2014 Stoeckel Inactivation of Bacillus spores

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ORIGINAL RESEARCH

Inactivation of *Bacillus* spores in batch vs continuous heating systems at sterilisation temperatures

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This study was performed to assess the heat resistance of spores of Bacillus *species in batch and continuous heating systems under commercial sterilisation conditions. Spores of thermophilic* Bacillus smithii *and mesophilic* Bacillus amyloliquefaciens *were found to be highly heat resistant in the batch system. They were able to withstand typical sterilisation temperatures.* B. amyloliquefaciens *showed tailing in the batch system and, before the onset of the tailing, a higher inactivation rate than in the continuous system at low temperatures. The reason for the tailing might be the presence of spore aggregates which are disrupted in the continuous system.*

Keywords *Bacillus amyloliquefaciens*, *Bacillus smithii*, Tailing, Spore aggregates, UHT milk, Heating system.

INTRODUCTION

Spores of mesophilic and thermophilic *Bacillus* and related species are ubiquitously present in the dairy environment. Raw milk usually contains 10^0 to 10^3 cfu/mL of spores (Te Giffel *et al.* 2002; Scheldeman *et al.* 2005). Sources of the spores in processed food are, amongst others, the soil, the farming environment and the feed (Carlin 2011). Additionally, spore-forming bacteria form biofilms in dairy processing lines which are difficult to remove. Thus, they can lead to the recontamination of heat-treated milk or milk products with spores (Scott *et al.* 2007; Shaheen *et al.* 2010). The occurrence of spores in long-life milk products causes the deterioration of the final product due to their ability to produce spoilage enzymes. Also, some *Bacillus* species and notably strains of *Bacillus cereus* are able to produce toxins (De Jonghe *et al.* 2010). Therefore, bacterial spores have to be inactivated when producing long-life milk products.

To inactivate spores, heat treatments such as UHT (ultra high temperature) processing and high temperature heating for ESL (extended shelf life) milk are commonly used in the dairy industry. UHT heating leads to commercial sterility of the final products (per definition: 9 log inactivation of *Geobacillus stearothermophilus* spores (Kessler 2002; Hinrichs and Atamer 2011)). ESL heating processes aim at the reduction of aerobic psychrotolerant spores to increase the shelf life (Rysstad and Kolstad 2006). Kinetic data for the thermal inactivation of *Bacillus* spores are determined to design and evaluate heating processes. Systems that are used to examine the inactivation of spores can be divided into two different types: batch heating systems and continuous heating systems. Batch systems such as glass capillaries and metal tubes (e.g. Iciek *et al.* 2006; Dogan *et al.* 2009) have the advantage of small volumes in closed vessels that heat up and cool down quickly. Only small numbers of spores are needed for the experiments. For the calculation of the kinetic parameters, one- or two-step modelling approaches can be used (Valdramidis *et al.* 2008). Large amounts of spores, however, have to be employed for continuous systems. Yet, these systems are closer to industrial heating processes. Only one-step modelling can be used because only dynamic temperature profiles are available (Valdramidis *et al.* 2008). It was recently demonstrated that kinetic parameters can be accurately estimated from dynamic and nonisothermal processes (Dogan *et al.* 2009; Dolan *et al.* 2013).

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Because kinetic data are more easily gained and interpreted using batch heating systems under isothermal conditions, it is common practice to use these results to design heating processes for the dairy industry. However, ambiguities exist in the literature regarding the effects of batch and continuous heating systems on the thermal inactivation of spores. Inactivation in a batch system compared with in a continuous system at UHT processing temperatures was found to be slower (Dogan *et al.* 2009) or faster (Burton *et al.* 1977; Wescott *et al.* 1995) for different *Bacillus* species. Also, the shape of the inactivation curves changed from tailing in batch systems to linear inactivation in continuous systems (Wescott *et al.* 1995). These findings indicate that the design of continuous heating processes using data from batch processes may not ensure product quality and safety.

