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A study on *Bifidobacterium lactis* Bb12 viability in bread during baking

Lu Zhang [a], Song Huang [a], Victoria Kristina Ananingsih [b], Weibiao Zhou [b], Xiao Dong Chen [a,*]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen City, Fujian 361005, PR China
[b] Food Science and Technology Programme, Department of Chemistry, National University of Singapore, 3 Science Drive 3 117543 Singapore, Singapore

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ABSTRACT

Bread was made from dough with an addition of freeze-dried culture of the probiotic strain *Bifidobacterium lactis* Bb12 (Bb12). The viable cell counts, specific volume, moisture content and water activity of the bread were determined after baking for 0, 3, 6, 9, 12 min at 165, 185, 205 °C, respectively. The viable counts of Bb12 in both the core and the middle sections of the bread declined significantly during baking. However, some bacteria survived in the bread even after baking for 12 min. Moreover, the specific volumes and moisture contents of the bread increased with baking, and water activity of bread remained almost the same during baking.

1. Introduction

Bread is the major type of bakery products and a staple food in most part of the world. A typical, traditional bread-making is largely wheat-flour based, involving dough mixing, proving (i.e., fermentation) and baking. Scientific issues to be addressed include textural development, bubble formation and growth, heat and mass transfer, structural transformation during processing and shelf life. A number of studies in the literature have been focused on mathematical modeling of bread baking process and functional bread development, validated with experimental testing (Purlis and Salvadori, 2009; Purlis, 2011; Therdthai and Zhou, 2003; Wang et al., 2008; Zhang and Datta, 2006).

A probiotic is a viable microbial dietary supplement that beneficially influences the host (human) through its effects in the intestinal tract (FAO/WHO, 2002). Moreover, probiotics are one of the three different types of food ingredients which can be used when developing nutritionally designed foods that promote health through gut microbial reactions (the other two ingredients are non-digestible carbohydrates and bioactive plant secondary metabolites) (Puupponen-Pimiä et al., 2002). However, functional bread containing the viable microorganisms has not been fully developed due to the high temperature reached during baking (Altamirano-Fortoul et al., 2012). The cell viability of many probiotic bacteria during processing and storage is often insufficient and thus limits their usefulness in food applications. Therefore, microencapsulation technique has been introduced to protect probiotics from environmental and physiological degradation (Ding and Shah, 2009; Lisboa et al., 2009; Manojlović et al., 2010; Poncelet et al., 2011). Altamirano-Fortoul et al. (2012) studied bread combining the microencapsulation of *Lactobacillus acidophilus* and starch based coatings, and different probiotic coatings were applied onto the outer surface of partially baked bread as well.

Considering the relatively complicated preparation and extra cost of making microcapsules, the aim of this work was to research the possibility of adding viable probiotics to dough directly at the very beginning of the bread manufacturing process from an experimental perspective, i.e., the probiotic bacteria participated in the whole process. Specifically, the deactivation process of Bb12 at high baking temperature was investigated. In addition, the physicochemical properties of bread contained with probiotics after the baking process were studied as well, which was a neglected issue by researchers in the literature. The results of this study may provide a relatively new perspective in thefield of probiotic bread research.

2. Materials and methods

2.1. Materials

Bifidobacterium animalis subsp. lactis Bb12 (here after termed Bb12) is a commercially available probiotic strain used throughout the world in a variety of functional foods and dietary supplements. The benefits of Bb12 have been documented in a number of independent clinical trials (Garrigues et al., 2010). *Bifidobacterium lactis* Bb12 in the form of Probio-Tec® BB-12® Stick-6 V1 as a commercial product was obtained from Chr. Hansen (Singapore). The stick

* Corresponding author. Tel.: +86 592 2186059; fax: +86 61 3 99055686.
E-mail address: xdc@xmu.edu.cn (X.D. Chen).

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contained 1.0 g of a standardized light beige fine powder consisting of a freeze-dried culture. It had a minimum viable count of 6 billion (6.0E+09) CFU (Colony Forming Units) per stick at the time of manufacturing.

The ingredients of dough are: bread flour (1 kg), sugar (40 g), fine salt (15 g), shortening (30 g), yeast (10 g), and UHT skim milk (650 g) with dissolved Bb12 powder (1 g), following a standardized bread formulation in Wang and Zhou (2004) except the milk and Bb12.

