ELSEVIER

Trends in Food Science & Technology 27 (2012) 73–82

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Single droplet drying for optimal spray drying of enzymes and probiotics

Maarten A.I. Schutyser*, Jimmy Perdana and Remko M. Boom

Wageningen University, Food Process Engineering Group, Bomenweg 2, 6703 HD Wageningen, The Netherlands (Wageningen University, Food Process Engineering Group, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Tel.: +31 317 488629; fax: +31 317 482237; e-mail: maarten.schutyser@wur.nl)

Spray drying is a mild and cost-effective convective drying method. It can be applied to stabilise heat sensitive ingredients, such as enzymes and probiotic bacteria, albeit in industrial practice for example freeze drying or freezing are often preferred. The reason is that optimum drying conditions and tailored matrix formulations are required to avoid severe heat damage leading to loss in enzyme activity or reduced survival of bacteria. An overview is provided on the use of protective carbohydrate-rich formulations in the spray drying of enzymes and probiotics. Subsequently, single droplet drying experimentation methods are reviewed for mapping drying trajectories of individual droplets. The advantage of these is to provide insight in inactivation kinetics of enzymes and probiotics and thus contribute to unravelling of stabilisation mechanisms. Finally, it is shown that detailed modelling of single droplet drying and insight in micro-structural changes during drying can be complementary to the experimental single droplet approaches.

Introduction

Many foods and food ingredients are dried in powdered form to provide shelf-life and retain activity of specific bioactive components. Amongst the convective air drying methods, spray drying is a mild technique due to its very short drying times and the relatively low temperatures to which the product is exposed (Kieviet & Kerkhof, 1995; Mazza, Brandão, & Wildhagen, 2003). However, compared to freeze or vacuum drying, the spray drying process is more prone to damaging heat sensitive components such as enzymes and probiotic bacteria. Because spray drying is much more cost effective as it can process larger volumes and operate at higher energy efficiency, many studies have been involved with optimising spray drying and product formulations towards minimal activity losses (Knorr, 1998). Although many successes have been reported, in practice optimisation results, especially from pilot-scale experiments, have been difficult to translate into general optimisation rules (Thybo, Hovgaard, Lindeløv, Brask, & Andersen, 2008). In this paper we make an appraisal for dedicated single droplet drying procedures and predictive models that can map drying behaviour and inactivation kinetics of enzyme and probiotic bacteria at the droplet level. For this we describe the state of the art of optimisation for spray drying as well as the most common stabilisation approach for enzymes and probiotics, i.e. using carbohydrates. Then, several single droplet drying procedures are discussed, followed by the most common approaches to model drying of sugar-containing single droplets. The coupling between drying and micro-structural properties is illustrated by projecting the drying history of single droplets on phase or state diagrams of specific solutes. Finally, some specific challenges are discussed to further develop single droplet studies into a practical tool for optimisation of spray-dried formulations.

Optimal spray drying of enzymes and probiotics

The drying conditions should be such that enzyme activity or survival of probiotic bacteria are retained as much as possible. Decreasing outlet temperatures and lower residence times are found to increase retention of enzyme activity and probiotic viability (Silva, Freixo, Gibbs, & Teixeira, 2011). Too low outlet temperatures may result in higher residual moisture contents, especially leading to loss of probiotic viability during subsequent storage of the powder. Increasing residence times can be detrimental to bioactive components; residence times in industrial dryers can increase, especially when following steps, such as fluidised bed drying, are included as well. Other drying

* Corresponding author.

0924-2244/$ - see front matter
doi:10.1016/j.tifs.2012.05.006

parameters of influence are the specific spray dryer configuration, such as nozzle type, positioning of air flow and injection of feed, and chamber design (Santivarangkna, Kulozik, & Foerst, 2007). For example, it is found that viability of spores of *Bacillus thuringiensis* slightly decreases with increasing nozzle pressure (Zhou, Dong, Gao, & Yu, 2008). At the scale of the droplet one can distinguish two distinct drying phases, i.e. the constant and falling rate periods. In the first phase the droplet is at the wet bulb temperature, whereas in the second phase the temperature increases and a moisture gradient develops across the droplet radius. It is reported that probiotic bacteria are very sensitive to the rapid changes that occur during the drying process, whereas the inactivation kinetics of the enzyme β-galactosidase was found not rate dependent (Chen & Patel, 2007; Perdana, Fox, Schutyser, & Boom, 2012b). Further it is observed that during the first phase inactivation of bacteria is mainly due to dehydration effects, whereas during the second phase it is due to a combination of thermal and dehydration effects (Chen & Patel, 2007; Lievense, Verbeek, Van 't Riet, & Noomen, 1994). Most enzyme inactivation takes place during the falling rate period due to elevated temperatures (Sloth *et al*., 2009).

Formulation can be adapted to increase stability of enzymes and probiotic bacteria during drying and subsequent storage. The most successful formulation for enzymes is concerned with the addition of carbohydrates, specifically sugars, maltodextrins, and polyols (Yamamoto & Sano, 1992). Carbohydrates contribute to the formation of glassy, amorphous powders. Two different mechanisms have been described explaining the improved stability of enzymes in the presence of carbohydrates during drying, viz. a thermodynamic mechanism involving hydrogen-bond formation affecting the equilibrium between native and unfolding state and kinetic stabilisation due to immobilisation of the enzyme in a glassy solid. To avoid excessive inactivation during drying and storage, it is desirable to use formulations with high glass transition temperature and store the formulations well below their T_g. In Table A1 in the Appendix an overview is given of anhydrous T_g for various carrier materials. It should be realised that in the presence of residual moisture content the T_g during for example storage is lower. Similar to enzymes, addition of carbohydrates is widely applied for probiotic bacteria, although in most cases in combination with other carriers, such as (reconstituted) skim milk. Two similar stabilisation mechanisms are described for probiotics, i.e. glass formation and stabilisation of the phospholipid cell membrane by hydrogen-bond formation, which effectuates a depression of the membrane phase transition temperature, i.e. the temperature at which the bilayer changes from the crystalline phase into the gel phase (Santivarangkna, Higl, & Foerst, 2008). During storage, sugars are reported to be effective protectants against oxidation damage, e.g. by scavenging free radicals (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). For stability during storage it is further required to maintain a constant and low final water content as an increase in water content may enhance the risk for glass/rubber transition with as a consequence loss of viability (Chavez & Ledeboer, 2007). Finally, storage under low temperatures is preferred as survival is inversely related to storage temperature (Wang, Yu, & Chou, 2004).

