule size post-homogenisation and post-reconstitution (12.5 g / 100 g), apparent viscosity pre- and post-homogenisation and post-reconstitution and creaming rate and layer of model infant formula containing intact (IF) and hydrolysed (HF) whey protein

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fat globule size</th>
<th></th>
<th></th>
<th>Viscosity</th>
<th></th>
<th></th>
<th>Creaming rate</th>
<th>Cream layer height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post- homogenisation</td>
<td>Reconst</td>
<td>Pre- homogenisation</td>
<td>Post- homogenisation</td>
<td>Reconst</td>
<td>Accept</td>
<td>Recon</td>
<td>mm / day</td>
</tr>
<tr>
<td></td>
<td>(D_{v,0.1})</td>
<td>(D[4,3])</td>
<td>(D_{v,0.9})</td>
<td>(D_{v,0.1})</td>
<td>(D[4,3])</td>
<td>(D_{v,0.9})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>0.30 ± 0.04(^a)</td>
<td>0.75 ± 0.13(^aC)</td>
<td>1.42 ± 0.30(^a)</td>
<td>0.11 ± 0.03(^b)</td>
<td>0.64 ± 0.06(^a)</td>
<td>1.46 ± 0.14(^a)</td>
<td>2.97 ± 0.07(^a)</td>
<td>3.02 ± 0.03(^a)</td>
</tr>
<tr>
<td>HF</td>
<td>1.13 ± 0.79(^a)</td>
<td>32.3 ± 15.1(^bC)</td>
<td>64.9 ± 33.4(^a)</td>
<td>0.14 ± 0.03(^b)</td>
<td>1.31 ± 0.24(^b)</td>
<td>3.38 ± 0.82(^b)</td>
<td>3.16 ± 0.08(^b)</td>
<td>3.85 ± 0.36(^b)</td>
</tr>
</tbody>
</table>

Results are shown as mean ± standard deviation for fat globule size and viscosity (n = 3) and for creaming rate and cream layer height (n = 2).

(\(^a\)-\(^b\)) Values within a column not sharing a common lower-case letter differ significantly (\(P < 0.05\)).

(\(^A\)-\(^C\)) For fat globule size (\(D[4,3]\)) and viscosity (pre, post and recon) values of parameters with the same units, not sharing a common upper-case letter differ significantly (\(P < 0.05\)).

Creaming rate corrected to normal gravity (1 x g)

\(^1\) Post-reconstitution
Figure 6.1 Fat globule size distribution profile of emulsions formulated with intact (••••) and hydrolysed whey (○○○○) protein, post-homogenisation (A) and on reconstitution (B). The y-axis gives % of total distribution per size interval (18 size intervals per decade).

6.4.2 Powder reconstitution properties

Powders were reconstituted to 12.5 g / 100 g prior to analysis, to reflect solids content in a typical reconstituted infant formula. No significant difference ($P > 0.05$) was found between the reconstituted D[4,3] and the post-homogenisation D[4,3] for IF (Table 6.1), showing that atomisation had no effect on FGS. For HF, the reconstituted D[4,3] was lower than that of the post-homogenisation D[4,3], indicating that a further
homogenisation effect occurred during atomisation, producing a mono-modal particle size distribution (Table 6.1) (graph not shown). This is a desirable result as a reconstituted powder with high FGS would separate rapidly upon rehydration. A reduction in FGS after atomisation can occur with 2-fluid-nozzle atomisation due to turbulence generated by the high shear forces between the liquid surface and high velocity air. McCarthy et al. (2012), in a study using the same atomiser type (2-fluid nozzle) observed significant FGS reduction for model infant formula. In the present study, the reduction in FGS (from a high initial value of 32.3 to 1.31 µm) may be related to greater flexibility/weaker interfacial layer dominated by peptides as opposed to intact proteins, and it may also be that atomisation is more effective at reducing the size of large fat globules. Viscosity of reconstituted IF and HF powders were not significantly different (P > 0.05). A small decrease in viscosity observed in HF on reconstitution in comparison to the post-homogenisation measurement, resulting in the reconstituted emulsions having similar viscosity. Again, this slight reduction in viscosity may be due to the decreased FGS. These minor effects are due to a combination of total solids (TS), temperature, and FGS; noting that analysis of viscosity pre-homogenisation was carried out at a higher temperature and TS (55 °C and 20%) compared to reconstituted product (20 °C and 12.5% TS). With regard to the lack of large differences in viscosity at different steps in the experiment, it should be noted that Dinkov et al (2008) found that the viscosity of whole milk increased from ~ 2.0 mPas at 20%/50°C to ~2.5 mPas at 11.2%/20°C. Thus, in this study, it can be inferred that the effect of decreasing total solids (reducing viscosity) largely outweighed the effect of a lower temperature (increasing viscosity).

