



Proceeding Paper The Potential Use of Synbiotic Combinations in Bread—A Review †

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Abstract: To date, the most commonly used probiotics in the potential synbiotic combinations (SC) in breads belong to the family of Lactobacillaceae and Bifidobacteriaceae. However, *Bacillus coagulans* as a heat-resistant bacteria, and *Saccharomyces boulardii* as a probiotic yeast could promising to use of SC in bread. Inulin is the most commonly direct-used prebiotic source in formulations of potential SC bread. Moreover, co-encapsulations of probiotics with prebiotics mainly including different hydrocolloids could be beneficial. Although the consumption of potential SC in bread generally including *Lactobacillus sporogenes* and inulin by diabetics had some health benefits, there is a need for more comprehensive clinical trials.

Keywords: Bacillus coagulans; hydrocolloid; co-encapsulation; edible film; type 2 diabetes

1. Introduction

Nowadays, the development of functional food products including probiotics, prebiotics, and synbiotics, which have an important role in the diet in terms of protecting and/or improving human health [1], come into prominence. Among those, synbiotics as a combination of probiotics and prebiotics have a synergetic health-promoting influence. In this regard, the synbiotic foods mainly include dairy (cheese, yoghurt, ice cream, and fermented milk, etc.), and non-dairy food products, such as confectioneries (chocolate, candy, etc.) [2], fermented or unfermented beverages (such as cereal-, legume- or fruitbased drinks, etc.) [3,4], and other food products such as mousse, salad dressing, and snacks [2]. However, there are limited studies in the literature regarding the potential synbiotic combinations in baked goods particularly bread as a staple food.

Inulin is the major directly-utilized prebiotic source in potential synbiotic combinations in bread, as seen in Table 1. However, there is a challenge in the production of a synbiotic bread which is generally related to the heat sensitivity of probiotic bacteria during the baking process. To protect the probiotic bacteria regarding their viability and stability during the heating process, they should be encapsulated and then added to the food structure, or should be covered with an edible film/coating on the bread crust structure [5]. In addition, PRO-PRE co-encapsulation, in other terms co-delivery or co-entrapment, of probiotics with prebiotics is a promising application regarding both stability and viability of living probiotics [6]. Moreover, recently it is revealed that some probiotic microorganisms particularly *Bacillus coagulans* could be also a promising for maintaining probiotic viability throughout thermal processes [7]. Although *B. coagulans*, which is a sporeforming probiotic [8], has resistance and/or tolerance to heat, conditions of the

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2. The Potential Synbiotic Combinations in Breads

2.1. The Influence of Potential Synbiotic Combinations in Breads on Probiotic Viability

Up to now, the most commonly used probiotic bacteria in potential synbiotic combinations in breads are *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, and *and Lacticaseibacillus rhamnosus*, which belong to Lactobacillaceae family and also *Bifidobacterium animalis*, *Bifidobacterium adolescentis* and *Bifidobacterium longum* which are species of Bifidobacteriaceae family (Table 1). Moreover, the mostly utilized wall materials are high-amylose maize starch (Hi-maize), chitosan, and some hydrocolloids such as pectin, xanthan gum, gum arabic, gellan gum, tragacanth gum, carboxymethyl cellulose, hydroxypropyl methylcellulose for preparing co-encapsulated probiotic bacterial strains in potential synbiotic combinations of breads, as shown in Table 1.