In Table 1 the survival and residual activity of two probiotic bacteria and two enzymes are shown under various drying and storage conditions and using different carrier formulations. Survival of *Bifidobacterium lactis* BBD2 appears correlated with T_g for some components used. Higher survival percentages For *Bifidobacterium longum* B6 dried with skimmed milk were explained by small cracks at the surface of the particles enhancing heat and moisture transfer (Lian, Hsiao, & Chou, 2002). It was explained that residual activity for β-galactosidase increased inversely with molecular weight, which is correlated to available stabilising hydroxyl groups (Yamamoto & Sano, 1992). Residual activities of 0% and 8.3% were found after drying and after storing lipase in aqueous solution without additives, respectively, compared to the survival percentages shown in Table 1 (Costa-Silva, Nogueira, Souza, Oliveira, & Said, 2011). Overall, high T_g of carrier materials is desired for stabilisation, but other factors need to be considered.

The application of a specific pre-treatment can enhance retention of probiotic stability during subsequent drying (Meng *et al*., 2008). For example, it is found that by exposing bacteria to sub-lethal stress conditions (e.g. heat shock) prior to spray drying, survival percentages are increased (Van de Guchte *et al*., 2002; Whitaker & Batt, 1991). Further, susceptibility of bacteria towards inactivation during drying is affected by the growth phase at which bacteria are harvested prior to drying (Peighambardoust, Tafti, & Hesari, 2011).

Establishing the optimum drying conditions, formulation, and pre-treatment is not straight forward. It is common practice to choose several formulations. Subsequently, pilot-scale drying experiments are carried out with varying inlet and outlet air temperatures to maximise capacity in combination with low activity losses. From cost perspective, only a limited set of variations in formulation is evaluated. Moreover, it appears difficult to translate optimum drying conditions determined with pilot-scale experiments to optimal industrial drying conditions (Thybo *et al*., 2008). An important difference between small scale and industrial spray dryers is the shorter residence time for small-scale dryers (typically 1–4 s versus 20–40 s for industrial dryers), which is correlated to the smaller height of its chamber (Filková, Huang, & Mujumdar, 2006; Fu *et al*., 2011). This is compensated for by reducing the droplet size, which again reduces the drying time (Goula & Adamopoulos, 2004). To produce these small (d_p = 1–25 μm) droplets a different atomisation principle is applied, viz. two fluid nozzle atomisation, whereas industrial spray dryers use either pressure nozzle or rotary disc atomisation (d_p = 80–400 μm) (Filková *et al*., 2006;

Table 1. Overview of residual activity of probiotic bacteria and enzymes after drying and storage in combination with different carrier materials and their corresponding glass transition temperatures.

Bioactive component	Drying conditions	Composition solid matrix	Residual activity (%)		T_g (°C)
			After drying	After storage	
Bifidobacterium lactis BBD2 (Chavez & Ledeboer, 2007)	Two step spray drying, T_{in} = 80 °C, T_{out} = 48 °C, followed by vacuum drying at 45 °C; solid carrier concentration = 20% with ratio protein:carbohydrate = 1:1, residual moisture content ~ 5%, storage for 90 days at 30 °C,	Soy protein isolate	28	1	95[a]
		Soy protein isolate + maltodextrin DE 20	33	1	85
		Soy protein isolate + lactose	100	50	74
		Skim milk powder + arabic gum	100	70	68
		Soy protein isolate + sucrose	46	0.01	62
		Skim milk powder + maltodextrin DE 20	100	1	60
		Skim milk powder + trehalose	56	0.1	49
		Skim milk powder	17	2	45
Bifidobacterium longum B6 (Lian, *et al.*, 2002)	One step spray drying, T_{in} = 100 °C, T_{out} = 50 °C; solid carrier concentration = 10%, residual moisture content = 6.2–10%	Soluble starch	29	ND	151[b]
		Gelatine	64		140
		Gum arabic	41		126
		Skim milk	83		92
β-galactosidase (Yamamoto & Sano, 1992)	Single droplet drying, 8 µL drop, T_{air} = 90 °C, air flow rate = 1 m/s; solid carrier concentration = 20%, residual moisture content ~ 7%	Maltodextrin DE 11	35	ND	160[b]
		Lactose	98		102
		Maltodextrin DE 40	50		100
		Maltose	30		87
		Sucrose	75		74
		Glucose	90		30
Lipase (Costa-Silva, Nogueira, Souza, Oliveira, & Said, 2011)	One step spray drying, T_{in} = 100 °C, T_{out} = 70 °C, solid carrier concentration = 10% with 0.1% Tween 80, residual moisture content 4.2–7%, storage for 8 months at 5 °C.	Mannitol	88	42	166[b]
		Maltodextrin DE 10	91	47	160
		Gum Arabic	88	64	126
		Trehalose	63	66	119
		Maltodextrin DE 20	91	67	141
		Lactose	100	69	102

[a] T_g is measured at 4% moisture content (Chavez & Ledeboer, 2007).
[b] T_g at 0% moisture content; data taken from Table A1.

Fyfe, Kravchuk, Nguyen, Deeth, & Bhandari, 2011). It is obvious that the differences in droplet size and drying time have significant effect on the temperature and moisture content history of particles, which will again influence the inactivation of enzymes and probiotic bacteria. Despite these differences, powders being produced with small-scale spray dryers typically exhibit vacuole formation, fat migration, and degradation reactions similar to industrially spray-dried products (Kim, Chen, & Pearce, 2009; Nijdam & Langrish, 2005; Walton, 2000).

Single droplet experimentation approaches can provide insight in the fate of well-defined individual drying droplets and the related inactivation processes (Adhikari, Howes, Bhandari, & Truong, 2000). A boundary condition is that single droplet drying experiments are carried out under representative conditions for spray drying. Subsequently, these can be used to assess inactivation kinetics of enzymes and probiotics during spray drying (Adhikari *et al.*, 2000; Li, Lin, Chen, Chen, & Pearce, 2006; Perdana *et al.*, 2012b; Yamamoto & Sano, 1992). The focus from this point forward is thus on single droplet drying and using this technique to evaluate inactivation of enzymes and bacteria in carbohydrate matrices under representative conditions.

Single droplet drying methods

Different single droplet drying methodologies exist, viz., levitation methods and free flight drying methods (Adhikari *et al.*, 2000). The first method requires immobilisation of single droplets through noncontact levitation (acoustic or aerodynamic) or through contact levitation (droplet pending on a glass filament or deposited on a flat surface). The second method involves the generation of a uniform stream of droplets, which are subsequently dried in a tall drying chamber during free fall.