Differences in emulsion stability were observed in the separation rate (creaming rate and cream layer height as measured by analytical centrifugation) of reconstituted powders in an accelerated storage test over 7.5 h, which simulates 3 months storage under gravity. The creaming rate and cream layer height were significantly (P < 0.05) higher for HF emulsion than IF, indicative of lower storage stability (Lajoie et al., 2001). This is consistent with Stokes’ law where particles of greater diameter separate from the continuous phase at a faster rate than smaller particles. Achieving a FGS < 1 µm has been identified as an important target for increasing emulsion stability and
reducing free fat level in powders (Hogan et al., 2001, Sheu and Rosenberg, 1995). FGS of 0.64 \( \mu \text{m} \) was observed for IF; however, this was significantly (\( P < 0.05 \)) greater for HF, with a value of 1.31 \( \mu \text{m} \). When using hydrolysed proteins, FGS could potentially be reduced through the use of different processing conditions e.g., the use of emulsifiers and higher homogenising pressure and passes (Danviriyakul et al., 2002, Keogh et al., 2006).

### 6.4.3 Powder properties

Both powders had similar protein, lactose and fat contents. Moisture content was 2.2 and 1.84 g / 100 g, for IF and HF, respectively, following drying (Table 6.2). A significantly (\( P > 0.05 \)) lower \( a_w \) of 0.15 was observed for HF compared to an \( a_w \) of 0.19 for IF. The ash content of HF was significantly (\( P < 0.05 \)) higher than IF, reflecting the higher mineral composition in hydrolysed whey starting material. Mean powder particle size, \( D[4,3] \), and tapped bulk densities of powders were not significantly (\( P > 0.05 \)) different (Table 6.3).

#### Table 6.2 Composition of model infant formula powders containing intact (IF) or hydrolysed (HF) whey protein

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein</th>
<th>Lactose</th>
<th>Fat</th>
<th>Moisture</th>
<th>Ash</th>
<th>( a_w )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g / 100 g</td>
<td>g / 100 g</td>
<td>g / 100 g</td>
<td>g / 100 g</td>
<td>g / 100 g</td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>12.4 ± 0.27(^a)</td>
<td>56.8 ± 0.14(^a)</td>
<td>27.6 ± 0.07(^a)</td>
<td>2.20 ± 0.23(^a)</td>
<td>1.86 ± 0.03(^b)</td>
<td>0.19 ± 0.00(^a)</td>
</tr>
<tr>
<td>HF</td>
<td>12.2 ± 0.18(^b)</td>
<td>57.0 ± 0.16(^a)</td>
<td>27.7 ± 0.08(^a)</td>
<td>1.84 ± 0.27(^a)</td>
<td>2.15 ± 0.05(^a)</td>
<td>0.15 ± 0.00(^b)</td>
</tr>
</tbody>
</table>

\(^a,b\) Values within a column not sharing a common letter differ significantly (\( P < 0.05 \)).

Generally, increasing viscosity gives rise to larger powder particles (Hogan et al., 2001); however, in this study the difference in viscosity was not enough to have a significant effect (Table 6.1 and Table 6.3). Solvent-extractable free fat (SFF) content of powders was significantly (\( P < 0.05 \)) lower in IF compared to HF powder (Table 6.3). It is suggested that this is due to the higher emulsification capacity of intact whey protein. Also, FGS for reconstituted IF was smaller than that for HF (Table 6.1), meaning that
the smaller oil droplets formed during spray-drying are more efficiently embedded within the powder wall matrix, and thus less likely to be extracted by solvent during testing (Maher et al., 2015). A significantly lower FGS was achieved when HF was reconstituted, suggesting that atomisation decreases FGS significantly, however FGS was still significantly larger compared to reconstituted IF. The effect of atomisation, may have led to rupture of the fat globule, however, inefficient emulsification occurred, leading to a higher SFF. Consequently, this had a positive impact on microencapsulation efficiency (ME), which was significantly higher \((P < 0.05)\) in IF (Table 6.3).