The aim of this study was to examine the inactivation of *Bacillus amyloliquefaciens* and *Bacillus smithii* spores isolated from the dairy environment in a batch heating system and to compare the inactivation of the *B. amyloliquefaciens* spores in a batch and a continuous heating system. The study was set out to determine the differences between the two systems. Special emphasis was put on the importance of the temperature profiles used for the calculation of the kinetic parameters. A one-step modelling approach considering the effects of time and temperature was applied.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The strains used in this study were isolated from ingredients or products of the dairy industry within the framework of a study supported by the German Ministry of Economics and Technology (project # AiF 16012 N). *B. amyloliquefaciens* strain F85 was obtained from an unheated wild garlic seasoning used as ingredient in dairy processing. *B. smithii* strain F64 originated from a quarg dessert product. The strains were isolated from the food products and identified as described in Lücking *et al.* (2013). They were chosen because of their spoilage potential and because little information is available on the apparently high heat resistance of their spores. In first experiments, the spores proved to be highly heat resistant and survived heating at 125 °C for 30 min (Lücking *et al.* 2013). Moreover, *B. smithii* F64 showed a high sporulation degree in comparison with other examined thermophilic strains which facilitated the production of the spores (data not shown).

Preparation of spore suspensions for the thermal treatments

For the production of the spores, a four-step procedure as described in Witthuhn *et al.* (2011) was used. Brain Heart Infusion agar and broth supplemented with 1 mg/L filter-sterilised vitamin B_{12} (Merck, Darmstadt, Germany) were used for the cultivation of the vegetative cells. *B. amyloliquefaciens* was incubated and sporulated at 30 °C, while for *B. smithii* 55 °C was used. *B. amyloliquefaciens* was sporulated on a sporulation medium with the following composition: 5 g/L peptone from casein (Merck), 3 g/L meat extract (Merck), 20 g/L agar agar (Roth, Karlsruhe, Germany), 1 g/L KCl (Roth) and 0.12 g/L $MgSO_4 \cdot 7\ H_2O$ (Merck). After autoclaving, 1 mL of each of the following, filter-sterilised solutions (1M $Ca(NO_3)_2$, 0.1 M $MnCl_2$, 1 mM $FeSO_4$) was added. The composition of the sporulation medium for *B. smithii* was described by Iciek *et al.* (2006).

After incubation for 4–5 days, the sporulation degree reached 99 and 90% for *B. amyloliquefaciens* and *B. smithii*, respectively. The spores were harvested and washed. The concentration of the final spore suspensions was determined as described below and was 9.2 x 10^8 cfu/mL for *B. amyloliquefaciens* and 1.6 x 10^8 cfu/mL for *B. smithii*.

Thermal treatment

Batch system

For the experiments performed in the batch heating system, the system described by Dogan *et al.* (2009) was used. Briefly, the self-designed system consisted of a pressure vessel with inlets for steam (up to 0.7 mPa) and iced water, and outlets for the same, each closed with a valve. The temperature of the steam was regulated using pressure reducing valves (Spirax Sarco GmbH, Konstanz, Germany). Up to six screw-capped stainless steel tubes (volume: 1.5 mL) with hermetic seals containing the spore suspension were placed in the vessel. For the experiments, the tubes in the pressure vessel were heated by direct contact with steam using saturated steam condensation and cooled by direct contact with iced water. The heating temperature was adjusted by opening and closing the valve for the steam manually. A temperature sensor (Pt100, 4L, Klasse B, NiCr-Ni; Ahlbom, Germany) connected to a data logger (Almemo, 2290-4, Ahlborn) was placed in an additional tube filled with water to record the temperature during processing directly in the medium. Spores of *B. amyloliquefaciens* or *B. smithii* were suspended in reconstituted ultrafiltration permeate, which was selected as the medium for the heating experiments (5.2 g/L, pH 6.6), in a ratio of 1:15 and given in the heating tubes. Spray-dried sweet whey permeate powder was supplied by Bayolan PT, Bayerische Milchindustrie eG, Landshut, Germany (dry matter 96.1 g 100/g, protein 3.5 g 100/g, fat <1.0 g 100/g, lactose 83.0–87.0 g 100/g and ash 6.5–8.0 g 100/g). Ultrafiltration permeate has the same composition as milk (without proteins and fat) and was used as a substitute for milk in the experiments. Temperatures from 110 to 140 °C and holding times from 4 s to 30 min were examined. Each heating experiment was conducted three times. In Fig. 1, a temperature profile is shown for the treatment at 130 °C for 4 s.