Un-encapsulated L. rhamnosus lost its viability after bread baking [11,12]. The viability of L. rhamnosus was increased by nearly 3% when incorporating baobap pulp (BP) into the culture medium. The inclusion of baobap pulp has a prebiotic potential in bread formulation resulting in a further increase in probiotic viability by about 3%. Those were interrelated with its higher pectin content which enhanced the metabolic activities of L. rhamnosus and also its interaction with BP due to an increase in hydrogen bonds, electrostatic forces, and steric hindrance. Throughout the resident time of simulated gastric- (90 min) and intestinal phase (180 min), the viability of encapsulated probiotic were significantly increased in BP-enriched bread. Further increase was also determined with precultured probiotics with BP powder. At the end of the simulated gastric phase, the viability of encapsulated probiotic was increased by nearly 6–11.5%. This was ascribed to the proliferation of probiotics because of the increase in carbon sourced from starch and phenolic contents of BP [11]. In another study, the number of viable encapsulated L. rhamnosus with different wall materials (sodium alginate, high-amylose maize starch, cassava starch, and chitosan) was in the range of nearly 4.94–9.2 log CFU/g after baking at different baking conditions (180 °C-30 min, 220 °C-20 min, 250 °C-15 min). The triple-layered capsules composed of sodium alginate combined with high amylose-resistant starch and chitosan had the highest probiotic viability after baking and in the simulated gastrointestinal system [12]. In simulated gastric conditions, the highest relative viability of L. acidophilus was obtained in the first layer composed of alginate using at 1%, and then, chitosan was significantly more effective than alginate as a second layer coating material at the same concentrations in fresh and 1-day stored bread. However, the relative viability of double-layered encapsulated probiotics was decreased with short-time storage (1 day) at room temperature irrespective of coating material [13]. The viability of probiotics (L. acidophilus, L. plantarum) irrespectively of strain was increased by microencapsulation independent of wall material throughout gluten-free bread baking and also storage ongoing more than 2 logarithmic cycles. However, this influence was more pronounced in encapsulated probiotics by tragacanth gum which is attributed to its higher prebiotic effect than sago starch [14]. In a similar vein, the viability of *L. casei* in the edible films based on konjac glucomannan was gradually reduced with a decrease of 2 log CFU/portion after 7-day storage at room temperature [15]. In another study, the higher probiotic viability in the edible coating was obtained in the combination of whey and sodium alginate than in high amylose maize starch and gelatin [16]. Although inulin concentration did not make a significant difference in Bacillus coagulans count in fresh and stored bread, it was more than 6 log CFU/ which was still met the minimum level complying with the WHO recommendation to provide health benefits [5].

2.2. The Influence of Potential Symbiotic Combinations in Breads on Technological Properties

The different encapsulation wall materials (sodium alginate individually, double or triple layered with chitosan, cassava starch, and Hi-maize resistant starch) for L. rhamnosus did not make a significant change in weight and specific volume of breads, and also its nutritional value (protein, fat, carbohydrate, ash, and fiber content) [12]. The higher specific volume and oven spring values in gluten-free bread including probiotics encapsulated with tragacanth gum were recorded. This could be explained by the hydroxyl group of tragacanth gum which has the capability of water absorption. Moreover, the hardness values did not significantly affect by probiotic sources (L. acidophilus, L. plantarum), but did by encapsulation wall material. In this regard, the softest in other terms the lowest hardness values were belong the gluten-free breads with probiotics encapsulated by tragacanth gum individually which is followed by using it combined with sago starch [14]. A significant moisture reduction was observed with increasing inulin levels in fresh bread consistent with water absorption values obtained from the farinograph. Increasing in content of inulin as a prebiotic source in bread formulation, resulted in a significant reduction in specific volume values. This could be attributed to the prohibition of proper expansion which is associated with gluten dilution, disruption of gluten-starch matrix, and lower water vapor because of the lower moisture content of the bread dough. A significant increase in hardness values in bread with increasing concentration of inulin was observed and several potential reasons were stated such as the formation of cross-linkages between inulin and starch and/or protein which led to a decrease in gas retention capacity, recrystallization of inulin while cooling, co-crystallization of inulin and also amylopectin, strengthen the solids around the gas cells and lower moisture content with inulin addition. While the L and b values of the crust part of the bread were significantly reduced with inulin addition, an opposite effect was observed in *a* values that resulted in darker and reddish color. This is related to a decrease in moisture content and partial hydrolyzation of inulin to glucose and fructose during the baking process which develops crust color via Maillard reaction and caramelization [5].