**Table 6.3** Physical properties of model infant formula powders containing intact (IF) and hydrolysed (HF) whey protein

<table>
<thead>
<tr>
<th>Sample</th>
<th>(D(v,0.1))</th>
<th>(D[4,3])</th>
<th>(D(v,0.9))</th>
<th>(\rho_{tapped})</th>
<th>Free fat</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu m)</td>
<td>(g / ml)</td>
<td>(g / 100 g)</td>
<td>powder</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>17.1 ± 3.04(^a)</td>
<td>64.9 ± 9.45(^a)</td>
<td>128 ± 34.03(^a)</td>
<td>0.32 ± 0.01(^a)</td>
<td>3.10(^b)</td>
<td>88.7(^a)</td>
</tr>
<tr>
<td>HF</td>
<td>16.6 ± 1.55(^a)</td>
<td>68.0 ± 24.2(^a)</td>
<td>161 ± 95.96(^a)</td>
<td>0.33 ± 0.01(^a)</td>
<td>5.82(^a)</td>
<td>79.0(^b)</td>
</tr>
</tbody>
</table>

\(\rho_{tapped}\) = tapped bulk density

\(^{a-b}\) Values within a column not sharing a common letter differ significantly \((P < 0.05)\).
It is desirable to maximise ME so that surface free fat is minimised in powders which increases their hydrophobicity, reduces solubility in water and promotes lipid oxidation (Písecký, 1997). There are suggestions in the literature as to how this might be achieved, e.g., involving altering molecular size, processing conditions, emulsifiers and protein source (Kelly et al., 2014, Keogh et al., 2006, Singh and Dalgleish, 1998). Singh and Dalgleish (1998) reported that hydrolysed whey protein with an average molecular mass of 0.52 kDa and a DH of 20% was sufficient to produce a mono-disperse emulsion with a D[4,3] <1 μm, with the current study using an average molecular weight of 5.84 kDa. The homogenisation mechanisms used in the Singh and Dalgleish (1998) study were different from ours. In the present study, a 2-stage valve homogeniser was used, whereas Singh and Dalgleish (1998) used a microfluidiser through which the emulsion was passed multiple times. In this context it should be noted also that DH is a crude predictor of hydrolysis profile, i.e., very different MW profiles may occur for the same DH which would result in different emulsification properties.

6.4.4 Differential scanning calorimetry

As expected, the \( T_g \) and \( T_{cr} \) values of powders (Table 6.4) decreased with increasing \( a_w \), due to the increased plasticization effects with increasing water content (Haque and Roos, 2004, Omar and Roos, 2007). \( T_g \) was not significantly (\( P > 0.05 \)) different for HF compared to IF, except at \( a_w = 0.23 \) (Table 6.4).

Powders with low \( T_g \) are more likely to present problems with sticking or caking. Stickiness occurs at a critical viscosity of \( 10^7 \) Pa.s (Bellows and King, 1973, Downton et al., 1982) at temperatures of 10 – 20 °C above \( T_g \) (Roos and Karel, 1991). Therefore, the temperature of the powder surface during spray-drying should be 10 – 20 °C below \( T_g \) to prevent stickiness. Netto et al. (1998) and Zhou et al. (2014) reported that moisture-induced decreases in \( T_g \) of lactose-free, hydrolysed protein powders are greater than that for non-hydrolysed powders. Although such findings suggest that hydrolysis of proteins increases their sensitivity to the plasticizing effects of moisture, presumably due to the greater mobility of shorter peptide chains, the \( T_g \) values
reported were for pure protein powders rather than the mixed protein/lactose/fat systems of the present study.

Molecular weight is related to \( T_g \), with a low molecular weight decreasing \( T_g \) value (Levine and Slade, 1986). Glass transition occurs at higher temperatures in proteins than in disaccharide sugars, as the plasticizing effects of moisture and heat are less pronounced in large macromolecules, with the effect that material relaxation and viscoelastic change are diminished in the presence of proteins (Hogan et al., 2010). Lactose, which was present at a constant level (~57 g / 100 g), was likely the dominant influence on \( T_g \), rather than the relatively minor contribution of proteins. Zhou and Roos (2011) showed that delays in crystallization were dependent on protein type, but that \( T_g \) was not significantly affected, either by type or molecular weight of proteins. Netto et al. (1998) and Mounsey et al. (2012) reported that hydrolysed casein did not significantly lower \( T_g \) in different hydrolysed protein powders. In the current study, \( T_g \) did not differ significantly \((P > 0.05)\) between model infant formula powders with intact and hydrolysed whey (IF and HF, respectively). It would appear that, at the protein/peptide to lactose ratio examined, it is lactose, rather than proteins, that determine the glass transition behaviour (Bhandari and Howes, 1999). A recent study of \( T_g \) in IMF examined selectively hydrolysed proteins and concluded that \( T_g \) was not affected by hydrolysis (Murphy et al., 2015).

