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Effect of non-thermal processing by High Hydrostatic Pressure on the survival of probiotic microorganisms: Study on *Bifidobacteria* spp.

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ABSTRACT

High Hydrostatic Pressure (HP) processing has been suggested as an alternative method to improve textural attributes of dairy products. Since, the global market seeks improved functional foods, it is important to investigate whether HP processing can be applied to fermented dairy probiotic products. The inactivation kinetics of *Bifidobacterium* spp. in a model system of acid pH value under high pressure (100–400 MPa) combined with moderate temperature (20–35 °C) was investigated. *Bifidobacterium* spp. inactivation followed first order kinetics at all pressure–temperature combinations used. Pressure and temperature were found to act synergistically on the viability loss of the bacterium. The corresponding z_T and z_P values of inactivation were also estimated and, values of 41.5 °C and 93.5 MPa at reference pressure of 200 MPa and reference temperature of 25 °C were estimated, respectively. HP treatment of 200 MPa at 20–25 °C for 10–15 min, recommended for textural modification, is not detrimental to the viability of the studied probiotic culture and would be suitable for respective fermented probiotic products.

1. Introduction

High Hydrostatic Pressure (HP) processing has been proposed as a method that could contribute to texture improvement of dairy products [4,8]. HP can be applied either to raw milk which is used for the production of dairy products, or to the final product. In recent years, an increasing number of dairy products include probiotic microorganisms since the global market focuses on functional foods. When HP treatment is used to improve the textural attributes of dairy products at their final structure, the common processing conditions ranged from 100 to 400 MPa and from 10 to 25 °C for process times in the range of 10–15 min. According to previous studies, a pressure range of 200–300 MPa and a temperature range of 20–25 °C for process time of 10 min can lead to a final product with significant improved texture and acceptable sensory characteristics, with limited inactivation of yogurt bacteria [1,2,6].

The objective of this work was to study the effect of HP treatment on the viability of probiotic microorganisms (*Bifidobacterium* spp.) and thus to evaluate the applicability of the HP process to a functional dairy product in order to improve its quality parameters and shelf life.

2. Materials and methods

2.1. Preparation of the inoculums

Stock culture of the microorganism was maintained in vials with the addition of glycerol (20%) as a cryoprotective fluid at −40 °C. For revival, one vial from the microorganism was transferred in 10 ml of MRS broth (Merck, 1.10661, Darmstadt, Germany) with the addition of 3% of L-cysteine HCl and incubated at 35 °C for 24 h. For growth, 100 μl of the above inoculums was transferred in 10 ml MRS broth/L-cysteine HCl and incubated at 35 °C for 18–20 h. The final suspensions were transferred into 90 ml MRS broth with modified pH value of 4.80 (HCl sol. 1 M) and served as the inoculums for all the experiments (initial count of approximately 10^8 CFU/ml).

2.2. High pressure treatment

The inoculums were placed into 5 ml pouches (laminate film: PP-aluminum–PE) for HP experiments. Inactivation experiments were conducted in duplicate at various combinations of pressure (100–400 MPa) and temperature (20–35 °C) for appropriate process times. The high pressure unit comprised a pressure intensifier and a multivessel system consisting of six vessels of 45 ml capacity each, and the pressure transmitting fluid used was polyglycol ISO viscosity class VG 15 (Resato International BV, Roden, Holland). Process temperature in the vessels was achieved by liquid

