Food Research International 155 (2022) 111040

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Bread as probiotic carriers: Resistance of *Bacillus coagulans* GBI-30 6086 spores through processing steps

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ARTICLE INFO

Keywords:
Sporeforming bacteria
Bakery product
Functional food
Beneficial microbes
Food processing

ABSTRACT

This study evaluated the resistance of *Bacillus coagulans* GBI-30, 6086 (BC) spores through the processing and storage of white and whole wheat bread. The physicochemical parameters of the probiotic bread formulations were also characterized. Loaves of bread containing or not BC were prepared. Throughout the processing, samples were collected (after mixture, after fermentation, and after baking) for enumeration of BC. In addition, BC was enumerated in different parts of loaves of bread (crust, crumb, and whole slice) collected after baking (day zero) and at different storage times (3, 7, and 10 days). The incorporation of BC did not affect the moisture, specific volume, texture and color parameters, water activity, and pH of loaves of bread. Mixing and fermentation steps did not reduce the BC survival in white or whole wheat bread. The highest ($p < 0.05$) number of decimal reductions (γ) was caused by baking in the crust for both loaves of bread. Baking caused around two γ of BC in the crust and 1.5 γ of BC in crumb and a whole slice of white and whole bread. Generally, storage did not increase the γ caused by baking, regardless of the evaluated part or type of bread. Results show the impacts of baking on BC and highlight the formulated white and whole wheat loaves of bread as suitable carriers for delivering the probiotic BC.

1. Introduction

Probiotics are defined as "*live microorganisms that, when administered in sufficient amounts, confer a health benefit on the host*" (Hill et al., 2014). Among the several microbial genera reported to present probiotic properties, some *Bacillus* strains have been claimed to present probiotic properties (Cutting, 2011; Cao et al., 2020; Nithya & Halami, 2013). The strain *Bacillus coagulans* GBI-30, 6086 (BC) received the GRAS status (FDA, 2016; FDA, 2017), being recognized as a non-toxic, non-pathogenic, and non-antibiotic resistant *Bacillus* strain (Endres et al., 2009).

In the last years, several health benefits of the administration of BC have been reported, including improvement in symptoms of irritated bowel syndrome (IBS), such as bloating and abdominal pain and decreased bowel movements (Dolin, 2009; Hun, 2009). The administration of BC was also shown to provide relief of abdominal pain and other symptoms of gastrointestinal disorders (Kalman et al., 2009). The ability of this strain to digest lactose, fructose, and milk protein observed *in vitro* has been related to its relief in lactose intolerance symptoms (Maathuis et al., 2010). Also, immune modulation and anti-inflammatory effects were already reported after BC administrations (Jensen, Benson, Carter, & Endres, 2010), as well as increased levels of immunological markers such as cytokines (IL-6, IL-8, and INF-γ) and CD3CD69 cells (Kimmel, Keller, Farmer, & Warrino, 2010). Pain reduction in patients with rheumatoid arthritis has also been associated with the administration of BC (Mandel, Eichas, & Holmes, 2010), and more recently, the administration of yogurt containing BC resulted in a decrease of glucose and triglycerides levels and a positive influence in the gut microbiota of healthy rats (Almada-Érix et al., 2021a).

For the successful application of probiotics in food matrices, in addition to the specific properties of the strain, the food matrix

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https://doi.org/10.1016/j.foodres.2022.111040
Received 3 November 2020; Received in revised form 10 February 2022; Accepted 17 February 2022
Available online 7 March 2022

composition is recognized to influence the survival during processing and storage and delivery of probiotics in the gastrointestinal tract (GIT) (Almada-Érix et al., 2021a; Guimarães et al., 2020; Soares et al., 2019a). Specific components of the food matrix may confer protection during the storage and when cells are exposed to several stresses such as low pH, bile acids, and digestive enzymes (Almada-Érix et al., 2021b; Fazilah, Ariffa, Khayat, Rios-Solisd, & Halima, 2018; Soares et al., 2019b). Even though *Lactobacillus* and *Bifidobacterium* are the most studied probiotic genera, their delivery in various processed foods is compromised because these bacteria do not survive under harsh processing conditions (Chan et al., 2021; Dantas et al., 2021; Mirzamani et al., 2021; Silva et al., 2021; Soares et al., 2019a). Besides, their functionality may also be affected (Almada-Érix et al., 2021a,b; Guimarães et al., 2020; Ranadheera, Baines, & Adams, 2010).

