Lactose/ β -Lactoglobulin Interaction During Storage of Model Whey Powders

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ABSTRACT

The objective of this study was to evaluate the presence or absence of interaction between lactose and β -lactoglobulin during storage of model whey powders at different water activities (a_w). Model whey powders were prepared by colyophilization of lactose with increasing quantities of β -lactoglobulin. These colyophilized β -lactoglobulin:lactose powders, assigned as BL powders, were stored from 0.11 to 0.95 a_w . The water sorption behavior of BL powders was studied gravimetrically, and the state of lactose was investigated using differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Before storage, BL powders were amorphous. After storage, a loss of water was observed on moisture sorption isotherms of BL powders. It was related to the formation of lactose crystals, detected by DSC and SEM analysis, and to the structural collapse of the powders. Water loss due to lactose crystallization was shifted to higher a_w with increasing β -lactoglobulin content in BL powders. Moreover, kinetics of moisture sorption demonstrated that β -lactoglobulin was also responsible for a slower crystallization process in BL powders. Then, the water sorption behavior of BL powders was very different from the behavior of the 2 compounds mixed after separate lyophilization. All these results pointed out interaction between lactose and β -lactoglobulin, which appeared during lyophilization and still occurred during storage. This lactose/ β -lactoglobulin interaction stabilized model whey powders against lactose crystallization.

(Key words: β -lactoglobulin, storage, lactose crystallization, co-lyophilization)

Abbreviation key: a_w = water activity, **BL powders** = colyophilized β -LG:lactose powders, **B + L powders** = powders composed of lactose and β -LG lyophilized separately and mixed, **BL(10:90)** = colyophilized β -LG:lac-

tose powder with 10% β -LG and 90% lactose, **BL(40:60)** = colyophilized β -lactoglobulin:lactose powder with 40% β -lactoglobulin and 60% lactose, **DSC** = differential scanning calorimetry, **SEM** = scanning electron microscopy.

INTRODUCTION

The production of whey and whey-derived powders is very important worldwide. In 2002, more than 1.6 million tonne of spray-dried whey were produced. Whey powders have interesting functional properties and are used as ingredients in the food industry (Huffman, 1996). Although their compositions become very specific, lactose and/or whey proteins (mainly β -lactoglobulin) are the principal compounds (Table 1).

The changes of lactose state particularly influence the shelf life of whey powders (Vuataz, 2002). In whey powders produced by spray drying or freeze-drying, lactose is in a meta-stable glassy state, very sensitive to changes in temperature and water content. During the storage of whey powders in severe conditions, the glass transition zone of lactose is passed. Viscosity strongly decreases, whereas molecular mobility highly increases (Champion et al., 2000; Mazzobre et al., 2001). This change in molecular mobility accelerates physico-chemical alterations. Lactose crystallization occurs and favors other damages, like particles caking, Maillard reaction, lipid oxidation, and so on (Burin et al., 2000; Özkan et al., 2003; Thomas et al., 2003). The preservation of the amorphous phase of lactose or the control of the crystallization process is thus very important for the dairy industry.

Lactose crystallization in milk powders differs from the crystallization of pure lactose, probably because of milk proteins (Jouppila and Roos, 1994; Knudsen et al., 2002). Lactose crystallization can also be inhibited in the presence of other proteins such as catalase (Forbes et al., 1998). According to Costantino et al. (1998), the development of interaction in the solid state between proteins and amorphous sugars could be responsible for the alteration of the crystallization. The fact that lactose is an efficient cryo-protectant for proteins also

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Table 1. Composition of powders derived from cheese whey and used as food ingredients.

Powders derived from cheese whey	Composition (%)				
	Proteins	Fat	Lactose	Minerals	Water
Whey powder	10-13	~1	70-75	7-11	3-5
WPC 80% ¹	79-81.5	0.4-6.2	4.7-10	2.7-3.3	4-4.5
WPC 35% ²	34-34.5	3.5	52-52.5	6-6.5	5
Whey protein isolate ²	91.4	~1	~1	1.5	5

¹Sammel and Claus, 2003. WPC = Whey protein concentrate.

²Chantrapornchai and McClements, 2002.

suggests that direct interaction, especially hydrogen bonds, can form in the solid state between lactose and the polar groups of proteins (Arakawa et al., 2001). Then, solid-state interaction may develop between lactose and milk proteins in whey powders and influence lactose crystallization.

