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Short communication

# Probiotic yogurt production under high pressure and the possible use of pressure as an on/off switch to stop/start fermentation

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ABSTRACT

This work intended to evaluate the effect of high hydrostatic pressure (HHP) on the lactic acid fermentation that occurs during yogurt production. The evaluated physicochemical parameters (pH, titratable acidity, and concentration of reducing sugars) indicated that HHP reduced the fermentation rate. Microbiological analysis (counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium lactis*) confirmed the inhibitory effect of pressure on the growth of these microorganisms. However, extension of the fermentation time at 5 MPa yielded a typical pH for yogurt with survival of the yogurt microorganisms. No fermentation was found in samples subjected to 100 MPa for 180 min, but these samples revealed normal metabolic activity when they were returned to atmospheric pressure. This finding indicates that the microorganisms did not undergo inactivation during this period at 100 MPa; instead, the microorganisms were metabolically inhibited, which did not hinder the subsequent fermentation at atmospheric pressure. This result opens the possibility of using pressure as an on/off switch to stop/start fermentation, similar to refrigeration.



## 1. Introduction

Yogurt is defined as an acidified and coagulated milk product (pH ≈ 4.5) that results from the lactic acid fermentation of milk, and this fermentation is performed by mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ($\geq 10^7$ CFU mL$^{-1}$ of yogurt) [1–3]. Yogurt supplemented with probiotic bacteria, particularly bifidobacteria, occupies a very strong position in the dairy market. Bifidobacteria can promote several health-related functions, including host resistance to infectious microbes and anticarcinogenic activities [4–6].

High hydrostatic pressure (HHP) is an emerging technology, with successful applications in the food industry, particularly as a non-thermal (or cold) pasteurization method [7]. Recently, interesting new applications of HHP in biotechnology have also been investigated and described [8], such as microbial fermentation at HHP conditions. When fermentative processes are performed under (sub-lethal) HHP, the involved microbial strains may develop specific stress response mechanisms, such as metabolic modulation (the suppression/reduction of some metabolic pathways or utilization of new ones). Therefore, it is possible to conduct fermentative processes under pressure and also obtain products with different characteristics and features, with great commercial potential interest. Bothun et al. [9] reported that the application of sublethal pressures (7–17 MPa) to *Clostridium thermocellum* growth redirected its fermentation products from acetate to ethanol, leading to a 60-fold increase in the ethanol:acetate ratio compared with the ratio obtained at atmospheric pressure. Similarly, other authors performed alcoholic fermentation by *Saccharomyces cerevisiae* under HHP and observed an increased fermentation rate (up to 3-fold) at 10 MPa relative to the results at atmospheric pressure [10].

This work intended to study the effect of HHP on food fermentation, using probiotic yogurt as a case study. It is noteworthy that the present study is the first in literature to ferment a food product under HHP conditions because previous studies on microbial fermentation under pressure were performed using synthetic culture media.

## 2. Materials and methods

### 2.1. Sample preparation

Commercial UHT treated semi-skimmed milk (1.6 g of lipids, 5.0 g of sugars, 3.2 g of proteins and 0.13 g of salt per 100 mL) was inoculated with a commercial plain probiotic yogurt (Danone, Paris, France) available in a local supermarket supplemented with

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*Bifidobacterium animalis* subsp. *lactis*. The microbial composition of the inoculum was 8.7 $\log_{10}$ CFU $g^{-1}$ of *S. thermophilus*, 5.3 $\log_{10}$ CFU $g^{-1}$ of *L. bulgaricus* and 7.3 $\log_{10}$ CFU $g^{-1}$ of *B. lactis*. Samples were prepared at a proportion of 80 mg of yogurt per mL of milk.

### 2.2. Fermentation under HHP

Fermentation was performed at 43 °C (the optimum process temperature at atmospheric pressure), at different HHP conditions (5, 15, 30, 50, 75 and 100 MPa). These experiments were conducted using HHP equipment (System U33, Unipress Equipment, Warsaw, Poland). As a control, fermentation was also performed at 0.1 MPa (atmospheric pressure), keeping all conditions equal to the conditions for fermentation under HHP. Samples were collected throughout the fermentation time, and each experiment was run in duplicate; the analyses were conducted in triplicate. Fermentation was stopped by immersing the samples in an ice bath.

### 2.3. pH and titratable acidity

pH was measured using a properly calibrated glass electrode (pH electrode 50 14, Crison Instruments, S. A., Barcelona, Spain) at 25 °C. Titratable acidity was quantified using a Titromatic 1S (Crison Instruments, S. A., Barcelona, Spain) with 1.50 mL of yogurt sample diluted in 10.5 mL of water; the sample was then titrated with a 0.1 N NaOH solution until reaching pH 8.9. The results are expressed in g of lactic acid $L^{-1}$ of yogurt.