Despite the vast consumption of bread since ancient times and the consumer's interest in functional bakery products (Pinto, Castro, Vicente, Bourbon, & Cerqueira, 2015), bread as a probiotic carrier has been limited by the baking step. Bread is prepared through the mixture of ingredients (flour, water, yeast, sodium chloride, with or without fat, sugar, and milk powder) followed by molding, fermentation, and baking in the oven at high temperatures (Pico, Bernal, & Gómez, 2015). Probiotic strains of *Lactobacillus* and *Bifidobacterium* cannot survive at the temperature achieved during baking (~200 °C) (Zhang, Huang, Ananingsih, Zhou, & Chen, 2014). Therefore, the application of *Bacillus* probiotics (Nithya & Halami, 2013; Soares et al., 2019b) can be a feasible alternative to develop functional loaves of bread processed at conditions that would kill *Lactobacillus* and *Bifidobacterium*. As spore-forming bacteria, *Bacillus* strains with claimed probiotic properties are resistant to harsh processing conditions and can remain viable during storage, as well as survive better the gastrointestinal tract conditions (Almada-Érix et al., 2021b; Cao et al., 2020; Soares et al., 2019a). The incorporation of BC in bread seems a strategy for functionalizing a worldwide daily-consumed food product.

Therefore, this study evaluated the fate of BC spores through the processing steps (mixture, fermentation, and baking) of white and whole wheat loaves of bread. The fate of BC in the crust, crumb, and loaves of bread was assessed during storage.

2. Materials and methods

2.1. Probiotic strain

A direct vat set (DVS) culture of *B. coagulans* GBI-30, 6086 spores (Ganeden Biotech Inc, Mayfield Heights, Ohio, USA) was inoculated at approximately 10^7 spores/g during the mixing stage of bread processing (as described in item 2.3).

2.2. Rheological properties of flours and bread formulations

Flours [refined (white) and whole-grain wheat] used in bread formulations were evaluated according to the official methods of AACCI (2010) as follow: for Farinographic (water absorption, stability) - method 54–21.02, using Brabender farinograph model 810,130 (Brabender, Duisburg, Germany); Alveografic (resistance and extensibility) - method 54–30.02, using Chopin alveograph, model MA95 (Chopin, Villeneuve-la-Garenne, France). Whole grain wheat and white wheat flours used for bread making presented 10 g and 10.9 g of moisture/100 g (dry basis). When analyzed in 100 g of dry sample, whole grain wheat and white wheat flours presented 13.3 ± 0.5 g and 12.7 ± 0.8 g of protein, 2.0 ± 0.2 g and 1.4 ± 0.2 g of fat, 1.6 ± 0.02 g and 0.7 ± 0.02 g of ash, 10.4 ± 1.3 g and 2.9 ± 0.2 g of fiber, and 72.7 g and 82.3 g of digestible carbohydrates, respectively. Farinographic analysis indicated a water absorption by whole grain wheat flour of 65.3% and stability of 15.5 ± 0.9 min, while in white wheat flour, the water absorption was 58.2% and stability of 23.1 ± 0.8 min (Wahanik et al., 2021).

Based on previous optimization experiments, white and whole wheat loaves of bread were formulated (Wahanik et al., 2021). Formulation of white bread included: white flour (100%), and other ingredients as % flour basis as following water (60%), sugar (4%), salt (2%), yeast (2%), whole milk powder (4%), hydrogenated vegetable fat (4%), sodium propionate (0.5%) and flavor enhancer (2%). Whole wheat bread ingredients were: whole wheat flour (51%), white flour (49%), and other ingredients as % flour basis: water (62%), sugar (4%), salt (2%), yeast (2%), whole milk powder (4%), hydrogenated vegetable fat (4%), sodium propionate (0.5%) and flavor enhancer (2%).

