Loss of solubility of α-lactalbumin and β-lactoglobulin during the spray-drying of whey proteins

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***Abstract*:**

A reversed phase HPLC technique (at pH 4.6) has been developed to measure the loss of solubility of α-lactalbumin and β-lactoglobulin resulting from the spray drying of whey protein isolate solution. Spray drying was performed in a pilot-scale co-current spray dryer with different feed concentrations (20 - 40% w/v) and outlet temperatures (60°C to 120°C). The study reveals that the solubility of both α-lactalbumin and β-lactoglobulin was not significantly affected at low outlet gas temperatures (60-80°C), but was strongly affected (up to 40%) at high temperatures (100-120°C). Significantly higher losses in solubility were observed for β-lactoglobulin compared to α-lactalbumin. Increasing the feed concentration at higher outlet temperatures also caused noticeable increases in insolubility. The reversed phase HPLC results were consistent with those from total protein nitrogen content (Kjeldhal) analysis.

Keywords: reversed phase HPLC, feed concentration, outlet temperature, denaturation, aggregation, milk.

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1. INTRODUCTION

Whey proteins are commonly used in food and formulated pharmaceutical products. There are two major whey proteins namely, α-lactalbumin and β-lactoglobulin. β-lactoglobulin accounts for 50% by mass of whey protein and has a molecular mass of 18 400 Da. It plays an extremely important role in the food industry, as a result of its gelation and emulsification properties (Schokker, Singh and Creamer, 2000; Anema, Stockman and Lowe, 2005; Anandharamakrishnan, Raghavendra, Barhate, Hanumesh and Raghavarao, 2005). α-lactalbumin is the second major globular whey protein (constituting 20% of the whey protein mass) which has a smaller molecular mass (14 200 Da) and can bind with Ca^{2+} ions. The biochemical and physicochemical properties of both these proteins have been extensively studied and their primary, secondary, tertiary and quaternary structures are well documented. The minor whey proteins include bovine serum albumin and various immunoglobulins.

The denaturation of whey proteins involves the disruption of protein structure. A change in conditions, such as temperature, unfolds the three-dimensional structure which allows cross-linking interactions to be made between protein molecules such as, protein-protein hydrophobic, electrostatic, hydrogen-bonding and disulfide-sulfhydryl interactions. These interactions result in aggregation, coagulation and finally precipitation (Creighton, 1992; Pelegrine and Gasparetto, 2005; Terebiznik, Buera and Pilosof, 1997). In the case of α-lactalbumin an increase (above 9.0) or decrease (below 4.0) in pH value results in the dissociation of Ca^{2+} ions from the molecule which destabilises the molecular configuration and thus leads to denaturation (Boye, Alli and Ismail, 1997). There are numerous studies of the effects of thermal treatments on the

denaturation of whey proteins during the heating of milk (Dannenberg & Kessler, 1988; Anema, 2000 & 2001; Oldfield, Singh, Taylor and Pearce, 2000; Ferreira, Mendes and Ferreira, 2001).

Solubility is an important property for the functional behaviour of whey proteins. Soluble proteins impart emulsion, foam, gelation and whipping properties; a decrease in solubility affects the protein functionality (Pelegrine and Gasparetto, 2005). The solubility depends on whether the proteins are in their native or denatured state but denaturation alone is not enough to cause a measurable loss of solubility, as the proteins must also aggregate. Aggregation is heavily influenced by pH as it affects the net charge on the protein molecule, and thus the electrostatic repulsive forces between molecules. At the isoelectric point (pH of 4.6) the net charge is zero, electrostatic repulsion forces are at a minimum and aggregation is easiest. The greater the deviation of pH from the isoelectric point, the greater are the repulsive forces and the less likely it is that aggregation will occur (Pelegrine and Gasparetto, 2005).

Protein solubility can be deduced from measurements of the concentration of proteins in a dissolved liquid phase in relation to the total amount of protein (both dissolved and undissolved) in a sample, such as used in the Kjeldahl total nitrogen method. A large number of chromatography methods are available to quantify protein concentrations in solution such as ion-exchange chromatography, gel electrophoresis, gel filtration chromatography and reversed phase high performance liquid chromatography (RP-HPLC). RP-HPLC is the principal technique for the quantitative composition analysis of protein mixtures, as the separation is based on hydrophobic interactions, which are the main forces that stabilize the three-dimensional structure of proteins (Ferreira, Mendes and Ferreira, 2001). Thus further denaturation does not occur

during analysis. However, the RP-HPLC method has not (to our knowledge) been used to study to solubilities of individual whey proteins in powdered product. We have thus used his technique to study the solubility of the individual whey proteins α-lactalbumin and β-lactoglobulin (using the Kjeldahl method as a check on the total protein solubility) in reconstituted powder that has been produced by spray drying.

Spray drying is a well established method for converting liquid feed materials into a dry powder form. During the spray drying process, the liquid feed is atomised and contacted with hot gas. Evaporation takes place to yield dried particles, which are subsequently separated from the gas stream by a variety of methods (Masters, 1991). Currently, spray drying is the preferred method for producing whey proteins in powdered form. However, spray drying can cause thermal denaturation of these proteins (Daemen and van der Stege, 1982).

It is well known that co-current is preferred to counter-current operation for protein based systems, as high rates of evaporative cooling from the wet droplet are able to maintain droplet temperatures far below those of the hot inlet gas. In contrast, in a counter-current operation the dry powder exiting the chamber will closely approach the inlet gas temperature (Masters, 1991). In a true co-current configuration the particle temperature will only rise as far as the gas outlet temperature, and this provides a better guide to the temperatures experienced by the particle than the inlet gas temperature. The gas outlet temperature is thus a better predictor of thermally-induced product degradation than gas inlet temperature, which explains, for example, why high outlet temperatures have been found to increase inactivation of enzymes (alkaline phosphatase, rennin and α-amylase), but the correlation with inlet gas

temperature is weak (Daemen and van der Stege,1982; Samborska, Witrowa-Rajchert and Gonçalves, 2005).