In the range of \( a_w \) levels of 0.1 to 0.33, \( T_{cr} \) was not significantly lower for IF compared to HF (Table 6.4). At an \( a_w \) of 0.44, \( T_{cr} \) was considerably lower for IF than for HF, which is consistent with DVS results in Figure 6.2, from which IF was seen to crystallise much more readily than HF. Hydrolysed whey has been shown to delay crystallisation in lactose/protein dispersions (Hogan and O’Callaghan, 2013); however, hydrolysed casein was shown to have no effect on crystallisation (Mounsey et al., 2012). It is also noted that there was a larger fat globule size (FGS) in HF post-homogenisation compared to the IF emulsion. The smaller fat globules in IF post-homogenisation may have provided more surface area for adsorption of protein, thus reducing the concentration of protein in the continuous phase, and thus the protein to lactose ratio, and consequently the lactose molecules would be freer to rearrange and crystallise upon heating (Maher et al., 2014).
Table 6.4 Glass transition ($T_g$) and crystallization temperatures ($T_{cr}$) of intact (IF) and hydrolysed (HF) whey model infant formulas humidified at different water activities (Mean values ± standard deviation for triplicate samples)

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>$T_g$ onset°C</th>
<th>$T_g$ mid°C</th>
<th>$T_{cr}$ onset°C</th>
<th>$T_{cr}$ peak°C</th>
<th>$T_g$ onset°C</th>
<th>$T_g$ mid°C</th>
<th>$T_{cr}$ onset°C</th>
<th>$T_{cr}$ peak°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>62.7± 2.5$^a$</td>
<td>67.6 ± 2.5$^b$</td>
<td>121.0 ± 2.0$^b$</td>
<td>124.0 ± 1.7$^b$</td>
<td>68.6 ± 2.9$^a$</td>
<td>74.5 ± 2.8$^a$</td>
<td>131 ± 5.3$^a$</td>
<td>136 ± 6.1$^a$</td>
</tr>
<tr>
<td>0.11</td>
<td>59.5 ± 2.2$^a$</td>
<td>64.3 ± 2.1$^a$</td>
<td>119.0 ± 1.7$^a$</td>
<td>122.0 ± 1.7$^a$</td>
<td>61.1 ± 1.7$^a$</td>
<td>66.9 ± 2.2$^a$</td>
<td>125 ± 5.0$^a$</td>
<td>129 ± 5.9$^a$</td>
</tr>
<tr>
<td>0.23</td>
<td>52.4 ± 1.1$^a$</td>
<td>57.5 ± 1.5$^a$</td>
<td>110.0 ± 2.8$^a$</td>
<td>112.0 ± 3.2$^a$</td>
<td>48.8 ± 0.2$^b$</td>
<td>54.1 ± 0.5$^b$</td>
<td>110 ± 4.7$^a$</td>
<td>114 ± 6.0$^a$</td>
</tr>
<tr>
<td>0.33</td>
<td>36.5 ± 0.4$^a$</td>
<td>39.7 ± 0.2$^a$</td>
<td>91.9 ± 3.5$^a$</td>
<td>93.3 ± 3.4$^a$</td>
<td>36.7 ± 0.6$^a$</td>
<td>40.0 ± 0.7$^a$</td>
<td>91.4 ± 1.2$^a$</td>
<td>95.4 ± 1.3$^a$</td>
</tr>
<tr>
<td>0.44</td>
<td>14.2 ± 0.7$^a$</td>
<td>18.5 ± 0.7$^b$</td>
<td>55.4 ± 3.0$^b$</td>
<td>56.2 ± 2.9$^b$</td>
<td>20.0 ± 1.6$^a$</td>
<td>23.0 ± 1.4$^a$</td>
<td>73.1 ± 3.9$^a$</td>
<td>75.3 ± 3.6$^a$</td>
</tr>
</tbody>
</table>

