Survival of Probiotic Lactic Acid Bacteria In Ice Cream During Storage: A Systematic Review

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Systematic Review

Keywords: Ice Cream, Probiotic, Lactic Acid Bacteria, Survival, Viability, Storage

Posted Date: April 14th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2693474/v1

Abstract

This systematic review aimed to synthesize the results of studies that investigated the survival of probiotic lactic acid bacteria (LAB) in ice cream, in order to determine the factors that enhance survival during storage. The most expressive factors in enhancing the survival of probiotic strains in ice cream were: (i) microencapsulation of LAB, (ii) addition of prebiotics, (iii) fruit and its derivatives, (iv) vegetable extracts, (v) flours, (vi) use of fat substitute for inulin, and (vii) and adaptation of LAB to cold or heat. The factors that have been shown to reduce the viability of probiotic strains were: (i) substitution of sugar for sweeteners and (ii) storage time. Still contradictory results were found regarding the addition of whey and vitamins and minerals, replacement of cow's milk by other ingredients, change in the amount of fat alone and together with sugar, the influence of the LAB species/subspecies and different methods ice cream manufacturing. Finally, different ice cream packaging materials had no significant effect on survival.

1 Introduction

Ice cream is a complex multiphase system consisting of dispersed air cells, fat globules, ice crystals and an aqueous phase (Marshall et al. 2003). It is a dairy product that contains milk, sweeteners, stabilizers, emulsifiers and flavorings, followed by pasteurization and freezing (Karaman and Kayacier 2012). Dairy-based products, such as ice cream, are considered poor in bioactive compounds, and one way found to improve the functionality of ice cream has been with the incorporation of probiotic bacteria (Sun-Waterhouse et al. 2013).

Probiotics are living microorganisms which when administered in adequate amounts confer benefits to health (FAO 2002). Some of the beneficial health effects promoted by probiotic intake include: reduced lactose intolerance, prevention of diarrhea, antimicrobial activity, prevention of inflammatory bowel disease and hypocholesterolemic effect (Vasiljevic and Shah 2008). To achieve these therapeutic effects, a daily intake of at least 100 g of a product containing between 10^6 and 10^7 CFU/g is recommended (Gomes and Malcata 1999).

According to Akalin et al. (Akalın et al. 2018), ice cream has a good potential as a probiotic carrier. This potential is associated with its composition, as well as its pleasant taste and attractive texture. In addition, probiotics are able to survive long periods of storage in frozen systems, such as the ice cream matrix itself, which includes nutritional constituents such as milk, fat and lactose proteins (Cruz et al. 2009). However, the loss of the viability of probiotics in the different stages of the ice cream production process (formulation, storage and thawing) cannot be avoided (Hanafi et al. 2022).

Currently, several studies have directed their research on the development of different ice cream formulations in order to add greater health benefits and disease prevention. Such research has aimed at the development of ice cream, for example, with reduction of fat or sugar (Prasertsiriphan and Kusump 2015), the use of fat or sugar substitutes (Hashemi et al. 2015), the addition of prebiotics (Kemsawasd and Chaikham 2020) and other products (Ahmad et al. 2020; Acu et al. 2021). The modification of the ice cream matrix, in the case of ice creams added from probiotics, can positively or negatively affect the survival of probiotic microorganisms during storage. In addition to changes in ice cream formulations, several studies have evaluated the effect of probiotic cell microencapsulation with the objective of reducing the loss of viability during ice cream storage (Karthikeyan et al. 2013; Ahmadi et al. 2014; Afzaal et al. 2019, 2020)

Although several studies showed ice cream formulations that emphasize the stability of the viability of probiotic strains in long-term, such as through microencapsulation (Homayouni et al. 2008) and the addition of prebiotics

(Akin 2005), there are still no conclusive studies demonstrating which ice cream formulation is better for incorporating these probiotic strains. Therefore, a systematic review on the survival of probiotic LAB in ice cream is important to achieve critical evidence about the survival of probiotics in different ice cream formulations. Thus, this is a systematic review that aims to summarize and critically evaluate the evidence on the survival of probiotic LABs strains in different ice cream formulations during the storage period.

2 Methodology

2.1 Search strategy

We implemented and report the current study according to the guidelines of the report items delivered for systematic reviews and meta-analyses (PRISMA). The review strategy was guided by PICO (population, intervention, comparator, results and configuration). The criteria within each category were as followed:

- Population: probiotic lactic acid bacteria.
- Intervention: original studies investigating the effects of probiotic ice cream supplementation (regardless of the supplementation agent) and microencapsulation of probiotic lactic acid bacteria during the storage period (regardless of time).
- Comparator: traditional probiotic ice cream (without supplementation or microencapsulation of the bacterium), considered in this case, the control of the experiment.
- Results: results include survival (log/CFU or CFU) of probiotic lactic acid bacteria in ice cream during storage.
- Environment: ice cream

2.2 Research in the literature

The survey of studies was conducted systematically during the months of March and April of 2021 in the following online databases: Google Scholar, PubMed, Taylor and Francis, Scielo, ScienceDirect, CrossRef Search and Microsoft Academic Search. The research was carried out using the following terms: ice cream, probiotic, lactic acid bacteria, survival, viability, storage. This phase was made by only one author, identifying by title possible studies related to the subject, without date restriction.

It is noteworthy that the research protocol was not registered in PROSPERO.

