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Application of Bifidobacterium spp in beverages and dairy food products: an overview of survival during refrigerated storage

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Abstract

The food industry has established a number of food and beverage products containing probiotic strains. A probiotic strain should maintain stability in the food product during processing and also during subsequent storage. Bifidobacteria are probiotic commonly found throughout the colon of both humans, animals and are considered normal residents of the gastrointestinal tract. These bacteria have important health functions. Thus, the incorporation of bifidobacteria has been shown to enhance the therapeutic value of food and beverage products. It is highly desirable that the viable counts of Bifidobacterium spp in the final product to be at least 10^6-10^7 cfu/ml to offering health benefits to the consumers. Therefore, the objective of this study is to review the applications of Bifidobacterium spp in beverages and dairy food products and the effect of different food matrices on their survival during the storage.

Keywords: beverages; yogurt; cheese; ice cream; Bifidobacterium spp; survival.

Practical Application: Bifidobacterium spp. carrier beverages and dairy foods are representing potential advantages and appreciated alternatives for the food industry.

1 Introduction

Probiotic is a preparation of viable microorganisms that are added to the diet of humans to control the growth of undesirable or less desirable microorganisms in the gastrointestinal tract that rich in the flora of more than 500 different bacterial species some of which have important health functions (Shori, 2016; Zendeboodi et al., 2020). A probiotic strain must maintain viability and functionality during manufacturing food products and subsequent storage without loss of the sensory properties of these foods (Shori et al., 2016; Muniandy et al., 2017; Champagne et al., 2018).

Factors such as diet, antibiotics, and stress can influence normal bifidobacteria concentrations in the human digestive system. Thus, incorporation of bifidobacteria has been shown to enhance the therapeutic value of foods and the survival of bifidobacteria in foods is varying depending on the food matrices (Adhikari *et al.*, 2000; Shori, 2015a). Several factors may affect the viability of Bifidobacterium spp. in food and beverage products including the time, pH and temperature of incubation and storage, ratio and strains in the inoculum, availability of nutrients, presence of undesirable microflora and their enzymes, the presence of hydrogen peroxide and dissolved oxygen, the concentration of metabolites (Donkor et al., 2006; Pereira et al., 2011; Shori, 2013a; Baba et al., 2014). Throughout the shelf life, it is highly desirable that the viable counts of Bifidobacterium spp in the final product to be at least 10^6-10^7 cfu/ml to offering health benefits to the consumers (Shori, 2013b). Therefore, the objective of this study is to review the applications of Bifidobacterium spp in food and beverage products and the effect of different food matrices on their survival during the storage.

2 Probiotic bacteria

Probiotics can be defined as "[...] live microbial, dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and or metabolic activity of the flora of the gastrointestinal tract [...]" (Fuller, 1989, p.366). Probiotics are present in fermented food products and known as lactic acid bacteria (LAB). These bacteria consist of several genera including *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* which have different beneficial effects on the host and has been used for decades in the food and natural sources to promote good health of human (Shori & Baba, 2013; Shori et al., 2020a, b).

3 Characteristics of Bifidobacterium spp as probiotics

Bifidobacteria are probiotic commonly found throughout the colon of both humans and animals which are considered normal residents of the gastrointestinal tract (Nielsen et al., 2003). They are once classified under the genus lactobacillus but they are found phylogenetically distinct from LAB and classified under phylum Actinobacteria. Out of 25 species, only ten are commercially used in conjunction with *L. acidophilus* as probiotics. For example, *B. bifidum, B. infants,* and *B. lactis*. Bifidobacteria usually appear in pairs with a V or Y-like shaped. These microbes are anaerobic, non-pathogenic, gram-positive, non-spore forming,