A dispensing or droplet generation method is required for single droplet drying experiments. This can be for example a micro syringe or a pneumatic needle dispenser. The pneumatic method can be used to dispense droplets down to approximately 150 µm in diameter (Chen, 2009; Perdana, Fox, Schutyser, & Boom, 2011). Drying of a stream of uniform droplets requires a continuous droplet generation method, for example piezo-electric atomisation. The principle of this method relies on the movement of a piezo-electric element creating a pressure wave. Patel and Chen (2007) reported production of powder particles using piezo-electric atomisation with an average diameter as low as 13 µm. Other continuous methods are for example electrostatic droplet generation or pulsed orifice atomisation.

During noncontact levitation methods, a single droplet is freely suspended in the air (Adhikari *et al*., 2000). To achieve this, gravitation needs to be counterbalanced by an external force such as an acoustic or aerodynamic force. The suspended drop can be as small as 100 μm. Using these noncontact levitation methods, the droplet evolution during drying can be visually monitored and the residence time can be varied easily (Sloth *et al*., 2006). Some limitations in the levitation methods are for example in the case of acoustic levitation, the acoustic field, being a sequence of sharp fluctuations in air pressure, alters the heat and mass transfer rate in the droplet. It is reported that the heat and mass transfer coefficients are larger than that with free falling droplet, as predicted with the Ranz–Marshal correlation (Ali Al Zaitone & Tropea, 2011; Yarin, Brenn, Kastner, Rensink, & Tropea, 1999). It is also important to understand the influence of the levitation streaming on the inactivation of heat sensitive products. For example acoustic streaming may influence the inactivation rate of micro-organisms (Dijkstra *et al*., 2011). Another practical challenge is the difficulty to accurately control the levitation of droplets.

The alternative is contact suspension, e.g. a droplet pending on a fine glass filament or thermocouple, or the deposition of the droplet on a hydrophobic flat plate (Perdana *et al*., 2011; Yamamoto & Sano, 1992). By connecting a mass balance to the filament, the mass of an individual droplet can be monitored during the drying. Additionally, a camera may be used to monitor the changing droplet morphology. The presence of the glass filament or surface to levitate the droplets leads to some unwanted effects, e.g. some additional heat transfer. Despite these unwanted effects the experimental set-up using a glass filament is practical and therefore preferred by the majority of the researchers (Adhikari *et al*., 2000). This method was even further improved by rotating droplets at the tip of a glass filament (Hassan & Mumford, 1993). Rotation of the droplet mimics the relative motion of droplets to the drying air and spin due to momentum transfer during spray drying. The drying of droplets on a hydrophobic surface has the advantage that very small (down to 150 μm) droplets can be deposited and that multiple droplets may be dried simultaneously, which facilitates a high throughput process. In Fig. 1, such a drying droplet drying on a hydrophobic surface is visualised at three time intervals. It is found that the conductive heat transfer via the surface is less than 5% compared to the convective heat transfer via the drying air and may thus be neglected (Perdana *et al*., 2012b). A disadvantage of this method is that the presence of the surface influences the air temperature and flow pattern of the drying air near the droplet, which reduces the drying rate.

Drying of a stream of uniform droplets can be carried out in a column dryer (Meerdink, 1993; Zbiciński & Piątkowski, 2004). The droplets are generated at the top of the column. Because of gravitational force, the droplets fall freely through the column and are dried through contact with the preconditioned air. During drying droplets fall under their terminal droplet velocity, which is approximately 1 m/s for a droplet with a diameter of 200 μm (Meyer, 2004). The advantages of this method are that it mimics quite well what happens to a drop during spray drying and relative large amount of sample material can be collected. An important disadvantage is that a tall column (30 m) would be needed to mimic typical residence times in spray dryers. The use of shorter columns (e.g. 6 m) does not provide enough time to simulate the complete drying of droplets. Other disadvantages are that it is not possible to monitor mass and temperature changes of individual droplets during drying (Adhikari *et al*., 2000), and that the typical gradient in temperature and humidity of the surrounding air in time, cannot be simulated.

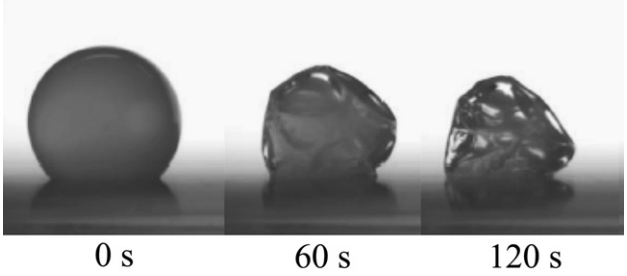

Fig. 1. Droplet drying containing 20% maltodextrin DE 4–7 on a hydrophobic surface at an air temperature of 80 °C, an absolute air humidity of 0 g/kg, dry air, a bulk air velocity of 0.20 m/s (from left to right), and an initial droplet height of 800 μm. Adopted partially from Perdana *et al*., 2012b.

All three different drying methods have their pros and cons. It may be concluded that single droplet drying methods with contact levitation are most practical. The glass filament method would be preferred for studying the drying of a single droplet, whereas the deposited droplet method offers opportunities for drying multiple droplets simultaneously. These methods can be applied to systematically study the influence of different drying parameters, viz. residence time, droplet size, and drying air temperature. Moreover, different product formulations and their impact on residual enzyme or microbial activity can be investigated if a high throughput approach is feasible.

Modelling the drying of single droplets

Modelling the drying of single droplets supports the interpretation of results from the experiments and the translation of these to the actual spray drying process (Adhikari *et al*., 2000). It is nearly impossible to measure the temperature and moisture profiles within such a small droplet over time, although several researchers have put a small temperature couple inside a droplet or even used an infrared camera to monitor the droplet surface temperature during drying (Fabien, Antoni, & Sefiane, 2011; Wulsten & Lee, 2008).

Several approaches exist for the modelling of the drying of a droplet containing dissolved solids (Mezhericher, Levy, & Borde, 2010; Patel & Chen, 2008). The most common approach assumes that there is no temperature gradient inside the drying droplet, although a small gradient usually develops during the first milliseconds of industrial spray drying (<2.3 °C) (Patel & Chen, 2008). An effective moisture diffusion coefficient is considered and Fick's law is applied to solve the spatial moisture distribution in the droplet. This modelling approach is frequently referred to as the effective diffusion model. The convective heat and mass transfer coefficients at the droplet surface are described with standard correlations (Ranz & Marshall, 1952). The effective moisture diffusivity is generally temperature and moisture content dependent (Labuza, Kaanane, & Chen, 1985; van der Sman & Meinders, 2011; Wang & Brennan, 1991; Yamamoto, 2001). This dependency should be validated with experimental data from controlled drying experiments under different conditions. Mostly used are thin film drying experiments during which the mass of the film is gravimetrically followed (Anandharamakrishnan, Rielly, & Stapley, 2007). Usually, the diffusion coefficient is a continuous function of the moisture content and thus does not explicitly include a glass transition.