2.3 Selection of studies

Eligible studies met the following inclusion criteria: experimental articles addressing the survival or viability of lactic acid bacteria in ice cream during the storage period; analysis of lactic acid bacteria survival only by the classical microbiological method (colony count in agar added culture medium); and articles or short communication in English. Reviews, summaries, theses, dissertations, conference documents and study protocols were excluded. Two authors (C and M) independently conducted eligibility screening. The initial screening was based on titles and abstracts according to the established eligibility criteria. The articles selected based on the eligibility criteria were independently reviewed by two authors (C and M). In this stage, the articles were read completely. Disagreements were managed by discussion to reach consensus or by a third reviewer (E. S. L.) when necessary.

2.4 Data extraction

The Microsoft Excel® platform was used to store the titles of the articles obtained from the search platforms and to eliminate the duplicate articles, and this step was done by only one author. After that, the abstracts of the articles selected in this stage were analyzed by another author and classified again for subsequent data extraction. The final extraction was done by inserting the data from the articles in the same platform and was checked in duplicate by two different authors.

3 Results And Discussion

3.1 Selection of studies

The detailed process of selection of the studies can be seen in Fig. 1, where 378 articles with titles that referred to the theme in the databases were found, and after removing the duplicates (n = 134), 244 remained for the abstract reading. After reading the abstracts, 134 were discarded because they were in disagreement with the eligibility criteria, and 63 were not available online for reading, 29 were not in English, 18 were frozen yogurt, 8 were literature reviews, 8 did not analyze ice cream, 4 analyzed yeasts, 2 isolated strains, 2 were dissertations, 1 did not evaluate survival by classical method and 1 was book chapter.

After this screening stage, 108 articles remained to be read in full, of which 24 were later excluded because they were incomplete (n = 14), were not related to the theme (n = 8) and were not completely in English (abstract in English only) (n = 3). Finally, 84 articles remained for the final extraction of the data.

3.2 Characteristics of the studies

The 86 articles analyzed are from experimental studies with publication dates between 1992 and 2021. Among these articles, 16 refer to ice cream with LAB microencapsulated, encapsulated or immobilized; 37 treats enriched, supplemented, fortified or added ice cream ingredients; 8 refer to ice cream with changes in the amount of sugar, fat and calories; 11 treat ice cream with substitutions of ingredients and 12 are other articles related to the survival of LAB in ice creams that did not fit in the previous categories.

Ice cream with LAB microencapsulated, encapsulated or immobilized

The results of the ice cream category with microencapsulated, encapsulated or immobilized LAB (19.0%, n = 16) demonstrate an improvement and increase in the survival of the BAL strains associated with microencapsulation or encapsulation in most articles (n = 14), as these processes seem to maintain viability stability bacteria involved in the capsule during food processing and storage (Vaniski et al. 2017). This further emphasizes why the microencapsulation method is effective and widely used in the food industry (Rajam and Anandharamakrishnan 2015).

Still within this category, the material that most demonstrated beneficial results for the survival of probiotics was calcium alginate. This material, although not one of the most commonly used in the industry, demonstrates a remarkable application (Rebello 2009) and still seems to have a protective effect against low freezing temperatures (Ahmadi et al. 2014; El-Sayed et al. 2014).

The articles that used microencapsulation with calcium alginate, acacia gum and resistant starch and those with immobilization with banana flour were the only ones in the category that exposed contradictory results to those found in other articles and in the literature. The probable explanation for this is that, although starch carbohydrate swells and acacia gum is consecrated as an encapsulating material superior to the others (Rebello 2009), the study by Jurkiewicz et al. (Jurkiewicz et al. 2011) had high amount of air incorporation (overrun), which caused oxidative damage in probiotic cells.

The article that immobilized the LAB with banana flour did not protect the strains to the point of increasing survival significantly as expected, but had a satisfactory survival (Phuapaiboon 2016), showing that it can be a method not as effective as microencapsulation but still interesting.