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pleomorphic rod, and catalase-negative (Nielsen et al., 2003; Zacarías et al., 2020). Unlike LAB, bifidobacteria have high G+C content (55% to 67 mol%) and plasmids are rarely found in the cytoplasm, except for *B. longum*. They grow optimally within temperature 37 °C to 41 °C and pH optima for growth ranges from pH 6.5 to pH7.0 (Sonomoto & Yokota, 2011). Bifidobacteria are nutritionally fastidious. *Bifidobacterium* spp. of human origin can utilize fructose, galactose, and lactose. They are the main saccharolytic bacteria found in the human colon, and able to utilize non-digestible oligosaccharides in the colon to produce acetic acid as well as lactic acid in the ratio of 3:2 through a unique fructose-6-phosphate phosphoketolase pathway (Toscano et al., 2015). Different species and/or strains of bifidobacteria may exhibit different beneficial health effects, such as regulation of intestinal microbial homeostasis, repression of procarcinogenic enzymatic activities within the microbiota, production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Toscano et al., 2015; Chugh & Kamal-Eldin, 2020). In addition, bifidobacteria enhance several immune functions i.e. the activation of macrophages and lymphocytes, antibody production, mitogenic response in the spleen, and Peyer's patches and function of natural killer cells (Marin et al., 1997).

4 Survival of Bifidobacterium spp in beverages

Beverages containing probiotics are a new and promising approach for therapeutic products (Shori, 2012). The success of probiotic beverages is often limited by the nature of the ingredients, the contamination, and the low viability of strains during storage (Shori et al., 2019; Jaimez-Ordaz et al., 2019; Bruno et al., 2020). One of the most commercial probiotics available in the beverages market is a species of Bifidobacterium. Several researchers have been studied the viability of Bifidobacterium spp. in dairy and non-dairy beverages (Table 1). For example, fermented whey-based goat milk beverage was prepared using *S. thermophilus* TA-40, *B. animalis* BB-12 and *L. rhamnosus* Lr-32 (Buriti et al., 2014). The viability of *B. animalis* BB-12 in whey-based goat milk beverages in the presence of guava or soursop pulps reduced (1 log cycle; $p < 0.05$) during 21 days of storage (Table 1). Allgeyer et al. (2010) found that the soluble corn fiber or inulin (5 g) did not affect the viability of *B. lactis* Bb-12 in yogurt-like drink during one month of storage. An earlier study showed that the inclusion of inulin (3 g/100 mL) and/or okara flour (5 g/100 mL) with ABT-4 culture (*L. acidophilus* La-5, *B. animalis* Bb-12, and *S. thermophilus*) in soymilk during fermentation have no influence on the viability of *B. animalis* during 28 days of storage (Bedani et al., 2013; Table 1). However, *B. animalis* B94 showed 1 log cycle increased in fermented soymilk after 28 days of storage (Donkor et al., 2007; Table 1).

Kun et al. (2008) found that Bifidobacterium strains (*B. lactis* Bb-12, *B. bifidum* B7.1 and B3.2) showed significant ($p < 0.05$) growth in pure carrot juice without nutrient supplementation during fermentation (Table 1). However, another study reported that *B. lactis* 420 and *B. lactis* Bb-12 have declined rapidly in carrot juice (without nutrient supplementation) after 7 days of refrigerated storage and they were untraceable after 2 months (Tamminen et al., 2013). In non-fermented probiotic milk/carrot juice drink with *L. acidophilus* LA5, *L. plantarum* and *L. rhamnosus* GG, *B. lactis* Bb-12, showed about 1 log cfu/ml reduction during 3 weeks of storage (Daneshi et al., 2013).

B. lactis Bb-12 has demonstrated good survival in orange juices during 6 weeks (6.9 log cfu/mL) whereas showed significantly ($p < 0.05$) decreased in pineapple juices to 3.0 log cfu/ml (Sheehan et al., 2007). Furthermore, the study found that the commercial probiotic (*B. lactis* Bb-12) cannot survive in cranberry juice (Sheehan et al., 2007). *B. animalis* B94 maintained a level of counts > 6.5 log cfu/ml after 72 hours of incubation in green tea extract (López de Lacey et al., 2014). Beverages could have positive health properties in the gut once the populations of Bifidobacterium spp. were beyond the minimum recommended level 7 log cfu/g for health benefits (Salva et al., 2011; Shori, 2015b).