The effect of process variables on the solubility of whey proteins during spray drying has been largely overlooked. One exception is Guyomarc'h, Warin, Muir and Leaver (2000) who found that denaturation of whey proteins takes place mainly during the pasteurisation stage, whereas denaturation occurs only to a small extent during spray drying at 160-190°C inlet and 65-90°C outlet air temperatures. Similar findings were reported more recently by Oldfield, Taylor and Singh (2005), who observed that varying the inlet and outlet gas temperatures (from 160 to 200 °C and 89 to 101 °C, respectively) did not significantly affect the amount of whey protein denaturation. They also found that most of the whey proteins had already been denatured in the previous stage in which the skimmed milk was preheated at 70°C to 120°C for 52 s, prior to evaporation and spray drying.

In this study we wish to use the RP-HPLC techniques to examine the effects of spray drying variables on the solubilities of individual whey proteins (α-lactalbumin and β-lactoglobulin) in spray dried product, using a wider spray drying temperature range (outlet temperatures 60°C to 120°C) than previously used and also varying the feed concentration (from 20% to 40% solids), which has not been previously reported. The Kjeldahl total nitrogen method will be used to check the overall protein solubility data.

2. MATERIALS AND METHODS

2.1. Whey Protein Solution Preparation

Whey protein isolate (WPI) powder was obtained from Ultimate Nutrition (Fleetwood, Lancashire, UK) with the following composition (per 100 g of dry powder): protein 92 g, carbohydrate 3 g, fat 3 g, fibre 1 g, lecithin 400 mg and calcium 200 mg. WPI powder is produced by microfiltration followed by spray drying, and the manufacturer claims that 99% of the whey proteins are undenatured and that the powder is lactose free. A nominal 20% (w/v) whey protein solution was prepared at room temperature by dissolving 400 g of powder in 1.5 litre of distilled water. This mixture was gently stirred in a laboratory mixer (Silverson) for 10 minutes to dissolve all the whey proteins and finally made up to 2 litres by addition of distilled water. The mixture was kept for a consistent period of 30 minutes before spray drying for the proteins to hydrate. The same procedure was followed for the nominal 30% and 40% (w/v) concentrations, using 600 g and 800 g of WPI powder respectively. The WPI powder has a moisture content of approximately 5% (see Table 1), and so the actual feed concentrations are slightly below the nominal values and are thus approximately 19%, 28.5% and 38% respectively.

2.2. Spray Drying

A tall-form co-current spray drier of 11 ft height x 3 ft diameter (Spray Processes, Bedford UK) was employed for the spray drying process. A peristaltic pump (Watson-Marlow 510U) was used to deliver the whey protein solution to the atomiser. The atomisation was performed by a twin-fluid nozzle, using compressed air at 45 psig as the atomising gas. The flow velocity of this air was measured at the inlet to the burner using a rotary vane anemometer

(Airflow LCA 6000VT, Airflow Developments Ltd, High Wycombe, UK). This was then used to calculate the air flowrate into the dryer (along with a small correction for additional air supplied to the combustion jets which was assumed to be in the stoichiometric ratio to the amount of natural gas burned). It was found that the gas flow rate was approximately constant at 227 kg/hr for all experimental conditions. Ambient air was directly heated in a burner using natural gas, allowing control of the inlet air temperature. The operation was started by feeding distilled water and the outlet temperatures were set by adjusting the liquid feed and air flow rate. Once the required outlet temperature was reached, the whey protein solution was fed into the drying chamber. The different feed concentrations of whey protein solution were spray dried over a range of gas inlet and outlet temperatures. The outlet temperature was effectively regulated by variation of the liquid feed flow rate. Four different outlet temperatures of 60°C, 80°C, 100°C and 120°C were used for each feed concentration. It was found that it was difficult to cover the whole range of gas outlet temperatures with a fixed gas inlet temperature. Instead the inlet temperature was set 100°C higher than the outlet temperature in each case with the exception of the 120°C outlet temperature for which an inlet temperature of 250°C was used. The measured liquid feed flow rates, inlet and outlet gas temperatures are given in Table 1. The particles were separated by a cyclone and collected in a receiving vessel. The final products were sealed immediately in glass bottles and stored in a refrigerator for later analysis. A second trial was run to assess repeatability of results and to confirm the observed trends of moisture content and loss of solubility with feed concentration. Three experiments (using an 80°C outlet temperature and each of the three feed concentrations) were repeated in trial 2, using the same operating conditions as

in trial 1, but with a different batch of WPI (Table 1). Similar trends and results were observed for both trials. Pooled standard errors for the percent loss of solubility over the three concentrations were calculated as 2.3 for α-lactalbumin and 1.5 for β-lactoglobulin.

2.3. Moisture Content

The average moisture content (wet basis) of the spray dried powder was measured gravimetrically. A known mass of sample (approximately 0.5 g) was placed in a glass sample bottle and dried in a vacuum oven at 105 ± 2 °C for a period of 24 hours. The sample was then removed and immediately weighed to limit water absorption from the atmosphere. The initial and final weights were then used to calculate the wet basis moisture content.

2.4. RP-HPLC analysis of whey proteins

The extent of protein denaturation can also be estimated from the loss of solubility at its isoelectric point (Fachin and Viotto, 2005). The proteins are more soluble at low pH (acidic) or high pH (alkaline) values as the molecules are charged and repel each other. When the pH approaches the isoelectric point (pI) the protein charges are progressively neutralised, reducing the repulsive forces which in turn allows protein aggregation to occur (Pelegrine and Gasparetto, 2005).

The amounts of native α-lactalbumin and β-lactoglobulin in the soluble fraction at pH 4.6 (whey protein isoelectric point) were determined by using reversed phase HPLC, based on the method described by Parris and Baginskl (1991), Ferreira, Mendes and Ferreira (2001) and Ferreira and Cacote (2003). A sample of 500 mg of spray dried whey protein was accurately weighed into a

100 ml beaker and a small amount of ultra-pure Milli-Q water was added to form a smooth paste. A further 40 ml water was then added to the paste. The beaker was placed on a magnetic stirrer and agitated vigorously. The dispersion was adjusted to pH 4.6 (the whey protein isoelectric point) with 0.1 M HCl solution and gently stirred for 30 min. The pH of the dispersion was monitored and maintained at 4.6 throughout the stirring period. The dispersion was then transferred to a 50 ml volumetric flask, diluted to the 50 ml mark with the addition of water and mixed by shaking. The dispersion was then centrifuged for 30 min at 20,000g and the resulting supernatant fraction was filtered through Whatman No.1 filter paper. The filtrate was diluted in water (1:1). Prior to RP-HPLC analysis, all samples (1.5 ml) were centrifuged in a microfuge at 15,000g for 3 minutes to remove any insoluble material.