Table 1
Data extraction from selected studies of the ice cream category with microencapsulated, encapsulated and immobilized cells.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period (days)	Main findings
Homayouni et al. 2008	*Lactobacillus casei* (Lc-01) and *Bifidobacterium lactis* (Bb-12)	Microencapsulation with calcium alginate and addition of resistant starch.	-20	180	Microencapsulations have increased survival.
Jurkiewicz et al. 2011	*Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* BI-04	Microencapsulation with calcium alginate, acacia gum or resistant starch	-18	180	Microencapsulation had no significant effect on bacteria survival.
Karthikeyan et al. 2013	*Lactobacillus casei* (NCDC-298) and *Bifidobacterium animalis* ssp. Lactis (BB-12)	Microencapsulation with calcium alginate and *whey protein.*	-23	180	Microencapsulation significantly increased the survival of the strains.
Sahitya et al. 2013	*Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231	Microencapsulation with alginate and addition of fructoligosaccharides.	-20	90	Microencapsulation increased the survival of strains during storage.
Ahmadi et al. 2014	*Lactobacillus acidophilus* (La-5)	Microencapsulation with calcium alginate microspheres and addition of different concentrations of fructoligosaccharides (FOS).	-18	60	Microencapsulation maintained survival and protected strains from injuries caused by freezing.
Karthikeyan et al. 2014	*Lactobacillus acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298)	Microencapsulation with calcium alginate and whey protein	-23	180	Both types of microencapsulation increased the survival of the strains.
Champagne et al. 2015	*Bifidobacterium longum* and *Lactobacillus rhamnosus*	Microencapsulation with whey protein and plus chocolate droplets	-16 and – 20	90 and 180	Increased survival of strains with microencapsulation in chocolate droplets and associated with tablets.
El-Sayed et al. 2014	*Lactobacillus plantarum*, *Lactobacillus casei* and *Bifidobaterium bifidum*	Encapsulation with calcium alginate and whey protein concentrate and addition of inulin, lactulose and fructoligosaccharides.	-20	90	Microencapsulation increased the survival of the strains.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period (days)	Main findings
Phuapaiboon 2016	*Lactobacillus casei* TISTR 1463 and *Lactobacillus acidophilus* TISTR 1338	Immobilization with banana flour.	-18	50	Microencapsulation did not influence the survival of bacteria.
Songtummin and Leenanon 2016	*Lactobacillus acidophilus* TISTR1338 and *Lactobacillus casei* TISTR390	Encapsulation with different concentrations of calcium alginate and addition of different concentrations of cryoprotectors.	-20	56	There was no significant difference between the survival of the bacteria between the concentrations of alginate and cryoprotectors.
Gonzalez-Cuello et al. 2018	*Lactobacillus bulgaricus*	Microencapsulation with oil-water emulsion and calcium carbonate.	Not quoted	20	Microencapsulation increased relatively the survival of the strains.
Kataria et al. 2018	*Bifidobacterium longum*	Microencapsulation with alginate starch capsules	-20	15	Microencapsulation increased the viability of bifidobacteria in ice cream.
Afzaal et al. 2019	*Lactobacillus acidophilus*	Encapsulation with sodium alginate or carrageenan.	-20	120	Microencapsulation significantly increased the survival of probiotics.
Farias et al. 2019	*Lactobacillus rhamnosus* ASCC 290 and *Lactobacillus casei* ATCC 334	Encapsulation with alginate-chitosan	-18	150	Lower loss in survival of *Lactobacillus rhamnosus* free form than in encapsulated form of Lactobacillus casei. However, microencapsulation reduced both losses.
Afzaal et al. 2020	*Lactobacillus casei*	Encapsulation with calcium alginate or concentrated whey protein	-20	80	Microencapsulation increased the survival of the strain.
Zaeim et al. 2020	*Lactobacillus plantarum*	Microencapsulation with calcium alginate and chitosan and addition of inulin and resistant starch.	-18, 4 and 25	90	Microcapsules containing inulin showed better performance than those containing starch.

Ice cream enriched, supplemented, fortified or added ingredients

Within the category of ice creams with added substances (44.0%, n = 37), it was found that the main exclusive additions or together were: prebiotics (n = 10), fruits and their derivatives (n = 12), whey (n = 5), vitamins and minerals (n = 5), extracts vegetables (n = 4), flours (n = 3) and other additions (n = 5). as additions that positively affected survival are prebiotics, fruits and their plant derivatives and extracts (Table 2).

Apparently, the addition of prebiotics - inulin, oligofructose and oligosaccharides - seems to be related to the greater survival of LAB in storage (Akin 2005; Akalin and Erişir 2008; Ayar et al. 2018; Kemsawasd and Chaikham 2020), providing an increase in growth (Pandiyan et al. 2012c, a) and maintaining the stability of viability during storage (di Criscio et al. 2010; Pandiyan et al. 2012b). Only Akalin and Erisir (Akalin and Erişir 2008) and Balthazar et al. (Balthazar et al. 2018) found different results of this, which may be explained by the different percentages of addition of probiotics, in addition to variations in temperature, species of lactic acid bacteria and mode of production among these ice creams.

The addition of fruits and their derivatives was positive in most articles, with satisfactory survival (Favaro-Trindade et al. 2006; Cruxen et al. 2017; Akalın et al. 2018) and significant increase in the survival of LAB (Sagdic et al. 2012; Ayar et al. 2018; Öztürk et al. 2018; Mahdian and Karazhian 2020; Kemsawasd and Chaikham 2020; Ahmad et al. 2020). This positive result can probably be explained by the fact that fruits and their derivatives present prebiotics (Mahdian and Karazhian 2020), fibers (Ayar et al. 2018; Mahdian and Karazhian 2020) and polyphenols (Ahmad et al. 2020), thus being a positive addition in this context.

The addition of whey appeared with positive results in all articles (Pandiyan et al. 2012a, c, b; Guler-Akin et al. 2016; Yaseen 2018), but only one of them analyzed the addition only of whey (Yaseen 2018), thus making it difficult to know if the positive effect is associated only with this increase.

Regarding the addition of vitamins and minerals, only the addition of vitamin C (Akın and Dasnik 2015) and zinc (Gheisari et al. 2016; Kozłowicz et al. 2019) showed to be positive for the survival of the strains, all others showed no significant differences. These contradictory results can possibly be explained due to the low success they have in trying to increase these micronutrients because of their specific characteristics, such as different solubility, being terms sensitive and others that make them vulnerable to manipulation (Penteado 2003).

In the articles that added vegetable extracts to ice cream, all formulations proved to be good means to have satisfactory amounts of viable strains (Aboulfazli et al. 2015a; Elsamani 2016; Kemsawasd and Chaikham 2020), and in some the higher the concentrations of plant extract were the better the results (Guler-Akin et al. 2016). This is probably due to the prebiotic properties of these ingredients (Kehinde et al. 2020) and these extracts generate a pH that maintains and increases the amount of living bacteria (Heenan et al. 2004).

