



# Proceeding Paper The Potential Use of Synbiotic Combinations in Cereal-Based Solid Food Products—A Review <sup>+</sup>

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Abstract: To date, the most commonly used probiotics in potential synbiotic combinations (SCs) in cereal-based solid food (CSF) products belong to the Lactobacillaceae family. On the other hand, *Bacillus coagulans* in pasta and *Saccharomyces boulardii* in cakes could be promising SCs in CSF. Inulin, followed by  $\beta$ -glucan, is the most commonly directly used prebiotic source of SCs in CSF. Although there are some promising results regarding the hypocholesterolemic effect of SCs in CSF, there is a need for more comprehensive in vivo and in vitro studies.

Keywords: cake; biscuit; pasta; co-encapsulation; wall material; hypolipidemic effect

# 1. Introduction

A synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" according to the consensus statement of the International Scientific Association for Probiotics and Prebiotics (ISAPP) [1]. Up to now, although there are many studies regarding potential synbiotic combinations in bread, there are limited studies in the literature based on baked goods (cake, biscuits/cookies/crackers) and other cereal-based solid foods (CSF) such as pasta/noodles, breakfast cereal, waffles, etc., as shown in Table 1. Until recently, the most commonly used probiotic bacteria in potential synbiotic combinations (SCs) in cereal-based solid food products have been *Lactobacillus acidophilus, Levilactobacillus brevis, Lacticaseibacillus casei, Limosilactobacillus fermentum, Lactiplantibacillus plantarum*, and *Lacticaseibacillus rhamnosus*, which belong to the Lactobacillaceae family (Table 1). Moreover, *Saccharomyces boulardii* and *Bacillus coagulans* are generally utilized in synbiotic combinations of cake and pasta formulations, respectively, as seen in Table 1. However, the potential of bacteria from the Bifidobacteriaceae family, except *Bifidobacterium bifidum*, has not been adequately evaluated.

The most utilized wall materials that have prebiotic potential in preparing coencapsulated probiotic bacterial strains to develop their stability and viability throughout the producing, storing, and handling processes in cereal-based solid food products are high-amylose maize starch (Hi-maize), chitosan, and some hydrocolloids such as pectin,  $\kappa$ -carrageenan, gum arabic, guar gum, xanthan gum, acacia gum, methylcellulose, and carboxymethylcellulose. Nevertheless, probiotic inclusions with prebiotic coating materials have not been sufficiently assessed in baked goods, as seen in Table 1. Therefore, this study aims to evaluate the potential synbiotic combinations in cereal-based solid food products, such as cake, biscuits, pasta/noodles, and breakfast cereal, summarized in Table 1, and their influence on health.



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# 2. The Effects of Potential Synbiotic Combinations on Some Cereal-Based Solid Food Products

2.1. Cake

A dramatic reduction ( $\approx$ 7 log CFU) was observed in unencapsulated *L. plantarum* after the cake-baking process. The encapsulated *L. plantarum* with pectin and maltodextrin, which are combined with calcium-alginate, led to a significant increase in probiotic protection capacity, and thus, viability rate after baking. Although there were no significant differences between the combination of pectin or maltodextrin with calcium-alginate regarding probiotic protection capacity after baking, the highest corresponding values were obtained when using an encapsulation wall material composed of calcium-alginate (2%) combined with both pectin (0.5%) and maltodextrin (0.5%), which potentially have higher thermal resistance. In simulated gastric fluids, no *L. plantarum* cells were detected in cakes with unencapsulated probiotics and those encapsulated with calcium-alginate individually. This could be attributed to wall materials having prebiotic potential, which assists in restricting the porous structure of the microbial composition, resulting in strengthening the gel network and thus restraining acid diffusion into the microbe, which could impact probiotic life. Probiotic viability was also developed by alginate-based microcapsules with pectin and maltodextrin in both simulated gastric and intestinal fluids [2].

No viable unencapsulated (*S. boulardii*, *L. acidophilus*, and *B. bifidum*) probiotics were detected irrespective of probiotic strain in cakes after the baking process. However, double-layered microcapsules, composed of gum arabic and  $\beta$ -glucan as the inner layer generated by spray-drying, and hydrogenated palm oil as an outer layer generated by spray chilling, enhanced the viability of *S. boulardii* and *L. acidophilus*, accounting for nearly 3 log CFU/g after baking. This was explained by the combination of hydrophilic and hydrophobic materials in the inner and outer layers, respectively. This was attributed to restricting conventional heat transfer by limiting the movement of water. However, no viable cells of single- or double-layered microencapsulated *B. bifidum* were defined after cake baking, which was interrelated with lower heat tolerance in comparison to other probiotics [3].