$^{(a,b)}$ Letters are used to compare different powders (HF and IF), i.e., values for a single parameter (onset, mid-point or peak) not sharing a common letter within a row (HF versus IF) differ significantly ($P < 0.05$)
6.4.5 Sorption isotherms

Powder composition and environmental RH play an important role in glass transition and lactose crystallisation behaviour of amorphous powders. These, in turn, directly affect functional characteristics such as flowability, stickiness, caking and storage stability (Shrestha et al., 2008). Respective sorption isotherms (0 - 90 % RH) of model infant formulas, containing intact and hydrolysed whey, are shown in Figure 6.2 (IF and HF), showing different water sorption characteristics. As fat does not absorb water the results are presented on a solids-non-fat (SNF) basis (Kelly et al., 2014).

A number of distinct stages can be observed on the isotherms. In both formulas, sorption of water from 0 – 30% RH, i.e., below T$_g$, occurred at a rate of 1.3 and 1.1 g / 100 g per 10% increase in RH for IF and HF, respectively. Maher et al. (2015) and Burnett et al. (2006) suggested that this occurs without change in structure and is mostly due to surface adsorption. It was postulated that HF would absorb more moisture during the initial stages of water sorption; Hogan and O’Callaghan (2013) and Mounsey et al. (2012) have shown that, as % DH increases, there is also a concomitant increase in moisture sorption in the lower RH range, due to more available sites for moisture molecules to associate with; however, these studies only showed sorption isotherms for non-fat dispersions containing protein and lactose.
Figure 6.2 Moisture sorption isotherms for powders with intact (●) and hydrolysed whey (○) protein. Powder moisture is expressed on a solids-non-fat basis.
At 30-40 % RH, there was an increase in the rate of moisture sorption to 3.15 and 3.7 g / 100 g, for IF and HF, respectively, indicative of water moving into the bulk of the powder as it approaches a glass transition region, prior to crystallisation. The dramatic influence of humidity on glass transition, even at ambient temperatures, is confirmed by Table 6.4, showing $T_g$ approaching 25°C at RH between 33% and 44% RH for powders. Crystallisation develops at temperatures above $T_g$ as amorphous materials relax to their more thermodynamically stable state.

Crystallization humidity ($R_{fc}$) is apparent when there is a sudden decrease in mass at a certain RH (Burnett et al., 2004, Kelly et al., 2014), which occurred for both powders upon humidification (0-90 %RH). $R_{fc}$ was higher for HF compared to IF (Figure 6.2), at 70% and 60% RH, respectively. This delay in crystallization in HF powder in relation to RH is also confirmed by DSC results (Table 6.4), this trend was also observed in Chapter 5, where crystallization temperatures are higher for HF at any RH. Examination of the DVS data in terms of time (min), showed HF equilibrated and crystallized at a faster rate in comparison to IF, ~ 440 min and ~ 980 min, respectively (data not shown). This means that HF powder is more stable and less likely to crystallize during storage.

A reduction in mass of sorbed water from ~ 10.2 to ~ 2.9 g / 100 g and 14.2 to 6.1 g / 100 g due to crystallization of amorphous lactose was observed for IF and HF, respectively (Figure 6.2). Post crystallization, the uptake in water increased to ~ 13.4 and ~ 16.6 g / 100 g at 90% RH for IF and HF, respectively. Maximum water sorption occurred more slowly for IF than for HF (~ 1465 min and ~ 439 min); this significant difference in equilibration time may be explained by the increase in hydrophilic charged peptides and amino acids due to hydrolysis, leading to rapid sorption of water molecules in HF powder (Mahmoud et al., 1992). Water diffusion increases in polar hydrophilic matrices (Palzer, 2010) and has been shown to increase with increasing water activity up to the point of lactose crystallization in skim and whole milk powder (Murrieta-Pazos et al., 2011) and hydrolysed protein/lactose dispersions (Hogan and O’Callaghan, 2013, Mounsey et al., 2012).
The GAB monolayer ($m_m$) values were 1.5 and 1.95 g water / 100 g solids corresponding to 15.8 and 18.4 % RH for HF and IF powders, respectively (Table 6.5).