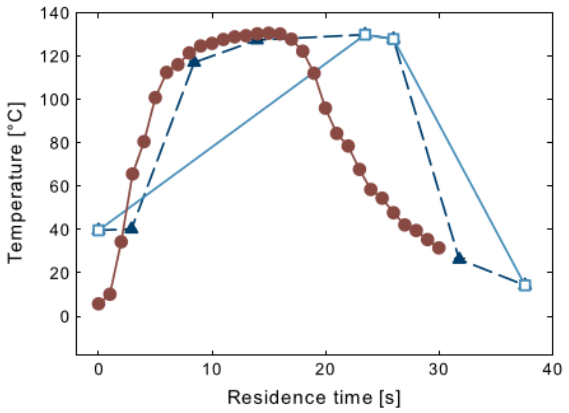

Figure 1 Different temperature profiles of the heating systems: continuous heating system with (1) permanently installed temperature sensors (solid line, □) and (2) four additional temperature sensors (broken line, ▲) for 130 °C/4 s and batch heating system (●) for 130 °C/4 s. Confidence intervals ($P < 0.01$) are given for the interpolated temperatures of profile 2.

Continuous system

Due to the high number of spores necessary for these experiments, only spores of *B. amyloliquefaciens* were examined in the continuous heating system. The spores suspended in ultrafiltration permeate were heated in the double pipe tubular heat exchanger (150 L/h) of a self-designed pilot plant (Asepto GmbH, Dinkelscherben, Germany). The heating system consisted of a sterilised feed tank (10 L; EB-1542-00, Bolz Intec GmbH, Argenbühl, Germany) in which spores and ultrafiltration permeate were mixed, then an eccentric screw pump (type NM021, Netsch, Waldkraiburg, Germany), a prewarming section with two plate heat exchangers, a heating section (double pipe tubular heat exchanger; $r = 3.5$ mm), a holding section (insulated tubes; $r = 3.5$ mm) and an ice water cooling unit (double pipe tubular heat exchanger; $r = 3.5$ mm). The product was heated with pressurised hot water (which was heated with steam) and cooled with iced water in counter-current flow. The holding time was adjusted by varying the length of the holding section using up to seven successively combinable holding tubes with lengths of 0–256 s at 150 L/h in an insulated vessel. When prolonging the holding time from 0 to 256 s, a maximum temperature difference of 5 °C at the exit of the holding section was observed. Temperature–time combinations of 110–140 °C and 0–256 s were examined using this system. For the experiments, the spores were mixed with the ultrafiltration permeate at 6 °C. Rapid heating, holding and immediate cooling of the sample to 10 °C followed. The heat-treated sample (500 mL) was taken after the cooling section under aseptic conditions and immediately cooled on ice before analysis. Each experiment was conducted with 10 L of spore suspension. Before the start of the experiments, the systems ran for approx. 15 min for temperature stabilising reasons.

Four temperature sensors (Pt100; Negele Messtechnik, Egg an der Günz, Germany) were installed permanently in the system which measured the temperature directly in the product. The positions of these temperature sensors were after preheating section, after heating section, after holding section and after cooling section (temperature profile # 1, Fig. 1). Additional temperature sensors (T/Couple, Labfacility, Bognor Regis, Great Britain) were installed on the surface of the heating and cooling sections in places where the outer tubes were missing to measure the temperature development in more detail (temperature profile # 2, Fig. 1). The installation of additional sensors was necessary as the imprecise knowledge of the temperature profile in continuous systems might lead to an over- or underestimation of the temperature effect. To determine the temperature in the product from the measured temperature on the surface, the temperature loss between the product and the surface of the inner pipe was assessed by measuring the surface temperature in places where a permanently installed sensor also measured the temperature directly in the product. A line of best fit describing this relation was acquired ($\text{temperature}_{\text{inside}} = 1.04 \pm 0.00 \times \text{temperature}_{\text{outside}} - 0.10 \pm 0.21$; $R^2 = 0.9995$). The temperature in the product was then interpolated from this information. The two different types of temperature profiles are shown in Fig. 1.

