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# Effect of baking conditions and storage on the viability of *Lactobacillus plantarum* supplemented to bread

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ABSTRACT

Bread is an interesting non-dairy-based vehicle for probiotics delivery given its daily consumption worldwide. The incorporation of probiotics in bread is challenging due to the high baking temperatures. In this study the influence of various baking conditions and subsequent storage on survival of a model strain *Lactobacillus plantarum* P8 is systematically investigated. Bread samples with varying dough weight (5, 30, and 60 g) were baked at different temperatures (175, 205, and 235 °C) for 8 min, and the residual viability of bacteria was determined every 2 min. Under all baking conditions, the viability of probiotics decreased from $10^9$ CFU/g to $10^{4\sim5}$ CFU/g after baking. For specific conditions a difference in bacterial viability between bread crust and crumb was observed, which was explained by the different temperature-moisture history and developed microstructure during baking. Remarkably, during storage bacterial viability increased by 2–3 log to $10^8$ CFU/g in crust and $10^6$ CFU/g in crumb, respectively. The re-growth of probiotics was accompanied by a decrease in pH of the bread and an increase of the total titratable acidity. The results of this work provide valuable experimental data for further modelling and optimization studies, which then could contribute to the development of probiotic bakery products.



## 1. Introduction

Probiotics are defined as live microorganisms that confer health benefits on the hosts when administered in adequate amounts (FAO/WHO, 2002). Most probiotic food available on the market are dairy fermented foods such as yoghurt, korut and kefir (De Prisco & Mauriello, 2016). However, there is an increasing consumer demand for non-dairy-based probiotic products, given the drawbacks of dairy products, such as the prevalence of allergy and lactose intolerance (Vijaya Kumar, Vijayendra, & Reddy, 2015).

Bread is a nutritious non-dairy based food containing carbohydrates, minerals, vitamins and dietary fibres (Pinto, Castro, Vicente, Bourbon, & Cerqueira, 2014). Non-digestible carbohydrates like oligosaccharides present in whole-wheat bread have been suggested to promote growth of probiotic bacteria (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Moreover, inoculation of lactic acid bacteria in dough can lead to high quality sourdough bread (Corsetti et al., 2008). Based on these perspectives, we identified bread as a potential food that can be enriched with probiotics.

Preservation of cell viability during baking and storage are essential as the minimum amount of live bacteria in the probiotic foods should be $10^6$~$10^7$ CFU/g to confer beneficial influence on consumer health (Ross, Desmond, Fitzgerald, & Stanton, 2005). However, incorporation of probiotics in bread is challenging because of the high temperatures during baking that negatively affect survival of the bacteria and additional loss of bacterial viability during subsequent storage at ambient temperatures (Soukoulis et al., 2014). Only few studies investigated strategies to improve bacterial survival in bread. Altamirano-Fortoul, Moreno-Terrazas, Quezada-Gallo, and Rosell (2012) micro-encapsulated *Lactobacillus acidophilus* in starch, applied it to the dough and obtained reasonably high viable counts after baking (about $10^6$ CFU/

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bread). However, drawback of this approach was that the probiotic coating negatively affected the physicochemical properties of the bread crust, leading to different color and crispness. Soukoulis et al. (2014) applied sodium alginate solutions with *Lactobacillus rhamnosus* GG (LGG) as a coating on the surface of pre-baked pan bread, and dried the crust at mild conditions. The viability of LGG was found approx. 7.6–9.0 log CFU in 30–40 g of bread slice. As stated above, only few studies have reported on the application of probiotics in bread or other bakery products (Espitia, Batista, Azeredo, & Otoni, 2016; Malmo, La Storia, & Mauriello, 2013; Reid, Champagne, Gardner, Fustier, & Vuillemard, 2007; Zhang, Huang, Ananingsih, Zhou, & Chen, 2014). However, none of these previous studies systematically evaluated the survival of bacteria during baking and storage conditions.

Therefore, the aim of this study is to better understand the impact of different bread sizes, baking conditions and subsequent storage on the survival of probiotic bacteria (*Lactobacillus plantarum* P8). The viability of bacteria was evaluated for both bread crust and crumb as they have a distinct temperature-moisture history during baking with corresponding microstructural properties.

## 2. Materials and methods

### 2.1. Probiotic strain and bacteria culture

The model probiotic strain *Lactobacillus plantarum* P8 (ATCC 14917) was provided by Inner Mongolia Agricultural University. The L. *plantarum* culture was routinely prepared using MRS broth (OXOID, United Kingdom) as the growth medium. A single colony of *L. plantarum* was inoculated in 10 mL sterile MRS broth and pre-cultured at 37 °C for 12 h. Subsequently, 1% v/v inoculum of *L. plantarum* was sub-cultured in 100 mL MRS broth at 37 °C for 24 h without agitation. Thirty millilitres of cell suspension were transferred into a centrifuge tube (50 mL), and the cell pellets were harvested by centrifugation (Thermo Fisher Scientific, USA) at 8000×*g* and 4 °C for 15 min, and were aseptically re-suspended in UHT skim milk for the baking experiment.