In another study, *S. boulardii* was inoculated into rice cake made from black glutinous rice, which has high antioxidant capacity and prebiotic potential, after starter addition to provide synbiotic characteristics. In the simulated gastrointestinal system, a higher survival rate accounting for nearly 97% of probiotic yeast was achieved in fermented rice cake inoculated with 10<sup>3</sup> *S. boulardii* when compared to a control cake consisting of only rice cake starter without probiotic yeast. This was attributed to the limiting or retardation of the influx of acidic fluids into the cells of probiotic yeast, thus preserving them throughout their gastrointestinal tract and also against bile attacks [4].

## 2.2. Biscuits

The initial viable probiotic counts were nearly 10 log CFU/g in cream biscuits including three encapsulated probiotic strains (mixture of *L. acidophilus, L. rhamnosus, B. bifidum*) with different wall materials (guar gum–inulin–dextrose mixtures or xanthan gum–maltodextrin–sucrose). Therefore, although the number of encapsulated probiotics was decreased by 2 log cycles after 8 weeks of storage, it still met the recommended probiotic level ( $10^6-10^8$  CFU/g). However, better probiotic viability was obtained in the cookies including encapsulated probiotics based on the mixture of guar gum–inulin than xanthan gum–maltodextrin. The scores of major sensorial properties such as taste and overall acceptability were higher in biscuits including probiotics encapsulated with a wall material mixture composed of guar gum–inulin than xanthan gum–maltodextrin. This was attributed to the aftertaste of xanthan gum. However, the encapsulated probiotic-supplemented biscuits remained acceptable for up to 8 weeks of storage, irrespective of the composition of the wall material mixtures [5].

In another study, sugar replacement in gluten-free cookies containing corn flour and buckwheat flour was conducted with inulin (a naturally soluble dietary fiber) and jocoque making up the cookie weight upon adding coated *L. brevis*. The *L* color values were reduced

because of the coating opacity when the biofilm formed. The firmness values were also decreased in the sugar-replaced cookies coated with a probiotic-forming film, and this was explained by the provision of moisture to the cookie structure by the coating related to moisture distribution [6].

#### 2.3. Pasta

The number of *Bacillus coagulans* in uncooked probiotic- and barley flour-supplemented pasta samples was nearly 7 log CFU/g, which is consistent with the qPCR data, but decreased with cooking depending on the cooking time, as expected. In this regard, consuming an average amount of 100 g of pasta in a meal could be accepted as sufficient to demonstrate beneficial effects on the gut microbiota. However, more satisfactory cooking quality of pasta that included barley flour and *B. coagulans* was obtained in 7 min than 5 min cooking time based on its higher weight and firmness values. From a nutritional point of view, there were no significant differences between the glycemic index values of the probiotic-enriched pasta including barley flour and the control pasta, and medium glycemic indexes were observed [7].

The viable L. plantarum cells encapsulated with fructooligosaccharides and denatured whey protein isolate accounted for 93.63% of encapsulated cell viability before cooking, and 62.42% of encapsulated cell viability was retained in raw noodles after cooking. This was attributed to the initial moisture content of raw noodles with encapsulated probiotics, which protects the cell membrane from osmotic shock and thermal damage throughout rehydration. Nevertheless, the cell viability was 80.29% and 64.74% in dried noodles at low and high temperatures, respectively. The decrease in probiotic viability, because drying was dedicated to heat stress throughout dehydration, resulted in damage to the cell wall and membrane. Moreover, the encapsulated cell viability was lost in dried noodles after cooking, which was attributed to thermal inactivation occurring throughout the rehydration of dried cells because of the leakage of fundamental cellular compounds. A shorter cooking time was needed for raw pasta including encapsulated L. plantarum when compared to the control pasta with no probiotic. This was explained by the decreasing gluten levels with the incorporation of probiotic microcapsules, which led to quicker starch gelatinization. The solid loss of both raw and dried pasta including encapsulated probiotics at low and high temperatures was higher than that of the control pasta. This was attributed to the discontinuous protein matrix, which occurred due to the distribution of probiotic microcapsules in the gluten network. No significant differences were determined in sensorial properties such as sweetness, firmness, and chewiness between raw noodles including probiotics with respect to the control [8].