2.2. Preparation of the bread samples

The Bb12 powder was dissolved in the UHT skim milk (viable count of Bb12 was about 5.0E + 07 CFU/ml), and incubated at 37 °C for 1 h. Dough was firstly made in a mixer (Varimixer Globe, USA) then molded to around 60 g each using an automatic dough divider and molder (DR ROBOT^2, Holland). After being proved at 40 °C/85% RH for 75 min in a prover (Binder KBF 115, Germany), the dough proceeded to baking in an oven (Eurofours®, France). The designated oven temperatures were 165 °C, 185 °C and 205 °C, respectively. Baking durations were 0, 3, 6, 9 and 12 min, respectively. Type T thermocouples were employed for temperature monitoring during the baking and cooling process. Specific volume, moisture content and water activity analyses were carried out immediately after each sampling. After cooling, the bread was packaged in sealed bags and was stored in a cabinet for up to 3 days (Temperature: 20.0 °C, Relative Humidity: 70.5%).

2.3. Microbiological analysis

The amount of viable Bb12 in the bread core and middle section (see Fig. 1) was determined after baking for 0, 3, 6, 9, 12 min. A bread portion (1 g) was taken from the core and middle of dough immediately, and then was aseptically diluted in 9 mL or 19 mL of 0.1% w/v sterile peptone water solution (OXOID CM009, United Kingdom) and homogenized for 1 min in a masticator (IUL, Spain). Serial dilutions were made in sterile peptone water and plated following the surface technique onto De Man, Rogosa and Sharpe Agar (M.R.S. Agar, OXOID CM0361, United Kingdom) supplemented with 50 ppm natamycin (Natamax®, 50% natamycin) (Danisco, Denmark) (Liu and Tsao, 2009) and 0.5% w/v L-cystein hydrochloride monohydrate (Sigma–Aldrich, USA). The agar plates were incubated at 37 °C for 48 h under anaerobic condition, i.e., the MRS agar plates were placed in anerobic jars with one or two sachets of AneroGen® (OXOID AN0025A, United Kingdom) in each of them. After incubation, the results were recorded as CFU per gram. Specifically, N represents the viable count value at different time, CFU/g, and N_0 represents the initial viable count value of Bb12, CFU/g. The data were expressed as averages of all available replicates (at least triplicate).

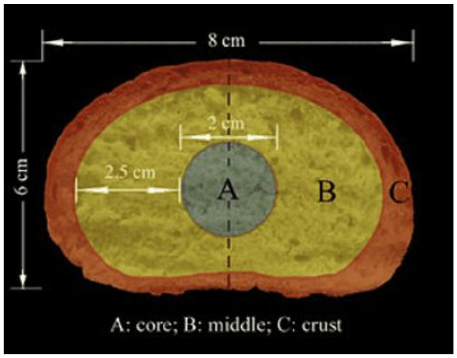

Fig. 1. Schematic to illustrate the cross section of the bread used in the current work.

2.4. Chemical and physical analysis

Specific volume, moisture content, water activity and pH of bread were measured during baking (0, 3, 6, 9 and 12 min) and storage (1 day and 3 days) respectively. Moisture content was analyzed according to AOAC method 925.10 (2002) (AOAC, 2002). Briefly, ca. 2 g of sample was accurately weighed to pre-dried and cooled dish, and then heated in an oven at 105 °C for 24 h. At the end of drying, the sample was immediately transferred to a desiccator and weighed after reaching room temperature. Hydrogen potential of the bread samples was quantified based on AOAC method 943.02 (2002). Specifically, bread crumb (10 g) was suspended in 100 ml of distilled water and the suspension was homogenized using a homogenizer (IUL, Spain) and then the pH value of the suspension was measured using a pH-meter (Metrohm 744 pH Meter, Switzerland). Specific volume was measured using a Volscan Profiler 600 (Stable Micro Systems, UK). Water activity was measured using a water activity meter AQUA LAB® Series 3 TE (Decagon Devices, Inc., USA).

2.5. Effect of proofing process on growth of Bb12

The proofing temperature was 40 °C and the relative humility was 85%, which was supposed to be conducive to the growth of Bb12. The amount of viable Bb12 in the bread core section was determined after 0, 15, 30, 45, 60, and 75 min of proofing, respectively.