In relation to studies with addition of flours, all articles showed good survival of the strains (Parussolo et al. 2017; Prashanth et al. 2018; Thaochalee et al. 2018). This is probably due to the characteristics of each flour, such as rice flour that like rice brings bioactive nutrients (Cho and Lim 2016) and is a source of prebiotics (Nealon et al. 2017) favoring the increase in the number of bacteria. Green banana flour is rich in complex carbohydrates and resistant starch, components that help decrease the pH of ice cream (Zhang et al. 2019) and yacon potato flour is an important source of fructoligosaccharides, containing once again prebiotics that aid in the growth of strains (Ojansivu et al. 2011).

Finally, still within this category had articles that analyzed the influence of other additions, such as cilembu sweet potato starch (Kusumah Dewi et al. 2015), cane and coconut sugar (Low et al. 2015), milk fat and sunflower oil (Calligaris et al. 2018), yeast (Varga and Andok 2018) and tapioca powder (Yadav et al. 2020). Of these, only coconut sugar, yeast and tapioca powder had a positive effect on the survival of LAB strains.

Table 2
Data extraction from selected studies of the category of ice cream enriched, supplemented, fortified or added of ingredients.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Akin 2005	*Streptococcus thermophilus,Lactobacillus delbrueckii* ssp. *Bulgaricus*, *Lactobacillus acidophilus LA-14 and* *Bifidobacterium lactis BL-01*	Addition of different concentrations of inulin and sugar	-18	90	Inulin increased the amount of viable probiotic cells. Sugar increased at the beginning and reduced in the end the amount of viable probiotic cells.
Favaro-Trindade et al. 2006	*Bifidobacterium longum* *Bi.lactis,* *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. Bulgaricus*	Addition of acerola pulp with pH variations	-18	105	Samples maintained a good amount of viable cells.
Favaro-Trindade et al. 2007	*Lactobacillus acidophilus 74 – 2, actobacillus acidophilus LAC 4* and Yoghurt starter culture	Addition of cajá fruit and cream with pH variations	-18	105	Satisfactory survival related to the addition. Samples with pH 4.5 had higher survival than pH 5.0.
Akalin and Erişir 2008	*Lactobacillus acidophilus La-5 and* *Bifidobacterium animalis Bb-12*	Addition of inulin or oligofructose	-18	90	Oligofructose improved the viability of the strains. Inulin decreased the amount of viable *B. animalis* cells.
di Criscio et al. 2010	*Lactobacillus casei and Lactobacillus rhamnosus*	Addition of different inulin concentrations.	-20	112	All samples preserved the amount of bacteria well and kept cells viable.
Pandiyan et al. 2012a	*Lactobacillus acidophilus*	Addition of inulin and whey protein.	-18 to -23	15	Addition of inulin increased the growth of the bacterium and thus generated a greater final amount of viable cells.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Pandiyan et al. 2012c	*Lactobacillus acidophilus*	Addition of whey protein and honey, fructoligosaccharides or inulin.	-18 to -23	15	Additions significantly increased the survival of the strain.
Pandiyan et al. 2012b	*Lactobacillus acidophilus and Saccharomyces boulardii*	Addition of fructoligosaccharides and whey protein	-18 to -23	15	Samples maintained a good amount of viable cells.
Sagdic et al. 2012	*Lactobacillus casei Shirota*	Addition of ellagic acid, gallic acid, grape seed extract, pomegranate bark extract and peppermint essential oil.	-18	60	All supplementations had positive effects on the survival of the strains. Pomegranate bark extract promoted the best result among supplementations
Senanayake et al. 2013	*Lactobacillus acidophilus (LA 5)*	Apple addition	-18	70	There was a large reduction in probiotics, but in the end the viable cells continued above the recommended.
Aboulfazli et al. 2015b	*Bifidobacterium bifidum (Bb-12)*	Formulation with addition of soybean and coconut vegetable milk and fermentation	-20	Not quoted	Vegetable extracts generated higher reproduction of probiotic cells than samples without after fermentation time. All samples had a higher amount of viable cells after fermentation.
Akın and Dasnik 2015	*Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. BB-12 lactis	Addition of different concentrations of glucose oxidase or ascorbic acid	-18	90	Only ascorbic acid kept a good amount of stipes.
Kusumah Dewi et al. 2015	Not quoted	Addition of different concentrations of cilembu sweet potato starch	Not quoted	14, 28 and 42	Higher survival was associated with lower concentrations of the additions and shorter storage time.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Low et al. 2015	*Lactobacillus acidophilus*	Addition of different concentrations of cane sugar or unrefined coconut sugar	-20	90	The samples with the highest survival were with coconut sugar. All samples maintained a good amount of viable cells.
Elsamani 2016	*Lactobacillus acidophilus* and *Bifidobacterium bifidum*	Formulation with addition of lupin and peanut vegetable milk	-18	30	Vegetable milks increased the growth of both species. All samples maintained a good amount of viable cells.
Gheisari et al. 2016	*Lactobacillus casei*	Zinc addition.	-18	90	Samples maintained a good amount of viable cells.