Determination of viable spore count

The samples were diluted with Ringer solution and then directly spread-plated on tryptic soy agar (TSA, Roth). The agar plates were incubated for 96 h at 30 °C for *B. amyloliquefaciens* and 55 °C for *B. smithii*. The number of viable cells was determined before and after the heat treatments. The limit of detection was 10 cfu/mL.

Calculation of the kinetic data

The kinetic data for the inactivation of *B. amyloliquefaciens* and *B. smithii* in the batch and the continuous heating system were calculated by applying the Arrhenius model using Matlab R2010a (The Mathworks, Natick, USA). The best-fit solution was found using the least squares method. The standard error of the parameters was calculated by bootstrapping (1000 repetitions). The temperature effects of the entire thermal process including heating and cooling were considered using the method of Dogan *et al.* (2009). For the experiments with *B. amyloliquefaciens* in the batch and the continuous heating system, a model covering nonlinear inactivation was used (Eq. 1). For the inactivation experiments with *B. smithii*, linear inactivation curves were assumed (Eq. 2) (Kessler 2002; Müller-Merbach *et al.* 2005). It was not possible to satisfyingly fit the Arrhenius model to the data from the continuous heating system. Therefore, for this system,

only D-values for certain temperatures were calculated (see below).

$$\frac{N_t}{N_0} = \left[1 + (n-1)\cdot N_0^{n-1}\cdot k_{ref}\cdot \exp\left(-\frac{E_a}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\cdot t\right]^{\frac{1}{1-n}} \quad for\, n \neq 1 \tag{1}$$

$$\frac{N_t}{N_0} = \exp\left(-k_{ref}\cdot \exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\cdot t\right) \quad for\, n = 1 \tag{2}$$

where N_t is the colony count at time t (cfu/mL), N_0 the initial colony count (cfu/mL), n the order of the reaction (), k_{ref} the rate constant at reference temperature (/s or /s (cfu/mL)$^{1-n}$), t the holding time (s), E_a the activation energy (J/mol), R the universal gas constant (8.314 J/mol/K), T the absolute temperature (K) and T_{ref} the reference temperature (394 K).

In the case of linear inactivation curves, the values obtained for E_a and k_{ref} were converted into D_ϑ- and z_ϑ-values using Eqs. 3 and 4 (Kessler 2002). As the reference temperature, 121 °C (394 K) was used.

$$D_\vartheta = \frac{2.303}{k_\vartheta} \tag{3}$$

$$z_\vartheta = \frac{\ln(10)\cdot R\cdot T_{ref}^2}{E_a} \tag{4}$$

In addition, for the experiments with *B. amyloliquefaciens*, the D-values for 110, 115 and 120 °C in the two systems were determined for the linear part of the inactivation curve to compare the inactivation effect. The following Eq. 5 was used (Kessler 2002).

$$D = \frac{-t}{(\log N - \log N_0)} \tag{5}$$

RESULTS

Temperature development in the batch and the continuous heating system

According to Burton *et al.* (1977), the differences in the inactivation behaviour of spores between batch and continuous heating systems are either due to a failure to characterise the heating plants correctly or due to differences in the spore destruction. Thus, emphasis was led on the correct description of the temperature development in the heating systems. For this reason, additional temperature sensors were installed in the continuous heating system. The temperature–time profiles were recorded for each experiment and were used to calculate the kinetic parameters of the inactivation. In Fig. 1, examples for the temperature–time profiles are given. In the batch system, approx. 10 s were needed to increase the temperature from 40 °C until the desired final temperature. For the continuous system, it was shown that the highest increase in temperature took place in the first third of the heating section. The desired temperature was reached after approx. two-thirds (approx. 14 s). Measuring the temperature only at the exit of the heating section was not sufficient.

Inactivation in the batch heating system

The inactivation of the two strains was examined in the batch heating system. In Figs. 2 and 3, the results for the inactivation of *B. amyloliquefaciens* F85 spores in ultrafiltration permeate are shown. The spores were able to survive treatments ranging from 130 to 140 °C for up to 33 s. Tailing of the inactivation curves was observed at 115, 120 and 125 °C. Figs. 4 and 5 illustrate the inactivation curves of *B. smithii* F64 spores. *B. smithii* showed linear inactivation curves. At high temperatures (≥130 °C), a behaviour similar to that of *B. amyloliquefaciens* was observed.