However, when drying amorphous products, glass transition in the outer layers will lead to the formation of a solid skin at the surface, avoiding further shrinkage. Using an effective diffusion modelling approach leads to under prediction of the droplet diameter after this. To describe morphologic changes at this point the effective diffusion modelling approach has been extended to a receding interface or shrinking core model. This modelling approach is particularly applied to sugar-rich solute matrices (Adhikari *et al*., 2000; Werner, Edmonds, Jones, Bronlund, & Paterson, 2008). The crust effectively separates the air and the liquid phase in the centre of the droplet. After the crust formation, evaporation is assumed to take place at the solution–skin interface instead of the surface of the particle; the evaporation front recedes to the centre of the particle during further drying. The crust is considered to be a porous layer through which water vapour diffuses and its total volume is assumed to remain constant. The moment at which the crust forms should be defined, i.e. usually by reaching a critical saturation moisture content (Perdana *et al*., 2012b). Recently, a critical temperature difference or $(T-T_g)_{crit}$ was defined for this point, which would be indicative for the mechanical stresses that develop during skin formation (Werner *et al*., 2008). This temperature difference is related to the stickiness point. The stickiness point of a powder can be determined by assessment of its flowability at increasing temperature and specific relative humidity. Following, two different morphologies have been modelled, i.e. a 'dense skin-porous crumb' or 'collapsed shell' (Adhikari *et al*., 2000; Werner *et al*., 2008). The receding model is handled mathematically within the diffusion model, where the radii of the numerical shells of the crust are fixed or are assumed to decrease depending on the morphology studied, i.e. 'dense skin-porous crumb' or 'collapsed shell'. The vapour diffusivity through the porous crust is calculated from the vapour diffusivity in air and the effective water diffusivity in the solute matrix. The experimental validation of such a modelling approach remains a challenge as Werner *et al*. (2008) did not provide experimental data for comparison. Despite this, an interesting aspect of their approach is that the glass transition is included in the modelling of the single droplet drying. As the glass transition is related to molecular mobility phenomena this may again offer opportunities to more explicitly consider structural transitions related to degradation reactions of bioactive components.

The loss of active enzyme or viable bacteria during drying is conventionally modelled by first order kinetics. The inactivation kinetics for a specific component–matrix combination can depend on temperature and moisture contents during drying, but also on the drying rates (Chen & Patel, 2007). Especially for probiotic bacteria it is suggested that the drying rate and rate of temperature change have influence on the inactivation process, which was explained as the imposition of stress on the bacteria (Li *et al*., 2006). Conventionally, Arrhenius type of equations are used (Yamamoto & Sano, 1992). Model parameters for inactivation kinetics are mostly obtained from constant heating experiments at different moisture contents (Liou, 1982; Meerdink, 1993; Perdana *et al*., 2012a). In such a steady-state approach, the potential influence of dehydration rate and rapid changes in temperature on activity loss are not included. It should be realised that the experimental time scale during the heating experiments is also much larger than during the actual drying process. This has again its effect on kinetic changes (e.g. glass transition, crystallisation) and on the magnitude of the inactivation rate, which is much lower during heating compared to drying. Single droplet drying experiments can thus be of value for establishing inactivation kinetics that have more predictive value (Perdana *et al*., 2012b). In Fig. 2, a schematic overview is given on how drying kinetics and inactivation kinetics of enzymes or probiotics are connected to provide model predictions on residual activity during the drying of a droplet.

Mapping the drying and inactivation history of single droplets

In Fig. 3, the temperature and moisture values history of an individual droplet of a maltodextrin (DE4-7) suspension during spray drying is plotted in the state diagrams of maltodextrin DE10 and whole milk, respectively. State diagrams are used to a.o. visualise glass transition as a function of temperature and moisture contents (Cuq, Abecassis, & Guilbert, 2003; Rahman, 2006; van der Sman & Meinders, 2011). The drying trajectories were calculated using an effective diffusion modelling approach

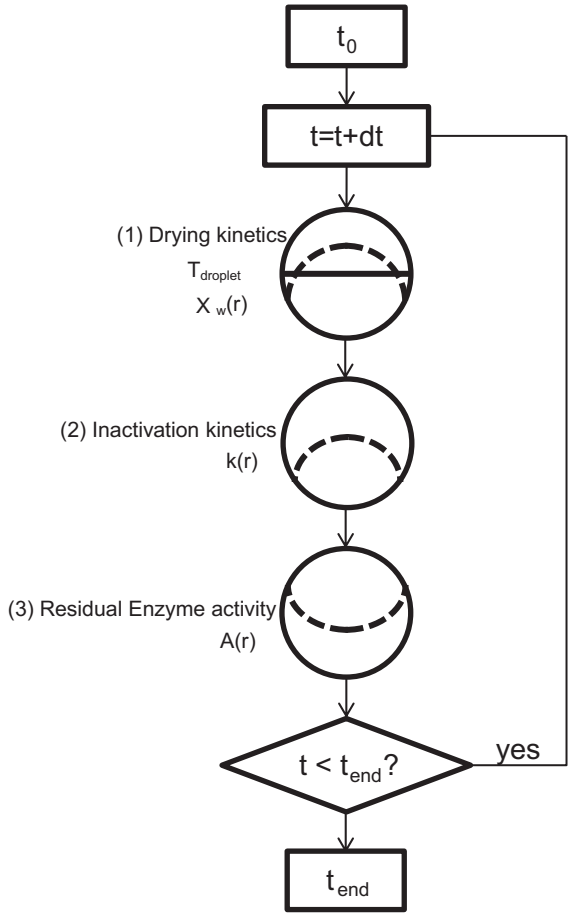

Fig. 2. Schematic overview of the modelling of the combined drying kinetics and the inactivation of enzymes or probiotics for a single droplet. Scheme adopted from Meerdink and Van 't Riet (1995).

assuming the drying of a droplet as described in our earlier work (Perdana *et al*., 2012b). Further, it is noted that the state diagram values (e.g. the glass transition curve) are dependent on the applied heating or cooling rates (normally approximately 20 K/min) during laboratory DSC measurements. One should thus be careful in interpreting this graph quantitatively; however a general notion can be obtained of the different states involved in the drying process, such as rubbery and glassy amorphous states. Additionally, the droplet drying history will deviate for whole milk as the physical properties of milk are different.

From the two plots it can be observed how the particle drying history intersects with the different states of the solute specific state diagrams. The surface and centre of the particle undergo different physical changes. In principle, for spray drying it is desired that the product surface quickly enters the glassy state to prevent a particle from sticking to the wall (Roos, 2002). Furthermore, the state diagrams provide insight into possible routes for stabilisation. The physical changes during the drying correspond to different physical properties, e.g. molecular mobility of the solute matrix. The latter is again coupled to different rates of inactivation. To quantify the drying history and inactivation of different matrices, relations should be available for the effective water diffusion coefficient, equilibrium moisture content, and inactivation kinetics as a function of temperature and moisture content. Unfortunately, these correlations are not widely available for any ingredient.