2.3. Bread processing steps

The loaves of bread were prepared using the modified straight dough method (Schmiele, Jaekel, Patricio, Steel, & Chang, 2012). The production was performed in different stages: ingredients mixture, dividing, resting, molding, fermentation (32 °C/60 min), and baking (200 °C/18 min). The dry ingredients were mixed in a vertical spiral kneading trough with two speeds A30 model mixer (Progresso, Colombo, Brazil) at slow speed (90 rpm, 1 min). The water was added at 4 °C, and the dough was mixed in slow (90 rpm, 7 min) and high speed (180 rpm, 6 min) to develop the gluten network. The dough was divided into 250 g that were left to rest (5 min), following molding in dough molder (Perfecta, Curitiba, Brazil). Afterward, the dough was placed in greased bread tins (10.25 × 6.75 × 22 cm) and further kept in evolution-CCKU-8040–182 model proofing chamber (Super Freezer, Poços de Caldas, Brazil) at 32 °C, for 60 min (controlled relative humidity 80%). Then, the doughs were baked (200 °C/18 min) in Vipinho-0448 trif model oven (Perfecta, Curitiba, Brazil). After baking, the loaves of bread were cooled down at room temperature, packaged, and further stored for ten days at 25 °C.

Two independent experiments (bread manufacture process) were done for each bread (white and whole wheat). BC spores were added at the mixture stage. Samples for enumeration of BC spores were collected at the following stages of the process: after mixture, after fermentation and, after baking in different parts of loaves of bread, namely crust, crumb, and whole slice (day zero). In addition, at different storage intervals (3, 7, and 10 days), the same parts of loaves of bread (crust, crumb, and whole slice) were collected for enumeration of BC spores (section 2.5). Samples (crust, crumb, and whole slice) were also collected at 1, 3, 7, and 10 days of storage to determine the quality characteristics of loaves of bread. Formulations without the addition of BC were similarly processed and used as controls for quality evaluation. The storage time evaluated was defined considering the shelf-life of loaves of bread.

2.4. Quality characteristics of bread

The bread quality characteristics analyzed on day 1 were moisture, specific volume, texture, and color. Water activity (a_w) and pH were measured on days 1, 3, 7, and 10. The pH was determined using a pHmeter Akso (AK103 model, Rio Grande do Sul, Brazil). The a_w was measured using the a_w analyzer, Aqualab digitaL 4TEV (Decagon, Pullman, USA), both according to the manufacturer's instructions. The color of the bread crumb was evaluated using the MiniScan XE portable spectrophotometer (Hunter Associates Laboratory, Inc, Reston, Virginia, USA) with D65 illuminant and 10° observation angle. The values of brightness (L*) ranging from 0 (black) to 100 (white), a* varying from green (negative) to red (positive), b* varying from blue (negative) to yellow (positive), C* representing the saturation and h* the tonality angle were evaluated. Moisture content was determined based on the 44–15.02 method, while the specific volume was determined according to the 10.05.01 method of AACCI (2010). All these analyses were made in triplicate.

The texture was determined with eighteen repetitions using the texture analyzer, the TAXT2i model (Stable Micro Systems, Haslemere/England), using the 74–09.01 method (AACCI, 2010). Two 12.5 mm

thick two-slice crumbs were compressed in each test (discarded bread slices). The test conditions were pre-test velocity = 1 mm/s, test velocity = 1.7 mm/s, post-test velocity = 10 mm/s, distance = 40% and time = 60 s. The measurements of firmness are expressed in N.