An analytical HPLC (Agilent 1100) unit equipped with a binary pump was used for the HPLC analysis. A 20µl sample was auto-injected into a polymeric reversed phase column containing a polystyrene-divinylbenzene copolymer-based packing (column length 250 mm; column diameter 4.1 mm; particle size 10µm, pore size 10nm). Gradient elution was carried out with a mixture of two solvents. Eluant A contained 0.1% trifluoroacetic acid (TFA) with 99.9% water; eluant B contained 0.1% trifluoroacetic acid (TFA), 19.9% water and 80% acetonitrile. The elution gradient was set as follows: 0-20 min 36-56% B; 20-30 min 56-60% B; 30-35 min 60-36% B; and, 3 min for column re-equilibration. The flow rate was 0.5 ml/min and the column temperature was maintained at 45°C. Concentrations were determined from the absorbance at a wavelength of 215 nm.

The whey proteins were identified by means of the retention time and peaks were quantified by comparing peak areas with the results of a calibration

series with pure native standards (supplied by Sigma Chemical Co.) for bovine α-lactalbumin in the range of 0.375 to 3 mg/ml and bovine β-lactoglobulin in the range of 0.65 to 5 mg/ml. The experiments were carried out in triplicate for each sample and average values were taken to calculate the loss of solubility from the following equation:

$$\%\,\text{loss of solubility} = \left(1 - \frac{SP}{SP_u}\right) \times 100 \quad (1)$$

where SP and SP_U are is the masses of soluble protein per unit mass of spray dried powder and untreated sample respectively. These values were normalised to dry powder equivalents using the measured moisture content.

2.5. Total protein solubility analysis

As a check on the RP-HPLC, solubility analysis, the total loss of whey protein (α-lactalbumin and β-lactoglobulin) solubility at pH 4.6, was determined following the procedure described by Morr *et al*. (1985). The soluble protein content of the supernatant was determined by a standard Kjeldahl method, using a nitrogen conversion factor of 6.38. The experiments were carried out in duplicate for each sample and average values were taken to calculate the solubility at pH 4.6 from the following equations:

$$Solubility = \frac{Mass\ of\ \ Soluble\ \ Nitrogen}{Total\ \ Nitrogen\ in\ Sample} \quad (2)$$

Thus the loss of solubility was calculated as:

$$\%\,\text{loss of solubility} = \left(1 - \frac{S}{S_u}\right) \times 100 \quad (3)$$

where S is the solubility of the spray dried sample and S_u is the solubility of the untreated sample.

2.6 Particle size

Volume mean particle sizes of the product powders were determined by a Coulter LS 130 (Coulter Corporation, USA) laser sizer, which measures particle sizes in the range of 0.4–800 μm using laser light scattering. Each sample was dispersed in a solvent (isobutanol) to perform the measurements. Reported values are the average of three independent measurements.

3. RESULTS

The main operating variables in the spray drying experiments were the feed concentration, the feed rate, the gas flow rate, the atomisation pressure, and the inlet and outlet temperatures. The full set of experimental operating conditions are presented in Table 1. The results for each measurement variable are given in the following subsections.

3.1. Moisture content

The values of average moisture contents (kg water / kg total) of the spray-dried powders are displayed in Table 1. The results reveal that increasing the outlet gas temperature (and inlet temperature) reduces the moisture content of the final products for all the feed concentrations. The same trend was shown in other published reports (Etzel, Suen, Halverson and Budijono, 1996; Samborska, Witrowa-Rajchert and Gonçalves, 2005). The driest product was found with initial feed concentrations of 30%.

3.2. RP-HPLC analysis of whey proteins

The RP-HPLC conditions were optimised for elution gradient, sample size, concentration and flow rate. The chromatographic system was calibrated

by the external standard method, with pure native standards for bovine α-lactalbumin and bovine β-lactoglobulin. Fig.1 shows the chromatographic patterns of the α-lactalbumin, β-lactoglobulin and a mixture of these two standards. The proteins were separated with retention times of (i) ~20 min for α-lactalbumin; (ii) ~24 and ~25 min (two peaks) for β-lactoglobulin B (β-lgB) and β-lactoglobulin A (β-lgA), respectively. The same trend of separation is observed in other reports (*e.g.* Elgar, Norris, Ayers, Pritchard, Otter and Palmano, 2000; Ferreira, Mendes and Ferreira (2001); Ferreira and Cocote (2003). The spray dried whey protein samples also exhibited similar retention times to the α-lactalbumin and β-lactoglobulin native standards. An example of a chromatogram of spray dried whey protein at 20% (w/v) feed concentration and at different outlet temperatures is compared with the untreated sample in Fig 2. The chromatogram of the untreated sample (feed solution) shows only two major peaks (α-lactalbumin and β-lactoglobulin) and thus indicates that other minor whey protein were rendered insoluble previously during the process. The decrease in the peak area in the RP-HPLC results shown in Fig. 2, for the native whey proteins (α-lactalbumin and β-lactoglobulin), indicates a decrease in solubility at the higher outlet temperatures, even when the feed flow rate remained approximately constant (which was the case for the 100 and 120 ^{o}C outlet temperatures – see Table 1).

3.3. Effects of outlet temperature and feed concentration on solubility of whey proteins

Table 1 represents the amount of soluble native α-lactalbumin and β-lactoglobulin present in the spray dried product, as determined from the RP-HPLC method. The loss of solubility of α-lactalbumin and β-lactoglobulin at

different outlet temperatures is plotted in Figs. 3 (a-c). The greatest loss in solubility of both α-lactalbumin and β-lactoglobulin is observed at the higher gas outlet temperatures (100 °C and 120 °C), so the spray drying operation has resulted in a significant amount of denaturation (Figs. 3 a-c). Greater losses in solubility were found for β-lactoglobulin compared to α-lactalbumin. In contrast, the lower outlet temperatures (60°C and 80°C) had less effect on protein insolubility.