In Fig. 4, the same droplet drying history as in Fig. 3 is combined with a contour plot for the inactivation rate constant of β-galactosidase in maltodextrin as a function of temperature and moisture content (Perdana *et al*., 2012a).

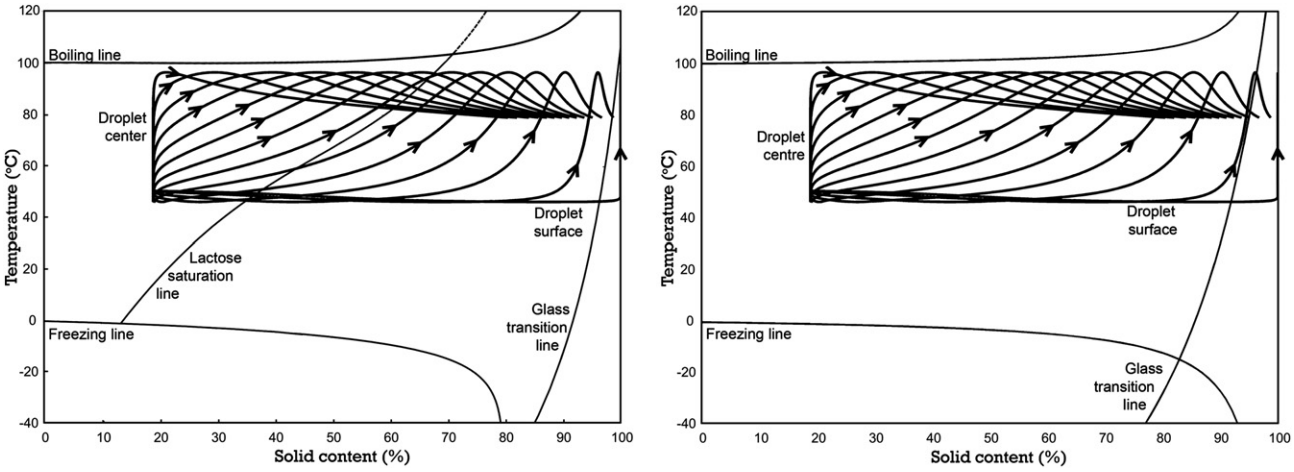

Fig. 3. Left: The state diagram of whole milk, adopted from Vuataz (2002). Right: The state diagram of maltodextrin DE10, partially adopted from van der Sman and Meinders (2011) and Roos and Karel (1991). The model-predicted drying history of a free flying droplet is projected on both state diagrams. Different drying histories are shown representing 14 radial layers of the particle between the centre of the particle and the surface. The applied drying conditions consisted of an inlet air temperature of 180 °C, an outlet air temperature of 78 °C, an initial droplet diameter of 20 μm, an initial droplet temperature of 50 °C, an initial solids content of 20 w/w%, and a residence time of 60 s.

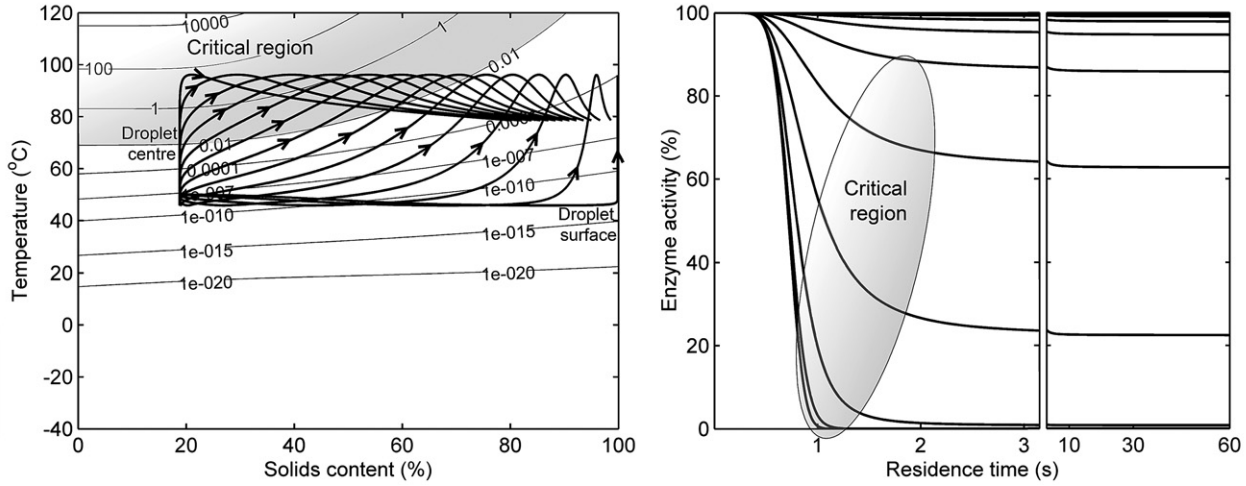

Fig. 4. Left: The drying history of the drying droplet for 14 radial layers plotted onto a contour plot of the inactivation rate constant (s^{-1}) of β-galactosidase as a function of temperature and solids content. Right: The decrease of enzyme activity during drying for the different sublayers, of which the lowest are the layers near the centre and the highest near the surface. The critical region in which the inactivation is most rapid is schematically indicated with a circle.

By comparing Figs. 3 and 4, it can be concluded that the glassy state is connected to lower inactivation rates of β-galactosidase, whereas the critical drying region (with a $k > 0.01$ s^{-1}) coincides with the rubbery phase. At the end of the drying process, the product is actually cooled down to approximately 25 °C in e.g. a fluidised bed. The cooling also contributes to a more even distribution of the water content in the powder particle. This process is not shown in Figs. 3 or 4. Depending on the water content and the final storage conditions the particle may become a glassy powder ($T_{storage} < T_g$) or not ($T_{storage} > T_g$). The latter may be expected especially at high moisture content in combination with low T_g, e.g. for the dried milk droplet in Fig. 3. When storing this milk particle above T_g, this may lead to undesirable crystallisation of lactose, resulting in poor solubility. If it is desired that the dried milk particle is a glassy powder with the absence of lactose crystals, the drying conditions should be adapted (e.g. higher air temperatures or extended drying time). From Fig. 4, the spatial differences in inactivation within the particle can be observed. It is clear that β-galactosidase present in the centre of the droplet is prone to the highest inactivation rates and will have the lowest residual activity. This might suggest that exploration drying of enzymes in a droplet that forms a hollow sphere upon drying, would be interesting (Etzel, Suen, Halverson, & Budijono, 1996), or that enzymes may be applied as a coating of a concentrated enzyme solution, sprayed on pre-dried primary particles.