5 Survival of Bifidobacterium spp. in yogurt

Yogurt is a semi-solid fermented milk product (Mahmood et al., 2019; Shori, 2020). It defined as a coagulated milk product that results from lactic acid bacteria i.e. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Shori & Baba, 2012; Coskun & Karabulut Dirican, 2019). Probiotics such as *Bifidobacterium* spp. are added to enhance the fermentation process for production bio-yogurt and can survive under the acidic conditions in the fermented milk (Donkor et al., 2006; Altuntas & Korukluoglu, 2019). However, these bacteria showed slow growth in milk and low survival rate during storage (El-Dieb et al., 2012). Therefore, active components such as plant extracts, milk proteins, inulin, and lactulose have been added to increase the growth and viability of *Bifidobacterium* spp. in yogurt (Table 2). Kailasapathy et al. (2008) reported that the amount of 5 or 10 g/100 g added fruit mixes (mango, mixed berry, passion fruit, and strawberry) in yogurt showed no effect on *B. animalis* ssp. *lactis* LAFTIs B94 growth. Similarly, inclusion of flavouring agents such as strawberry (0.08 & 0.16; w/w), vanilla (0.07 & 0.14; w/w), peach (0.08 & 0.16; w/w) and banana (0.1 & 0.2; w/w) essences did not affect the viability of bifidobacteria in yogurt (Vinderola et al., 2002). The presence of pectins and fructooligosaccharides in either apple or banana fibers showed an increase in the numbers of *B. animalis* subsp. *lactis* BL04 by 1 log cfu/ml in skim milk yogurt compared to the absence (Espírito Santo et al., 2012). A reduction in the viability of *B. animalis* Bb-12 was shown in stirred fruit yogurt made from goat's milk during one month of storage but it still in the recommended level of 7 log cfu/g for health benefits (Ranadheera et al., 2012). Another study reported that supplementation of whey protein concentrate (WPC; 3%-5%) in fermented goat milk sustained a high population of *B. lactis* Bb-12 even after 21 days of refrigerated storage (Martín-Diana et al., 2003; Table 2). The high growth of *B. lactis* from 7.41 log cfu/ml to 10.3 log cfu/ml was significantly associated with the presence of lactulose in milk during fermentation (Oliveira et al., 2011a). Likewise, Oliveira et al. (2011b) found that the growth of *B. lactis* in fermented skim milk dramatically stimulated ($p < 0.05$) by 40 mg/g inulin during 7 days of storage at 4 °C (Table 2). Counts of 7.52 cfu/ml were recorded for *B. longum* in fermented milk with *S. thermophilus* TA040, *L. delbrueckii ssp. bulgaricus* LB340 and *L. acidophilus*

Table 1. Survival of *Bifidobacterium* spp. in beverages during refrigerated storage.