The loss of solubility of α-lactalbumin and β-lactoglobulin at different feed concentrations is plotted in Figs. 4 (a-b). This study reveals that the loss of solubility of both α-lactalbumin and β-lactoglobulin noticeably increases (by about 10%) with these increase in liquid feed concentration at the higher outlet gas temperatures (100 and 120 °C) but, at lower temperatures this effect is difficult to distinguish.

3.4. Total protein solubility

Figure 5 shows the results obtained for the loss of total protein solubility at pH 4.6, using (a) the sum of the α- lactalbumin and β- lactoglobulin results from RP-HPLC analysis and (b) based on their total nitrogen content, from the Kjeldahl method. The two graphs are in broad agreement.

3.5 Particle size

The average volume mean particle diameters over all four outlet temperatures was 17.2 μm, 1.6 μm and 9.6 μm for nominal feed concentrations of 20%, 30% and 40% respectively.

4. DISCUSSION AND CONCLUSIONS

The similarity of the results for RP-HPLC and total nitrogen in Figure 5 gives confidence to the RP-HPLC method of analysing protein solubility. The RP-HPLC method also has distinct advantages in that it is much quicker, more straightforward and can resolve individual proteins. The experiments reported here show that β-lactoglobulin consistently suffers a greater loss in solubility than α-lactalbumin during these spray drying experiments. α-lactalbumin is reported to be the more heat stable whey protein (Ferreira, Mendes and Ferreira, 2001), as it is the only milk protein which binds with the Ca^{2+} ion (present in the whey used in these experiments) and this binding increases the stability of α-lactalbumin at higher processing temperatures (Permyakov and Berliner, 2000). Dissociation of the Ca^{2+} ion is indeed one of the steps in the denaturation process of α-lactalbumin (Boye, Alli and Ismail, 1997). Furthermore, it has been found that α-lactalbumin does not aggregate by itself, but relies on forming aggregates with β-lactoglobulin (Schokker, Singh and Creamer, 2000).

The strongest influence on solubility is undoubtedly temperature. High outlet (120°C) and inlet gas (250°C) temperatures produce very significant losses of the order of 40%; it is the outlet conditions that provide the better indication of the temperatures experienced by the droplet during spray drying (Oldfield, Taylor and Singh, 2005). The published literature indicates that the degree of denaturation of proteins increases with increasing temperature and holding time (Dannenberg and Kessler, 1988; Law and Leaver, 1997). Oldfield, Taylor and Singh (2005) found that the rates of denaturation (measured by a gel electrophoresis method) of both α-lactalbumin and β-lactoglobulin substantially increased over the range 80-120°C during the preheating of skim milk. They

also found that spray drying between 89°C and 101°C produced only small amounts of denaturation. As denaturation is a prerequisite for solubility loss, these results are in agreement with the trends found here.

Noticeable variations in solubility were found when the inlet feed concentration was varied. In general, feed concentrations of 40% produced the greatest loss in solubility. Two factors may be causing this: (i) a direct effect of concentration on the loss of solubility and/or (ii) an indirect effect from droplets of different initial concentrations undergoing different temperature histories during drying.

A direct effect of concentration on loss of solubility could either be caused by an influence on denaturation, or on aggregation. Law and Leaver (1997) found that the rate of “denaturation” of whey protein (based on their loss of solubility at pH 4.6) increased with increasing total whey protein concentration in milk. Kessler, Plock and Beyer (1992) also observed the same trend in heated milk. However, Anema (2001) also found that α-lactalbumin denaturation was unaffected by varying solid concentration from 10 to 39% in skimmed milk during heating over a temperature range of 75 to 100 °C for 15 min. Anema (2000) even found that increased feed concentration had a retardation effect on the denaturation of β-lactoglobulin in reconstituted skim milk, but this may be influenced by the presence of lactose. Protein-bound lactose groups increase the active radius of the β-lactoglobulin molecules and thus leads to a decrease in the thiol-disulfide exchange reaction rate which in turn slows the irreversible denaturation of β-lactoglobulin. The same trend was shown by (McKenna and O’Sullivan, 1971) who found that increasing the concentration of skim milk solids from 9% to 40% during heating at 80°C for 20 min reduced the degree of denaturation.

The two apparently contradictory results can be reconciled by noting that Law and Leaver's in fact measured solubility, and this is influenced by aggregation as well as denaturation. Aggregation, however, is more likely to be affected by concentration, as this process requires neighbouring molecules to collide (Law and Leaver, 1997). The protein molecules are able to aggregate in the denatured state as the hydrophobic and thiol groups are exposed, which were previously buried inside the molecules (Hoffmann and van Mil, 1997). Anema (2000) observed that although β-lactoglobulin denaturation was retarded at higher milk solid concentrations, decreases in solubility were observed.

There may also be a second indirect influence of feed concentration on solubility through its effect on the particle temperature. Upon atomisation, the liquid droplets contact the inlet hot gas and water evaporation takes place rapidly from the droplet surface, cooling the droplets initially to their wet bulb temperature (44-48°C), as well as cooling the surrounding hot gas (Oldfield, Taylor and Singh, 2005). This stage of drying is believed to last only for a relatively short period of time. A crust is then likely to form on the surface of the droplet, which reduces the surface water activity and increases the droplet temperature.

It is likely that the more concentrated feed will form a dry crust more quickly and this will elevate the particle temperature away from the wet-bulb temperature. There is evidence for greater crust formation at 40% feed concentration, as drier product was obtained in experiments with 30% feed concentration. One might expect that the feeds with the highest solids content would produce the driest product, as less moisture removal is required. However, the reverse appears to be true, suggesting that the drying of the 40% feed concentration solutions may somehow be impeded. This could occur if the

crust that forms dries to such an extent that water diffusion is hampered by a severe reduction in diffusivity. The variation in mean diameter may be due to higher liquid feed rates (Masters, 1991) and/or bubble inflation of particles (Etzel, Suen, Halverson and Budijono, 1996). In the case of 40% feed concentration, the formation of a crust may restrict bubble inflation and thus result in smaller particle sizes. Thus, to summarise, the effect of increasing the feed concentration would be to promote crust formation, which would raise particle temperatures and lead to greater levels of denaturation and aggregation.

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REFERENCES

Anandharamakrishnan, C., Raghavendra, S. N., Barhate, R. S., Hanumesh. U & Raghavarao, KSMS. (2005). Aqueous two-phase extraction for recovery of proteins from cheese whey. *Food and Bioproducts Processing,* 83(C3),191-197.