Inactivation in the continuous heating system

The spores of *B. amyloliquefaciens* F85 were additionally examined in the continuous heating system. The results are shown in Fig. 6. At 110 °C, no inactivation occurred in the continuous system due to the short holding times. At 115, 120 and 125 °C, log-linear inactivation was observed. At 130, 135 and 140 °C, complete inactivation occurred already after a holding time of 4 s (data not shown).

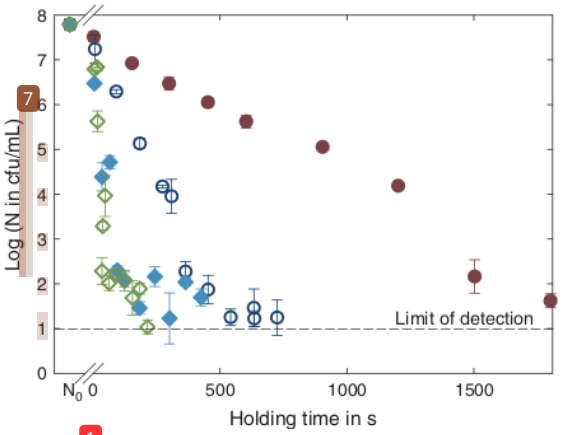

Figure 2 Inactivation of *Bacillus amyloliquefaciens* F85 spores in the batch heating system. Heating medium: ultrafiltration permeate. Temperatures: 110 °C (●), 115 °C (○), 120 °C (♦), 125 °C (◇,). Limit of detection: 10 cfu/mL.

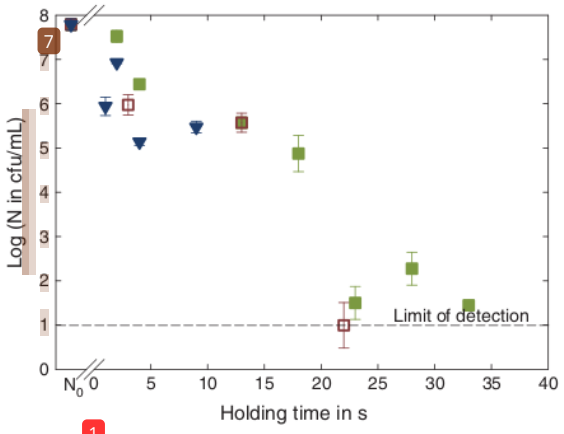

Figure 3 Inactivation of *Bacillus amyloliquefaciens* F85 spores in the batch heating system. Heating medium: ultrafiltration permeate. Temperatures: 130 °C (■), 135 °C (□), 140 °C (▼). Limit of detection: 10 cfu/mL.

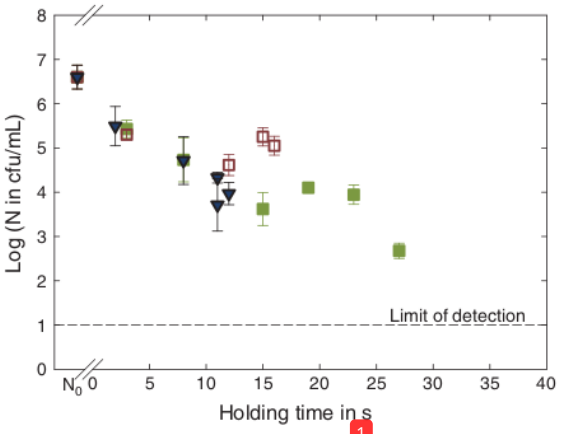

Figure 5 Inactivation of *Bacillus smithii* F64 spores in the batch heating system. Heating medium: ultrafiltration permeate. Temperatures: 130 °C (■), 135 °C (□,), 140 °C (▼). Limit of detection: 10 cfu/mL.