Guler-Akin et al. 2016	*Lactobacillus acidophilus and* *Bifidobacterium* BB-12	Addition of different concentrations of carob extract and whey powder	-18	90	Samples supplemented with carob extract and whey powder had a higher amount of viable cells. The higher the amounts of extract and whey, the greater the growth of both species.
Purahmad and Golestani 2017	*Lactobacillus acidophilus* and *Bifidobacterium lactis*	Addition of inulin and fermented milk and fermentation.	-20	112	Fermented milk had the highest survival.
Cruxen et al. 2017	*Bifidobacterium lactis (Bl-04)*	Addition of butiá pulp	-18	90	Addition did not negatively affect survival and viable cells were above recommended.
Parussolo et al. 2017	*Lactobacillus acidophylus* NCFM	Addition of different concentrations of yacon flour.	-18	150	All samples maintained a good amount of viable cells. The higher the concentration of flour, the higher the survival.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Akalın et al. 2018a	*Lactobacillus acidophilus* and *Bifidobacterium lactis*	Addition of apple fiber, orange, oats, bamboo and wheat.	-18	180	All samples were survival above the recommended, except those with orange and bamboo fiber. Control sample had a higher amount of stipes followed by samples plus wheat fiber.
Ayar et al. 2018	*Lactobacillus acidophilus (ATCC 4357D-5)* and *Bifidobacterium animalis* subsp. lactis *(ATCC 27536)*	Addition of different concentrations of rice, corn, sunflower, barley, grape, apricot, apple and inulin.	-20	60	Addition of fruit and grain fibers increased the survival of probiotics overall, except for spent grains. Samples with the addition of inulin had a higher amount of viable cells.
Balthazar et al. 2018	*Lactobacillus casei 01*	Formulation with sheep's milk and addition of inulin	-18	150	Addition did not increase the number of bacteria.
Góral et al. 2018	*Lactobacillus rhamnosus B 442, Lactobacillus rhamnosus 1937 eLactococcus lactis JBB 500*	Addition of magnesium ions.	-30	2	Addition decreases strain count.
Öztürk et al. 2018	*Lactobacillus casei 431*	Formulation with goat's milk and addition of blue myrtle fruit and white myrtle fruit	-20	56	Increase in survival related to additions.
Prashanth et al. 2018	*Bifidobacterium bifidum* and *Lactobacillus acidophilus*	Formulation with buffalo milk and addition of green banana flour	-26	60	The higher the addition, the greater the survival.
Calligaris et al. 2018	*Lactobacillus rhamnosus*	Addition of anhydrous fat from milk and sunflower oil pure or previously inoculated.	-18 and − 30	14	Probiotics previously inoculated in oil or fat had higher survival.
Thaochalee et al. 2018	*Lactobacillus acidophilus LA-5*	Addition of different concentrations of germinated brown rice flour and corn flour.	-20	30	Satisfactory survival in all samples.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Varga and Andok 2018	*Bifidobacterium animalis* subsp. lactis Bb-12	Addition of different yeast concentrations (*Saccharomyces cerevisiae*).	-13	7	Increase in survival related to additions.
Yaseen 2018	*Lactobacıllus reuteri*	Addition of different concentrations of sweetened whey.	-25	21	Addition increased the amount of strains.
Kozłowicz et al. 2019	*Lactobacillus Rhamnosus B 442*	Addition of zinc ions and fermentation or electrification.	-30	90	Probiotics in fermented samples were significantly higher than in non-fermented samples. Satisfactory survival in all samples.
Ahmad et al . 2020a	*Bifidobacterium lactis and Lactobacillus acidophilus*	Formulation with buffalo milk and polyphenol addition of apple peel extract	-20	90	The higher the addition, the greater the survival.
Kemsawasd and Chaikham 2020	*Lactobacillus casei 01 and Lactobacillus acidophilus LA5*	Addition of inulin, riceberry and sesame milk.	4	60	Increase in survival related to additions. All samples had a great decrease in viable cells, but samples with the addition of vegetable milks and inulin showed higher survival of the strains in the long term.
Mahdian and Karazhian 2020	*Lactobacillus casei LC-01*	Addition of different concentrations of apple fiber, banana and mango.	-18	60	Increase in survival related to additions.
Pankiewicz et al. 2020	*Lactobacillus rhamnosus B 442*	Addition of calcium ions and electroporization.	-30	1	There was no significant difference between the additions. Electrical pulses have increased survival.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Yadav et al. 2020	*Lactobacillus casei*	Addition of different concentrations of tapioca powder.	-21 to -29	Not quoted	The highest survival was obtained in the sample with 15% tapioca and 3% *L. casei*. The greater the amount of bacterial inoculation, the greater the amount of final probiotic cells.
Acu et al. 2021	*Bifidobacterium bifidum,* *Lactobacillus paracasei* and *Bifidobacterium longum*	Formulation with goat's milk and addition of tagatose, litesse ultra and polydextrose, frozen raspberries, raspberry puree and blackberry puree	-18	120	Satisfactory survival in all samples.

Ice cream with changes in the amount of sugar, fat and calories

The modifications found within the ice cream category with modifications in amounts of its ingredients (9.5%; n = 8), were in the amount of fat (n = 6), of sugars (n = 5) and calories (n = 1), both alone and together (Table 3).

