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REVIEW ARTICLE

Synbiotics: a technological approach in food applications

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Abstract The purpose of the present review is to explore the research about synbiotic food diversity, as well as the probiotics and prebiotics concentration used there in, and the different tests that this type of food is subjected to. The interaction probiotic–prebiotic–food is complex. The role of probiotics is clear, but the role of prebiotics is variable, as they are used for different purposes. The required doses in order to exert beneficial effects upon consumer health are adequate for probiotics, but not for prebiotics. The food processes affect in different ways the probiotics growth. It is essential to carry out more studies with protection technologies for probiotics, on the other hand, it is needed to assess if probiotics survival is because of the food matrix or because of the prebiotic with further experimentation.

Keywords Synbiotics · Probiotics · Prebiotics · Food technology

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Introduction

The former way of life has changed, nowadays, lifestyle in general is more sedentary than it used to be, people spend more time seated, whether for office work or entertainment activities, every day less strength and/or physical exercise is done, which leads to adverse alterations in the body and therefore in people's general health (Christmann 2020). Families lifestyle has also been altered in recent years, since it is increasingly common for mothers to have jobs with extended working time, whereby, family which is the core of society no longer eat traditionally, with home-cooked, healthy and balanced foods. On the contrary, family dynamics are more accelerated where there is not only little time to prepare food but also to eat, hence the stages of digestion are not properly done (Kristo et al. 2020). In this way the quality of what is consumed has been decreasing, so that, people are looking for the fastest and easiest to get options, leading to ingesting less healthy or unhealthy food, either by fast food chains or by the increase of convenience stores. Because of the above, nowadays, thriving of food research focuses on finding the way or methods to make healthier foods. Amongst the options there are added foods with bioactive components, so that, in addition to provide the nutrients of their nature, provide direct beneficial effects on the consumer's health. Amongst the bioactive components of interest are probiotics, prebiotics and synbiotics (Pandey et al. 2015). However, regarding its incorporation in food until now it is a great challenge, since the following aspects have to be taken into account: (1) type of bioactive compound (probiotic, prebiotic, synbiotic), (2) food matrix (solid or liquid), in addition to its complexity or simplicity, (3) the food process, since it is important the moment of incorporation of the bioactive component in it, so that, it does not lose its chemical function and properties, and (4) finally but not least, sensorial acceptability, since in many investigations only care is taken that the bioactive compound is in the minimum quantities necessary to confer a benefit to the host, being able to harm the sensory behaviour of the product. In the light of the above, the objective of the present review is to explore the research about the synbiotic food diversity, as well as the concentration used, and the different tests this kind of foods are subjected to.

Probiotics

The intestinal human microbiota and its modification by supplementation with beneficial microorganisms to the consumers health, nowadays is a thriving topic, such microorganisms are so called probiotics (Suez et al. 2019). Probiotics were defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Amongst the most used probiotic bacteria for human consumption can be found the lactic acid bacteria that include the following: *Lactobacillus acidophilus, L. plantarum, L. casei, L. casei spp rhamnosus, L. delbrueckii spp bulgaricus, L. fermentum, L. reuteri, Lactococcus lactis spp lactis, L. lactis spp. cremoris, Bifidobacterium bifidum, B. infantis, B. adolecentis, B. longum, B. breve, Enterococcus faecalis, E. faecium*, amongst other (Redondo-Useros et al. 2019).

The selection of probiotic lactic acid bacteria are: human origin, non-pathogenic behaviour, resistance to technological processes, resistance to gastric acidity and biliary toxicity, adhesiveness to the epithelial tissue of the intestine, capability to persist within the gastrointestinal tract, antimicrobial substances production, immune response modulation and capacity of influencing metabolic activities (Dlamini et al. 2019).

The conservative action of lactic acid bacteria is due to the inhibition of a great number of pathogenic microorganisms, which are harmful, because of a variety of final fermentation products; such substances are lactic acid, acetic acid, hydrogen peroxide, diacetyl, bacteriocins, and by-products generated by the action of lactoperoxidase enzyme on hydrogen peroxide and tiocianate. Bacteriocins are molecules that have peptide type structures or biologically active proteins, that present bactericide action on specific cellular receptors, besides their chemical composition and their specific mode of action are very varied (Sarwar et al. 2018; Zhang 2019).

Amongst the attributed benefits to probiotics, the following are mentioned: effect against lactose intolerance, diarrhoea control, effect against constipation, pro-carcinogens neutralization, anti-inflammatory action of the intestine, antagonist effect against pathogens, enhancing the epithelial structure, beneficial effects on liver disease, on immunity, on host mental, oral and vaginal health (Rajput and Li 2010; Cheng et al. 2019).