2.6. Statistical analysis

All data were presented as mean values of at least three replicates, and error bars represent ± 1 standard error (SE). Statistical analysis of the results was performed using Microsoft Excel (Microsoft®, USA). Data were analyzed by nonparametric one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Variation of viable counts in bread during baking

It was envisaged that the survival rate of bacteria was mainly affected by heating temperature and heating time. As such, the viable counts inside the dough were measured every 3 min during baking at three different baking temperatures, i.e., 165 °C, 185 °C, and 205 °C. The initial viable count of Bb12 in the dough was 2.1E + 06 CFU/g, which was the mean value of all valid data measured in this experiment. As can be seen in Fig. 2, the bacteria population decreased slightly after 3 min of baking (about 10^6 CFU/g), and then reduced substantially from 3 min to 6 min of baking (about 10^3 CFU/g). It is obvious that the amount of Bb12 in bread baked at 205 °C had the largest reduction during the first 3 min (see Figs. 2 and 3). This inactivated "shoulder" indicates that there might be a thermal death point or the temperature reached the lethal temperature during this period of time (Borges et al., 2012). However, there was just a slight difference between the viable count values of Bb12 after 9 min and 12 min of baking (about 10^2 CFU/g) and the viable counts in the bread baked at the three different temperatures had no significant difference after baking for 6 min. This phenomenon might be because that heat resistant genes or heat shock proteins might be activated or formed in great

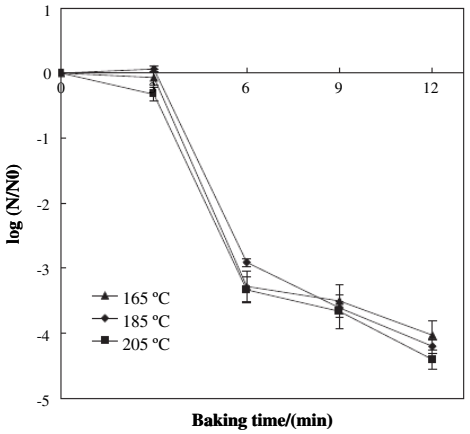

Fig. 2. Viable counts of Bb12 in the core section of the bread during baking at three different conditions.

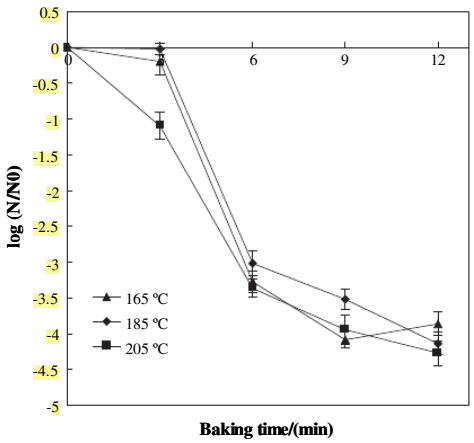

Fig. 3. Viable counts of Bb12 in the middle section of the bread during baking at three different conditions.

amounts when Bb12 cells were exposed to heat, which had a positive effect to improve the thermo tolerance of Bb12 (Desmond et al., 2004; Ding and Shah, 2007). Therefore, heat inducible thermo tolerance allows bacteria, after a non-lethal heat shock, to tolerate a second heat stress higher in intensity. Furthermore, the viable count values of the middle section were less than that of the core section under all baking conditions. This may be because that the dough in the middle reached the lethal temperature faster than the one at the core. In brief, although skim milk is known as an adequate protectant of microorganism in thermal drying process (Fu and Chen, 2011), the bread matrix with skim milk was not effective enough to protect Bb12 from thermal death during baking in this study.

3.2. Variation of the viable counts in bread during proofing

In order to investigate the behavior of Bb12 when they was together with yeasts during proofing process, the viable counts inside the dough were measured every 15 min during proofing. It can be seen from Fig. 4 that the amount of Bb12 in the dough did not change significantly. The order of magnitude almost remained the same. This might be because Bb12 was still in the lag phase of bacterial growth during proofing. Therefore, 75 min proofing did not have a significant impact on its amount detected at these timings.

3.3. Temperature profiles during baking

The temperature variations at two positions of the dough (i.e., core and middle) were recorded by a data logger every 9 s during baking, and the temperature profiles of the air inside the oven were also measured. The temperature profiles of bread without addition of Bb12 (Control) were recorded as well. However, for simplicity, we just show the profiles of bread with Bb12 (Sample) because there was no significant difference between those two groups according to the results of statistical analysis.

Fig. 5 indicates that the temperatures in both core and middle sections of the bread baked at 205 °C were higher than the other two samples. For each baking temperature, the middle section of the bread had a higher temperature than the core section, which

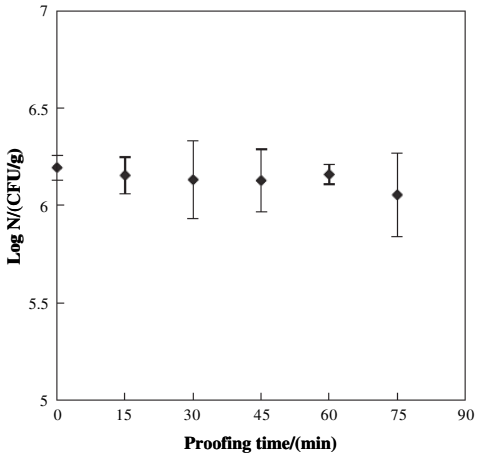

Fig. 4. Viable counts inside the dough during proofing.