Anema, S. G. (2000). Effects of milk concentration on the irreversible thermal denaturation and disulfide aggregation of β-lactoglobulin. *Journal of Agricultural and Food Chemistry*, 48(9), 4168-4175.

Anema, S. G. (2001). Kinetics of the irreversible thermal denaturation and disulfide aggregation of α-lactalbumin in milk samples of various concentrations. *Journal of Food Science,* 66(1)*,* 2-9.

Anema, S. G., Stockmann, R., & Lowe, E. K. (2005). Denaturation of β-lactoglobulin in pressure-treated skim milk. *Journal of Agricultural and Food Chemistry*, 53(20), 7783-7791.

Boye, J. I., Alli. I., & Ismail, A. A. (1997). Use of differential scanning calorimetry and infrared spectroscopy in the study of thermal and structural stability of α-lactalbumin. *Journal of Agricultural and Food Chemistry*, 45(4), 1116-1125.

Creighton, T. E. 1992. *Protein Folding*. W. H. Freeman and Company: New York.

Daemen, A. L. H., & van der Stege, H. J. (1982). The destruction of enzymes and bacteria during the spray drying of milk and whey. 2. The effect of the drying conditions. *Netherlands Milk Dairy Journal*, 36(3), 211-229.

Dannenberg, F., & Kessler, H. (1988). Reaction kinetics of the denaturation of whey proteins in milk, *Journal of Food Science,* 53(1)*,* 258- 263.

Elgar, D. F., Norris, C. S., Ayers, J. S., Pritchard, M., Otter, D. E., & Palmano, K. P. (2000). Simultaneous separation and quantisation of the major bovine whey protein including proteose peptone and caseinomacroptide by reversed phase high-performance liquid chorography on polystyrene-divinylbenzene. *Journal of Chromatography A*, 878(2)*,* 183-196.

Etzel, M. R., Suen, S, Y., Halverson, S. L., & Budijono,S. (1996). Enzyme inactivation in a droplet forming a bubble during drying. *Journal of Food Engineering,* 27(1),17-34.

Fachin, L., & Viotto, W. H. (2005). Effect of pH and heat treatment of cheese whey on solubility and emulsifying properties of whey protein concentrate produced by ultrafiltration. *International Dairy Journal,* 15(4), 325-332.

Ferreira, I. M. P. L. V. O., Mendes, E., & Ferreira, M. A. (2001). HPLC/UV Analysis of protein in dairy products using a hydrophobic interaction chromatographic column. *Analytical Science*, 17(4), 499-501.

Ferreira, I. M. P. L. V. O., & Cacote, H. (2003). Detection and quantification of bovine, ovine and caprine milk percentage in protected denomination of origin cheese by reversed-phase high-performance liquid chromatography of beta-lactoglobulins. *Journal of Chromatography A*, 1015(1-2), 111-118.

Guyomarc'h, F., Warin, F., Muir, D. D., & Leaver, J. (2000). Lactosylation of milk proteins during the manufacture and storage of skim milk powders. *International Dairy Journal,* 10(12), 863-872.

Hoffmann, M. A. M., & van Mil, P. J. J. M. (1997). Heat-induced aggregation of β-lactoglobulin: Role of free thiol group and disulfide bonds, *Journal of Agricultural and Food Chemistry*, 45(8)*,* 2942-2948.

Kessler, H. G., Plock, J., & Beyer, H. J. (1992). Influence of composition and concentration of milk proteins on whey protein denaturation and gel forming characteristics. *IDF Special Issue, 9303*, 216-226.

Law, A. J. R., & Leaver, J. (1997). Effects of protein concentration on rates of thermal denaturation of whey proteins in milk, *Journal of Agricultural and Food Chemistry*, 45(11)*,* 4255-4261.

Masters, K. (1991). Spray Drying Handbook*,* Longman Scientific and Technical, Harlow.

McKenna, B. M., & O'Sullivan, A. C. (1971). Whey protein denaturation in concentrated skim milks. *Journal of Dairy Science,* 54(7),1074-1077.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A., & Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science,* 50(6), 1715-1719.

Oldfield, D. J., Singh, H., Taylor, M. W., & Pearce, K. N. (2000). Heat-induced interactions of β-lactoglobulin and α-lactalbumin with casein micelle in pH-adjusted skim milk. *International Dairy Journal,* 10(8), 509-518.

Oldfield, D. J., Taylor, M. W., & Singh, H. (2005). Effect of preheating and other process parameters on whey proteins reactions during skim milk powder manufacture. *International Dairy Journal,* 15(5), 501-511.

Parris, N. & Baginski, M. A. (1991). A rapid method for the determination of whey protein denaturation. *Journal of Dairy Science,* 74(1), 58-64.

Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey protein solubility as function of temperature and pH. *Lebensmittel-Wissenschaft und -Technologie*, 38(1), 77-80.

Permyakov, E. A., & Berliner, L. J. (2000). α-lactalbumin: Structure and function. *FEBS Letters*, 473(3)*,* 269-274.

Samborska, K., Witrowa-Rajchert, D., & Gonçalves, A. (2005). Spray-drying of alpha amylase - The effect of process variables on the enzyme inactivation. *Drying Technology,* 23(4), 941-953.

Schokker, E. P., Singh, H., & Creamer, L. K. (2000). Heat-induced aggregation of β-lactoglobulin A and B with α-lactalbumin. *International Dairy Journal,* 10(12)*,* 843-853.

Terebiznik, M. R., Buera, M. P., & Pilosof, A. M. R. (1997). Thermal stability of dehydrated α-amylase in trehalose matrices in relation to its phase transitions. *Lebensmittel-Wissenschaft und -Technologie*, 30(5), 513-518.

Table 1. Experimental conditions used in the spray drying experiments and their effect on moisture content and soluble native whey protein content.