Prebiotics

In 2017, the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Gibson et al. 2017) issued an expert consensus on the definition and scope of prebiotics. Some of the conclusions are the following: (1) Prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit", (2) benefits of prebiotics upon health are constantly evolving, however until now the next are considered: benefits on gastrointestinal tract (pathogens inhibition or immune response stimulation), cardio metabolism (reduction of lipid levels in blood, effects on insulin resistance), mental health (metabolites with influence on brain function, energy and cognition), bones (minerals bioavailability), amongst other benefits, (3) currently prebiotics are established as carbohydrate based, but also other substances as polyphenols and polyunsaturated fatty acids converted to their respective conjugated fatty acids could belong to the updated definition, assuming overwhelming evidence on the host.

Synbiotics

The term synbiotic is used when a product contains both probiotics and prebiotics. Because the word refers to synergism, hence it must be reserved for products in which the prebiotic component selectively favours the probiotic compound (Mishra et al. 2018). In fact, it is considered that a synbiotic has more beneficial effects on human health than probiotics and prebiotics alone (Bandyopadhyay and Mandal 2014). The synbiotic is destined to enhance the promoting health bacteria survival in order to modify the intestinal flora and its metabolism. The term synbiotic should be kept for products possessing scientific validation of synergism exclusively in which prebiotics selectively favour the added probiotics within the particular synbiotic (Nicoleta-Maricica 2019). They also help to reduce undesired metabolites concentration including nitrosamines, to inactivate carcinogens, to prevent constipation and diarrhoea from various aetiologies (Wasilewski et al. 2015). It should be mentioned that the synbiotic effect on health is probably associated with the individual combination of a probiotic and a prebiotic, considering a wide range of possible combinations. Its application in modulating the human intestinal microbiota is promising (Markowiak and Slizewska 2017).

Dairy products

Yoghurt

Bahrudin et al. (2020) conducted an investigation with the aim to assess whether a synbiotic yoghurt confers benefits to constipation-predominant irritable bowel syndrome patients. The formulations used were: (1) Yoghurt with *L. helveticus*–1.7% polydextrose, and (2) Yoghurt with *L. helveticus* without prebiotic. As the research is about a disease, analyses to the yoghurt were not done. In both formulations, the yoghurts were sterilized, so the probiotic was not viable. In this research was found that the yoghurt without polydextrose alleviated the disease symptoms after a week of consumption. Regardless finding a benefit upon consumers with a disease, the yoghurt does not meet the established criteria to be considered potentially synbiotic.

Three different methodologies were developed by Li et al. (2020) in order to obtain a synbiotic yoghurt. The first methodology was to incorporate free *L. casei* and 1.5% lactitol. The second methodology was to incorporate 10% encapsulated *L. casei* with lactitol, and the third methodology was to incorporate 10% doble encapsulated *L. casei*, the first encapsulation was made with lactitol and alginate; then these capsules were encapsulated with chitosan. Briefly, in the yoghurt production process pasteurized milk was inoculated with the starter culture, then incubated, and finally the probiotic was incorporated either in the three different ways aforementioned. The final products were subjected to viable count, texture and syneresis analyses. In all cases, the obtained probiotic concentration meets the requirements for a probiotic yoghurt. In the case of the prebiotic for the second and the third methodology, the final concentration is not specified, but it can be inferred that it does not meet the minimum amount needed in order to confer a health benefit on the consumer or to have a prebiotic effect in the yoghurt. In this investigation the capsules were subjected to an in vitro gastrointestinal simulation, but the simulation was not done for the yoghurt. The prebiotic activity was done only for lactitol, but not for yoghurt.

Yoghurt: based drink

Haji-Ghafarloo et al. (2020) carried out an investigation with the aim to evaluate the prebiotic potential of a synbiotic yoghurt—based drink. The formulations were made using two prebiotics: arabic gum with four levels of variation (0, 0.25, 0.5 and 1%) and ginger extract with three levels of variation (0, 0.25 and 0.5%); as probiotic *B. bifidum* was used, giving a total of twelve experiments. Skim milk was heated, cooled and the starter cultures added, then incubated, cooled, then the prebiotics were added to the yogurt within 50%v/v water; after, the product was pasteurized, cooled, and then the probiotic was added, incubated and finally cooled for its storage. The tests performed to the final product were titratable acidity, pH, apparent viscosity, phase separation, probiotic viability and sensory analyses.