**Table 6.5** Guggenheim-Anderson-de Boer (GAB) isotherm constants C, K, and monolayer value ($m_m$) of model infant formula powders containing intact (IF) and hydrolysed (HF) whey protein powders

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>K</th>
<th>C</th>
<th>$m_m$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF</td>
<td>-0.71</td>
<td>0.25</td>
<td>0.07</td>
<td>1.87</td>
<td>4.22</td>
<td>2.04</td>
<td>0.97</td>
</tr>
<tr>
<td>HF</td>
<td>-1.04</td>
<td>0.37</td>
<td>0.07</td>
<td>2.00</td>
<td>5.00</td>
<td>1.48</td>
<td>0.99</td>
</tr>
</tbody>
</table>

$a_w$, range of all powders was 0.1-0.4 for all experimental data; four data points were taken for all powders. The terms C, K, and $m_m$ (g water / 100 g dry weight) are derived from constants $\alpha$, $\beta$, and $\gamma$.

*Root mean square error.*
6.4.6 Scanning electron microscopy
Powders were imaged at two levels of RH (Figure 6.3), to show the effect of RH on powder surface morphology. At 0% RH (before lactose crystallisation), IF and HF powders appeared similar in shape and structure and had a smooth surface (Figure 6.3; A and E). Powders examined at a higher magnification showed similarities in wall structure (Figure 6.3; B and F). In contrast, both powders stored at 54.4% RH (post lactose crystallisation) were noticeably rougher, possibly due to protruding lactose crystals (Figure 6.3; C and G). At higher magnification, it was apparent that HF had a noticeably rougher surface in comparison to IF, suggesting that low molecular mass amorphous proteins are susceptible to plasticisation and deformation (Netto et al., 1998). It is proposed that the high proportion of peptides < 1 kDa contributed significantly to the material behaviour of these powders.
Figure 6.3 Scanning electron microscopy (SEM) images of IF (A and B, pre-crystallization, low and high magnification; C and D, post-crystallization, low and high magnification) and HF (E and F, pre-crystallization, low and high magnification; G and H, post-crystallization, low and high magnification). Scale bar indicates 10 µm for low magnification and 2 µm for high magnification.
6.5 Conclusions

Model IMF powders were successfully produced using intact and hydrolysed whey protein ingredients with no added emulsifiers. The composition of model IMF emulsions had a significant effect on their physical stability throughout processing. By changing whey protein source from intact to hydrolysed whey, emulsions had larger fat globule sizes and were less stable to creaming. Spray-dried HF had higher free fat and FGS in reconstituted emulsion. Large FGS had negative impacts on emulsion stability, with creaming rate and cream layer height being significantly ($P < 0.05$) higher for HF than IF. A lower RH$_c$ was observed for IF, which is indicative of a higher susceptibility to crystallisation, conducive to a shorter shelf-life compared to HF. $T_g$ was affected primarily by lactose level rather than protein type. This research has shown that the use of hydrolysed whey protein in IMF reduces the emulsion stability, but increases the storage stability in terms of lactose crystallisation of the spray-dried emulsion. However, higher free fat associated with HF powder would lead to increased lipid oxidation and reduced flowability.
6.6 References


Chapter 7: Overall Discussion
7.1 Influence of protein content on physical and dissolution properties

In the first part of this thesis (Chapter 2 and 3), a series of commonly used defatted dairy powder ingredients, namely MPC powders, over a wide range of protein levels (35.4-85.6 g / 100 g) were examined. In the experimental design, as protein content increased there was concomitant decrease in lactose. This allowed for examination of changing powder composition (increasing protein/ decreasing lactose) on surface characteristics and physico-chemical properties. Factors such as surface morphology and chemical composition were examined by SEM and XPS, respectively.

Surface morphology of MPC powders changed as protein content increased, with low protein powder (MPC35) found to be wrinkled and becoming smoother and dimpled as protein content increased to > 60 g / 100 g protein (MPC 60). This was expected, as low protein powders (MPC35) showed similar surface morphology to SMP examined in the literature (Kim, Chen, & Pearce, 2003), considering that SMP and MPC35 have similar composition. The change in surface morphology as protein content increased has been linked to the difference in diffusivity of molecules, (see Chapter 1; Section 1.3.2).

The surface coverage of protein exceeded the bulk percentage of protein in all cases, with a concomitant increase in protein on the surface as protein content of powder increased. From XPS, it could be deduced that casein was the dominant protein on the surface due to the high mineral content on the surface associated with casein, i.e., Ca and P, and also from the hypothesis that the soluble fraction of milk migrated from the surface to the core.