Nominal feed Concentration (w/v)	**Inlet air temperature**** (°C)	**Outlet air temperature**** (°C)	**Liquid feed rate** (kg/hr)	**Moisture Content** (wet basis) (kg water / kg total)	**Individual soluble whey protein content** (g per 100g of dry powder)	
					α-la	**β-lg**
WPI (untreated, Trial1)	–	–	–	5.8	23.5	46.4
WPI (untreated),Trial 2)				5.4	22.5	46.4
20%	161±0.2	60±0.9	11.4	10.6	22.8	45.1
	180±1.6	80±0.4	6.1	10.0	23.2	45.2
	180±0.2 *	80±0.5	6.8	8.1	21.4	44.3
	206±0.3	100±0.2	5.1	7.7	22.2	42.7
	255±1.0	120±1.0	4.7	7.0	17.2	29.9
30%	160±0.9	60±0.6	14.7	16.0	22.7	45.5
	181±0.9	80±0.5	7.3	8.5	23.0	45.4
	180±1.0 *	80±0.4	8.5	6.9	21.3	44.6
	203±0.8	100±0.9	5.4	5.3	21.7	40.4
	250±0.5	120±0.5	4.8	5.0	18.2	29.8
40%	160±0.4	60±0.4	17.5	15.3	22.7	45.0
	184±0.6	80±1.0	9.1	11.4	22.5	43.9
	180±0.1 *	80±0.5	10.1	11.2	21.4	43.1
	202±0.5	100±0.8	5.7	9.0	20.1	33.4
	252±1.5	120±1.0	5.2	7.0	15.2	25.5

Trial 2: Same conditions of first trial, but with different batch of feed material

** Values reported are average values observed, and ± values indicate the observed variation between minimum and maximum values

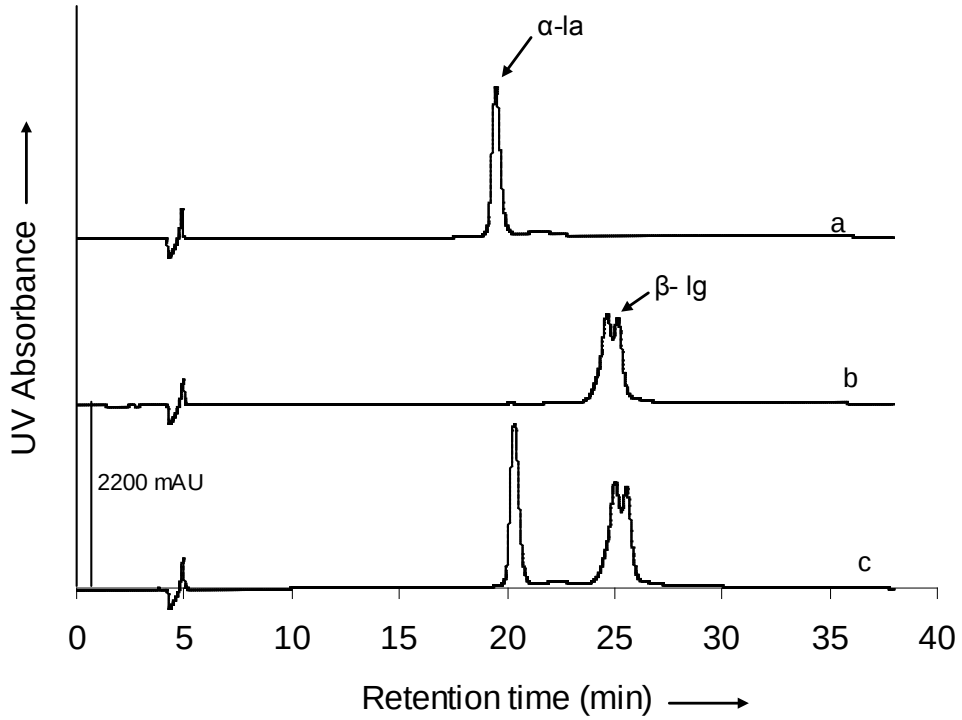


Fig. 1. RP-HPLC chromatograms of whey protein standards. (a). α-lactalbumin (b) β-lactoglobulin and (c) mixed standard (α-lactalbumin and β-lactoglobulin at the ratio of 1:2).