Scientific challenges

Fundamental insight in structural changes during drying is complementary to single droplet drying studies and can provide better understanding on drying behaviour and stabilisation mechanisms of enzymes and probiotics during drying and storage. That being said, it should be realised that in practice there is not always a good correlation between state changes and stability of components (Chang & Pikal, 2009; Santivarangkna *et al*., 2008). As long as the stabilisation mechanisms are not fully understood, the formulation development for spray-dried powders remains mostly empirical and use of experimental screening methodologies is a must. This leads to the formulation of several scientific challenges:

1. Representative single droplet drying procedures should be developed, which mimic the actual drying conditions during spray drying. Following, the method could also be used to evaluate the influence of different storage conditions, e.g. temperature, low oxygen environment, and moisture content, on activity loss. Automation of single droplet drying and storage experiments could allow quick screening of stable product formulations (Perdana *et al*., 2012b). The latter is of interest to explore the large number of variations in drying and storage conditions in combination with specific formulations in a systematic and fast way.
2. Single droplet modelling approaches should include state structural changes, e.g. crust formation. The finding that a critical temperature difference ($T-T_g$) can be taken as the onset of crust formation could be a starting point for further development of the receding model (Werner *et al*., 2008). A prerequisite for the development of such more complex models is the availability of accurate experimental data for validation.
3. Analytical techniques should be developed for measuring drying kinetics and activity loss during drying of

small single droplets. When drying a droplet with an initial diameter of 200 μm and 40% solids content, only 2 μg of sample remains. Even a simple analysis such as a dry weight analysis may thus become problematic. Because of the lack of such methods most researchers use mostly larger single droplets (up to 500 μm–2 mm in diameter). The assessment of activity is usually possible for a small sample volume, but requires more tedious experimentation, e.g. plating or enzyme activity assays.

4. The structural state changes could be described as a function of the specific properties of the solute. An example is the work of van der Sman and Meinders (2011), in which the state diagram of starch water mixtures was predicted using the Flory–Huggins free volume theory. Their modelling approach can be used to predict and construct state diagrams, avoiding tedious experimentation.

Conclusions

The spray drying of heat sensitive products can be accelerated if a more systematic analysis is made of varying product formulations in relation to their effect on the active component and well-defined drying and storage conditions. This can be realised by single droplet drying procedures combined with predictive models that can map drying behaviour and inactivation kinetics of components at the particle level. It is emphasised that single droplet drying experiments are not an alternative to pilot-scale drying experiments, but a valuable addition. Single droplet procedures should be complemented with increased fundamental insight on stabilisation mechanisms and phase transitions during drying and storage. Specific scientific challenges include amongst others the development of single droplet drying approaches that allow the screening of various drying and storage conditions and formulations in a high throughput manner.

Acknowledgements

The authors thank Advanced Chemical Technologies for Sustainability (ACTS)–Netherlands Organization for Scientific Research (NWO) for financial aid through the Process on a Chip (PoaC) program. Additionally, the authors would like to thank Dr. M.B. Fox from NIZO food research for his contribution to the discussions on single droplet drying.

Appendix.

Table A1. An overview of carrier materials and their anhydrous glass transition temperature.

Component	T_g (°C)	Reference	Additional information
Amorphous potato starch	245	(Benczedi, Tomka, & Escher, 1998)	Extrapolated value; at $x_w = 4$ wt%, $T_g = 178.7$
Starch	243	(Bhandari & Howes, 1999)	
Maltodextrin:			
DE 5	188	(Roos & Karel, 1991)	
DE 20	141	(Roos & Karel, 1991)	
DE 36	100	(Roos & Karel, 1991)	
Maltohexose	175	(Orford, Parker, Ring, & Smith, 1989)	
Amylopectin	151	(Kalichevsky, Jaroszkiewicz, Ablett, Blanshard, & Lillford, 1994)	
Gelatine	120 & 180–190	(Fraga & Williams, 1985)	Two T_g values were found.
α-Casein	132	(Mizuno, Mitsuiki, Motoki, Ebisawa, & Suzuki, 2000)	Extrapolated value; at $x_w = 6$ wt%, $T_g = 100$ °C
Gum arabic	126	(Mothé & Rao, 2000)	
Trehalose	119	(Simperler *et al.*, 2006)	
Raffinose	103	(Liu, Bhandari, & Zhou, 2006)	
Lactose	102	(Haque, Kawai, & Suzuki, 2006)	
Skim milk powder	92	(Roos, 2002)	
Sodium alginate	83	(Nakamura, Hatakeyama, & Hatakeyama, 1991)	
Whey protein isolate	76	(Ghanbarzadeh & Oromiehi, 2008)	
Sucrose	74	(Simperler *et al.*, 2006)	
Galactose	32	(Roos, 1993)	
Glucose	30	(Noel, Parker, & Ring, 1996)	
Fructose	20	(Roos, 1993)	
Xylose	13	(Liu *et al.*, 2006)	
Glycerol	−76	(Win & Menon, 2006)	
Water	−137	(MacFarlane & Angell, 1984)	

References

Adhikari, B., Howes, T., Bhandari, B. R., & Truong, V. (2000). Experimental studies and kinetics of single drop drying and their relevance in drying of sugar-rich foods: a review. *International Journal of Food Properties, 3*, 323–351.

Ali Al Zaitone, B., & Tropea, C. (2011). Evaporation of pure liquid droplets: comparison of droplet evaporation in an acoustic field versus glass-filament. *Chemical Engineering Science, 66*, 3914–3921.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2007). Effects of process variables on the denaturation of whey proteins during spray drying. *Drying Technology, 25*, 799–807.

Benczedi, D., Tomka, I., & Escher, F. (1998). Thermodynamics of amorphous starch–water systems. 1. Volume fluctuations. *Macromolecules, 31*, 3055–3061.

Bhandari, B. R., & Howes, T. (1999). Implication of glass transition for the drying and stability of dried foods. *Journal of Food Engineering, 40*, 71–79.

Chang, L. Q., & Pikal, M. J. (2009). Mechanisms of protein stabilization in the solid state. *Journal of Pharmaceutical Sciences, 98*, 2886–2908.

Chavez, B. E., & Ledeboer, A. M. (2007). Drying of probiotics: optimization of formulation and process to enhance storage survival. *Drying Technology, 25*, 1193–1201.

Chen, X. B. (2009). Modeling and control of fluid dispensing processes: a state-of-the-art review. *International Journal of Advanced Manufacturing Technology, 43*, 276–286.