2.5. Enumeration of BC spores

Samples collected at different processing steps and storage were submitted to heat shock (80 °C/10 min) as indicated by the supplier of BC strain. Then, decimal dilutions were prepared, and samples were inoculated through the Pour-plate technique in BC agar [g/L: yeast extract powder (5 g), peptone (5 g), glucose (5 g), potassium phosphate dibasic (0.5 g), potassium phosphate monobasic (0.5 g), magnesium sulfate (0.3 g), trace mineral solution (1 mL)] (Kasvi, Curitiba, PR, Brazil) formulated according to guidelines of the supplier of the BC strain used. All reagents were from Dinâmica Quimica (Diadema, SP, Brazil), except yeast extract powder and peptone that were from Kasvi® (Curitiba, PR, Brazil). Then, the inoculated Petri dishes were incubated at 42 °C for 48 h, the colonies were counted, and the results were expressed as log CFU/g. The analyses were done in duplicate, and the results were expressed as the logarithm of the number of spores/g. The number of decimal reductions (γ) of BC in each stage of processing or storage was calculated based on the difference between the initial inoculum (N_0) and final count (N_f) (equation 1) after each stage of the process (Soares et al., 2019b).

$$\gamma = \log N_0 - \log N_f. \quad (1)$$

2.6. Statistical analysis

All data were presented as means ± standard deviation and analyzed by ANOVA, followed by the *t*-test and Scott-Knott using the Sisvar software 5.6 (Lavras, MG, Brazil). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Quality parameters of flours and probiotic loaves of bread

The water absorption, stability, resistance, and extensibility of flours are shown in Fig. 1. Whole wheat flour showed higher water absorption and lower stability, resistance, and extensibility than white refined flour ($p < 0.05$).

The quality characteristics of bread (moisture, specific volume, texture, and color) are presented in Table 1. The addition of BC did not affect any of the evaluated quality parameters in white or whole wheat bread.

Moisture and texture did not vary significantly between bread's loaves added to BC (probiotic) and control loaves of bread (without BC). Whole wheat loaves of bread (probiotic and control) showed lower volume values ($p < 0.05$) when compared to white loaves of bread (probiotic or control) (Table 1; Supplementary Fig. 1 and 2). The color parameters b* and C* did not differ significantly among the loaves of