### 2.2. Bread samples preparation

Bread samples were made following a recipe from Zhang et al. (2016): wheat flour (100 g), sugar (4 g), salt (1.5 g), instant yeast (1 g), butter (3 g), and UHT skim milk (65 g) with *L. plantarum* added. Bread without bacteria addition was made as the control. Dough was prepared in a stand mixer (Hauswirt® HM730, China) by mixing the dry ingredients at speed 1 for 1 min and mixing at speed 3 for 7 min after milk was added. After resting for 5 min, dough was divided into balls of 5, 30 and 60 g, respectively. The dough was proofed at 40 °C, 85% RH in a climate chamber (Yiheng Scientific Instruments Co., Ltd., China) for 1 h (or 45 min for 5 g dough). Subsequently, bread samples were baked for 8 min in an electric oven (Changdi® CRTF30W, China) at 175 °C, 205 °C and 235 °C, respectively.

After baking at 175 °C for 8 min, bread samples with an initial weight of 30 g were sealed in polyethylene bags and stored at 25 °C, 55% RH in the climate chamber for 5 days, to investigate the impact of storage on the viability of *L. plantarum* in bread. Some properties, i.e. viability of bacteria in bread, moisture content of bread matrix, pH of crumb, etc. were determined according to the methods described in the following section.

### 2.3. Physicochemical properties analysis

Temperature profiles of the bread crust and crumb during baking were monitored by inserting K-type thermocouples (Omega®, USA) into the top surface and core sections of the dough. The thermocouples were connected to a computer via a Picometer TC-08 (Pico Technology, UK), and the sampling interval was 1 s.

Moisture contents (% kg/kg wet base) of the bread crust (top surface) and crumb (centre) during baking and storage were determined according to Eqn. (1).

$$Moisture\ content(\%) = \frac{W_1 - W_2}{W_1} \quad (1)$$

in which, $W_1$ (kg) is the weight of the sample after sampling and $W_2$ (kg) the weight after dehydration at 105 °C for 24 h in an oven.

The pH and total titratable acidity (TTA) of the bread crumb were measured before and after baking, as well as during storage (Palacios, Sanz, Haros, & Rosell, 2006). Bread dough or crumb (10 g) was mixed with 100 mL acetone/water (5/95, v/v) in a stomacher (iMix®, Interlab, France), and the pH of the suspension was determined using a pH meter (Thermo Scientific, USA). The same suspension was titrated against 0.1 N NaOH to a final pH value of 8.5 to determine the TTA. The TTA was expressed as the amount (mL) of NaOH used for titrating 10 g of sample.

### 2.4. Microbiological analysis

Viable cell counts of *L. plantarum* in bread were determined after baking for 0, 2, 4, 6, 8 min and after storage for 1, 2, 3, 4, 5 days. Samples of bread crust and crumb were obtained from the top surface and the core section of the bread, respectively. For bread samples with an initial weight of 30 g or 60 g, samples of bread crust or crumb (5.0 g) aseptically homogenized with 95 mL sterile peptone water (0.1% w/w, Solarbio®, China) in a stomacher (iMix®, Interlab, France) for 1 min, while 1.0 g of crust or crumb were homogenized in 49 mL peptone water for those 5 g bread samples. Subsequently, serial dilutions of the cell suspension were made in 9 mL sterile peptone water (0.1% w/w), and 100 μL suspension was spread onto a modified MRS agar plate.

To inhibit the growth of Bacillus strains and yeast on the MRS agar plate, vancomycin (20 ppm, Shyuanye®, China) and natamycin (200 ppm, Antai®, China) were added into the MRS agar broth (MRS: Oxoid, UK; agar powder: Solarbio, China) through a 0.22 μm polyethylene sulfone filter (Millipore®, USA) (Hartemink, Domenech, & Rombouts, 1997; Liu & Tsao, 2009). The growth of *L. plantarum* was not affected by the addition of vancomycin and natamycin (Zhang et al., 2014). The agar plates were statically incubated at 37 °C for 48 h. *L. plantarum* colonies were counted and the viability ($N$) was expressed as colonies forming units per gram of sample (CFU/g). The semi-logarithmic survival curves of *L. plantarum* were presented by plotting the residual viability, i.e. $\log(N/N_0)$, against baking time for each baking condition, in which $N_0$ was the initial viable count (CFU/g) of *L. plantarum* in the dough before baking.

### 2.5. Microstructure analysis

Microstructure of the bread with or without (control) *L. plantarum* addition was monitored using scanning electron microscopy (SEM). Samples from the crust and crumb of the 30 g bread baked at 175 °C were stored at − 80 °C overnight, and were dried in a lyophilizer (Sihuan Scientific Instruments Co., Ltd., China). The dried samples were cut into small pieces with a knife blade and fixed on an aluminium stub using a conducting carbon tape, and then coated with gold using a sputter. SEM images were recorded using a JEOL 7001 F (Jeol Ltd, Tokyo, Japan).