Bifidobacterium strains	Type of food	Additive	Co-culture	Initial population	Final population	Storage period	Storage temperature	Reference
B. animalis Bb-12	fruit juice	Orange juice	*Lactobacillus salivarius ssp. salivarius* UCC118, *L. salivarius ssp. salivarius* UCC500, and *L. paracasei ssp. paracasei* NFBC43338, *L. rhamnosus* GG and *L. casei* DN-114 001	7.6 log cfu/mL	6.9 log cfu/mL	6 weeks	4 °C	(Sheehan et al., 2007)
		pineapple juice		7.6 log cfu/mL	3.0 log cfu/mL			
B. animalis B94	fermented soymilk	-	*L. acidophilus* L10 and *L. casei* L26	7 log cfu/g	8 log cfu/g	28 days	4 °C	(Donkor et al., 2007)
B. lactis Bb-12	carrot juice	-	-	7.21 log cfu/ml	7.88 log cfu/mL	1 day	37 °C	Kun et al. (2008)
B. bifidum B78.1				7.14 log cfu/ml	8.82 log cfu/ml			
B. bifidum B38.2				7.64 log cfu/ml	8.71 log cfu/mL			
B. animalis Bb-12	non-fermented milk drink	carrot juice	*L. acidophilus* LA5, *L. plantarum* and *L. rhamnosus* GG	6.29 log cfu/mL	5.78 log cfu/mL	20 days	4 °C	(Daneshi et al., 2013)
B. lactis 420	carrot juice	-	*L. plantarum* Lp-115, *L. paracasei* Lpc-37, *Lactobacillus rhamnosus* GG	7 log cfu/mL	2 log cfu/ml	4 weeks	4 °C	Tamminen et al. (2013)
B. lactis Bb-12				> 6 log cfu/mL	~2 log cfu/mL			
B. animalis Bb-12	fermented soy product (FSP)	Plain with inulin, 3 g/100 ml of soymilk with okara, 5 g/100 ml with inulin˥ okara, ratio 3:5 g/100 mL	*L. acidophilus* La-5 and *S. thermophilus*	8.88 log cfu/g	8.84 log cfu/g	28 days	4 °C	(Bedani et al., 2013).
				8.86 log cfu/g	8.84 log cfu/g			
				8.97 log cfu/g	8.81 log cfu/g			
				8.95 log cfu/g	8.81 log cfu/g			
B. animalis Bb-12	Fermented whey-based goat milk beverage	guava or soursop pulps	*S. thermophilus* TA-40 and *L. rhamnosus* Lr-32	8.05 log cfu/mL	7 log cfu/mL	21 days	4 °C	(Buriti et al., 2014)
B. animalis LAFTI-B94	green tea extract	-	*L. paracasei* LAFTI-L26 and *L. acidophilus* LAFTI-L10	6.0 log cfu/mL	> 6.5 log cfu/mL	72 hours	37 °C	(López de Lacey et al, 2014)
B. animalis Bb-12	Blend beverage	20% Frozen juçara pulp; 40% frozen strawberry pulp; 40% banana pulp	-	7.0 log cfu/mL	3.8 log cfu/mL	90 days	4 °C	(Oliveira Ribeiro et al., 2020)

Table 2. Survival of *Bifidobacterium* spp. in yogurt during refrigerated storage.

Bifidobacterium strains	Type of food	Additive	Co-culture	Initial population	Final population	Storage period	Storage temperature	reference
B. lactis Bb-12	Yogurt	3%WPC	*S. thermophilus, L. delbrueckii subsp. bulgaricus, L. acidophilus*	7.22 log cfu/g	7.13 log cfu/g	21 days	4 °C	(Martín-Diana et al., 2003)
		5%WPC		7.64 log cfu/g	7.28 log cfu/g			
B. animalis ssp. *lactis* LAFTIs B94	Yogurt	-	*L. acidophilus* LAFTIs L10, *S. thermophilus* DD 224	8.19 log cfu/g	7.74 log cfu/g	35 days	4 °C	Kailasapathy et al., (2008)
B. animalis subsp. lactis BL 04	Fermented milk	lactulose	Yogurt culture, *L. acidophilus* LAC4 and *L. rhamnosus* LBA	7.41 log cfu/mL	10.3 log cfu/mL	during fermentation	4 °C	Oliveira et al. (2011a)
B. animalis subsp. lactis BL 04	Fermented skim milk	Inulin (40 mg/g)	*L. rhamnosus* and *L. bulgaricus*	7.5 log cfu/mL	9.1 log cfu/mL	7 days	4 °C	Oliveira et al. (2011b)
B. animalis Bb-12	Stirred yogurt	fruit	*Propionibacterium jensenii* 702 and L. *acidophilus* La-5	~10^8 log cfu/ g	~10^7 cfu/g	4 weeks	4 °C	Ranadheera et al. (2012)
Bifidobacterium longum BL05,	Yogurt	-	*S. thermophilus TA040, L. delbrueckii ssp. bulgaricus LB340 and L. acidophilus La14*	7.52 log cfu/mL	6.41 log cfu/mL	30 days	3-5 °C	(Cruz et al., 2012)
B. animalis Bb-12	Yogurt	Green tea infusion (5%, 10% or 15%)	*S. thermophilus, L. acidophilus* (LA-5),	> 7 log cfu/g	> 7 log cfu/g	2 weeks	4 °C	Najgebauer-Lejko, (2014)
B. bifidum	Yogurt	*Allium sativum*	Yogurt culture, *Lactobacillus acidophilus LA-5and Lactobacillus casei LC-01,*	9.91 log cfu/ml	8.68 log cfu/ml	21 days	4 °C	(Shori and Baba, 2015)
		Cinnamomum verum		9.82 log cfu/ml	9.0 log cfu/mL			
B. lactis HN019	Simbiotic yogurt	*7% tupinambor flour and 10% sugar*	*S. thermophiles* and *L. delbrueckii subsp. bulgaricus,*	9.50 log cfu/g	8.23og cfu/g	30 days	5 °C	(Ribeiro et al., 2019)
Encapsulated B. animalis subsp. lactis BB-12	Goat milk yoghurt	Inulin 2% (w/v)	*S. thermophilus, L. delbrueckii subsp. bulgaricus*	9.5 log cfu/ml	8.8 log cfu/mL	28 days	4 °C	(Pradeep Prasanna & Charalampopoulos, 2019)
B. bifidum Bb-12	Synbiotic yogurt	*0.05% Auricularia auricula* aqueous extract (AAE)	*S. thermophilus, L. delbrueckii subsp. bulgaricus*	8.23 log cfu/g	7.94 log cfu/g	28 days	4 °C	(Faraki et al., 2020)
		0.1% AAE		8.1 log cfu/g	7.94 log cfu/g			