The change in fat and sugar concomitantly seemed to be associated both with having no effect on the survival of probiotic LAB and to have a negative effect on survival and to have a positive effect as well. This is probably due to the different ice cream formulations of these articles, where the concentrations of 0.15% fat and sugar at 6.37% were the only ones to be associated with the positive effect (Villalva et al. 2017) and the other concentrations above that seemed to have no effect (Alamprese et al. 2002, 2005) or have a negative effect (Shahsavan et al. 2018). In addition, some of these articles had inulin addition and do not have the same amounts of ingredients in the formulations, which may also have created this discrepancy in the results.

The only fat modification appeared as positive, but the results were contradictory, since in one of the articles the higher the amount of fat the greater the survival (Turgut and Cakmakci 2009) while in the other the survival was satisfactory in an ice cream decreased in fat (Prasertsiriphan and Kusump 2015). Another article found that the change in fat for both higher and lower values did not have an effect on survival, showing that there are still contradictory and inconclusive results.

To finish this category, Chiquetti et al. (Chiquetti et al. 2016) found that the decrease solely in lactose sugar did not increase the count of probiotic LABs, but maintained satisfactory amounts to be considered a functional product.

Table 3
Extraction of data from the selected studies of the ice cream category with changes in the amounts of sugar, fat and calories.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Alamprese et al. 2002	*Lactobacillus johnsonii* La1	Change in the amount of sugar and fat	-16 and – 28	30 (at −16 ºC) and 240 (at −28 ºC)	Modifications maintained survival.
Alamprese et al. 2005	*Lactobacillus rhamnosus* GG	Change in the amount of sugar and fat	-16 and – 28	30 (at −16 ºC) and 365 (at −28 ºC)	No significant effect of the modifications.
Turgut and Cakmakci 2009	*Lactobacillus acidophilus* and *Bifidobacterium bifidum*	Change in the amount of fat	-20	90	The higher the amount of fat, the greater the survival.
Leandro et al. 2013	*Lactobacillus delbrueckius* UFV H2b20	Change in the amount of fat and fat replacement by inulin	-16	40	No significant effect of modifications and replacement.
Prasertsiriphan and Kusump 2015	*Lactobacillus acidophilus* BCC51147, *Lactobacillus rhamnosus* DSM20021 and *Lactobacillus casei* 01	Change in the amount of fat	-20	280	Satisfactory survival.
Chiquetti et al. 2016	*Lactobacillus acidophilus* La-5	Change in the amount of lactose	-18	28	No significant effect of modification.
Villalva et al. 2017	*Bifidobacterium lactis* Bb-12	Change in the amount of calories, fat and sugar and addition of inulin.	-18	21	Satisfactory survival.
Shahsavan et al. 2018	*Lactobacillus acidophilus* La-5	Change in the amount of sugar and fat and addition of inulin	-24	90	The higher the amount of sugar and fat, the lower the survival.

Ice cream with ingredient substitutions

Regarding the category of ice cream with ingredient substitutions (13.1%; n = 11), seen in Table 4, the main substitutions were cow's milk for other milks or ingredients (n = 5), of sugar for other sweeteners (n = 3), of fat for inulin (n = 2) and other substitutions (n = 2).

The substitution of cow's milk with goat's milk appeared to increase the count of probiotic LABs at the end of the storage period, as well as in the substitution of that same milk by vegetable extracts of soybean and coconut. These substitutions of cow's milk arise with positive results probably because the new ingredients have compounds that enhance the growth of LAB, such as vegetable extracts that have prebiotic substances (Kehinde et al. 2020). The replacement of cow's milk appeared to be negative when replaced by water and as neutral when replaced by sweet potato.

Other sweeteners as a way to replace sugar were shown to have contradictory results, as they had both articles with positive results, as neutral and negative. However, it seems that this substitution may not be beneficial in the long term since the only article that had a positive result had a shorter storage period than the other articles, being 28 days (Kalicka et al. 2019), while the others were 90 days (Hashemi et al. 2015) and 180 (Başyiğit et al. 2006). In addition, sugar is important for LAB because it is one of the carbohydrates used for fermentation of these and soon to keep the cells alive (Teuber 2001), different from sweeteners, so is not an interesting replacement.

Both articles that replaced fat with inulin obtained positive results in the survival of the strains, although fat is known to be protective for LAB (Calligaris et al. 2018). The possible explanation is that inulin acts as a prebiotic that promotes the growth of strains during storage, generating higher count of LABs at the beginning and consequently higher count of LABs at the end of storage (Akin 2005).

On the other hand, the substitution of goat's cream with inulin showed no significant effect on the survival of the LAB analyzed, while the article that replaced the aqueous phase with tiger nut extract had a positive effect. These substitutions were to be possibly beneficial, exposed that both inulin and plant extract have prebiotic properties (Akin 2005; Kehinde et al. 2020).