In the present work, model whey powders containing lactose and β -lactoglobulin were prepared by colyophilization. The behavior of moisture sorption of the model powders was studied, and the state of lactose was determined according to the water activity of storage. The objective of our investigation was to determine the influence of β -LG on lactose crystallization and to evaluate the presence or absence of interaction between lactose and β -LG during the storage of model whey powders at different water activities.

MATERIALS AND METHODS

Preparation of the Model Whey Powders

Amorphous systems were obtained by freeze-drying solutions of lactose, β -LG, and the 2 compounds at different ratios. Solutions of 10% (wt/vol) alpha-monohydrate lactose (Prolabo, France) and β -LG (Daisco, Eden Prairie, MN) were prepared in distilled water. The solutions were stirred until complete dissolution. The pH of lactose solution was 4.07, and the pH of all solutions containing β -LG was between 7.30 and 7.56. The solutions were mixed to obtain β -lactoglobulin:lactose ratios of (10:90), (20:80), (30:70), and (40:60). Solution samples of 75 mL were immediately dispensed into 500-mL round-bottomed flasks and quickly frozen at -30°C in a refrigerating bath. Frozen samples were stored at -30°C at least 12 h and then freeze-dried 48 h using a Lyovac GT3, Leybold-Heraeus, Orsay, France). The powders were stored in vacuum desiccators over P_2O_5 at 20°C for 1 wk to complete the dehydration. The powders composed of the 2 compounds were assigned a colyophilized β -LG:lactose powder with 10% β -LG and 90% lactose (**BL[10:90]**), **BL(20:80)**, **BL(30:70)**, and colyophilized β -LG:lactose powder with 40% β -LG and 60% lactose (**BL[40:60]**).

Storage of the Model Whey Powders and Moisture Sorption Isotherms

Samples were stored for 3 mo over the saturated salt solutions described in Table 2. The solutions corresponded to water activity (a_w) ranging from 0.11 to 0.95.

Approximately 0.5 g of powder samples was placed at each a_w and stored at $20 \pm 1^{\circ}\text{C}$. Two replicates were done. The moisture sorption was monitored gravimetrically. Moisture sorption isotherms were drawn up from the percentage of water adsorbed at each water activity (g of water/100 g of dry solid) when equilibrium was reached.

Differential Scanning Calorimetry (DSC)

The state of lactose in model whey powders was determined using a DSC analysis system (Pyris 1 and Pyris manager 2.0 software, Perkin Elmer). The instrument was calibrated using indium. Analysis involved 50- μL open aluminum pans (Perkin Elmer) containing powder samples of 3 to 5 mg. An empty pan was used as a reference. Two replicates of each sample were analyzed. Each sample was heated at a temperature rate of $10^{\circ}\text{C}/\text{min}$ in a temperature range from 25 to 250°C .

Scanning Electron Microscopy (SEM)

Samples of the moisture sorption equilibrium were used for SEM investigation. Thin layers of powder samples were mounted on aluminum stubs, using double-sided adhesive tape. The mounted samples were first sputter-coated with carbon and then with gold/palla-

Table 2. Saturated salts solutions used for moisture sorption isotherms, and corresponding water activity (a_w). (Data from Greenspan, 1977)

Salt	Corresponding a_w	Salt	Corresponding a_w
LiCl	0.113	$\text{Mg}(\text{NO}_3)_2$	0.545
CH_3COOK	0.231	NaBr	0.587
MgCl_2	0.331	NaCl	0.754
NaI	0.392	KCl	0.851
K_2CO_3	0.432	KNO_3	0.946

Figure 1. Moisture sorption isotherms of all lyophilized powders after 12-wk storage. The 3 arrows on the graph indicate a sharp water loss, respectively, from lactose above 0.39 water activity (a_w); from BL(10:90), BL(20:80), and BL(30:70) above 0.44 a_w ; and from BL(40:60) above 0.55 a_w .

dium. The coated samples were examined with a Hitachi S2500 scanning electron microscope operating at 17 kV accelerating voltage.

RESULTS AND DISCUSSION

Moisture Sorption Isotherms

The moisture sorption isotherms of lactose, β -LG, and model whey powders (BL powders) with the 2 compounds are shown in Figure 1. The average standard deviation was 0.37% and corresponded to the size of the symbols used in Figure 1. The moisture sorption isotherms of all the powders were thus clearly different.