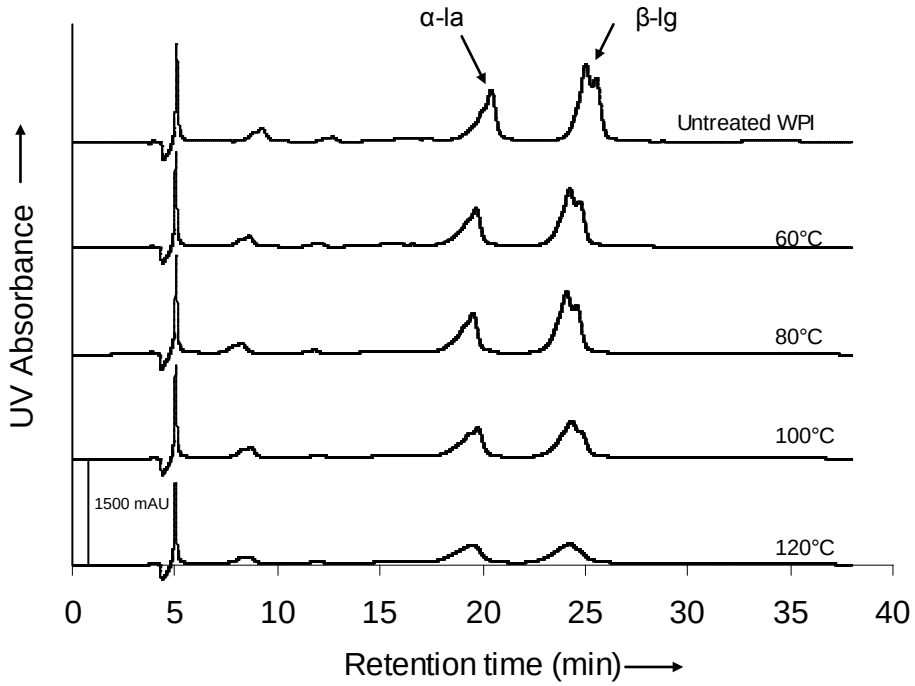
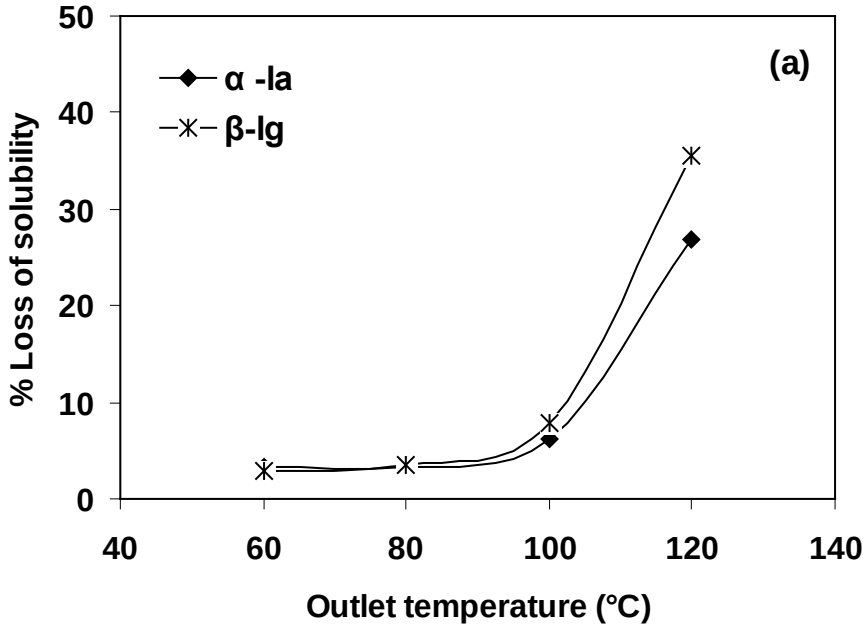
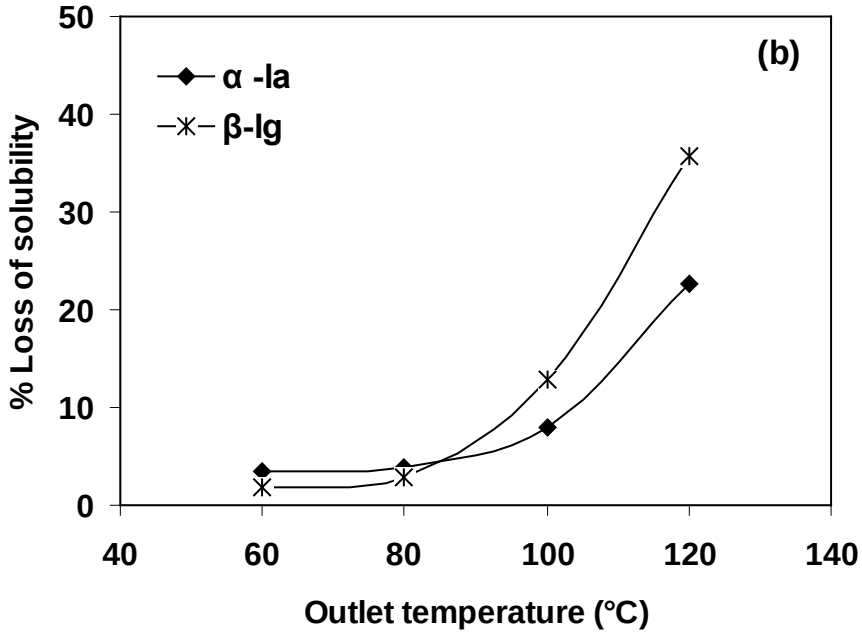


Fig. 2. RP-HPLC Chromatograms of spray dried whey protein powder (20%w/v) feed concentration after spray drying with different outlet temperatures.


(a)
α -la
β-lg
% Loss of solubility
Outlet temperature (°C)
0
10
20
30
40
50
40
60
80
100
120
140

1
2
3
4
5
6


(b)
α -la
β-lg
% Loss of solubility
Outlet temperature (°C)
0
10
20
30
40
50
40
60
80
100
120
140

7
8
9

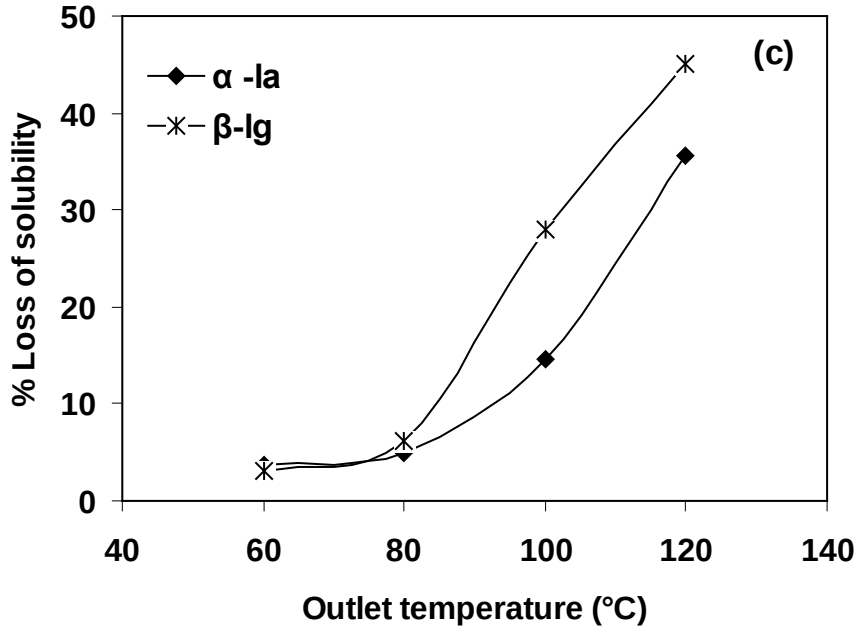
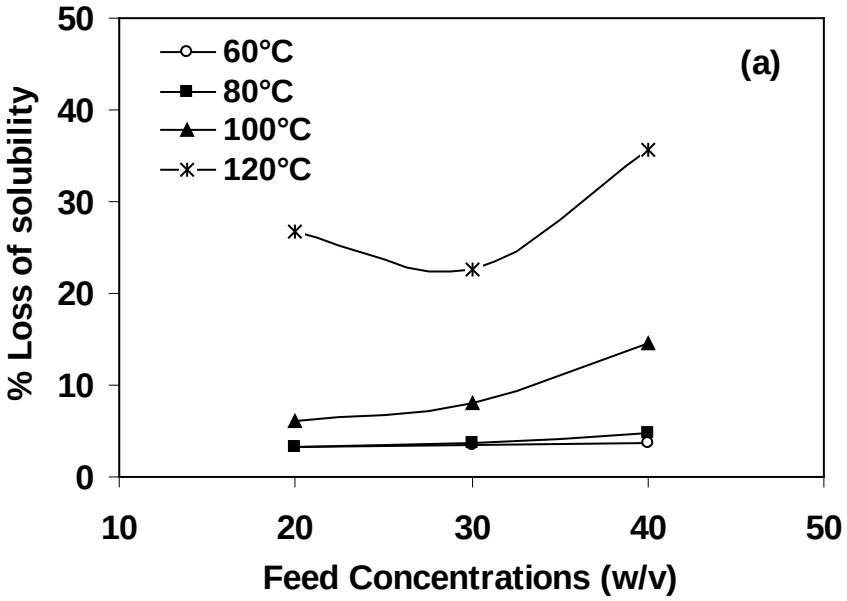