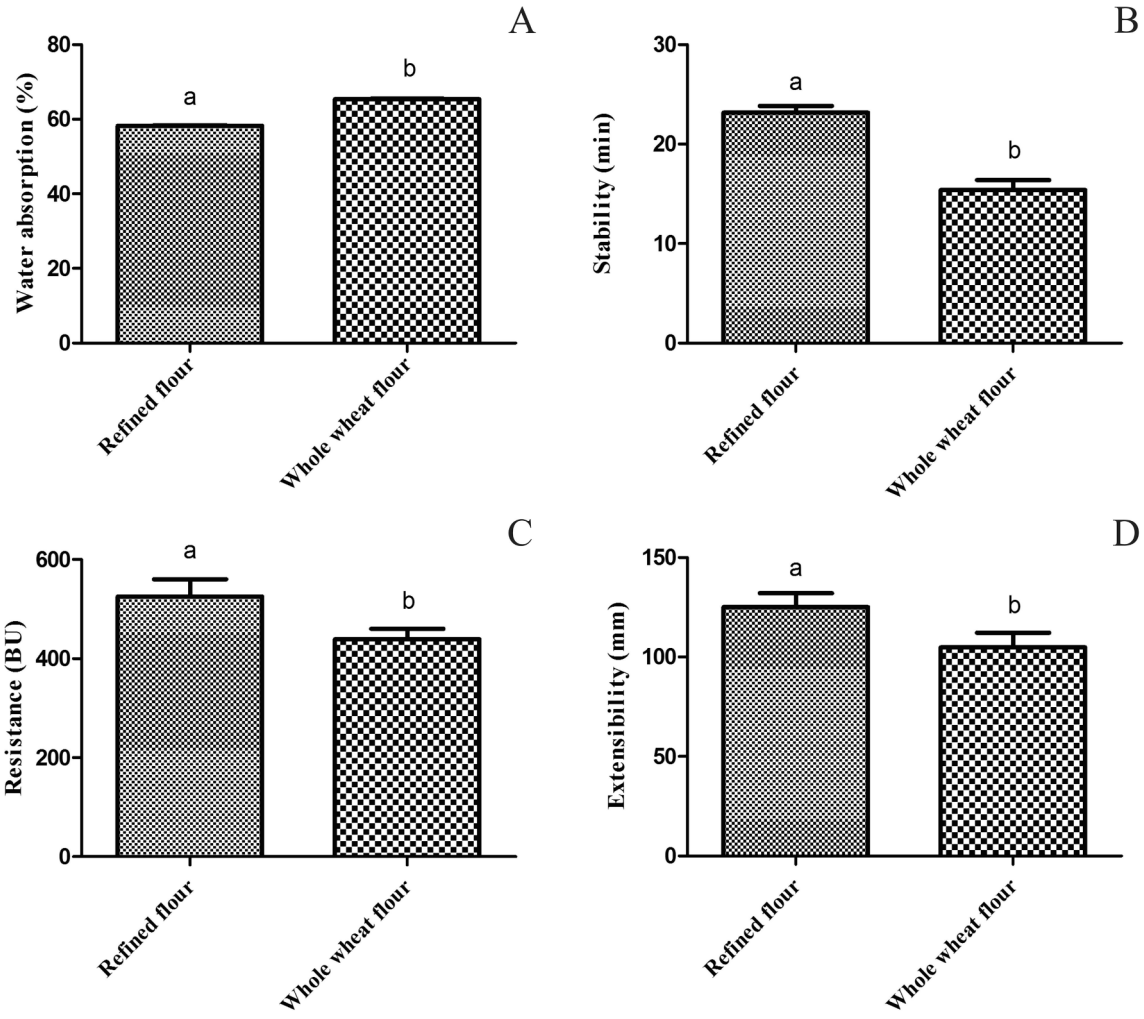

Fig. 1. Rheological properties (water absorption (A), stability (B), resistance (C), and extensibility (D)) of white and whole flours used for bread making.

Table 1
Moisture, specific volume, texture and color in different bread types.

Bread type				Color				
	Moisture (%)	Volume (L/Kg)	Texture (Firmness; N)	L*	a*	b*	C*	h*
Wheat bread	34.6 ± 0.17^{a}	4.2 ± 0.15^{a}	1.7 ± 0.10^{a}	84.5 ± 0.60^{a}	1.6 ± 0.02^{a}	19.7 ± 0.2^{a}	19.8 ± 0.23^{a}	4.8 ± 0.01^{a}
Wheat probiotic bread	34.8 ± 0.85^{a}	4.1 ± 0.28^{a}	1.9 ± 0.19^{a}	84.8 ± 0.42^{a}	1.6 ± 0.03^{a}	18.8 ± 0.00^{a}	18.8 ± 0.00^{a}	4.9 ± 0.09^{a}
Whole wheat bread	35.8 ± 0.38^{a}	3.4 ± 0.02^{b}	2.4 ± 0.16^{a}	75.2 ± 0.37^{b}	4.5 ± 0.03^{b}	18.8 ± 0.01^{a}	19.4 ± 0.01^{a}	13.5 ± 0.08^{b}
Whole wheat probiotic bread	35.5 ± 0.56^{a}	3.3 ± 0.16^{b}	2.2 ± 0.77^{a}	74.9 ± 0.31^{b}	4.6 ± 0.23^{b}	18.5 ± 0.15^{a}	19.1 ± 0.09^{a}	14.1 ± 0.79^{b}

Means ± standard deviation of two replicates (3 replicates); Means with different superscript letters in the same column indicate significant difference between samples, by Scott-Knott ($p < 0.05$);.

bread analyzed. However, a* and h* parameters increased, while the L* parameter reduced ($p < 0.05$) in whole wheat loaves of bread compared to white loaves of bread (Table 1; Supplementary Fig. 1 and 2).

The a_w and pH of loaves of bread were 0.94–0.95 and 5.7–5.9, respectively. The pH did not vary among the bread formulations (probiotics and controls) analyzed or throughout the ten days of storage in the same bread (Supplementary Table 1). The a_w did not vary in the same formulation between different days analyzed (1, 3, 7, and 10). However, it varied ($p < 0.05$) among the four bread formulations (probiotics and controls) analyzed on the same day (1, 3, and 7) (Supplementary Table 1). The whole wheat probiotic bread showed minor increases in water activity on days 3, 7, and 10, respectively, compared with white probiotic bread.