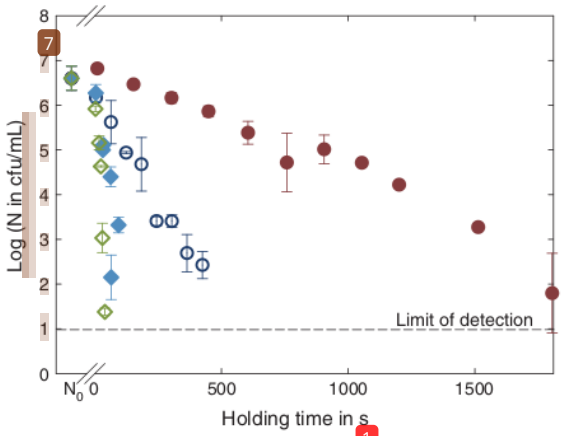

Figure 4 Inactivation of *Bacillus smithii* F64 spores in the batch heating system. Heating medium: ultrafiltration permeate. Temperatures: 110 °C (●), 115 °C (○,), 120 °C (♦), 125 °C (◇). Limit of detection: 10 cfu/mL.

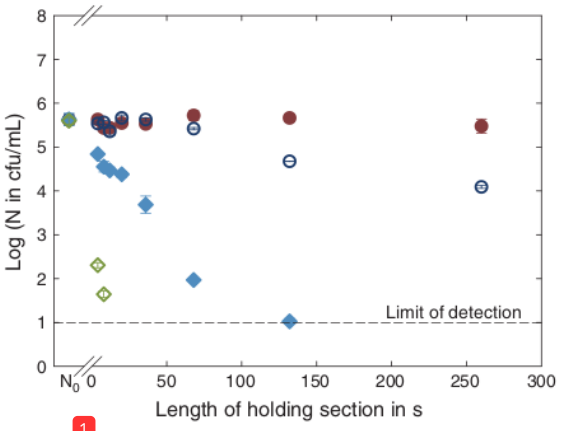

Figure 6 Inactivation of *Bacillus amyloliquefaciens* F85 spores in the continuous heating system. Heating medium: ultrafiltration permeate. Temperatures: 110 °C (●), 115 °C (○,), 120 °C (♦), 125 °C (◇). Limit of detection: 10 cfu/mL.

Calculation of the kinetic parameter

In Table 1, the kinetic parameters calculated according to the Arrhenius model are summarised for the batch heating system. For the determination of the kinetic parameters, the entire temperature profiles recorded in a reference tube during the experiments were used applying one-step modelling. For the continuous heating system, it was not possible to fit the model to the data. Therefore, kinetic parameters according to the Arrhenius model are not given for this system. Instead, *D*-values for the linear parts of the inactivation curves of *B. amyloliquefaciens* in the two systems were calculated additionally and are summarised in Table 2.

DISCUSSION

Inactivation of *B. smithii* spores

Spores of the thermophilic *B. smithii* strain showed a high heat resistance in the batch heating system. The spores survived heat treatments of 130, 135 and 140 °C applied for up to 30 s. *B. smithii* F64 showed a linear inactivation behaviour. To our knowledge, no data are available concerning the thermal resistance of *B. smithii* spores. In comparison with the results from Dogan *et al.* (2009) for *G. stearothermophilus*, a thermophilic species whose spores

Table 1 Kinetic parameters for the inactivation of *Bacillus amyloliquefaciens* F85 and *Bacillus smithii* F64 spores in ultrafiltration permeate in the batch heating system determined using one-step modelling

Strain	*Heating system*	ϑ_{ref} [°C]	E_a[kJ mol^{-1}]	k_{ref} [s^{-1}]	n[–]	z_ϑ-value[K]	D_ϑ-value [min]
F85	Batch	121	181.7 ± 18.1	0.009 ± 0.003	1.16 ± 0.03	–[a]	–[a]
F64	Batch	121	187.9 ± 8.5	0.035 ± 0.004	1	15.8	1.1

ϑ_{ref}, Reference Temp; E_a, Activiation Energy; k_{ref}, Rate constant; n, Reaction Order, z_ϑ, Z value; D_ϑ, D value.
[a]Not calculated due to nonlinear inactivation curves.