La14 after one day of storage (Cruz et al., 2012). This viable cell count of *B. longum* has decreased about 1 log cfu/ml after 30 days of storage (Table 2). Recently found that green tea infusion (5%, 10%, or 15%) has no influence on the viability of *B. animalis* ssp. *lactis* BB-12 in yogurt during 3 weeks of storage (Najgebauer-Lejko, 2014). However, green tea sustained the viability of bifidobacteria in yogurt above 7 log cfu/g for more than 2 weeks compared to plain yogurt. The presence of *Allium sativum* and *Cinnamomum verum* extracts in cow and camel milk yogurts provided higher viable cells of *B. bifidum* and continued to survive for 21 days of refrigerated storage (Shori & Baba, 2015; Table 2).

6 Survival of Bifidobacterium spp. in cheese

Cheese is one of the best carriers for probiotics (Pivetta et al., 2020). The development of probiotic cheeses can be very strain-dependent as many of the probiotic strains showed insufficient performance in the cheese environment (Shori et al., 2018; Silva et al., 2018; Prezzi et al., 2020). Several studies have reported the applications of *Bifidobacterium* spp. in different types of cheeses (Table 3). Tharmaraj & Shah, (2004) studied the changes in the population of *B. animalis* in cheese-based dips with different bacterial combinations over 10 weeks of storage. The authors found the best combination of probiotic bacteria can be used when combined *B. animalis* with *L. acidophilus* and *L. paracasei* subsp. *paracasei,* together (inoculation at 9 cfu log/g). *B. animalis* showed a high level of the population required for health benefits through 10 weeks of storage. Cheddar cheeses inoculated with *B. longum* 1941 or *B. animalis* subsp. *lactis* B94 used as an adjunct with starter lactococci showed high viability of *Bifidobacterium* spp. cells during 24 weeks ripening period (Ong & Shah, 2009; Table 3). Despite, *B. lactis* Bb-12 can survive in a high level of cheddar cheese (≥10^8 cfu/g) during six months of ripening, the increase was associated with a high level of moisture content (40%) which considered slightly above the legal limit permitted for Cheddar cheese (Mc Brearty et al., 2001). *B. longum* 15708 showed slow growth in salted cheddar cheese with about 3 log cfu/g loss of their viable cells after 3 days of storage (Fritzen-Freire et al., 2010a). A previous study demonstrated high survival of *B. lactis* Bb-12 in cheddar cheese followed by Bifidobacterium sp. DR10 and *B. lactis* B94 respectively with viable cell counts above 7 log cfu/g (Phillips et al., 2006; Table 3). *B. lactis* registered high cell counts (7.5 log cfu/g) in semi-hard cheese incubated with *L. acidophilus* and *L. paracasei* for 60 days (Bergamini et al., 2009). However, inulin or oligofructose (10%) had no significant effect on the survival of *B. animalis* subsp. *lactis* in petit-suisse cheese during 30 days of storage (Cardarelli et al., 2008; Table 3). Likewise, the presence of fructooligosaccharides or a mixture of inulin and fructooligosaccharides (50:50) in the synbiotic cheeses has not affected the viability of *B. lactis* B94 during 60 days of ripening period (Rodrigues et al., 2012).

