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Determination of the effects of environmental conditions on the survival of probiotic bifidobacteria in fermented milk and development of polynomial survival models

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Bifidobacteria are used as probiotic bacteria in fermented milk products and are reported to have health benefits on humans if consumed in sufficient numbers. However, its survival in many probiotic foods is poor due to many environmental conditions, pH, storage temperature and oxidation reduction potential (E_h) being the most important. Therefore, determination of the effects of these environmental conditions as well as development of mathematical models to describe the survival, are of importance to the food industry.

The effects of storage temperature, pH and E_h on the survival of *Bifidobacterium longu* were determined in a 3×4×3 factorial design using three storage temperatures (4, 8, 12^oC), four pH (4.0, 4.25, 4.5, 4.75) and three E_h (0, 200, 400 mV) values. Experimental data were fitted to the Baranyi survival model using the software DMFit Ver 2.1 to generate survival curves. Analysis of variance (ANOVA) was performed using SAS Ver 8.0 in order to determine the single and combined effects of the environmental conditions on bifidobacteria. Polynomial multiple regression equations were calculated using Micromath Scientist Ver 1.05 to quantify the effects of environmental conditions on the survival of bifidobacteria and to generate the secondary polynomial survival model.

B. longum was susceptible to acidity and survival was log-linear at low pH values (pH 4.0, 4.25) whereas at high pH (pH 4.75) pronounced shouldering and tailing effects were apparent forming sigmoid survival curves. Both individual environmental conditions (T, pH, E_h) as well as interactions between E_h and pH affected survival and death was always more rapid at low pH and high E_h values. Storage temperature, pH and E_h could be manipulated to enhance survival. Of the three, E_h reduction by addition of reducing agents appears to be the best way to enhance survival without adversely affecting other properties. The proposed polynomial survival model (T_{4D}=23.896+1.919*T+33.0104*pH- 0.120*E_h-1.227*T*pH+0.00135*pH*E_h+0.00227*T*E_h+0.0175*T^2-1.564*pH^2 + 0.0000415 *E_h^2) is the first of its kind developed to describe the survival kinetics of bifidobacteria in fermented milk. Moreover, this study is the first to incorporate E_h in a modeling experiment on the survival of a bacterium. The proposed polynomial survival model can be used by the bio-yoghurt manufacturers in determining the shelf-life and the expiry date of their products.

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