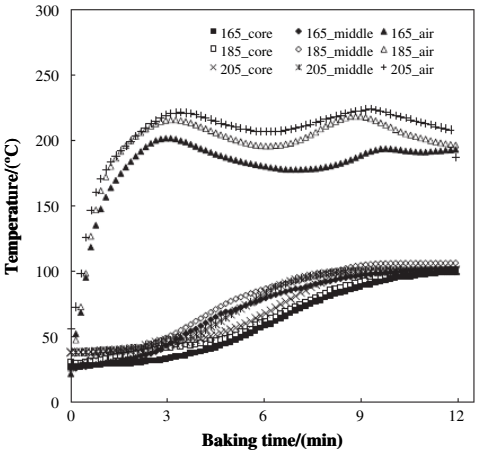

Fig. 5. Temperature profiles and differences between the core and the middle of the bread baked at 165, 185 and 205 °C vs. baking times.

was because the middle section was closer to the hot air, so it needed less time for heat to transfer from the air to this section. This is consistent with the results of viable counts described earlier. In addition, it is noticeable that the temperatures gaps among the bread baked at different temperatures were small, which might be the reason why the viable counts of Bb12 in each piece of bread showed no significant difference.

3.4. Chemical and physical properties of bread

A series of physical and chemical transformations is caused by the heat and mass transfer in bread during baking (Zanoni et al., 1993). Some properties of bread with addition of Bb12 were also measured during baking and storage. Generally, the specific volume of bread increased with baking time due to the expansion of the crumb (Fig. 6). Furthermore, the moisture content at core and middle sections of bread also showed an increasing trend during baking, but decreased during storage (Figs. 7 and 8). This result can be explained utilizing "evaporation–condensation" mechanism (De Vries et al., 1989). To be specific, during baking, the temperature gradient inside the bread induces a partial water vapor pressure gradient, via the saturating water vapor pressure. Beneath the drying region, water vapor migrates through the gas phase towards the thermal core of the bread, where the partial water vapor pressure is at its lowest (Wagner et al., 2007).

Remarkably, as can be seen in Figs. 5 and 7, the moisture content and temperature of the bread both increased with baking time. Specifically, temperatures reached the lethal temperature of

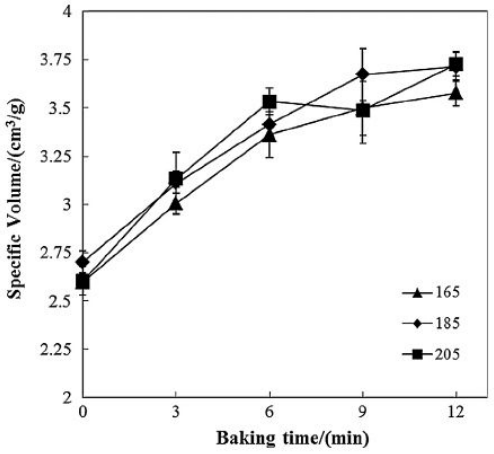

Fig. 6. Specific volume of the bread during baking at 165, 185 and 205 °C.

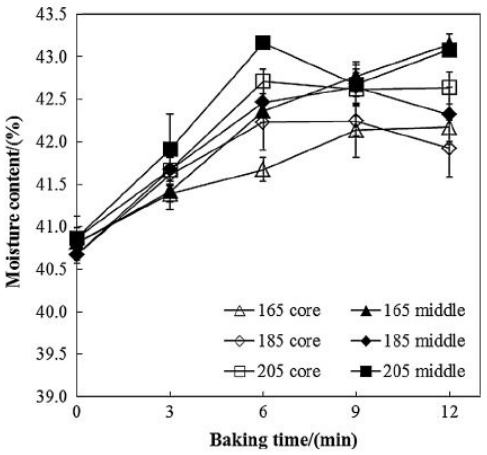

Fig. 7. Moisture content of the bread (core and middle sections) during baking at different baking temperatures.

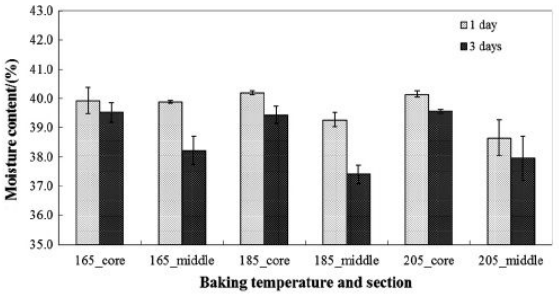

Fig. 8. Moisture content of the bread (core and middle sections) during storage.