Table 2 *D*-values for the inactivation of *Bacillus amyloliquefaciens* S85 spores in the batch and the continuous heating system for the linear part of the curves

	D-value [min]		
Heating system	*110 °C*	*115 °C*	*120 °C*
Batch	5.13 ± 0.30	1.41 ± 0.09	0.60 ± 0.12
Continuous	–[a]	2.75 ± 0.33	0.53 ± 0.06

[a]No inactivation observed.

are used to define UHT heating regions (Kessler 2002), the spores have a higher heat resistance. According to the results from this study, *B. smithii* spores are able to survive temperature–time combinations of UHT milk and other sterilisation processes.

The strain used in this study was isolated from an unspoiled quarg dessert product with chocolate. It was not possible to further trace the source of the spores. The origin of the strain could be the raw milk, but also the other ingredients used for the dessert product. In addition, in this study, spores of other *B. smithii* strains were isolated from spices (caraway and ginger; data not shown).

Inactivation of *B. amyloliquefaciens* spores

Also the spores of the mesophilic *B. amyloliquefaciens* strain showed a high heat resistance in the batch heating system. The spores survived heat treatments of 130, 135 and 140 °C for up to 30 s (Fig. 3). In contrast to the spores of *B. smithii*, *B. amyloliquefaciens* spores exhibited tailing at temperatures varying from 115 °C to 125 °C. The apparent order of reaction n was calculated as 1.16. The detectable tailing appeared in the concentration range of 10^1 to 10^2 cfu/mL, which is just above the limit of detection. At 110 °C, tailing was not detected because the holding times in the heating system were not long enough to reach very low cell numbers (Fig. 2). Because *B. amyloliquefaciens* strain F85 showed tailing, *D*- and *z*-values were not calculated for the entire inactivation curves. Instead, *D*-values were calculated for 110, 115 and 120 °C for the linear parts of the inactivation curves to compare these with the results from the continuous heating system.

Few literature data are published regarding the heat resistance of *B. amyloliquefaciens* spores. However, it is possible that *B. amyloliquefaciens* was misidentified as *Bacillus subtilis* in former studies (Valerio *et al.* 2012) which would explain the few reports in the literature on the heat resistance of *B. amyloliquefaciens* spores, a species frequently associated with spoilage of bread (ropiness) (Valerio *et al.* 2012). In a previous study, we examined *B. amyloliquefaciens* spores of strain HRSF 298 (own isolate from a dairy product) and found a similar resistance at sterilisation temperatures (Witthuhn *et al.* 2011). The spores were able to withstand UHT processing conditions. Also for strain HRSF 298, nonlinear inactivation behaviour was observed at some temperatures (105, 110 and 115 °C) in the batch heating system. Ahn *et al.* (2007) observed linear inactivation at 105 °C and tailing at 121 °C. Tailing of the survival curves at 120 °C was also noticed by Margosch *et al.* (2006) and at 121 °C by Rajan *et al.* (2006). In all of these studies, batch heating systems such as glass capillaries (Margosch *et al.* 2006) or metal tubes (Rajan *et al.* 2006; Ahn *et al.* 2007) were used for the thermal inactivation experiments.

For the calculation of the kinetic parameters in the continuous heating system, the temperature development in the system was determined using two different methods. It was shown that recording the temperature only at the exit of the heating section is not sufficient to accurately describe the temperature development. The desired holding temperature was reached already after approx. two-thirds of the heating section (Fig. 1) which prolonged the measured holding time of approx. 10 s. In the continuous heating system, tailing of the inactivation curves was not observed. It was not possible to fit the employed model (linear and nonlinear) to the data from the continuous system.

In general, it can be said that when a linear temperature rise is assumed (profile # 1), the inactivation effect of the high temperatures is overestimated. In consequence, this might lead to nonsterile products if the kinetic parameters are determined using this kind of temperature profiles. These findings illustrate the importance of (i) recording the temperatures also in the heat exchangers during heating and cooling and (ii) using the entire (dynamic) temperature profiles for the calculation of the kinetic parameters. One-step (or global) modelling is necessary to calculate the kinetic data.