Product	Probiotic Source(s)	Prebiotic or Potential Prebiotic Source(s)	References
Bread	Lactobacillus acidophilus	Inulin ^{a, b} , carboxymethylcellulose ^{,b} , pectin ^{a, b} , fresh agave sap ^{a, b}	[17]
Bread	Lactobacillus acidophilus	Xanthan gum ^b , gellan gum ^b , chitosan ^b	[13]
Cream bread	Lactobacillus acidophilus	Xanthan gum ^b , maltodextrin ^b	[18]
Gluten-free "Barbari" bread	Lactobacillus acidophilus, Lactiplantibacillus plantarum	Tragacanth gum $^{\rm b}$, sago starch $^{\rm b}$	[14]
Bread	Lactiplantibacillus plantarum	Inulin ^b , gum arabic ^{a,b} , maltodextrin ^{a, b}	[19]
Pan bread	Lacticaseibacillus rhamnosus	Baobab pulp ª, high- amylose maize starch ʰ, chitosan ʰ	[11]
Pan bread	Lacticaseibacillus rhamnosus	High-amylose maize starch ^b , cassava starch ^b , chitosan ^b	[12]
Pan bread, hamburger bread	Lactobacillus acidophilus, Lacticaseibacillus casei	Inulin ª, high-amylose maize starch ʰ, chitosan ʰ	[20]
Bread bun	Lacticaseibacillus casei	Inulin [,] konjac glucomannan [,]	[15]
Bread	Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, Acetobacter aceti, Bifidobacterium bifidum, Bifidobacterium adolescentis, Bifidobacterium longum,	Whey, glycerol ^b ; high amylose maize starch ^b	[16]

Table 1. The potential use of synbiotic combinations in bread.

	Bifidobacterium animalis, Lactobacillus acidophilus,		
	Lactococcus lactis subsp. cremoris,		
	Propionibacterium freudenreichii, Enterococcus		
	faecium, Streptococcus salivarius subsp.		
	thermophilus		
Bread	Bacteroides ovatus, Bifidobacterium adolescentis	Arabinoxylan ^a	[21]
Bread	Lactobacillus acidophilus, Bifidobacterium animalis	Apple pomace ^a	[22]
Bread	Bifidobacterium animalis spp. lactis	Hydroxypropyl cellulose ^b	[23]
Steamed bread	Bifidobacterium longum	Gellan gum ^b	[24]
Bread	Bacillus coagulans	Inulin ^a	[5]

a: direct usage, b: coating.

2.3. The Influence of Potential Synbiotic Combinations in Breads on Human Health

There are limited clinical trials based on the potential synbiotic bread consumption on human health which is mainly focused on the effect on patients with type 2 diabetes mellitus (T2DM) regarding insulin metabolism and serum high-sensitivity C-reactive protein [25], blood lipids [26,27], apolipoproteins [26], nitric oxide, biomarkers of oxidative stress, and serum liver enzymes [28]. According to results of an randomized, doubleblind, controlled clinical trial, the consumption of synbiotic bread consisting of L. sporogenes and inulin through 3 times/day in a 40 g per serve for 8 weeks by T2DM patients (n = 27), led to a significant decrease in the levels of serum insulin, and thus beneficial for insulin metabolism [25]. Ghafouri et al. [26] revealed that a decrease in total cholesterol levels and Apo A1 was observed in the patients with T2DM (n = 25) who consumed synbiotic bread, which is composed of *B. coagulans*, β -glucan, and inulin, for 3 times in a day for 8 weeks. However, while triacylglycerol(s), and very low-density lipoprotein cholesterol were decreased, the high-density lipoprotein-cholesterol was increased, but the levels of total cholesterol and low-density lipoprotein-cholesterol were not significantly influenced (p > 0.05) with consumption of synbiotic bread including *Lactobacillus sporogenes* and inulin by diabetic patients (n = 26) [27]. The consumption of a total of 120 g per day of the same content of synbiotic bread for 8 weeks gave rise to a significant increase in nitric oxide, and a decrease in malondial dehyde was observed in patients with diabetes (n = 27). On the other side, no significant difference was defined in terms of blood pressure, liver enzymes, plasma glutathione, plasma total antioxidant capacity, and the levels of iron, calcium, and magnesium [28].

3. Conclusions

To sum up, the following studies should focus on the survival of more probiotic microorganisms, especially *Bacillus coagulans* and *Saccharomyces boulardii*, optimization of different encapsulation techniques, wall materials, film/coatings together with different types and concentrations of prebiotic sources used in other cereal-based food products, and also in gluten-free bread. Moreover, the viability of probiotics with prebiotics which are used directly, or as a wall material for encapsulation or edible film/coating should be assessed from a holistic perspective regarding the nutritional, technological, and sensorial properties of bread.

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