## 2.4. Other Cereal-Based Solid Foods

*S. boulardii* viability was highest with acacia gum due to thermal protection and preventing oxidative damage, but the lowest with carboxymethylcellulose and methylcellulose compared to other coating agents, such as modified starch and maltodextrin, when coated breakfast cereals were exposed to pre-heated-milk at 70 °C and 80 °C. The low viability of the coated probiotics on breakfast cereals with cellulose-derivative hydrocolloids was attributed to their higher viscosity at low concentrations, resulting in the formation of a permeable surface after drying. An increase in the coating concentration of acacia gum as a coating material from 2.5% to 10% led to an increase in viability and thermal protection of *S. boulardii* when exposed to pre-heated milk at different temperatures (50, 60, 70, and 80 °C). This was explained by layer formation, which makes a barrier for heat penetration around *S. boulardii*. Coating with acacia gum showed approximately 12% times higher viability compared to coating *S. boulardii* without acacia gum in simulated intestinal fluid. Therefore, it was stated that the coating not only preserved *S. boulardii* but also enhanced its viability [9].

A probiotic culture-inoculated yoghurt at four different concentrations (0.5, 1.5, 3, and 4.5%) enriched with inulin and lactulose (disaccharide derivative of lactose) as a prebiotic

source at 3% each was used in tarhana production. The number of probiotics was increased with concentrations of probiotic culture, and the highest values were acquired after 2 days of fermentation irrespective of the probiotic strain. On the 2nd day of fermentation, the highest microbial count generally belonged to *L. acidophilus*, followed by *S. thermophilus* and *B. bifidum*, respectively, in tarhana dough. Although the microbial counts of each probiotic strain were increased with the rise in probiotic concentration in dried tarhana, the drying process negatively influenced the microbial counts compared to tarhana dough. However, the sensorial attributes such as flavor and texture were still above 4.5 on a 5-point hedonic scale [10].

	Product	Probiotic Source(s)	Prebiotic or Potential Prebiotic Source(s)	References
	Cupcake	Lactiplantibacillus plantarum	Pectin <sup>b</sup> , maltodextrin <sup>b</sup>	[2]
	Cupcake	Lactiplantibacillus plantarum	к-carrageenan <sup>b</sup>	[11]
	Cream-filled cake	Lacticaseibacillus casei Saccharomyces boulardii,	High-amylose resistant starch <sup>b</sup>	[12]
CAKE	Cake	Lactobacillus acidophilus, Bifidobacterium bifidum	Gum arabic <sup>b</sup> , $\beta$ -cyclodextrin <sup>b</sup>	[3]
	Fermented rice cake (Khao-Maak)	Saccharomyces boulardii	Germinated black glutinous rice <sup>a</sup>	[4]
	Muffin	Lactiplantibacillus plantarum	Stevia rebaudiana <sup>a</sup>	[13]
	Gluten-free cake mix	Bacillus coagulans	Inulin <sup>a</sup> , resistant starch <sup>a, x, z</sup> , maltodextrin <sup>a, x</sup>	[14]
BISCUIT/ COOKIE/ CRACKER	Cracker	Lacticaseibacillus casei	Inulin <sup>b</sup> , whey <sup>b</sup>	[15]
	Biscuit cream	Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Bifidobacterium bifidum	Inulin <sup>b</sup> , guar gum <sup>b</sup> , xanthan gum <sup>b</sup> , maltodextrin <sup>b</sup>	[5]
ЩСО	Gluten-free cookie	Levilactobacillus brevis	Inulin <sup>a, x</sup>	[6]
	Gluten-free biscuit	Lactobacillus acidophilus	Inulin <sup>b</sup> , fructooligosaccharide <sup>b</sup>	[16]
ΕE	Pasta	Bacillus coagulans	Barley flour <sup>a</sup>	[7]
PASTA/NOODLE	Pasta	Lactiplantibacillus plantarum, Lactobacillus acidophilus, Limosilactobacillus fermentum	β-glucan <sup>a</sup>	[17]
AS'	Noodles	Lactiplantibacillus plantarum	Fructooligosaccharide <sup>b</sup>	[8]
$\mathbf{P}_{t}$	Whole-grain pasta	Bacillus coagulans	β-glucan <sup>a</sup>	[18]
S	Breakfast cereal	Saccharomyces boulardii	Acacia gum <sup>b</sup> , methylcellulose <sup>b</sup> , carboxymethylcellulose <sup>b</sup> , modified starch <sup>b</sup> , maltodextrin <sup>b</sup>	[9]
OTHERS	Waffle filling	Lactobacillus acidophilu, Bifidobacterium bifidum	Inulin <sup>a, x</sup> , pectin <sup>b</sup> , lactulose <sup>a, y</sup>	[19]
Ō	Traditional fermented food (tarhana) <sup>t</sup>	Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacterium bifidum	Inulin <sup>a</sup> , lactose <sup>a</sup>	[10]