β -Lactoglobulin had a sigmoid moisture sorption isotherm, which is typical of food proteins. β -Lactoglobulin was very hygroscopic and adsorbed more water than the other powders studied. In BL powders above 0.70 a_w , the higher the β -LG content, the more the powder adsorbed water. For instance, lactose, BL(10:90), BL(20:80), BL(30:70), BL(40:60), and β -LG stored at 0.85 a_w adsorbed 4.2, 6.1, 6.7, 12.0, 13.5, and 26.2% water, respectively.

The isotherms of all powders containing lactose were irregular between 0.39 and 0.55 a_w : they released water and their sorption isotherms thus showed a "break." Lactose powder released water at 0.39 a_w . The water release occurred at higher a_w with increasing β -LG content in BL powders. The BL powders with 10 to 30% β -LG released water at 0.43 a_w . The sorption isotherms of BL(40:60) with 40% β -LG only showed a "break" at 0.55 a_w .

According to the literature, the release of water by powders containing lactose is due to lactose crystallization (Berlin et al., 1968; Jouppila and Roos, 1994). Wa-

Figure 2. Differential scanning calorimetry profiles of lyophilized lactose samples before and after a 3-mo storage at water activities between 0.11 and 0.95 at 20°C.

ter is released when the hygroscopic amorphous lactose crystallizes into its less hygroscopic crystalline forms. The "break" in the sorption curves of BL powders between 0.39 and 0.55 a_w could thus correspond to lactose crystallization. However, sorption isotherms alone were not sufficient to assert the amorphous or crystalline state of lactose. β -Lactoglobulin, which is strongly hygroscopic, could adsorb the water released at the beginning of the crystallization and thus conceal the phenomenon. Then, complementary methods were used to determine the amorphous or crystalline state of lactose at these critical water activities.

Determination of the State of Lactose in Model Whey Powders

Visual observations. After 3 mo of storage, the particles of lactose powders were agglomerated, and a collapse of their structure was clearly observed at 0.43 a_w and above. Similar alterations were observed from 0.55 a_w in powders containing 10 to 30% β -LG and from 0.59 a_w in BL(40:60). Since collapse and crystallization of lactose occur simultaneously in milk powders, the visual observations of the samples were consistent with the results from sorption isotherms (Newell et al., 2001).

Differential scanning calorimetry. The characterization of lactose state in the lyophilized powders before and after storage was undertaken using DSC (Figures 2, 3, and 4).

Figure 3. Differential scanning calorimetry profiles of BL(10:90) after a 3-mo storage at water activities between 0.11 and 0.95 at 20°C.

The DSC curves of lyophilized lactose before storage showed an exothermic peak around 175°C and an endothermic peak at 218°C (Figure 2). These peaks corresponded to the recrystallization of amorphous lactose during analysis and to melting of crystalline α -lactose produced, respectively. These DSC profiles were in

Figure 4. Differential scanning calorimetry profiles of BL(40:60) after a 3-mo storage at water activities between 0.11 and 0.95 at 20°C.

agreement with those obtained by Corrigan et al. (2002) for spray-dried amorphous lactose. Before storage, lyophilized lactose samples were thus amorphous.

The DSC curves obtained after a 3-mo storage at a_w between 0.11 and 0.95 are reported in Figure 2. From 0.11 to 0.33 a_w , exothermic peaks of lactose crystallization were observed at 175°C, showing that lactose was amorphous.

The DSC trace of lactose sample stored at 0.39 a_w presented a α -lactose melting peak (222°C) and an endothermic one at 84°C, with irregular shape. According to Darcy and Buckton (1997), this peak could correspond to the water released from a collapsed structure of lactose.

In the DSC profile at 0.43 a_w , a small shoulder at 150°C indicated the presence of α -lactose monohydrate, and a melting peak of anhydrous β -lactose was shown at 242°C (Figure 2). Lactose samples stored at 0.43 a_w had thus crystallized to form mainly anhydrous β -lactose and α -lactose monohydrate. This is in agreement with Buckton et al. (1998) who demonstrated that samples that began to crystallize at 44% relative humidity very clearly exhibited the characteristic peak of β -lactose (DSC and infrared spectral shape). From 0.55 to 0.95 a_w , lactose samples contained α -lactose monohydrate, since all DSC curves showed an endothermic peak at 150°C. Moreover, the melting peaks of crystalline lactose were irregular with temperatures between 220 and 240°C. This could indicate that different α -lactose and β -lactose crystalline forms were produced in addition to α -lactose monohydrate. Although mutarotation could occur during DSC analysis and modify the final proportion of lactose crystalline forms (Angberg, 1995), these results were consistent with those of Darcy and Buckton (1997), Buckton et al. (1998), and Corrigan et al. (2002). The authors demonstrated that at a_w above 0.55, lactose crystallized in mostly crystalline anhydrous α - and β -lactose, and subsequently mutarotated in the solid state to crystalline α -lactose monohydrate during storage.

