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Impact of Heat Stress on the Cellular Activity and Culturability of Probiotic Microorganisms

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The essential manufacturing step of probiotic products involves dehydration of microorganisms, resulting in cellular stabilization and improved formulation and storage characteristics. Spray drying is one of the most common dehydration techniques used for the preparation of pharmaceutical formulations containing probiotics. This method is less time- and cost- consuming as compared to other drying techniques such as lyophilization [1, 2]. However, spray drying of probiotics involves the challenge of maintaining viable microorganisms in spite of high temperatures involved in the process.

The aim of the present study was investigation of the impact of heat on the cellular activity and culturability of probiotic strains *Enterococcus faecium* M 74 and *Bifidobacterium bifidum* 12. Alterations in membrane permeability, esterase activity and production of superoxide radicals were investigated after exposure of cells to heat at different time intervals. The measurements were obtained using fluorimetry and flow cytometry after staining of cells with fluorochromes.

The results of heat stress showed deleterious alterations in the membrane integrity and esterase activity of both strains after exposure to temperatures of 60°C to 90°C. However, *B. bifidum* 12 cells were more affected than *E. faecium* M 74. The maximum damage of cell membrane and active metabolism occurred after shorter periods of heat exposure in the case of *B. bifidum* 12. In addition, increasing the temperature or extending the exposure time resulted in a remarkable impairment of the esterase activity of *B. bifidum* 12. The obtained results were thereafter used for the improvement of cell stabilization during spray drying of *Enterococcus faecium* M 74 and *Bifidobacterium bifidum* 12.

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