Fig. 3. Effect of spray dryer outlet temperature on the loss of solubility of α-lactalbumin and β-lactoglobulin (a) 20% feed concentration (b) 30% feed concentration (c) 40% feed concentration.



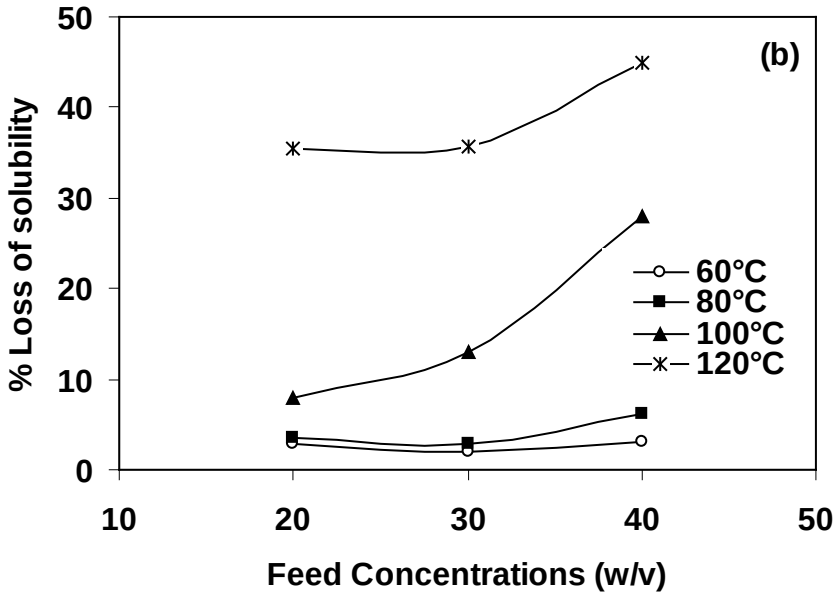
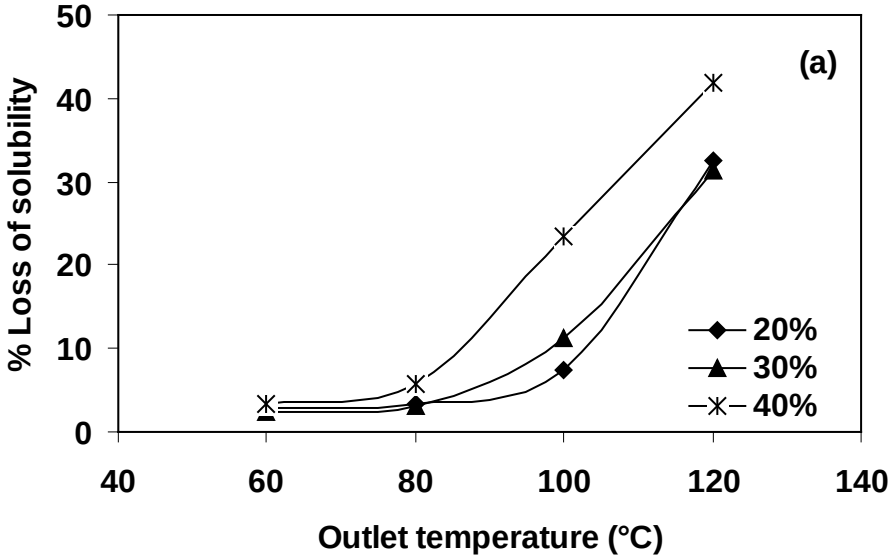


Fig. 4. Effect of spray dryer feed concentration on loss of solubility of the spray dried product. (a) α-lactalbumin and (b) β-lactoglobulin.



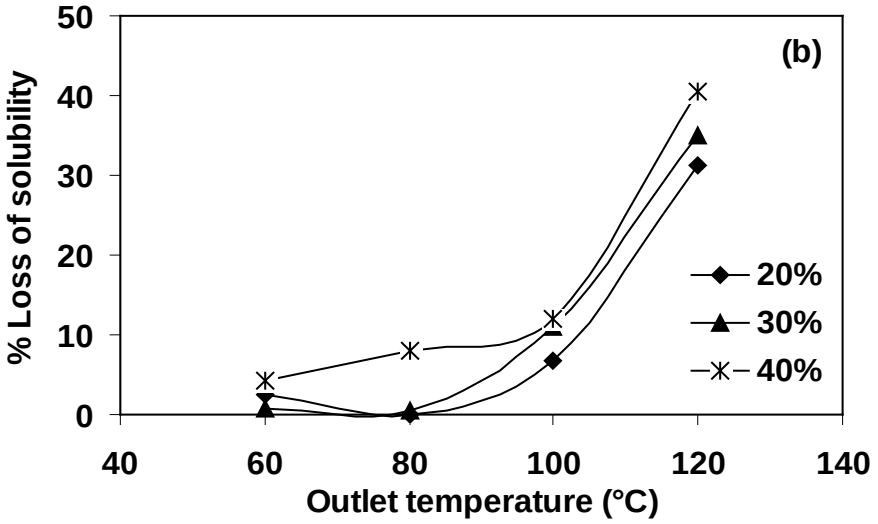


Fig. 5. Effect of spray dryer outlet temperature and feed concentration on the loss of solubility of the total protein (a) combined RP-HPLC analysis, (b) total nitrogen content analysis.