3.2. BC survival throughout the processing and storage of loaves of bread

The survival of BC through different steps of bread processing is shown in Table 2. Mixture and fermentation steps did not affect ($p \geq 0.05$) the survival of BC in bread, regardless of the formulation.

Generally, BC showed high resistance to baking (≤2 γ) in white and whole wheat bread. In the white probiotic bread, the γ of BC caused by baking was 1.9, 1.6, and 1.5 log CFU/g in the crust, crumbs, and the whole slice of white bread. In whole wheat bread, baking led to 1.6 γ of BC in crust and 1.5 and 1.5 γ in crumb and whole slice, respectively. There was no significant difference ($p < 0.05$) in the survival of BC in the crust, crumb, and whole slice in the probiotic loaves of bread (white or whole wheat bread) on day 0, as well as no differences in the survival of BC were observed when comparing the results of white with whole wheat bread making (Table 2). Thus, considering the processing steps, only baking led to a significant reduction in the counts of BC ($p < 0.05$) when compared to the reduction in the counts that occurred during mixture and fermentation steps, for both white and whole wheat bread ($p < 0.05$) (Table 2).

The γ of BC caused by storage in the crust, crumb, and whole slice of white and whole wheat probiotic bread are shown in Table 3. The γ of BC observed in the crust, crumb, and the whole slice varied within 1.9–2.9, 1.5–2.0, 1.5–2.2, respectively. For whole wheat bread, the γ of BC in the crust, crumb, and whole slice of whole wheat probiotic observed varied within 1.6–2.1, 1.2–1.6, and 1.3–2.2 CFU/g, respectively. Regarding the part of bread samples (in the form of crust, crumb, and whole slice) compared individually during storage, in the whole wheat probiotic bread, day 7 differed significantly ($p < 0.05$) from the other days (0, 3, and 10) in the whole slice, showing higher γ of BC (Table 3). However, no increase in γ was observed during storage of the white and whole wheat loaves of bread, regardless of the part of bread evaluated.

Table 2
Fate of *Bacillus coagulans* GBI-30, 6086 throughout processing of breads with different formulations.

Processing steps	White probiotic bread γ (log CFU/g)	Whole probiotic bread γ (log CFU/g)
After mixture	0.02 ± 0.01^{aA}	0.01 ± 0.00^{aA}
After fermentation	0.05 ± 0.09^{aA}	0.20 ± 0.13^{aA}
After baking (crust) day 0	1.9 ± 0.84^{aB}	1.6 ± 0.15^{aB}
After baking (crumb) day 0	1.6 ± 0.18^{aB}	1.5 ± 0.09^{aB}
After baking (whole slice) day 0	1.5 ± 0.54^{aB}	1.5 ± 0.02^{aB}

(γ = No-Nf); (No) Initial count; White probiotic bread: 7.2 log CFU/g; Whole wheat probiotic bread 7.2 log CFU/g. Means ± standard deviation of two replicates; Means with different superscript lowercase letters on the same line indicate significant difference between samples of white and whole wheat breads by test t ($p < 0.05$). Means with different superscript capital letters on the same column indicate significant difference between samples, by Scott-Knott ($p < 0.05$).

4. Discussion

In the last years, an expansion of the diversity in the bread market and bakery industry has been observed in response to the consumer's demand for healthy products with functional ingredients. Remarkably, probiotic bacteria and whole-grain flour that contains a higher content of fibers, proteins, minerals (e.g., calcium, magnesium, and potassium), and phytochemicals have been suggested as alternative ingredients for the functionalization of loaves of bread (NithyaBalaSundari et al., 2020; Zhang, Taal, Boom, Chen, & Schutyser, 2018).