Comparison of the inactivation in the batch and the continuous heating system

In the batch heating system, tailing was observed (n = 1.16), whereas in the continuous heating system, log-linear inactivation occurred. Hence, we compared the inactivation rates (D-values) in the two systems at 110, 115 and 120 °C during the linear part of the inactivation curves (Table 2). At 110 and 115 °C, the batch heating system was more lethal than the continuous heating system. The D-values were lower. At 120 °C, the inactivation rates in the two systems were similar. It was not possible to calculate the inactivation rates at higher temperatures because the spores were immediately inactivated in the continuous heating system. But it seems that at temperatures of 125 °C and higher, the continuous system was more lethal because spores were not detected anymore, whereas at the corresponding holding times in the batch system, spores were still viable. To summarise, the major differences between the two systems were the higher lethality of the batch system at low temperatures (110 and 115 °C), the higher lethality of the continuous system at high temperatures (>125 °C) and especially the occurrence of tailing in the batch system.

Burton *et al.* (1977) and Wescott *et al.* (1995) found the inactivation of *G. stearothermophilus* spores in a batch heating system to be faster than in a continuous heating system in the temperature range studied here. Contrarily, the continuous system was more lethal than the batch system for spores of *G. stearothermophilus* and *Bacillus flexus* (Dogan *et al.* 2009) as well as for spores of *B. cereus* (Wescott *et al.* 1995). Similar to the results from this study, also Burton *et al.* (1977) observed that at some point, this behaviour inversed. From approx. 145 °C on, they found the continuous system to be more lethal. However, the authors from the other studies did not find satisfactory explanations for the observed differences. Facts such as the residence time distribution of the spores in the continuous heating system or the temperature drop in the holding section can be a reason for a higher apparent heat resistance of the spores in this system, but not for a lower. In the employed continuous system, shearing takes place. Considering the volumetric flow rate (150 L/h) and the radius of the pipes (5 mm), an apparent shear rate of 424/s was present in the system (Arellano *et al.* 2013). In the eccentric screw pump which was installed in the continuous system, even higher shearing took place. In a recent study, we were able to show that shear stress in this range triggers the inactivation of spores (M. Stoeckel, A.C. Westermann, Z. Atamer and J. Hinrichs, unpublished data). This might be a reason for the higher lethality of the continuous system.

The second difference that was observed between the two systems in this study was the occurrence of tailing in the batch heating system, but not in the continuous system. As mentioned before, tailing of *B. amyloliquefaciens* spores in batch systems was also detected by other authors (Margosch *et al.* 2006; Rajan *et al.* 2006; Ahn *et al.* 2007). Yet, in these studies, continuous systems were not examined. In a recent study with spores of *B. cereus*, we showed that tailing only appeared in nonsheared batch heating experiments (Stoeckel *et al.* 2013). Tailing in the batch system, but not in the continuous system, was also observed by Wescott *et al.* (1995) for *B. cereus* spores. The authors explained this phenomenon with the presence of spore aggregates in the spore suspension that are disrupted during processing in the continuous system. One spore aggregate always leads to one colony when determining the spore count in colony-forming units, independent of the size of the aggregate (Mathys *et al.* 2007). Thus, a disrupted aggregate produces more colony-forming units than the aggregate itself. Because aggregates with unknown numbers of spores produce one colony until the last spore is inactivated (Mathys *et al.* 2007), tailing is detected when aggregates are present.

According to Burton *et al.* (1977), the differences in the inactivation behaviour of spores between batch and continuous heating systems are either due to a failure to characterise the heating plants correctly or due to differences in the spore destruction. The first reason can be neglected as we described the temperature development in both systems correctly. We propose another possible reason for the observed differences, that is, the disaggregation of spore aggregates due to shear stress.

CONCLUSION

Spores of *B. smithii* and *B. amyloliquefaciens* isolated from the dairy environment were examined regarding their heat resistance in a batch (both species) and a continuous (only *B. amyloliquefaciens*) heating system at UHT processing temperatures. Both strains were highly heat resistant and are able to withstand temperature–time combinations of UHT milk processing. Differences were observed between the inactivation behaviour in the batch and in the continuous system for *B. amyloliquefaciens*. The inactivation curves in the batch system exhibited tailing and had a higher inactivation rate in the log-linear part of the curve at 110 and 115 °C. This behaviour might be due to the disruption of spore aggregates present in the spore suspension. In addition, it was shown that the precise recording of the temperature development in the continuous system, especially in the heating section, is of importance for the correct calculation of kinetic parameters.

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