Then, lactose samples stored above 0.43 a_w were crystallized. This was consistent with the moisture sorption isotherm of lactose (Figure 1). The loss of water at a_w above 0.39 corresponded to lactose crystallization. The lactose samples stored at 0.39 a_w were thought to be amorphous. The DSC analysis showed that a very early step of alteration already occurred, since lactose started to collapse. According to state diagrams of lactose and results of the literature, the collapse temperature of lactose is known to be 10 to 15°C above glass transition temperature (T_g), whereas the crystallization temperature is sometimes 50°C higher than T_g (Jouppila and Roos, 1994; Champion et al., 2000). As a consequence, the water activity of collapse is lower than the a_w of

lactose crystallization. Collapse occurs prior to crystallization (Newell et al., 2001). Corrigan et al. (2002) noticed that a lactose-collapsed structure could be stable more than 1 yr, since collapsed amorphous lactose desorbed water very slowly (Darcy and Buckton, 1997). This explains why the moisture sorption isotherm of lactose was stable.

The DSC curves for BL(10:90) and BL(20:80) powders stored from 0.11 to 0.43 a_w presented a small exothermic peak at 180°C, which meant that all samples contained amorphous lactose. From 0.55 to 0.95 a_w , DSC traces showed a clear endothermic peak at 140 to 150°C, corresponding to the release of water by α -lactose monohydrate (Figure 3). Thus, BL(10:90) and BL(20:80) samples were crystallized at water activities above 0.55 after 3 mo of storage.

The higher content of β -LG in BL(30:70) and BL(40:60) partly concealed the DSC analysis of the state of lactose. The DSC traces of BL(40:60) are shown Figure 4. The DSC curves were relatively flat for samples stored below and at 0.55 a_w . However, above 0.55 a_w , DSC traces exhibited melting peaks of crystalline lactose around 200°C. This melting temperature, which did not correspond to pure α - or β -lactose crystals, may be due to the specific crystallization products formed in the presence of proteins. Then, the lactose contained in BL(40:60) seemed to be amorphous until 0.55 a_w and to crystallize above 0.59 a_w .

The break observed in the moisture sorption isotherms of the BL powders (Figure 1) may correspond to the water release upon lactose crystallization. The more the model whey powders contained β -LG, the higher the a_w of lactose crystallization.

Scanning electron microscopy. Scanning electron microscopy was used as a complementary technique to confirm the DSC analysis of model whey powders, especially with high β -LG content. Micrographs in Figure 5 show particles of (a) lactose, (b) β -LG, and (c) BL(10:90) stored 3 mo at 0.23 a_w . The particles of all powders studied had miscellaneous irregular size and shape. However, the surfaces of all particles were very smooth, which is characteristic of amorphous systems (Lai and Schmidt, 1990; Aguilera et al., 1994). The critical water activity of crystallization was determined from the micrographs. Below this a_w , particle surfaces were smooth, since lactose remained amorphous. Above the critical a_w , lactose crystals clearly appeared on particle surfaces (Figure 6) (Lai and Schmidt, 1990). The critical a_w of crystallization was 0.43 in lactose powders and 0.55 in BL powders containing 10 to 30% β -LG. The critical a_w of BL(40:60) with 40% β -LG was 0.59, since lactose crystals were observed on particle surfaces only at this water activity and above (Figure 7).

Figure 5. Scanning electron micrographs of (a) β -LG, (b) lactose, and (c) BL(10:90) stored 3 mo at 20°C and 0.23 water activity ($\times 1000$ magnification).

Figure 6. Scanning electron micrograph of BL(10:90) stored 3 mo at 20°C and 0.55 water activity. Lactose crystals are clearly observed on particle surfaces.

