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Thermal treatment of skim milk concentrates in a novel shear-heating device: Reduction of thermophilic spores and physical properties

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ABSTRACT

Endospores of thermophilic bacilli are a major concern for producers of dairy powders. In this study, we heat treated 10 different spore suspensions at 110 °C in skim milk and skim milk concentrate (36% dry matter) of the species *Geobacillus stearothermophilus* (10 min) and *Anoxybacillus flavithermus* (5 min) in a new shear-heating device. The highest log reduction in skim milk concentrate was 3.5. The death behavior of the spores was strain dependent. Particle formation and Maillard reaction were observed. By increasing the shear-rate up to 1500 s^{-1} the particle size was reduced for both heating times (D90 reduction: 57.4 and 77.0%, respectively). The particle size was lessened by a reduction of dry matter of 27%, compared to 36%. This work emphasizes, that heat treatment of concentrated dairy products represents a technological option to reduce thermophilic spores in skim milk concentrate and powders produced thereof.

1. Introduction

Evaporation and subsequent spray drying of milk products, such as skim milk or whey, are technologies to store these food ingredients over a long period by reduction of the free water. The loss of favorable ingredients during the production is comparable low (Schuck, 2002). Though the export of powdered milk products globally has increased over the last years, the demand for stricter quality standards is high. One parameter to value the quality of milk powders is the count of thermoresistant endospores from thermophilic microorganisms (Burgess, Lindsay, & Flint, 2010; Hill & Smythe, 2012; Watterson, Kent, Boor, Wiedmann, & Martin, 2014). The total aerobic spore count for the Chinese market should not exceed 10^3 cfu·g^{-1} for infant formula (Yuan et al., 2012). During the production of powdered products (separation, pasteurization, evaporation) the temperatures are in an optimal range for the growth and sporulation of thermophilic spore formers, such as *Geobacillus* and *Anoxybacillus* sp. (Scott, Brooks, Rakonjac, Walker, & Flint, 2007). Furthermore, we showed recently that some thermophilic strains are capable of surviving a high-temperature-short-time pasteurization in their vegetative form (Reich et al., 2017). The vegetative cells can sporulate later on and remain in the production site. This leads to the problem that a milk powder product with very low counts of thermophilic spores is hard to achieve for manufacturers worldwide (Kent, Chauhan, Boor, Wiedmann, & Martin, 2016; Sadiq et al., 2016; Witthuhn, Lücking, Atamer, Ehling-Schulz, & Hinrichs, 2011).

One technological option to overcome this problem is the heat treatment of the concentrated milk product after or between the evaporation steps. Until now, there is very little research about possible reduction of bacterial endospores in concentrated milk products available as experimental set-ups to investigate these questions are lacking. A problem that arises is the coagulation of concentrate milk or whey at higher total solids due to protein aggregation (Dumpler & Kulozik, 2015; Havea, Watkinson, & Kuhn-Sherlock, 2009; Hinrichs, 2000). Inactivation of bacterial endospores are however often studied in glass capillary or stainless steel tubes (Berendsen, Zwietering, Kuipers, & Wells-Bennik, 2015; Dogan, Weidendorfer, Müller-Merbach, Lembke, & Hinrichs, 2009; Mathys, Heinz, Schwartz, & Knorr, 2007). Hereby and in the case of a highly viscous heating medium, the thermal transfer is not homogenous throughout the sample. Reasons for the coagulation of skim milk concentrates are dissociation of the κ-casein at higher temperatures and concentration factors (Anema, 1998). For concentrated whey products, crosslinking between the main whey proteins occurs, depending on pH-level and mineral composition (Havea, Singh, & Creamer, 2002). When casein micelles interact with denatured whey proteins, gel formation occurs at high temperatures and it is difficult to study the effect of the media on the death behavior of bacterial spores.

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Therefore, the aim of the study was to establish an experimental set-up to heat (> 100 °C) concentrated skim milk with high dry matters (DM) while simultaneously shearing the sample and avoid an inhomogeneous heating of the product. The influence of the heating media (skim milk or skim milk concentrate) on the log-reduction value of thermophilic spores isolated from milk powder environments should be investigated in a first step. A process slot for heating concentrated products should be furthermore established focusing on the two aspects: (1) particle size distribution of the skim milk concentrate due to gel formation and (2) color development. The heating time as well as the shear rate should be varied and the effect on the media skim milk concentrate studied.

2. Material and methods

2.1. Design of the shear-heating test system

The measuring of thermal death data for certain microorganisms is a crucial step for process optimization and adaption. In the past, log-reductions values were requested depending on the product (e.g. 9-log for thermophilic spores for UHT-processing) and the process was designed considering the product quality (e.g. brown color). To establish process parameters for the heating of concentrated milk products with respect to microbiological death data and product parameters a system for the experiments needs to be established. The used systems, capillary heating device or tubes, have limitations regarding a mixing during the heating-up and cooling phase. In fact, the heat transfer is not comparable to an industrial process where a continuous heating takes place. For the design of an experimental set-up, the following requirements had to be fulfilled:

(1) the whole device should be autoclavable and cleanable with chemicals and disinfectants;
(2) the shear rate, simulating flow conditions, of the whole system should be varied easily by an electronic motor;
(3) the performance of the electric motor should stay stable at high temperatures;
(4) the whole unit should be heated with steam for a fast heat transfer and cooled with ice water;
(5) temperature development in the shear cell should be monitored precisely;