The viability of *B. animalis* Bb-12 in Minas Frescal cheese did not effect by the presence of lactic acid (Fritzen-Freire et al., 2010b). This may explain the high viability of Bifidobacterium in Minas cheeses. The viable cell counts of *B. animalis* Bb-12 added to Minas fresh cheese with *L. acidophilus* La-5 and *S. thermophilus* increased significantly ($p < 0.05$) during 21 days of storage (Buriti et al., 2007; Table 3). *B. animalis* subsp. *lacti*s showed the highest counts (~8.43 log cfu/ml) when combined with *S. thermophilus* in Minas frescal cheese whey as compared with other probiotics such as *L. acidophilus* and *L. rhamnosus* (Almeida et al., 2008).

7 Survival of Bifidobacterium spp in ice cream

Ice cream is a frozen dairy product made from a combination of served ingredients other than milk (Shori et al., 2018). The effectiveness of probiotics ice cream consumption on consumer's health is related to bacterial viability. The incorporation of *Bifidobacterium* spp. has been studied in ice cream (Table 4). It is very important to maintain the stability of *Bifidobacterium* spp. in ice cream during storage. Silva et al. (2015) found that *B. animalis* had the ability to maintain satisfactory viability in goat's milk ice cream during 120 days of frozen storage (Table 4). Akın et al. (2007) reported that the addition of 2% of inulin in ice cream containing 10% w/w of fermented milk increased the viability of *B. lactis* from 10^5 cfu/g to 10^6 cfu/g compared to the absence. Akalin & Erisir (2008) found that the presence of 4% inulin or oligofructose did not influence the viability of *B. animalis* BB-12 in low-fat ice cream stored at -18 °C for 90 days. Another study reported that non-fermented soymilk ice cream improved the viability of *B. lactis* during 30 days of storage at -20 °C (Aboulfazli et al., 2016; Table 4). Bifidobacterium species (*B. lactis* BBDB2 and *B. lactis* Bb-12) showed initial populations of 10^7-10^8 cfu/g in frozen soy dessert (Heenan et al., 2004). Besides, both probiotics species maintained sufficient viability of 10^7 cfu/g after 28 weeks of storage at -20 °C. Fermented acerola (Malpighia emarginata) ice cream has demonstrated a good carrier of Bifidobacterium where *B. lactis* and *B. longum* showed viability of 8.65 log cfu/g and 7.51 log cfu/g respectively over 15 days of storage (Favaro-Trindade et al., 2006).

The viable cell counts of *B. animalis* (Bb-12) in synbiotic ice cream showed a decrease by 2.9 log after 6 months of storage (Homayouni et al., 2008; Table 4). However, microencapsulated of *B. animalis* showed a reduction by only 0.7 log and maintained *B. animalis* viability between 10^8 and 10^9 cfu/g overall shelf. This indicated that encapsulation can significantly maintain the high viability of probiotic bacteria in ice cream over storage (Shori, 2017). A previous study revealed that encapsulated *B. bifidum* 231 in ice cream increased significantly ($p < 0.05$) the viability of probiotic (8.06 log cfu/g) compared to non-encapsulated bacteria (6.33 log cfu/g) after 90 days of storage (Sahitya et al., 2013). In addition, *B. bifidum* 231 has been improved after co-encapsulated with 3% Fructooligosaccharides during storage at -20 °C (Sahitya et al., 2013). Similar behavior has been shown by *B. Lactis* Bb-12 with 30% rise in their viability in ice cream microencapsulated with calcium alginate and whey protein after 6 months of storage (Karthikeyan et al., 2013).