Chen, X. D., & Patel, K. C. (2007). Micro-organism inactivation during drying of small droplets or thin-layer slabs – A critical review of existing kinetics models and an appraisal of the drying rate dependent model. *Journal of Food Engineering, 82*, 1–10.

Costa-Silva, T. A., Nogueira, M. A., Souza, C. R. F., Oliveira, W. P., & Said, S. (2011). Lipase production by endophytic fungus *Cercospora kikuchii*: stability of enzymatic activity after spray drying in the presence of carbohydrates. *Drying Technology, 29*, 1112–1119.

Cuq, B., Abecassis, J., & Guilbert, S. (2003). State diagrams to help describe wheat bread processing. *International Journal of Food Science and Technology, 38*, 759–766.

Dijkstra, C. E., Larkin, O. J., Anthony, P., Davey, M. R., Eaves, L., Rees, C. E. D., et al. (2011). Diamagnetic levitation enhances growth of liquid bacterial cultures by increasing oxygen availability. *Journal of the Royal Society Interface, 8*, 334–344.

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*, 17–34.

Fabien, G., Antoni, M. l., & Sefiane, K. (2011). Use of IR thermography to investigate heated droplet evaporation and contact line dynamics. *Langmuir, 27*, 6744–6752.

Filková, I., Huang, L. X., & Mujumdar, A. (2006). Industrial spray drying systems. In *Handbook of industrial drying* (3rd ed.). CRC Press.

Fraga, A. N., & Williams, R. J. J. (1985). Thermal properties of gelatin films. *Polymer, 26*, 113–118.

Fu, N., Zhou, Z., Jones, T. B., Tan, T. T. Y., Wu, W. D., Lin, S. X., et al. (2011). Production of monodisperse epigallocatechin gallate (EGCG) microparticles by spray drying for high antioxidant activity retention. *International Journal of Pharmaceutics, 413*, 155–166.

Fyfe, K., Kravchuk, O., Nguyen, A. V., Deeth, H., & Bhandari, B. (2011). Influence of dryer type on surface characteristics of milk powders. *Drying Technology, 29*, 758–769.

Ghanbarzadeh, B., & Oromiehi, A. R. (2008). Studies on glass transition temperature of mono and bilayer protein films plasticized by glycerol and olive oil. *Journal of Applied Polymer Science, 109*, 2848–2854.

Goula, A. M., & Adamopoulos, K. G. (2004). Influence of spray drying conditions on residue accumulation—simulation using CFD. *Drying Technology, 22*, 1107–1128.

Haque, M. K., Kawai, K., & Suzuki, T. (2006). Glass transition and enthalpy relaxation of amorphous lactose glass. *Carbohydrate Research, 341*, 1884–1889.

Hassan, H. M., & Mumford, C. J. (1993). Mechanisms of drying of skin-forming materials .1. Droplets of materials which gelatinized at high-temperature. *Drying Technology, 11*, 1713–1750.

Kalichevsky, M. T., Jaroszkiewicz, E. M., Ablett, S., Blanshard, J. M. V., & Lillford, P. J. (1994). The glass transition of amylopectin measured by DSC, DMTA and NMR. *Carbohydrate Polymers, 18*, 77–88.

Kieviet, F., & Kerkhof, P. J. A. M. (1995). Measurements of particle residence time distributions in a co-current spray dryer. *Drying Technology, 13*, 1241–1248.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2009). Surface composition of industrial spray-dried milk powders. 2. Effects of spray drying conditions on the surface composition. *Journal of Food Engineering, 94*, 169–181.

Knorr, D. (1998). Technology aspects related to microorganisms in functional foods. *Trends in Food Science & Technology, 9*, 295–306.

Labuza, T. P., Kaanane, A., & Chen, J. Y. (1985). Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *Journal of Food Science, 50*, 385–392.

Li, X., Lin, S. X. Q., Chen, X. D., Chen, L., & Pearce, D. (2006). Inactivation kinetics of probiotic bacteria during the drying of single milk droplets. *Drying Technology, 24*, 695–701.

Lian, W.-C., Hsiao, H.-C., & Chou, C.-C. (2002). Survival of bifidobacteria after spray-drying. *International Journal of Food Microbiology, 74*, 79–86.

Lievense, L. C., Verbeek, M. A. M., Van 't Riet, K., & Noomen, A. (1994). Mechanism of dehydration inactivation of Lactobacillus plantarum. *Applied Microbiology and Biotechnology, 41*, 90–94.

Liou, J. K. (1982). *An approximate method for nonlinear diffusion applied to enzyme inactivation during drying*. Unpublished Proefschrift Wageningen, Liou, Wageningen.

Liu, Y., Bhandari, B., & Zhou, W. (2006). Glass transition and enthalpy relaxation of amorphous food saccharides: a review. *Journal of Agricultural and Food Chemistry, 54*, 5701–5717.

MacFarlane, D. R., & Angell, C. A. (1984). Glass transition for amorphous solid water. *The Journal of Physical Chemistry, 88*, 759–762.

Mazza, M. G. G., Brandão, L. E. B., & Wildhagen, G. S. (2003). Characterization of the residence time distribution in spray dryers. *Drying Technology, 21*, 525–538.

Meerdink, G. (1993). *Drying of liquid food droplets: enzyme inactivation and multicomponent diffusion*. Unpublished Proefschrift Wageningen, Meerdink, [S.l.].

Meerdink, G., & VantRiet, K. (1995). Prediction of product quality during spray drying. *Food and Bioproducts Processing, 73*, 165–170.

Meng, X. C., Stanton, C., Fitzgerald, G. F., Daly, C., & Ross, R. P. (2008). Anhydrobiotics: the challenges of drying probiotic cultures. *Food Chemistry, 106*, 1406–1416.

Meyer, W. J. (2004). *Concepts of mathematical modeling*. Dover Publications.

Mezhericher, M., Levy, A., & Borde, I. (2010). Theoretical models of single droplet drying kinetics: a review. *Drying Technology, 28*, 278–293.

Mizuno, A., Mitsuiki, M., Motoki, M., Ebisawa, K., & Suzuki, E.-i. (2000). Relationship between the glass transition of soy protein and molecular structure. *Journal of Agricultural and Food Chemistry, 48*, 3292–3297.

Mothé, C. G., & Rao, M. A. (2000). Thermal behavior of gum arabic in comparison with cashew gum. *Thermochimica Acta, 357–358*, 9–13.

Nakamura, K., Hatakeyama, T., & Hatakeyama, H. (1991). Formation of the glassy state and mesophase in the water–sodium alginate system. *Polymer Journal, 23*, 253–258.

Nijdam, J. J., & Langrish, T. A. G. (2005). An Investigation of milk powders produced by a laboratory-scale spray dryer. *Drying Technology, 23*, 1043–1056.