The SEM analysis was consistent with the results obtained from moisture sorption isotherms, visual observations, and DSC analysis. Model whey powders were stabilized against lactose crystallization. In BL powders containing from 10 to 30% β -LG, lactose crystallization was delayed to 0.55 a_w , and BL powders with 40% β -LG were stabilized until 0.59 a_w .

Kinetics of Moisture Sorption

The kinetics of moisture sorption were considered in order to better understand the delayed crystallization of lactose in BL powders. The moisture sorption isotherms of (a) BL(10:90) and (b) BL(40:60), determined after 1, 2, 4, and 9 wk of storage, are shown in Figure 8.

The BL(10:90) and BL(20:80) reached the equilibrium of moisture sorption from the first week of storage (Figure 8a). On the contrary, the percentage of water adsorbed by BL(30:70) and BL(40:60) gradually decreased until the ninth week of storage at 0.55 and 0.59 a_w , respectively, (Figure 8b). There was a time-delayed crystallization in model whey powders containing 30 and 40% β -LG, whereas lactose crystallization occurred faster in BL powders with less β -LG.

Knudsen et al. (2002) previously noticed that a more gradual (i.e., slower) lactose crystallization occurred in whole milk powder with 26 to 29% milk protein than in infant formula with 12% milk protein. However, this phenomenon is not fully explained yet. Forbes et al. (1998) reported that the protein hygroscopicity could

Figure 7. Scanning electron micrographs of BL(40:60) after 3-mo storage at 20°C and at (a) 0.55 water activity (a_w) and (b) 0.59 a_w . Particle surfaces were smooth at 0.55 a_w , whereas lactose crystals were clearly observed at 0.59 a_w .

be involved in the inhibition of lactose crystallization. In our study, β -LG adsorbed water more rapidly than lactose did, and the equilibrium of moisture content was almost reached after 24 h exposure of the β -LG samples to moisture. The amplification of crystallization might thus be limited because the water normally required for the growth of lactose crystals could be preferentially adsorbed by β -LG.

Figure 8. Kinetics of moisture sorption of (a) BL(10:90) and (b) BL(40:60). The graphs collect the moisture sorption isotherms of the powders after 1, 2, 4, and 9 wk of storage at 20°C.

Finally, β -LG could physically limit the access of water vapor to the molecules of amorphous lactose, generate a delay in saturating the powder bed with moisture, and thus cause a gradual crystallization of lactose (Stubberud et al., 1996). The steric hindrance of β -LG could also limit the propagation of lactose crystals.

Lactose/ β -LG Interaction

The moisture sorption isotherms of 2 powders mechanically mixed after separate lyophilization (B + L [10 + 90] and B + L [40 + 60]) were drawn up and compared to the corresponding theoretical curves calculated from the sorption isotherms of lyophilized lactose and β -LG, and to the sorption curves of BL(10:90) and BL(40:60) (Figure 9a, b). The water sorption curves of powders composed of lactose and β -LG lyophilized separately and mixed (B + L powders) with the 2 components lyophilized separately were similar to the theoretical curves. The behavior of water sorption of these mixed powders corresponded to the addition of the behavior of each compound taken separately, neglecting β -LG/lactose interaction. Lactose crystallization (confirmed by DSC and SEM analysis) occurred at the water activity predicted from individual lactose and

Figure 9. Theoretical and experimental moisture sorption isotherms of (a) BL(10:90) and (b) BL(40:60) after a 9-wk storage. Theoretical curves were calculated from the moisture sorption isotherms of each component considered separately. B + L powders were composed of lactose and β -LG mixed after separate lyophilization. BL = β -LG.

β -LG powders. Contrary to the mixed powders, the model whey powders BL(10:90) and BL(40:60) had specific sorption curves that differed from the theoretical curves, especially between 0.39 and 0.60 a_w . The BL powders adsorbed more water than predicted at the a_w before lactose crystallized—i.e., at 0.43 a_w for BL(10:90) and at 0.43 and 0.55 a_w for BL(40:60). Moreover, in BL powders, water release during lactose crystallization occurred clearly at higher water activity than predicted. The obvious differences between theoretical and experimental moisture sorption isotherms of BL powders showed evidence of solid-state interaction between lactose and β -LG. This molecular interaction stabilized model whey powders against lactose crystallization. Jouppila and Roos (1994) noticed a delayed crystallization of lactose in milk powders compared with pure lactose, and they reported that milk proteins could be responsible for the phenomenon. Although suspected, the result we obtained for model whey powders was, to our knowledge, not clearly demonstrated between 2 milk components.