Table 1. The potential use of synbiotic combinations in some cereal-based solid food products.

<sup>a</sup>: direct usage; <sup>b</sup>: coating; <sup>x</sup>: used as a fat replacer; <sup>y</sup>: used as a sugar replacer; <sup>t</sup>: prebiotics were used in yoghurt for tarhana production; <sup>z</sup>: type of resistant starch is not defined.

# 3. The Effects of Potential Synbiotic Combinations in Some Cereal-Based Solid Food Products on Health

The feeding of experimental rats with synbiotic biscuits (5 g or 10 g in 10 mL aquadest) including *L. acidophilus*, inulin, and fructooligosaccharide led to a significant decrease in total blood cholesterol levels. Moreover, an increase in HDL levels was observed, and this was explained by the fermentation ability of probiotics, which caused a decrease in pH values, and thus, an increase in H+ ions in the intestine, which led to an increase in water links with lipids through lipoprotein. In contrast, a decrease in LDL levels was recorded and attributed to a decrease in triglyceride synthesis due to the inhibition effect of inulin

on lipogenic enzymes in the liver, and also the fermentation of inulin by probiotics which induce the generation of short-chain fatty acids such as propionic acid [16]. According to the results of a single-blind, parallel, randomized placebo study, upon the consumption of one serving/day of potential synbiotic whole-grain pasta composed of *B. coagulans* and  $\beta$ -glucans for 12 weeks by healthy overweight or obese volunteers (n = 41), their plasma LDL/HDL cholesterol ratios were decreased [18]. In another study, the consumption of 200g/day of dried tarhana, which is prepared from yoghurt containing inulin (3%) and lactulose (3%) fermented by 4.5% probiotic culture, for 45 days led to a significant decrease in total plasma cholesterol and triglycerides in hyperlipidemic volunteers (n = 15). Therefore, it was declared that the potentially synbiotic tarhana has a significant hypocholesterolemic effect regarding its influence on the plasma lipid profiles of human subjects. This was mainly attributed to its behavior as a soluble fiber and resistance against hydrolyzation by the human digestive system, and thus, its hypolipidemic effect. Moreover, the lowering cholesterol effect was also attributed to  $\beta$ -glucan from wheat flour, and other tarhana ingredients such as onion, green pepper, and tomato, due to its lycopene content [10].

#### 4. Conclusions

The Lactobacillaceae family is the most commonly employed probiotic in potential synbiotic combinations in cereal-based solid foods (cake, biscuits/cookies, pasta/noodles, etc.). On the other hand, promising probiotics such as *Saccharomyces boulardii* and *Bacillus coagulans* were evaluated in cakes and pasta, respectively, with prebiotics/potential prebiotics which could have synbiotic potential. In this regard, the major directly used prebiotic sources were inulin and  $\beta$ -glucan regarding their potential synbiotic combinations in cereal-based solid foods. Consequently, future in vivo and in vitro studies should be centered around the survivability of more probiotic microorganisms, especially the Bifidobacteriaceae family, for which studies are lacking, and the optimization of the encapsulation process, with different prebiotic sources at different levels utilized, particularly gluten-free cereal-based solid food products. Moreover, not only should the viability of probiotics with prebiotics be evaluated, but so should the nutritional, technological, and sensorial properties of cereal-based solid food products regarding their synbiotic potential. The potential synbiotic combinations in cereal-based liquid food products such as juices/beverages should be addressed in other studies. From a human health perspective, there is a requirement for more comprehensive in vivo and in vitro studies regarding the hypocholesterolemic effect of potential symbiotic combinations in cereal-based solid foods.

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