\( T_{gr} \) increased with increasing protein content, possibly due to interactions between large protein molecules and lactose, and, as % RH increased, there was a decrease in \( T_{gr} \). Sorption isotherm of powders showed that MPC35 displayed lactose crystallisation, whereas all other powders did not show crystallisation and increased in mass as % RH increased. Higher protein powders have a lower concentration of lactose, which reduces the tendency to powder crystallisation during storage. Lactose crystallisation during storage is a negative attribute of powders as it results in increased free fat levels (McCarthy, et al., 2013) and caking in powders.
Complete rehydration and solubilisation of these MPCs was also examined in Chapter 3. It was found that an elevated reconstitution temperature (50 °C) was needed to solubilise high protein MPC powders. Dairy protein fractions have different solubility, with whey proteins being found to be more soluble than caseins; the casein fraction may cause a decrease in solubility by forming a hydrophobic skin layer.

A technique was developed using static light scattering (SLS) to monitor the solubilisation of powder particles during rehydration. Data was then classified according to size to describe the rate of rehydration.

Defatted dairy powders such as SMP, MPC and MPI have been examined in the literature; however, such powders were typically either studied in isolation with no comparison of different milk sources, or from different manufacturing companies. These two chapters (Chapter 2 & 3) examined the effect of increasing protein content on MPC powders from a common milk source and were produced under constant spray-drying conditions.

Recently, there has been emphasis on powder surface characterisation as determined by XPS and SEM; this section, therefore, examined these powder characteristics using these techniques. It can be summarised that increasing protein content resulted in a changed powder surface morphology, with potential implications for texture and flow properties. Fat was over-represented on the surface of all MPC powders, with the previous authors citing the presence of surface fat having links to decreased flowability. Chapter 3 has added to the literature as it evaluated the rehydration and solubilisation properties of MPC, using a novel technique to characterise particle size and then subsequently classifying these sizes into groups.

7.2 Effect of formulation and processing conditions on physical properties of powders

Oil type (PO or SO) had no significant effect on the principal physical characteristics of fat-filled dairy-based powders, i.e., bulk density, particle size, water sorption isotherm characteristics, moisture and $a_w$. Likewise, oil type did not affect fat globule size of the emulsion or of the reconstituted formula (Chapter 4). Thus, while the oil type, PO or SO, affected viscosity of the emulsion, this effect did not impact on physical
characteristics of the powders. Oil type also did not influence glass transition or water sorption characteristics. Conversely, outlet temperature influenced each of the parameters tested, except for fat globule size in the reconstituted product. Free fat level increased, as expected, with increasing outlet temperature, but was not influenced significantly by oil type used in the emulsion. This work has shown that the use of fat blends did not impact significantly on physical properties of sodium caseinate stabilised emulsions/spray-dried powders.

### 7.3 Effect of protein type on water diffusivity within a powder formulation

As with two previous chapters (Chapters 2 & 3), Chapter 5 also examined protein/lactose dispersions, but investigated the effects of including hydrolysed whey protein in the formulations. The casein: whey ratio in this chapter was altered to reflect that of an infant formula (60: 40; whey: casein), rather than the naturally occurring ratio of that in bovine milk (20: 80, whey: casein). Chapter 5 examined the effect of including a hydrolysed whey protein, with a DH of 12%, on surface and physico-chemical properties. A DH of 12% was chosen, as it was known to have good emulsification capacity, which was relevant to the study in Chapter 6. The processing conditions were kept constant in producing the powders in this chapter. Sorption isotherm results showed that inclusion of hydrolysed whey protein caused lactose to crystallise at higher humidity than powders with intact whey protein, showing that the presence of hydrolysed whey protein interferes with the crystallisation of lactose.

A technique was developed to determine the diffusivity of moisture in the powders using DVS data together with particle size distribution data, applying Fick’s second law (Equation 5.11; Section 5.2.8). It was found that a higher diffusivity was associated with the hydrolysed whey protein, which can be associated with the higher amount of available sites for moisture to bind to, reflecting the lower molecular weight peptides. The delay in crystallisation may be due to increased competition for moisture between the hydrolysed whey protein and lactose.