### 2.4. Reducing sugar concentration

The reducing sugar concentration was assessed with the colorimetric method using the 3,5-dinitrosalicylic acid reagent (DNS), described by Miller [11]. The absorbance values (at 540 nm) were measured in a Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, USA). The concentration values were calculated using a calibration curve, which was obtained using standard equimolar glucose/fructose solutions because it was reported that the use of different monosaccharides for reducing sugar quantification has no effect on the results [12]. The results are expressed in g of reducing sugars $L^{-1}$ of yogurt.

### 2.5. Microbiological analysis

The microbial enumerations were performed using the pour plate technique. *L. bulgaricus* counts were determined on double-layer agar plates of MRS medium (Lactobacillus Agar acc. de Man, Rogosa and Sharpe – Merck, Munchen, Germany) at pH 5.7 ± 0.2 that were previously sterilized according to the manufacturer's instruction. The cultures were enumerated after incubation at 30 °C for 5 days [13]. *S. thermophilus* counts were conducted in M17 (Liofilchem, Rosetodegli Abruzzi, Italy) medium at pH = 7.2 ± 0.2 that was sterilized according to the manufacturer's instruction after incubation at 37 °C for 72 h [14]. *B. lactis* counts were performed according to Darukaradhya [15]. Plates containing 15–300 colonies were enumerated, and the counts are expressed as the $\log_{10}$ CFU $mL^{-1}$ of probiotic yogurt.

### 2.6. Activation volumes calculation

The reaction rate constants ($k$) for the pH decrease, reducing sugar decrease and titratable acidity increase with time were calculated for different pressure values using 4–5 data points. These values were further used to estimate the activation volume ($Va$) using a linear form of the Eyring Law according to Eq. (1):

$$\ln(l) = \ln(A) - Va \times \frac{p}{R_p \times T} \quad (1)$$

where $k$ is the reaction rate constant (h), $A$ is a constant, $Va$ is the activation volume ($cm^3\ mol^{-1}$), $p$ is the pressure (MPa), $Rp$ is the universal gas constant (8.314 $cm^3$ MPa $(K\ mol)^{-1}$), and $T$ is the absolute temperature (K).

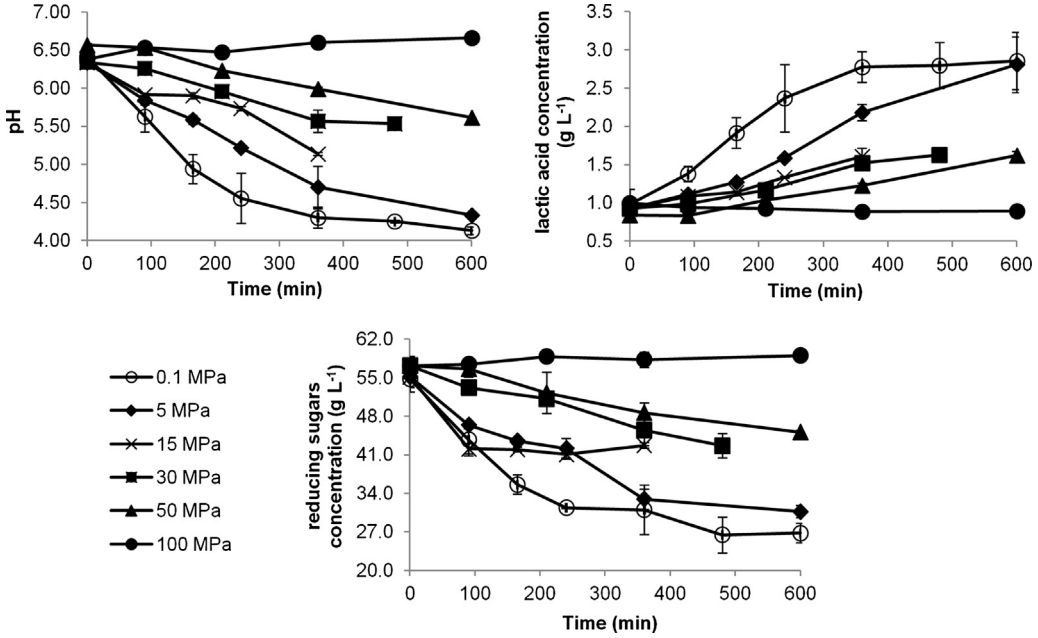


**Fig. 1.** Variation of pH, lactic acid concentration and reducing sugar concentration during the fermentation period, as measured on samples exposed to different pressure conditions ranging from 0.1 to 100 MPa. Vertical error bars represent the standard deviation.