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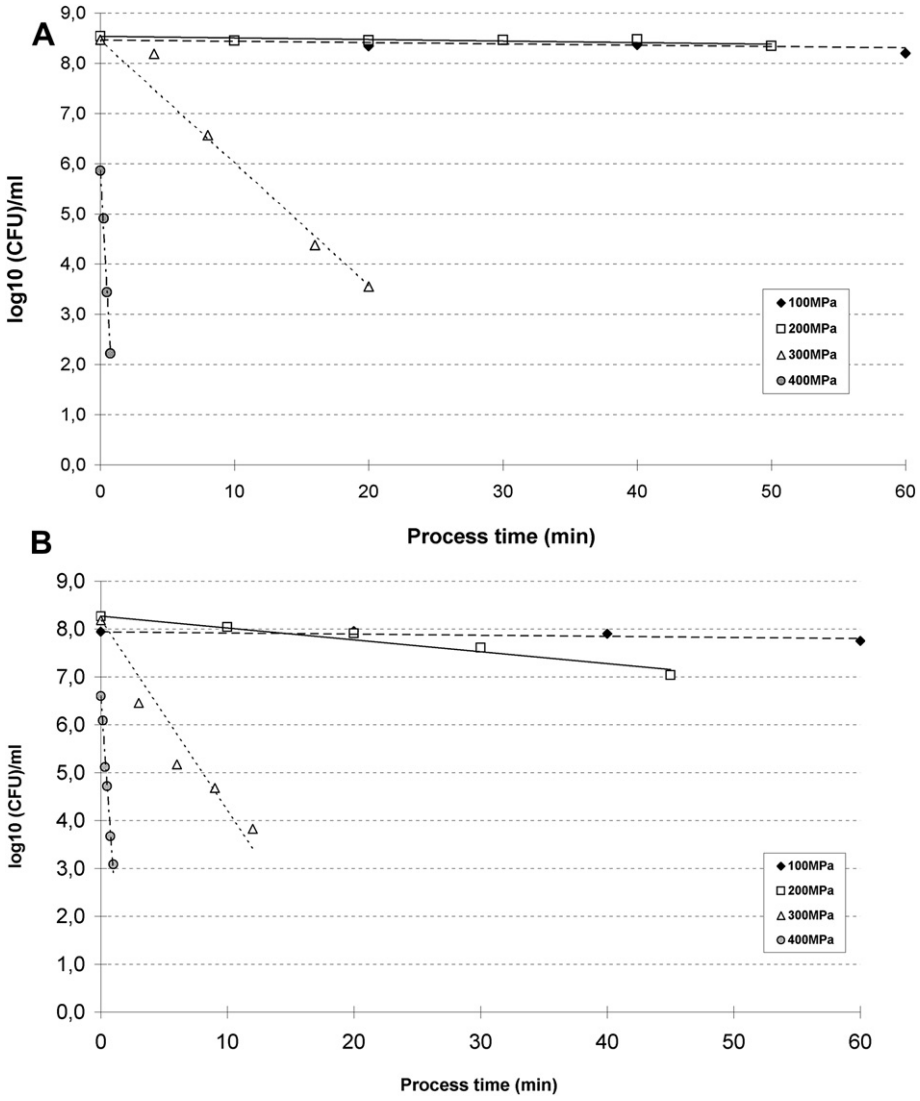

Fig. 1. *Bifidobacterium* spp. inactivation during HP processing at 100–400 MPa at 20 °C (A) and 25 °C (B).

circulation in the outer jacket controlled by a heating–cooling system. The initial temperature increase during pressure build-up (about 3 °C per 100 MPa) was taken into consideration in order to achieve the desired operating temperature. Pressure and temperature were constantly monitored (intervals of 1 s) and recorded during the process.

2.3. Enumeration of remaining viable cells

Enumeration of remaining viable cells was conducted with the appropriate plate methodology. The growth medium used was MRS agar (Merck, 1.10660, Germany) modified with 5% NNLP sol. and 3% L-cysteine HCl sol., which were filter-sterilized. Incubation of Petri dishes was carried out in anaerobic jars (Merck, 1.16387, Germany) using Anaerocult A (Merck, 1.13829, Germany) as a catalyst at 35 °C for 72 h [7].

2.4. Data analysis

First order kinetics was fitted to the logarithm of the viable cell concentration [5]. The decimal reduction times (D, min) were estimated at all studied conditions to describe the effect of process time on the inactivation. The effect of temperature was expressed

Table 1
Decimal reduction times, D (min), of *Bifidobacterium* spp. z_P and z_T values are also presented.

	20 °C	25 °C	30 °C	35 °C	z_T (°C)
100 MPa	–	–	263	250	–
200 MPa	–	40.3	18.6	16.2	41.5
300 MPa	4.08	2.53	1.43	0.71	19.8
400 MPa	0.25	0.27	0.24	0.11	25.2
z_P (MPa)	–	93.5	98.0	87.7	

Table 2
Parameters of the model of *Bifidobacterium* spp. inactivation as a function of pressure and temperature

Parameter	Estimated value
P_{ref} (MPa)	300
T_{ref} (°C)	30
D_o (min)	1.72
z_T (°C)	26.8
z_P (MPa)	90
A (MPa^{-1})	0.003
R^2	0.98