A visual investigation of the effects of water vapour sorption on surface morphology was then examined. SEM showed surface morphology of powders pre- and post-crystallisation by exposure of powders to 0 or 54.4 % RH. At an elevated %
RH, powder surface was seen to dramatically change; there were observable differences in powders containing intact whey protein to that containing hydrolysed whey protein. From SEM images of powders before and after lactose crystallisation, powders containing hydrolysed whey displayed a more agglomerated structure in comparison to powders containing intact whey dispersion after crystallization. The higher water diffusivity of hydrolysed whey-protein-based powders can lead to powder handling and quality issues related to caking, stickiness and flowability in humid environments. Consequently, these issues need to be considered in the design of packaging and conditions of storage for these powders.

The diffusivity of moisture through powder particles has implications on storability of powders. Dairy powders of different formulation have been studied before (SMP and WMP), to examine the diffusivity of moisture; however, the effect of hydrolysed whey protein was never examined. Also, this chapter added to the literature as it did not assume one particle size but rather included the particle size distribution for calculations of moisture diffusivity through powders (Chapter 5).

7.4 Effect of protein type on surface morphology and physical properties of a model infant formula

Chapter 6 examined the effect of hydrolysed whey protein within a model IMF powder, in comparison to an IMF powder made with intact whey protein. Following changing whey protein source from intact to hydrolysed whey, emulsions had larger fat globule sizes and were less stable to creaming. Spray-dried powder with hydrolysed whey had higher free fat and FGS in reconstituted emulsion. Large FGS had negative impacts on emulsion stability, with creaming rate and cream layer height being significantly ($P < 0.05$) higher for powder containing hydrolysed whey protein compared to intact whey protein.

Very different surface morphology were obtained after storage at elevated RH for model IMF containing hydrolysed whey having a rougher and agglomerated surface. Also, during storage, a lower RH$_c$ was observed for IMF with intact whey protein, which is indicative of a higher susceptibility to crystallisation, conducive to a shorter shelf-life as compared to powder containing hydrolysed whey. This research
has shown that the use of hydrolysed whey protein in IMF reduces emulsion stability, but increases storage stability, in terms of susceptibility to lactose crystallisation of the spray-dried emulsion.

7.5 Overall conclusions

Overall, the research presented in this thesis explored the effects of changing powder composition and processing conditions on the surface characteristics, functional properties and storage stability of dairy powders. For MPC powders, it was shown that increasing protein concentration, in parallel with reducing lactose concentration, resulted in increased storage stability in terms of delays in lactose crystallisation meaning powders can withstand higher humidity environments without being structurally deformed. These higher protein powders also take more time to rehydrate; therefore, it is important to know the optimum conditions (temperature, time, agitation rate etc.) at which proteins are fully solubilised. This work has shown that the casein fraction of MPC requires a higher temperature for solubilisation than the whey fraction.

Fat globule size was significantly higher in reconstituted model IMF incorporating hydrolysed whey protein than in model IMF with intact whey protein, resulting in reduced emulsion stability and the undesirable consequence of higher free fat content in powders. Free fat in IMF is organoleptically unappealing to consumers. The atomisation process during spray-drying resulted in a decrease in FGS in IMF containing hydrolysed whey protein, meaning that emulsifiers, e.g., lecithin or monoglycerides were not required to produce reasonably stable emulsions. Hydrolysed whey protein IMF powders were more stable to lactose crystallisation due to more binding sites for water associated with the lower molecular weight of the whey protein peptides.

In conclusion, this research has provided theoretical and practical implications relevant to powder formulation and processing on powder surface characteristics and physico-chemical properties. The research is particularly relevant to the IMF industry, in which MPC powders and hydrolysed proteins are used.
7.6 Future research

Some suitable follow-up studies to the work presented in this thesis include:

- Varying total solid content of mixes before spray-drying to examine the effect of total solids on surface characteristics and storage properties of powders;

- Changing the ratio of specific protein fractions within a formulation, i.e., to be closer in composition to that of human breast milk. This could potentially be achieved by altering the whey protein fraction, α-lactalbumin, initially in isolation and then with a combination of other protein fractions.

- Using statistical mixture design software to vary protein and lactose content, keeping to codex regulations while optimising physico-chemical properties of IMF. This could be achieved by varying protein and lactose as found in commercial IMF powders. Increase protein content (1.3 to 1.6 g/ 100g) and decrease lactose (7 to 6 g / 100g) as stage in IMF increases.

- Study in more detail the physical and surface characteristics of powders during storage and how this affects their functionality.
7.7 References


