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The role of the glassy state in production and storage of freeze-dried starter cultures

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Abstract

The inactivation rates k of the model microorganism F19 (Chr. Hansen A/S, Hørsholm, Denmark) as well as the spin-spin relaxation time, T_2,were determined in the area around the glass transition temperature T_g. By keeping cell-sugar lyophilisates at defined temperatures and water activities, storage above and below T_g could be realized. In order to characterize the temperature dependence of T_2 and k, the results were fitted using the Williams-Landel-Ferry (WLF) and Arrhenius models. T_2 was found to be solely dependent on the temperature interval between T_g and the storage temperature, T_p. In case of inactivation kinetics this temperature interval could not fully explain the results. In contrast to molecular mobility, the microbial inactivation was supposed to be influenced additionally by the absolute storage temperature. This is due to fact that microbial inactivation is a highly complex process which is dependent on a number of physico-chemical reactions with some being more while some are less diffusion limited. In terms of inactivation kinetics and molecular mobility, T_g did not act as an absolute threshold but could divide the results into weakly and strongly temperature-dependent fractions. Within the freeze-drying process the glassy state of the sample matrix did not show a protective effect.

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Keywords: Freeze drying; Glass transition; Molecular Mobility; Inactivation kinetics; WLF;

1. Introduction

Starter cultures play an important role throughout the whole food industry. Thanks to their fermentative activity, many food products obtain their characteristic taste, aroma and structure. Moreover probiotic strains can add an additional health effect to the food products. A common way of stabilizing the cultures is drying [1]. However, during drying and subsequent storage, the culture suffers a significant loss in survival and activity. In order to minimize the detrimental effects, protectants (e. g. disaccharides) are added to the formulation prior to the drying step. Generally, this addition leads to a higher yield and improved storage stability. Due to the high complexity of the microorganisms, the underlying protection

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mechanisms determined from model proteins or liposomes cannot be simply applied to this system. One of the known protection mechanisms is the formation of a glassy matrix. Due to the restricted mobility within this matrix, the rates of detrimental physical, chemical and biochemical reactions are strongly depressed. Our aim was therefore to elucidate the role of the glassy state and molecular mobility in terms of microbial stabilization during freeze-drying and storage.

2. Material & Methods

2.1. Sample preparation and drying

Probiotic starter culture Lactobacillus. *paracasei* (F19) from Chr. Hansen A/S (Hørsholm, Denmark) was chosen as a model microorganism. The cells were provided as a frozen concentrate with a titer of around 10^{11}cfu/g (colony forming units). Cells were grown in a 4L batch fermentation process in Scharlau MRS broth (Barcelona, Spain) at 37°C, starting with an optical density (OD600) of about 0.3. The fermentation process was carried out without neutralization. After 10h, fermentation was stopped in the late exponential phase by cooling the cell suspension to 4°C. Cells were harvested by centrifugation (10 min, 4000g, 4 °C) to a final cell density of 10^{11}cfu/mL and washed twice with phosphate buffer saline (0.1M K_2HPO_4/KH_2PO_4 with 0.15M NaCl, pH 7.0). After washing, lactose (Merck, Darmstadt, Germany) was added to the cell suspension with 50 %(w/w) of the bio dry mass. This corresponds to an initial protectant concentration of 60 mg/mL. Exact aliquots of 1 mL of the sample suspension were transferred to 10 mL injection vials (Schärf Präzision Europa GmbH, Meiningen). The sample height was 2 mm. Samples from one fermentation were dried within one batch in a Delta 1-24 LSC freeze dryer (Martin Christ, Osterode, Germany). The shelves of the dryer were precooled to -20 or -40 °C depending on the chosen process. Samples were frozen to a final temperature of -60 °C by dipping vials into liquid nitrogen for 30 s. In case of storage test all samples were dried with the same three-step program (step 1: 14h 0.37 mbar/0 °C; step 2: 5.5h 0.12 mbar/15 °C; step 3: 3.5h 0.12 mbar/25 °C). In order to determine the influence of the glassy state on the drying result, process parameters were chosen according to Table 1. Due to the different process parameters the time to reach the glassy state of the sample matrix could be varied.

2.2. Determination of cell viability

Inactivation of the culture was quantified by determination of colony-forming units (cfu) before and directly after freeze drying and after certain time intervals during storage at defined conditions (see Table 2). The dry samples were rehydrated with sterile double-distilled water at 25 °C. Decimal dilutions of the samples were prepared with Ringer's solution. The number of viable cells was determined by the spread plate technique using MRS agar (1.5 %) and subsequent incubation at 37°C for 48 h under anaerobic conditions. Survival after freeze drying was defined as the percentage ratio of cells before and after drying. The survival after storage was defined as the percentage ratio of cells after drying and storage. Inactivation rates (k) were determined on basis of 4-6 weeks storage tests.

2.3. Lyophilization and microbalance

In order to investigate the influence of the glassy state on drying, different drying protocols with different residence times outside the glassy state were developed. Therefore, a freezing temperature of -20 °C were chosen. With this freezing condition the drying process starts in the non-glassy state whereupon the sample reaches the glassy state during drying due to further water removal. The transition of the sample into the glassy state, and thus the residence time in the non-glassy state, could be detected by introducing the microbalance CWS40 (Christ, Osterode, Germany) in the freeze-dryer according to the