Noel, T. R., Parker, R., & Ring, S. G. (1996). A comparative study of the dielectric relaxation behaviour of glucose, maltose, and their mixtures with water in the liquid and glassy states. *Carbohydrate Research, 282*, 193–206.

Orford, P. D., Parker, R., Ring, S. G., & Smith, A. C. (1989). Effect of water as a diluent on the glass transition behaviour of malto-oligosaccharides, amylose and amylopectin. *International Journal of Biological Macromolecules, 11*, 91–96.

Patel, K. C., & Chen, X. D. (2007). Production of spherical and uniform-sized particles using a laboratory ink-jet spray dryer. *Asia-Pacific Journal of Chemical Engineering, 2*, 415–430.

Patel, K. C., & Chen, X. D. (2008). Surface-center temperature differences within milk droplets during convective drying and drying-based Biot number analysis. *AIChE Journal, 54*, 3273–3290.

Peighambardoust, S. H., Tafti, A. G., & Hesari, J. (2011). Application of spray drying for preservation of lactic acid starter cultures: a review. *Trends in Food Science & Technology, 22*, 215–224.

Perdana, J., Fox, M. B., Schutyser, M. A. I., & Boom, R. M. (2011). Single-droplet experimentation on spray drying: evaporation of a sessile droplet. *Chemical Engineering & Technology, 34*, 1151–1158.

Perdana, J., Fox, M. B., Schutyser, M. A. I., & Boom, R. M. (2012a). Enzyme inactivation kinetics: coupled effects of temperature and moisture content. *Food Chemistry, 133*, 116–123.

Perdana, J., Fox, M. B., Schutyser, M. A. I., & Boom, R. M. (2012b). Mimicking spray drying by drying of single droplets deposited on a flat surface. *Food and Bioprocess Technology*. doi:10.1007/s11947-011-0767-4.

Rahman, M. S. (2006). State diagram of foods: Its potential use in food processing and product stability. *Trends in Food Science & Technology, 17*, 129–141.

Ranz, W. E., & Marshall, J. M. (1952). Evaporation from drops, Part 1. *Chemical Engineering Progress, 48*, 173.

Roos, Y. H. (1993). Melting and glass transitions of low molecular weight carbohydrates. *Carbohydrate Research, 238*, 39–48.

Roos, Y. H. (2002). Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait, 82*, 475–484.

Roos, Y., & Karel, M. (1991). Water and molecular weight effects on glass transitions in amorphous carbohydrates and carbohydrate solutions. *Journal of Food Science, 56*, 1676–1681.

Santivarangkna, C., Higl, B., & Foerst, P. (2008). Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. *Food Microbiology, 25*, 429–441.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress, 23*, 302–315.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2008). Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. *Journal of Applied Microbiology, 105*, 1–13.

Silva, J., Freixo, R., Gibbs, P., & Teixeira, P. (2011). Spray-drying for the production of dried cultures. *International Journal of Dairy Technology, 64*, 321–335.

Simperler, A., Kornherr, A., Chopra, R., Bonnet, P. A., Jones, W., Motherwell, W. D. S., et al. (2006). Glass transition temperature of glucose, sucrose, and trehalose: an experimental and in silico study. *The Journal of Physical Chemistry B, 110*, 19678–19684.

Sloth, J., Jorgensen, K., Bach, P., Jensen, A. D., Kiil, S., & Dam-Johansen, K. (2009). Spray drying of suspensions for pharma and bio products: drying kinetics and morphology. *Industrial & Engineering Chemistry Research, 48*, 3657–3664.

Sloth, J., Kiil, S., Jensen, A. D., Andersen, S. K., Jørgensen, K., Schiffter, H., et al. (2006). Model based analysis of the drying of a single solution droplet in an ultrasonic levitator. *Chemical Engineering Science, 61*, 2701–2709.

Thybo, P., Hovgaard, L., Lindeløv, J., Brask, A., & Andersen, S. (2008). Scaling up the spray drying process from pilot to production scale using an atomized droplet size criterion. *Pharmaceutical Research, 25*, 1610–1620.

Van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S. D., & Maguin, E. (2002). Stress responses in lactic acid bacteria. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 82*, 187–216.

van der Sman, R. G. M., & Meinders, M. B. J. (2011). Prediction of the state diagram of starch water mixtures using the Flory–Huggins free volume theory. *Soft Matter, 7*, 429–442.

Vuataz, G. (2002). The phase diagram of milk: a new tool for optimising the drying process. *Lait, 82*, 485–500.

Walton, D. E. (2000). The morphology of spray-dried particles a qualitative view. *Drying Technology, 18*, 1943–1986.

Wang, N., & Brennan, J. G. (1991). Moisture sorption isotherm characteristics of potatoes at four temperatures. *Journal of Food Engineering, 14*, 269–287.

Wang, Y.-C., Yu, R.-C., & Chou, C.-C. (2004). Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. *International Journal of Food Microbiology, 93*, 209–217.

Werner, S. R. L., Edmonds, R. L., Jones, J. R., Bronlund, J. E., & Paterson, A. H. J. (2008). Single droplet drying: transition from the effective diffusion model to a modified receding interface model. *Powder Technology, 179*, 184–189.

Whitaker, R. D., & Batt, C. A. (1991). Characterization of the heat-shock response in Lactococcus-Lactis subsp lactis. *Applied and Environmental Microbiology, 57*, 1408–1412.

Win, K. Z., & Menon, N. (2006). Glass transition of glycerol in the volume-temperature plane. *Physical Review E, 73*, 040501.

Wulsten, E., & Lee, G. (2008). Surface temperature of acoustically levitated water microdroplets measured using infra-red thermography. *Chemical Engineering Science, 63*, 5420–5424.

Yamamoto, S. (2001). A short-cut method for determining concentration dependent diffusivity in liquid foods and polymer solutions from regular regime drying curves. *Drying Technology: An International Journal, 19*, 1479–1490.

Yamamoto, S., & Sano, Y. (1992). Drying of enzymes: enzyme retention during drying of a single droplet. *Chemical Engineering Science, 47*, 177–183.

Yarin, A. L., Brenn, G., Kastner, O., Rensink, D., & Tropea, C. (1999). Evaporation of acoustically levitated droplets. *Journal of Fluid Mechanics, 399*, 151–204.

Zbiciński, I., & Piątkowski, M. (2004). Spray drying tower experiments. *Drying Technology, 22*, 1325–1349.

Zhou, X., Dong, J., Gao, J., & Yu, Z. (2008). Activity-loss characteristics of spores of *Bacillus thuringiensis* during spray drying. *Food and Bioproducts Processing, 86*, 37–42.