Both white and whole flours used in formulations in the present study displayed rheological properties appreciable for bread manufacture, despite de distinct characteristics (Ortolan et al., 2015). The higher values of water absorption and lower values of stability, resistance, and extensibility observed in the whole wheat flour are mainly explained by the higher content of fibers compared to refined flour (Schmiele et al.,

Table 3
Fate of *Bacillus coagulans* GBI-30, 6086 in bread formulations during shelf-life.

Storage time	White probiotic bread γ (log CFU/g)	Whole probiotic bread γ (log CFU/g)
Crust (day 0)	1.9 ± 0.84^{aA}	1.6 ± 0.15^{aA}
Crumb (day 0)	1.6 ± 0.18^{aA}	1.5 ± 0.09^{aA}
Whole slice (day 0)	1.5 ± 0.54^{aA}	1.5 ± 0.02^{aA}
Crust (day 3)	2.9 ± 0.05^{aA}	1.9 ± 0.62^{aA}
Crumb (day 3)	2.0 ± 0.34^{aA}	1.2 ± 0.08^{aA}
Whole slice (day 3)	2.2 ± 0.33^{aA}	1.3 ± 0.20^{aA}
Crust (day 7)	2.6 ± 0.20^{aA}	1.8 ± 0.61^{aA}
Crumb (day 7)	1.5 ± 0.56^{aA}	1.6 ± 0.31^{aA}
Whole slice (day 7)	1.7 ± 0.70^{aA}	2.2 ± 0.08^{aB}
Crust (day 10)	1.9 ± 0.23^{aA}	2.1 ± 0.10^{aA}
Crumb (day 10)	1.9 ± 0.05^{bA}	1.2 ± 0.20^{aA}
Whole slice (day 10)	1.7 ± 0.36^{aA}	1.4 ± 0.31^{aA}

(γ = No-Nf); (No) Initial count; White probiotic bread: 7.2 log CFU/g; Whole wheat probiotic bread 7.2 log CFU/g. Means ± standard deviation of two replicates; Means with different superscript lowercase letters on the same line indicate significant difference between samples of white and whole wheat by test t ($p < 0.05$). Means with different superscript capital letters on the same column indicate significant difference between days in samples of crust, crumb and whole slice, respectively, by Scott-Knott ($p < 0.05$).

2012). Other nutrients of the whole grains, such as proteins and lipids lost during refining, may have influenced the rheological parameters assessed (Ortolan et al., 2015; Schmiele et al., 2012).

The loaf bread is characterized by a thin crust and a crumb with regular porosity, besides the soft and elastic texture. The quality of this type of bread is mainly defined by texture, moisture, specific volume, crust color, crust/crumb ratio, crumb firmness, and flour characteristics (Besbes, Jury, Monteau, & Bail, 2014). The addition of BC in loaves of bread did not affect any of the quality parameters evaluated. This result is significant for the bakery industry since it has been reported that the addition of non-spore-forming probiotic bacteria may change texture parameters in foods (Guimarães et al., 2020).

As expected, the whole wheat loaves of bread showed a lower volume than white loaves of bread based on flour properties. The fibers present in whole wheat flour extensively compete for water with other polymers resulting in the dilution of the gluten and weakening of the gluten network formed that confers the viscoelastic properties leading to weaker and more compact doughs (Khalid, Ohm, & Simsek, 2017; Schmiele et al., 2012;). Also, whole wheat loaves of bread present darker coloring (as indicated by L*, a*, and h* parameters). The presence of fibers confers a darker color to the whole wheat flour color compared to white flour and consequently a different color to the derived bread. Overall, other whole grain components that participate in the Maillard reaction during baking may have also influenced the darker color of whole loaves of bread (Khalid et al., 2017).

The pH and a_w characterize the loaves of bread as foods with high water activity and low acidity (Geng, Harnly, & Chen, 2016). No changes in pH also suggest that the strain did not germinate and multiply in the loaves of bread during the storage. No changes in pH of baking products (chrysanthemum cookies, egg pastry cakes, mooncakes, muffins, polo loaves of bread, soda cookies, sponge cakes, and toasts) containing *B. coagulans* GBI-30, 6086 during storage has also been reported previously (Jao et al., 2011). Otherwise, the higher a_w in whole loaves of bread compared to white loaves of bread can be justified by the presence of fibers, which retain the moisture content.