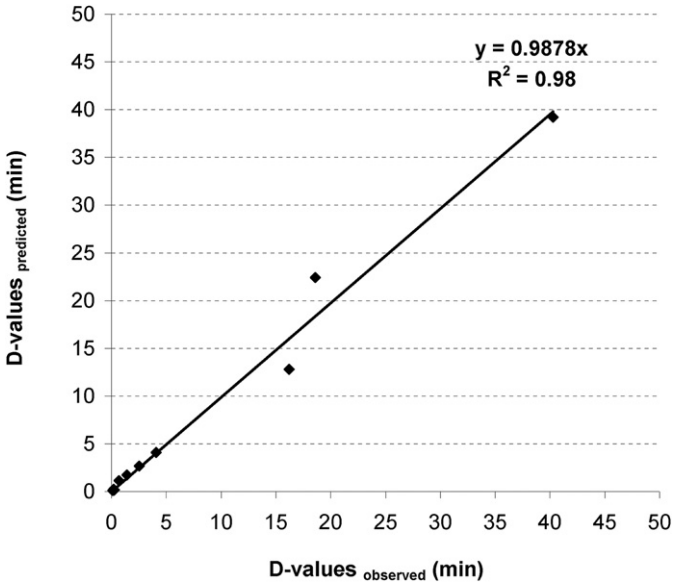

Fig. 2. Comparison of experimental and predicted from the model decimal reduction times (D, min).

through the z_T value and the effect of pressure was described by the z_P value [3]. The parameters of the mathematical model, which describes the inactivation of the microorganism as a function of pressure and temperature, were estimated using SYSTAT 8.0 (SYSTAT 8.0 Statistics, 1998, SPSS Inc., Chicago, USA) software.

3. Results

The effect of processing at various combinations of pressure (100–400 MPa) and temperature (20–35 °C) on the viability of *Bifidobacterium* spp. was measured as a function of time. In Fig. 1(A,B) the effect of process pressure at 20 and 25 °C on the viability of *Bifidobacterium* spp. is shown. HP inactivation was described by first order kinetics (R^2 ranged from 0.84 to 0.99). The D-values were estimated at all studied pressure and temperature combinations (Table 1). No effect on the viability of the tested microorganism was observed when low pressure (100 MPa) and low temperatures (20 and 25 °C) were used. The D-values decreased with increasing processing pressure and temperature at all temperature and pressure levels tested respectively and thus, a synergistic effect of temperature and pressure was observed.

At each temperature the effect of pressure on the inactivation rate constant was expressed through the z_P value (Table 1) which remained almost constant, indicating the same dependence from pressure in all tested temperatures. The effect of temperature on the D-values was expressed by the z_T value for all pressures tested (Table 1). Results indicate that at low process pressures, temperature has a significant effect on the viability of the bacterium while at high pressures (400 MPa) a lower effect of temperature on the viability is observed.

A single multi-parameter equation was applied to describe the effect of pressure and temperature process conditions on the D-value of *Bifidobacterium* spp. The above equation takes into account the effect of pressure on the z_T-value, while the z_P-value does not depend on process temperature. The parameters of the model were estimated (Table 2), using non-linear regression (SYSTAT 8.0). The predicted D-values from the model were well correlated with the corresponding D-values obtained from the experimental data (Fig. 2).

$$D = D_0 \cdot \left(\exp\left\{ -\frac{2.303 \cdot T \cdot T_{ref}}{z_T} \cdot \exp\left[-A\left(P - P_{ref}\right)\right] \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right) + \frac{2.303}{z_P} \cdot \left(P - P_{ref}\right) \right\} \right)^{-1}$$

4. Conclusions

Based on literature data on the application of HP process in dairy products, the recommended conditions of HP treatment (200 MPa at 20–25 °C for process time of 10–15 min [1,6]) for texture improvement are not detrimental to the viability of the examined probiotic bacterium. The maximum calculated inactivation due to the HP process is reduction of about 0.4 log 10 CFU/ml in viable cells of the bacterium and hence, it is possible to be applied in products with this kind of probiotic culture. As it is proposed in these studies, more intense HP conditions can be also applied (e.g. 200 MPa at 30–35 °C for process time less than 30 min or 300 MPa at 20 °C for process time less than 10 min) maintaining sufficient numbers of *Bifidobacterium* spp. while at the same time improving viscosity and flavor of dairy products. Further research is needed to determine whether HP process can be applied in dairy food systems which comprise starter cultures along with probiotic bacteria and to examine the interactions between these microorganisms and the viability of both kinds of bacteria, in order to achieve dairy products with improved textural attributes while maintaining their functionality.

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