GENERAL DISCUSSION

The delay of lactose crystallization to higher water activity was previously observed in the presence of high molecular weight compounds (Berlin et al., 1968; Karmas et al., 1992). This effect was attributed to the higher glass transition temperature (T_g) of the mixture with respect to pure lactose. However, some molecules like poly(vinyl pyrrolidone), trehalose, or human growth hormone delay sugar crystallization without affecting the T_g value of the systems (Costantino et al., 1998; Mazzobre et al., 2001; Stubberud and Forbes, 1998). In these combined systems, other factors, like the modification of molecular environment, might be involved.

First, noncovalent interaction between lactose and β -LG, especially hydrogen bonds, could develop from freeze drying and direct the repartition of the 2 compounds within the system. Kim et al. (2002) and Millqvist-Fureby et al. (1999) demonstrated that proteins were largely located on the particle surfaces of industrial spray-dried dairy powders and lyophilized lactose/bovine serum albumin systems. In BL powders, β -LG could thus largely cover the surface of powder particles and limit the access of water vapor to amorphous lactose. This spatial organization may be responsible for the shift of lactose crystallization to higher water activity.

Covalent interactions may also develop between lactose and β -LG. Maillard reaction probably occurred during storage of BL powders, since the white BL powders became more yellow, especially between 0.59 and 0.85 a_w . Lactosylation may have occurred in the powder state, since a restricted water environment increases the rate of Maillard reaction (Morgan et al., 1999; Schebor et al. 1999; Burin et al., 2000). Moreover, the lactosylation of β -LG by 10 lactose molecules, which corresponds to a solid-state glycation (Morgan et al., 1999) increases the surface hydrophilicity of the protein, and this could modify the hydrophilic interaction within the BL powders (French and Harper, 2001).

Other investigations will be carried out to pinpoint the nature of the lactose/ β -LG interaction. First, the development of browning in BL powders will be studied. The repartition of β -LG and lactose on the particle surfaces of the BL powders could also be evaluated by electron spectroscopy for chemical analysis. Moreover, ^1H -RMN or infrared spectroscopy techniques could be undertaken to characterize the nature of the molecular bonding between the 2 compounds.

Then, the stability of lactose-rich powders used as food ingredients, in which a small part of lactose often remains amorphous, could be improved by complementation in β -LG before spray drying.