8 Conclusion

The utilization of beverages and dairy foods as *Bifidobacterium* spp. carrier is representing potential advantages and appreciated alternatives for the food industry. In order to improve the survival rates of *Bifidobacterium* spp. in beverages and dairy foods during refrigerated storage, ingredients with active components such as plant phytochemicals, milk proteins, inulin, and lactulose could be a promising approach for controlled *Bifidobacterium* spp. viability. Furthermore, microencapsulation is good processing to maintain the high viability of *Bifidobacterium* spp. Encapsulation materials are known as safe ingredients and can be used in food applications. Therefore, there is a big interest in the improvement of the physical properties and mechanical stability of the polymers used in probiotics encapsulation, to ensure a high population of probiotics not only in food during storage but also after gastrointestinal digestion.

Table 3. Survival of *Bifidobacterium* spp. in cheeses during refrigerated storage.

Bifidobacterium strains	Type of food	Additive	Co-culture	Initial population	Final population	Storage period	Storage temperature	Reference
B. lactis Bb-12	cheddar cheese	-	-	8.9 log cfu/g	≥10^8 cfu/g	six months of ripening	8 °C	Mc Brearty et al. (2001)
B. longum BB536				6.8 log cfu/g	10^5 cfu/g			
B. longum 1941	cheddar cheese	-	*Lactococcus lactis* subsp. *Lactis, L. lactis* subsp. *cremoris, Lb. acidophilus* 4962 and *Lb. casei* 279	8.9 log cfu/g	8 log cfu/g	24 weeks	4 °C	(Ong et al., 2006)
B. lactis B94	cheddar cheese	-	*L. acidophilus* (L10 and La5), *L. paracasei* L26, *L. casei* Lc1 and *L. rhamnosus* DR20	8.0 log cfu/g	7.4 log cfu/g	32 weeks	9-10 °C	Phillips et al. (2006)
B. lactis Bb12				8.0 log cfu/g	8.14 log cfu/g			
Bifidobacterium sp. DR10				8.9 log cfu/g	8.5 log cfu/g			
B. animalis Bb-12	Minas fresh cheese	-	*L. acidophilus* La-5, *Streptococcus thermophilus*	6.13 log cfu/g	6.56 log cfu/g	21 days	5-7°C	(Buriti et al., 2007)
B. animalis subsp. *lactis*	Minas frescal cheese whey	-	*L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. rhamnosus* and *S. thermophilus*	10^8 log cfu/mL	~ 8.43 log cfu/mL	1 day	4 °C	(Almeida et al., 2008)
B. animalis subsp. *lactis*	petit-suisse cheese	10% Oligofructose	*S. Thermophilus* and *L. acidophilus*	7.60 log cfu/g	7.34 log cfu/g	28 days	4 °C	(Cardarelli et al., 2008)
		10% Inulin		7.69 log cfu/g	7.35 log cfu/g			
		10% Honey		7.44 log cfu/g	7.21 log cfu/g			
B. longum 1941	cheddar cheese	-	*Lactococcus lactis* subsp. lactis and *L. lactis* subsp. *cremoris*	9.72 log cfu/g	7.15 log cfu/g	24 weeks	4 °C	Ong & Shah (2009)
B. animalis B94				9.57 log cfu/g	7.53 log cfu/g			
B. lactis	semi-hard cheese	-	*L. acidophilus*, *L. paracasei*	7 log cfu/g	7.5 log cfu/g	60 days	12 °C	(Bergamini et al., 2009)
Bifidobacterium longum 15708	salted cheddar cheese	-	*L. helveticus* R0052, *B. longum* R0175, *B. lactis* BB-12 and *B. infantis* 15697	6.8 log cfu/g	3.6 log cfu/g	3 days	4 °C	(Fortin et al., 2011)
B. lactis B94	Synbiotic cheese	Fructooligosaccharides Inulin and fructooligosaccharides (50:50)	*L. casei* 01	8.0 log cfu/g	9.9 log cfu/g	60 days	12 °C	(Rodrigues et al., 2012)
B. bifidum ATTC-29521	cheddar cheese	-	-	9.13 log cfu/g	8.1 log cfu/g	35 days	4 °C	(Afzaal et al., 2020)