Table 4
Data extraction from selected studies of the ice cream category with ingredient substitutions.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Başyiğit et al. 2006	*Lactobacillus acidophilus,L actobacillus agilus* and *Lactobacillus rhamnosus*	Replacement of sugar with aspartame and fermentation	-20	180	Substitutions maintained survival. There was no difference in survival between fermented and unfermented.
Bolanõs et al. 2012	*Streptococcus thermophilus,Lactobacillus delbrueckii subesp. Bulgaricus and* *Bifidumbacterium animalis BB-12*	Replacement of cow's milk with goat's milk	-18	Not quoted	Increase in survival related to substitution.
Nurliyani et al. 2013	*Lactobacillus acidophilus*	Replacement of skimmed milk with sweet potatoes	-20	30	No significant effect related to substitution.
Aboulfazli et al. 2015a	*Lactobacillus acidophilus (La-05; L)* and *Bifidobacterium bifidum (Bb-12; B)*	Formulation with soybean and coconut vegetable milk to replace cow's milk	-20	90	Increase in survival related to substitutions.
Hashemi et al. 2015	*Bifidobacterium lactis*	Replacement of fat with inulin and sugar by lactulose	-18	90	Increase in survival related to inulin replacement and decreased survival related to sugar replacement.
El-Shenawy et al. 2016	*Lactobacillus acidophilus La-5 and* *Bifidobacterium bifidum Bb-12*	Replacement of the aqueous phase with tiger nut extract	-20	90	Increase in survival related to substitution.
Fragoso et al. 2016	*Pediococcus pentosaceus UAM22*	Replacement of fat with inulin	-23	21	Satisfactory survival related to substitution.
Guerra et al. 2018	*Lactobacillus paracasei*	Replacement of cow's milk with water and addition of acerola	-18	21	Decrease in survival related to substitution.
Kalicka et al. 2019	*Bifidobacterium BB-12*	Replacement of sugar by different sweeteners (xylitol, erythritol, maltitol and isomalt)	-22	28	Satisfactory survival related to substitutions.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
de Paula et al. 2020	*Lactobacillus rhamnosus and* *Lactobacillus paracasei*	Formulation with goat's milk and replacement of goat's cream with inulin	-18	84	No significant effect related to substitution.
Homayouni et al. 2021	*Lactobacillus casei*	Formulation with soybean vegetable milk to replace cow's milk	-25	180	Satisfactory survival related to substitution.

Other articles related to the survival of LAB in ice cream

Finally, the fifth and last category (14.3%; n = 12) seen in Table 5, it was of articles found that analyzed survival related to storage time and temperature (n = 6), the LAB species/subspecies (n = 3), the ice cream manufacturing method (n = 5), the different BAL treatments (n = 1) and different ice cream packages (n = 1).

According to the articles found, storage time has a negative influence on survival, since the longer the time, the shorter the survival (Hekmat and McMahon 1992; Kalandaragh et al. 2016) which was expected, since probiotics are losing their viability over time (Fenster et al. 2019). Nevertheless, some articles found that the time associated with the right temperature did not negatively influence (Abghari et al. 2011; Ghosh and Chattopadhyay 2011) or that did not significantly influence the survival of LAB (Magariños et al. 2007; Nousia et al. 2011).

As for the influence of the species and subspecies of LAB on survival, Freeman and Scholar (Freeman and Scholar 2009) found that survival does not depend on the LAB subspecies, while Coman et al. (Coman et al. 2012) e Kalandaragh et al. (Kalandaragh et al. 2016) found that there was a satisfactory survival, but that it was related to the elaborate set of LAB species. These results and others show that there may be an influence of the species and subspecies of LAB on survival during storage, but it is not yet clear.

The methods of making ice cream have several effects on survival, as Ranadheera et al. (Ranadheera et al. 2013) and Da Silva et al. (Silva et al. 2015) found that the ice cream formulation had both a positive and neutral influence on survival, as did Ergin et al. (Ergin et al. 2016) and Arslan et al. (Arslan et al. 2016) found that treatments such as ice cream fermentation have a positive effect on survival. But Kalandaragh et al. (Kalandaragh et al. 2016) found that ice cover and different percentages of LAB inoculation in ice cream had no significant effect.

Finally, subjecting LAB to processes that promote adaptation to cold and heat proved desirable to accentuate survival during storage (Ergin et al. 2016), but different packages (polypropylene, polyethylene and glass) had no significant effect on their survival (Ranadheera et al. 2013).

Table 5
Data extraction from selected studies in the category of other articles related to the survival of LAB in ice cream.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Hekmat and McMahon 1992	*Lactobacillus acidophilus and* *Bifidobacterium bifidum*	Survival analysis in flavorless ice cream	-29	119	The longer the storage time, the lower the survival.
Magariños et al. 2007	*Lactobacillus acidophilus La-5 and* *Bifidobacterium animalis subsp. Bb-12 lactis.*	Survival analysis in flavorless ice cream	-25	60	No significant effect on survival related to temperature and storage time.
Freeman and Scholar 2009	*Bifidobacterium animalis ssp. animalis ATCC 25527 and* *Bifidobacterium animalis ssp. DSMZ 10140 lactis*	Survival analysis in unspecified ice cream	-25	11	Survival does not depend on the subspecies.
Abghari et al. 2011	*Lactobacillus acidophilus and Lactobacillus rhamnosus*	Survival analysis in flavorless ice cream	-19	84	Satisfactory survival related to temperature and storage time.
Ghosh and Chattopadhyay 2011	*Lactobacillus casei and* *Lactobacillus acidophilus*	Survival analysis in vanilla ice cream	-20	60	Satisfactory survival related to temperature and storage time.
Nousia et al. 2011	*Lactobacillus acidophilus LMGP21381*	Survival analysis in flavorless ice cream	-15° and 25°	315	No significant effect on survival related to temperature and storage time.
Coman et al. 2012	*Lactobacillus rhamnosus IMC 501* and *Lactobacillus paracasei IMC 502*	Survival analysis of a probiotic product in flavorless ice cream	-18 to -20	52	Satisfactory survival related to probiotic product.
Ranadheera et al. 2013	*Lactobacillus acidophilus LA-5,* *Bifidobacterium animalis subsp. lactis* BB-12 and *Propionibacterium jensenii* 702	Survival analysis in ice cream with formulation with goat's milk and different packages	-20	364	Satisfactory survival related to formulation and no significant packaging-related effect.