REFERENCES

- Angberg, M. 1995. Lactose and thermal analysis with special emphasis on microcalorimetry. *Thermochim. Acta* 248:161–176.
- Arakawa, T., S. J. Prestrelski, W. C. Kenney, and J. F. Carpenter. 2001. Factors affecting short-term and long-term stabilities of proteins. *Adv. Drug Deliv. Rev.* 46:307–326.
- Berlin, E., B. A. Anderson, and M. J. Pallansch. 1968. Water vapor sorption properties of various dried milks and wheys. *J. Dairy Sci.* 51:1339–1344.
- Buckton, G., E. Yonemochi, J. Hammond, and A. Moffat. 1998. The use of near infra-red spectroscopy to detect changes in the form of amorphous and crystalline lactose. *Int. J. Pharm.* 168:231–241.
- Burin, L., K. Jouppila, Y. Roos, J. Kansikas, and M. P. Buera. 2000. Color formation in dehydrated modified whey powder systems as affected by compression and T_g . *J. Agric. Food Chem.* 48:5263–5268.
- Champion, D., M. Le Meste, and D. Simatos. 2000. Towards an improved understanding of glass transition and relaxations in foods: Molecular mobility in the glass transition range. *Trends Food Sci. Technol.* 11:41–55.
- Chantrapornchai, W., and D. J. McClements. 2002. Influence of NaCl on optical properties, large-strain rheology and water holding capacity of heat-induced whey protein isolate gels. *Food Hydrocoll.* 16:467–476.
- Corrigan, D. O., A. M. Healy, and O. I. Corrigan. 2002. The effect of spray-drying solutions of polyethylene glycol (PEG) and lactose/PEG on their physicochemical properties. *Int. J. Pharm.* 235:193–205.
- Costantino, H. R., J. G. Curley, S. Wu, and C. C. Hsu. 1998. Water sorption behavior of lyophilized protein-sugar systems and implications for solid-state interactions. *Int. J. Pharm.* 166:211–221.
- Darcy, P., and G. Buckton. 1997. The influence of heating/drying on the crystallization of amorphous lactose after structural collapse. *Int. J. Pharm.* 158:157–164.
- Forbes, R. T., K. G. Davis, M. Hindle, J. G. Clarke, and J. Maas. 1998. Water vapor sorption studies on the physical stability of a series of spray-dried protein/sugar powders for inhalation. *J. Pharm. Sci.* 87:1316–1321.
- French, S. J., and W. J. Harper. 2001. Theoretical representation of surface properties on hypothetical Maillard modified β -lactoglobulin using GRASP/GRASS. *Milchwissenschaft* 56:310–313.
- Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Nat. Bureau Standards A* 81:89–93.
- Huffman, L. M. 1996. Processing of whey proteins for use as food ingredient. *Food Technol.* 2:49–52.
- Jouppila, K., and Y. H. Roos. 1994. Glass transition and crystallization in milk powders. *J. Dairy Sci.* 77:2907–2915.
- Karmas, R., M. P. Buera, and M. Karel. 1992. Effect of glass transition on rates of nonenzymatic browning in food systems. *J. Agric. Food Chem.* 40:873–879.
- Kim, E. H.-J., X. D. Chen, and D. Pearce. 2002. Surface characterization of four industrial spray-dried dairy powders in relation to chemical composition, structure and wetting properties. *Colloids Surf. B Biointerfaces* 26:197–212.
- Knudsen, J. C., H. S. Antanuse, J. Risbo, and L. H. Skibsted. 2002. Induction time and kinetics of crystallization of amorphous lactose, infant formula and whole milk powder as studied by isothermal differential scanning calorimetry. *Milchwissenschaft* 57:543–546.
- Lai, H.-M., and S. J. Schmidt. 1990. Lactose crystallization in skim milk powder observed by hydrodynamic equilibria, scanning electron microscopy and ^2H nuclear magnetic resonance. *J. Food Sci.* 55:994–999.
- Mazzobre, M. F., G. Soto, J. M. Aguilera, and M. P. Buera. 2001. Crystallization kinetics of lactose in systems co-lyophilized with trehalose. Analysis by differential scanning calorimetry. *Food Res. Int.* 34:903–911.
- Millqvist-Fureby, A., M. Malmsten, and B. Bergenstahl. 1999. Surface characterization of freeze-dried protein/carbohydrate mixtures. *Int. J. Pharm.* 191:103–114.

- Morgan, F., J. Léonil, D. Mollé, and S. Bouhallab. 1999. Modification of bovine β -lactoglobulin by glycation in a powdered state or in aqueous solution: Effect on association behavior and protein conformation. *J. Agric. Food Chem.* 47:83–91.
- Newell, H. E., G. Buckton, D. A. Butler, F. Thielmann, and D. R. Williams. 2001. The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the processes of collapse and crystallisation. *Int. J. Pharm.* 217:45–56.
- Özkan, N., B. Withy, and X. D. Chen. 2003. Effects of time, temperature, and pressure on the cake formation of milk powders. *J. Food Eng.* 58:355–361.
- Sammel, L. M., and J. R. Claus. 2003. Whey protein concentrates effects on pink color development in a cooked ground turkey breast model system. *Meat Sci.* 65:1293–1299.
- Schebor, C., M. P. Buera, M. Karel, and J. Chirife. 1999. Color formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems. *Food Chem.* 65:427–432.
- Stubberud, L., and R. T. Forbes. 1998. The use of gravimetry for the study of the effect of additives on the moisture-induced recrystallisation of amorphous lactose. *Int. J. Pharm.* 163:145–156.
- Stubberud, L., H. G. Arwidsson, V. Hjortsberg, and C. Graffner. 1996. Water-solid interactions. III. Effect of glass transition temperature, T_g, and processing on tensile strength of compacts of lactose and lactose/polyvinyl pyrrolidone. *Pharm. Dev. Technol.* 1:195–204.
- Thomas, M. E. C., J. Scher, and S. Desobry. Milk powders aging: Effect on physical and functional properties. *Crit. Rev. Food Sci. Nutr.* (accepted).
- Vuataz, G. 2002. The phase diagram of milk: A new tool for optimising the drying process. *Lait* 82:485–500.