Table 4. Survival of *Bifidobacterium* spp. in ice creams during refrigerated storage.

Bifidobacterium strains	Type of food	Additive	Co-culture	Initial population	Final population	Storage period	Storage temperature	Reference
B. lactis BBDB2 *B. lactis* Bb-12	non-fermented frozen soy dessert	-	*L. acidophilus* MJLA1, *L. rhamnosus* 100-C, *L. paracasei* ssp. *paracasei* 01 and *Saccharomyces boulardii* 74012	10^7–10^8 cfu/ g	10^7 cfu/g	28 weeks	-20 °C	Heenan et al. (2004)
B. longum *B.lactis*	Fermented acerola ice cream	acerola pulp	*S. thermophilus* and *L. bulgaricus*	8.41 log cfu/g 8.04 log cfu/g	7.51 log cfu/g 8.65 log cfu/g	15 days	-18 °C	(Favaro-Trindade et al., 2006)
B. lactis BL-01	Ice cream	2% of inulin	*S. thermophilus*, *L. bulgaricus* and *L. acidophilus* LA-14	> 8 log cfu/g	> 6 log cfu/g	90 days	−18 °C	(Akın et al., 2007)
B. animalis BB-12	Ice cream	Plain 4% Oligofructose 4% Inulin	*L. acidophilus* La-5	6.27 log cfu/g 6.60 log cfu/g 5.96 log cfu/g	5.94 log cfu/g 6.25 log cfu/g 5.47 log cfu/g	90 days	−18 °C	Akalin & Erisir, (2008)
B. animalis BB-12	synbiotic ice cream	-	*L. casei* (Lc-01)	> 10 log cfu/g	> 7 log cfu/g	180 days	−20 °C	(Homayouni et al., 2008)
B. animalis subsp. *lactis* BB-12	Ice cream	-	*L. acidophilus* *LA-5* and *Propionibacterium jensenii* 702	> 8 log cfu/g	> 7 log cfu/g	52 weeks	−20 °C	(Ranadheera et al., 2013)
B. animalis BB-12	Ice cream	-	*Lactobacillus casei (NCDC-298)*	9.86 log cfu/ml	7.21 log cfu/ml	180 days	− 23 °C	(Karthikeyan et al., 2013)
B. animalis subsp. lactis BLC1	Ice cream	-	-	7 log cfu/g	≥ 6.5 log cfu/g	120 days	−18 °C	Silva et al. (2015)
B. animalis BB-12	Non-fermented soy milk ice cream Non-fermented coconut milk ice cream	-	*L. acidophilus*	7.53 log cfu/g 7.45 log cfu/g	7.77 log cfu/g 7.37 log cfu/g	30 days	−20 °C	(Aboulfazli et al., 2016)
B. animalis Bb-12	Skimmed milk powder ice cream	19.40% Concentrated apple juice 6% FOS	*Lactobacillus acidophilus La-5*	8.59 log cfu/g	8.37 log cfu/g	84 days	−18 °C	(Matias et al., 2016)
B. animalis subsp. *lactis* *BB-12*	Non-fermented ice cream	7.5% xylitol	-	8.37 log cfu/g	8.24 log cfu/g	28 days	−22 °C	(Kalicka et al., 2019)

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