Reference	LAB strain	Procedure done	Temp. of storage (ºC)	Storage period. (days)	Main findings
Silva et al. 2015	*Bifidobacterium animalis subsp. BLC1 lactis*	Survival analysis in ice cream with formulation with goat's milk	-18	120	No significant effect on formulation-related survival.
Arslan et al. 2016	*Lactobacillus acidophilus (ATCC 4356)*	Survival analysis in flavorless ice cream and fermentation.	-20	90	Increase in survival related to different fermentation methods.
Ergin et al. 2016	*Lactobacillus acidophilus*	Survival analysis in unspecified ice cream, treatment of adaptations and fermentation.	37 and – 20	90	Increase in survival related to cold and heat adaptation treatments, as well as the fermentation stage in ice cream processing.
Kalandaragh et al. 2016	*Bifidobacterium lactis* and *Lactobacillus acidophilus*	Survival analysis in vanilla and vanilla coated ice cream	-18	150	Satisfactory survival related to strains. No significant effect of the different inoculation percentages and ice cover on survival. The longer the storage time, the lower the survival.

4 Conclusion

Microencapsulation, addition of prebiotics, fruits and their derivatives, plant extracts and flours in ice cream, and the substitution of fat by inulin and adaptation to cold and heat are factors that seem to potentiate the survival of probiotic LAB during storage. The addition of whey and vitamins and minerals, the change in the amount of fat alone and associated with sugar, the species/subspecies of LAB and different methods of ice cream manufacture have still contradictory results. Different materials of the ice cream packaging were shown to have no significant effect on survival. Finally, the substitution of sugar by sweeteners and longer storage time were shown to be factors that decrease the survival of LAB during the storage period.

Declarations

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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Figures

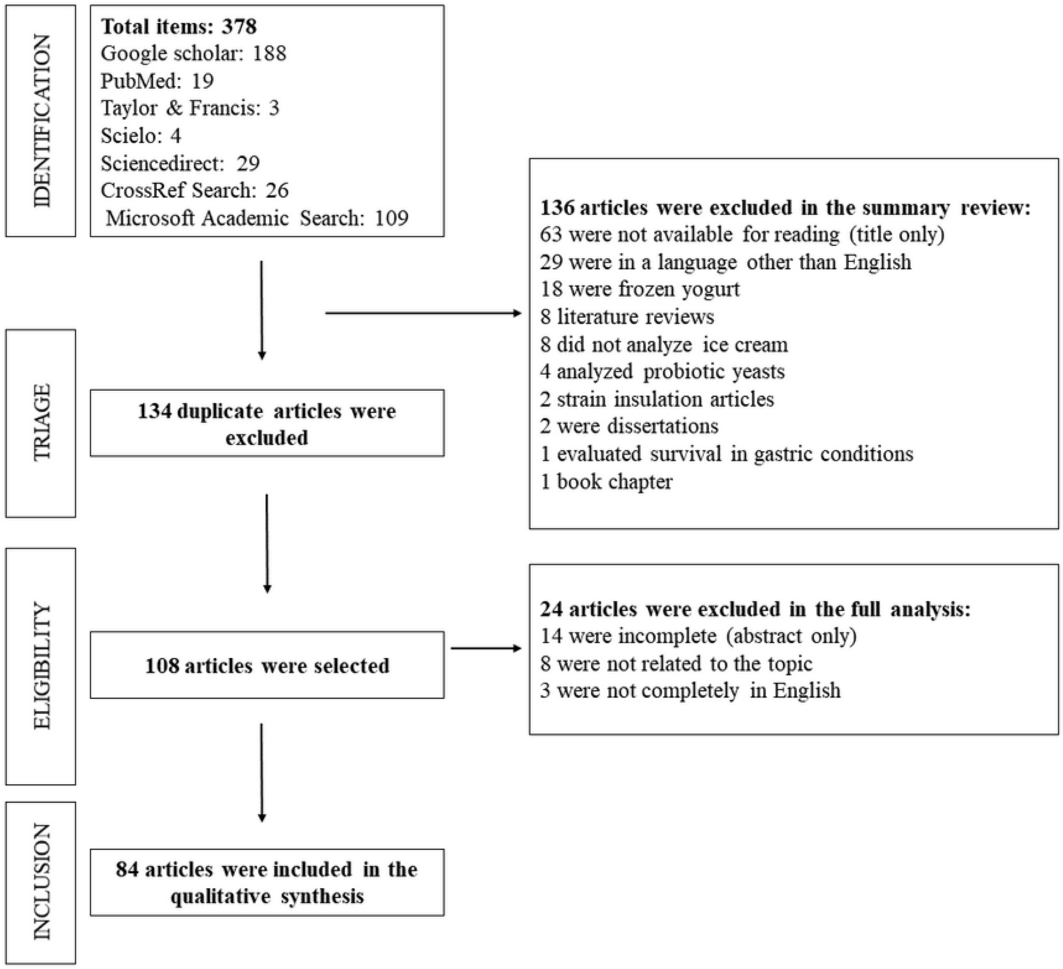

Figure 1

Flowchart of the study design of systematic review.