Mixing and fermentation steps did not affect the survival of BC that presented excellent resistance to baking in both white and whole wheat loaves of bread with reduction $\leq$ 2 log CFU/g. On the other hand, the viable counts of *Bifidobacterium lactis* BB12 declined 4 log cycles after baking at various temperatures (165, 185, 205 °C) for 12 min (Zhang et al., 2014). Particularly, baking caused 2 log reductions of BC in the crust of loaves of bread, which is the portion most directly exposed to a high temperature (~190–200 °C), while the crumb reaches temperature up to 70–80 °C. Moreover, the counts of BC in white and whole wheat bread did not increase during ten days of storage, indicating that this microorganism could not germinate and outgrow in the product under the storage conditions studied. This outcome is of great relevance because it shows that BC cannot grow, and consequently, cannot spoil loaves of bread during storage. The spoilage potential of sporeforming bacteria is a significant concern when dealing with their use as probiotic microorganisms. Some *Bacillus* species such as *B. licheniformis, B. subtilis, B. pumilus, B. megaterium, B. amyloliquefaciens*, and *B. cereus* represent a risk for the microbiological stability of bakery products due to their ability to cause spoilage known as "rope" (Li et al., 2020; Pereira et al., 2020; Valerio et al., 2015). Rope spoilage is characterized by the sweet-smelling, softening, and sticking of bread crumbs and the presence of filaments and "ropes" when bread is stretched out in two parts (Pereira et al., 2020; Valerio et al., 2015). Therefore, BC is appropriate for producing probiotic loaves of bread because it can withstand bread baking while it does not grow in loaves of bread during storage. As a result, the application of BC in loaves of bread expands the options for new probiotic foods. BC was found to survive baking of eight different products and the viability during the storage at 4 or 25 °C for 15 and 6 days, respectively (Jao et al., 2011). Nonetheless, it is essential to consider the γ during the process for the calculation of the needed initial inoculum. This information is critical so the concentration of probiotics required for beneficial effects to the host to be reached appropriately (Granato, Branco, Cruz, Faria, & Shah, 2010; Cao et al., 2020; Nithya & Halami, 2013).

5. Conclusions

B. coagulans GBI-30, 6086 presented high resistance to the bread manufacturing process, particularly the baking process conducted at very high temperatures. Besides, white and whole wheat bread carrying *B. coagulans* GBI-30, 6086 was stable during storage for ten days. Incorporating *B. coagulans* GBI-30, 6086 to bread formulations did not change any of the quality parameters evaluated. Therefore, this study shows that finding probiotic strains that can withstand harsh processing conditions (i.e., probiotic sporeforming bacteria) will allow the expansion of probiotic foods with potential health benefits to consumers.

CRediT authorship contribution statement

Carine N. Almada-Érix: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Caroline N. Almada:** Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Geany T. Souza Pedrosa:** Writing – review & editing, Visualization, Formal analysis, Investigation. **João Paulo Biachi:** Formal analysis, Investigation, Visualization. **Mariane S. Bonatto:** Formal analysis, Investigation, Visualization. **Marcio Schmiele:** Methodology, Resources, Validation, Formal analysis, Investigation, Supervision. **Elizabeth H. Nabeshima:** Methodology, Resources, Validation, Formal analysis, Investigation, Supervision. **Maria Teresa P.S. Clerici:** Methodology, Resources, Validation, Formal analysis, Investigation, Supervision. **Marciane Magnani:** Visualization, Validation, Formal analysis, Investigation, Writing – review & editing. **Anderson S. Sant'Ana:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the "*Conselho Nacional de Desenvolvimento Científico e Tecnológico*" (CNPq, Grants #302763/2014-7 and #305804/2017-0) and "*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*" (CAPES; Finance code 001). Authors thank to Ganeden Biotech Inc, Mayfield Heights, Ohio, USA, for the donation of BC strain used in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111040.

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