To fulfill the requirements, a device was built that can be inserted in a heating (steam up to $6 \cdot 10^5$ MPa) and cooling (ice water) vessel, described in detail elsewhere (Dogan et al., 2009). Furthermore, a system consisting of stainless steel was constructed with a stainless steel rotor. To stabilize the rotor, two polymer ball-bearings were inserted so that the rotor levitates in the outer body. The gap between rotor and outer body is 1 mm. Two temperature sensors (OMEGA Deckenpfronn, Germany, Pt100) go succinctly in the shear-heating gap and are fixed with a compression fitting. In order to allow the motor to hold the performance at high temperatures, a magnetic coupling consisting of samarium-cobalt (DST Magnetic coupling, Neuenrade, Germany) was chosen. The rotational speed was measured with a photoelectric guard, positioned on the top of the electric motor. The whole system is shown in Fig. 1 with two photographs and a schematically drawing.

2.2. Microorganisms, preparation of spore suspensions and spore count determination

2.2.1. Bacterial test strains

The microorganisms used in this study were isolated from milk and whey products (intermediate products and powdered products) and are listed in Table 1. All strains belong to the family *Bacillaceae* and the two species used were *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus*. Their corresponding food sources were described in detail in Reich et al. (2017). The microorganisms were stored at −20 °C in glycerol (30% (w/v), Merck, Darmstadt, Germany). The strains were sequenced via their 16S rDNA prior to this study.

2.2.2. Preparation of spore suspensions

The method for the preparation of the spore suspension was used as described in Witthuhn et al. (2011) with slight modifications. Briefly, prior to the preparation of suitable spore suspensions a dilution streak on tryptic-soy-agar (TSA, Roth, Karlsruhe, Germany) for 12–24 h (55 °C) was prepared. Afterwards, the cells were grown in a liquid culture in tryptic-soy-broth (TSB, Roth, 55 °C, 20 mL) for 8 h at 110 rpm. From the first culture, 100 µL were transferred into a second liquid culture (20 mL, TSB, 55 °C) for approx. 14 h. By using two liquid cultures, a denser culture and thus a better spore yield was achieved. 1 mL of the culture was given on TSA-plates (diameter 140 mm) to which 0.1% (*v*/v) of sterile 0.1 M $MnCl_2$, 1 mM $FeSO_4 \cdot 7\ H_2O$ and 1 M $Ca(NO_3)_2 \cdot 4\ H_2O$ (Roth,) were added. The sporulation was monitored via phase contrast microscopy and after 2–8 days of incubation, the spores were harvested. After pasteurization (80 °C, 10 min) the suspension was washed 4 times with cold phosphate buffer (0.002 M KH_2PO_4 and 0.008 M K_2HPO_4, Roth) through centrifugation (2218 *g*, 7 min). After the washing steps, the spores were stored up to 3 days in ethanol (35%, Roth) and were finally resolved in phosphate buffer at 2 °C before using them. The step of spore suspension preparation was conducted three times in independent biological replicates. For each spore suspension, the spore titer was adjusted dependent on the suspension with the lowest count. The initial average spore counts for each strain are given in Table 1.

2.2.3. Determination of spore count

For the determination of the spore count (before and after heat treatment), a serial dilution in a quarter Ringer's solution (Merck KGaA) was prepared and spread plated on TSA plates. The plates were incubated at 55 °C for 24–48 h, counted and the concentrations were calculated by the weighted arithmetic mean.

2.3. Heating media and heating experiment

2.3.1. Preparation of the heating media

As heating media, UHT-treated skim milk (0.3% fat) from a local distributor was used as reference media. For the preparation of the concentrated skim milk, raw milk from the Meiereihof Research Station (University of Hohenheim) was separated (total fat content < 0.3%, SA 10-T, Frautech, Schio, Italy), pasteurized (72 °C, 32 *s*, 150 $L \cdot h^{-1}$, ATS-Südmo, Feldkirchen, Germany) and stored at 4 °C over night. Afterwards, the pasteurized skim milk was concentrated via reverse osmosis (3839 HRX-NYV, Koch Membrane Systems Inc., Stafford, GB, at 50 °C). The transmembrane pressure was increased stepwise during filtration to a maximum value of 40 MPa. The filtration process was stopped as the final flux was < 2 $L \cdot m^{-2} \cdot h^{-1}$. The skim milk concentrate was frozen at −20 °C until further use for the experiments. The dry matter content was determined after the sea sand method (VDLUFA, 1985). The protein content in the media was calculated by measuring the nitrogen content in the sample (LecoFP-528, LecoInstruments, Mönchengladbach, Germany) according to the method of Dumas (IDF, 2002). The dry matter of the skim milk concentrate after reverse osmosis was 32.4% ± 0.1, the protein content 12.47% ± 0.04. A higher dry matter (40%) for the experiments was adjusted with skim milk powder (VWR, Radnor, USA). For heating experiments, 1 mL spore suspension was added to 9 mL of the heating media to a dry matter of 36%. The pH-value of the skim milk and the skim milk concentrate was measured (WTW, Weilheim, Germany) and was 6.7 and 6.3, respectively. For lower dry matters, the skim milk concentrate was diluted and the dry matter content was controlled again via the sea sand method described above.