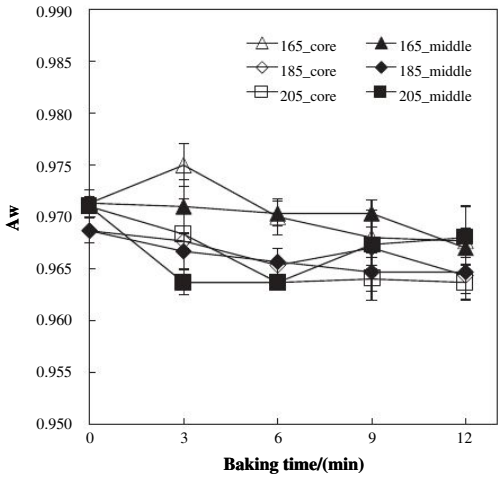

Fig. 9. Water activity (A_w) of the bread (core and middle sections) during baking at different baking temperatures.

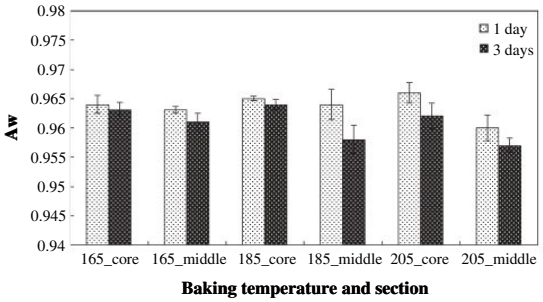

Fig. 10. Water activity (A_w) of the bread (core and middle sections) during storage.

Table 1
Comparison of pH between control group and samples with Bb12[a].

Storage days	Control			Sample with Bb12		
	165 °C	185 °C	205 °C	165 °C	185 °C	205 °C
1	5.91 ± 0.01	5.99 ± 0.11	5.86 ± 0.02	5.88 ± 0.02	5.93 ± 0.04	5.90 ± 0.03
3	6.11 ± 0.01	5.83 ± 0.01	5.84 ± 0.01	5.86 ± 0.01	5.87 ± 0.04	5.94 ± 0.01

[a] Result = mean ± SE, n = 3.

Bb12 after baking for about 6 min and the moisture content became higher than the initial stage of baking at the same time. This relatively high level of moisture content, coupled with a high temperature, formed a hygrothermal environment in the middle section of the dough, just like "moist heat sterilization", as dry heat delivered by superheated steam or hot air is not as effective as moist heat at the same temperature for sterilization (Ansari and Datta, 2003). This may be another reason why the viable counts of Bb12 in bread declined substantially after baking for 3 min. In terms of water activity (A_w), the results seem to indicate that the ratio of "free" to "bound" water was not changed to a significant extent during both baking and storage (Figs. 9 and 10) (Czuchajowska and Pomeranz, 1989). Therefore, water activity was not a critical factor which decisively influenced the survival rate of Bb12 during baking in the present study.

The pH values of bread made with or without addition of Bb12 are shown in Table 1. Generally, the pH values of these two kinds of bread baked at different temperatures barely changed with the storage days. The slight variations of pH could not demonstrate whether the survived bacteria of Bb12 still grew during storage or not. There are several possibilities: (a) Bb12 did not keep growing during storage due to the enlarged lag time caused by heat injury (Busta, 1976; Huang and Chen, 2013); (b) Bb12 kept dying during storage; (c) Bb12 kept growing, but the unfavorable substrates and environment resulted in a slow metabolism, i.e., bacteria could not produce enough lactic acid to reduce the pH of the bread. However, it needs further investigations on the storage behavior of this baked probiotic-fortified bread.

4. Conclusion

In the present study, the viable counts of Bb12 in bread during baking were measured. The results demonstrated that there were still some survival probiotic bacteria after baking for 12 min at three different baking temperatures. However, this study had a limitation: Bb12 is a kind of anaerobic bacteria, but the whole experimental process was carried out in an aerobic environment except for the measurement of viable counts, so it was unable to completely eliminate the interference of oxygen. To solve this problem, other aerobic (or facultative anaerobic) probiotic bacteria, such as Lactobacillus strains may be used to replace Bb12. Further studied are needed to reveal the inactivation kinetics of Bb12 in bread during baking and whether the survival bacteria would keep growing during storage or not. The possibility of adding viable probiotics to the dough may be studied from a theoretical perspective through emulation in the near future.

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