The principal objective of this project was to evaluate the prebiotic potential of a synbiotic drink. Nevertheless, it was not observed an increment in *B. bifidum* during the product storage; besides, there was not carried out an in vitro gastrointestinal simulation in order to assess the prebiotic potential of the drink. The probiotic concentration meets the requirements in all experiments for a probiotic product, as well as the prebiotics concentration is not enough in order to exert a potential benefit on consumers health.

Ice cream

A chocolate ice cream was developed by Peres et al. (2018) with the objective to evaluate its sensory profile and the influence of the label information on the acceptance by the consumer. The formulation was as follows: inulin (6.7%) and *L. acidophilus*.

The prebiotic was mixed at first with all the ingredients, an artisanal ice cream machine was used. The mixture was pasteurized and cooled. Then the probiotic was added under stirring until a desired creaminess. Finally, the ice cream was packaged and stored at − 18 °C.

The prebiotic concentration meets the required doses to exert benefits upon consumer health. On the other hand, the probiotic concentration in final product is not mentioned. There are no physicochemical nor microbiological analyses as the aim of the project is about sensory evaluation.

Di Criscio et al. (2010) conducted an investigation which objective was to elaborate functional ice creams. The following formulations were used: (1) *L. casei* (6LogCFU/g)–3% inulin, (2) *L. casei* (6LogCFU/g)–6% inulin, (3) *L. rhamnosus* (7LogCFU/g)–3% inulin, and (4) *L. rhamnosus* (7LogCFU/g)–6% inulin. A control ice cream without probiotics nor prebiotics was elaborated. For its production a laboratory scale plant was used. Probiotics were added as freshly-grown liquid culture after pasteurization. Samples were frozen at − 20 °C, and pH, acidity, total solids content, fat, protein, firmness, deformation of maximum load, microbiological and sensory analyses were carried out on 0–1 day, and after weeks 1, 3, 7 and 16. All formulations presented a probiotics viability higher than 7LogCFU/g at the end of week 16, complying with the recommendation for these type of food. Adding 3% Inulin did not significantly affected texture, whilst adding 6% inulin increased firmness between 20 and 30%. In regard to sensory attributes addition of microorganisms and inulin did not significantly affect consistency, flavour intensity and homogeneity; nevertheless, colour showed adverse results. The most accepted ice creams were the *L. rhamnosus*—6% inulin, followed by *L. casei*—6% inulin formulations.

In this investigation the freezing process seemed not to affect the cellular membrane, because instead of decreasing the microorganisms concentration, they got increased by one logarithmic cycle. Also, the ice cream fulfils the daily recommended doses for probiotics and prebiotics. In this case, the interest is in the technological functionality of the prebiotic, without considering its possible prebiotic effect and/or prebiotic potential. The moment at which the prebiotics were added is not indicated.

In order to evaluate the feasibility to include yacon flour as prebiotic and *L. acidophilus* as probiotic in strawberry ice cream elaboration process (Parussolo et al. 2017) on its physicochemical, microbiological and sensorial attributes, as well as its probiotic potential for a 150 days storage period, four formulations were developed: (1) 0.06% *L. acidophilus*—1.5% yacon, (2) 0.06% *L. acidophilus*—3% yacon, (3) 0.13% *L. acidophilus*—1.5% yacon y (4) 0.13% *L. acidophilus*—3% yacon, besides the control ice cream without prebiotic nor probiotic. The yacon flour was added to the ice cream base mixture. After the homogenization the freshly-grown liquid culture was added and then transferred to an ice cream machine. The ice cream was kept at -18 °C. A period of 150 days storage was established; pH, acidity and viable count were analysed. The initial analyses were 5 days after its elaboration and then in intervals of 30 days. All formulations presented a viability of 7LogCFU/g at the end of the 150 days. The yacon flour addition increased the viable microorganisms concentration through 120 days storage, and later appreciating a decrease of them. Acceptability of sensory attributes including appearance, colour, aroma, flavour and texture, confirm that addition of probiotics and prebiotics is feasible in the development of nutritionally and functionally enriched foods. This product complies with the specifications for a potential probiotic food. It should be mentioned that the prebiotic potential is not determined. Also, it is noteworthy that there is an increase of microorganisms concentration during 120 days, despite being at freezing temperature.

Pandiyan et al. (2012) elaborated a synbiotic ice cream with 0.3% *L. acidophilus* and 0.4% inulin. The steps for its production were: heating, homogenization, heating with addition of inulin, pasteurization, ripening, heating, cooling with addition of 4% lyophilized *L. acidophilus*, incubating, and finally frozen at − 18 to − 23 °C. The ice cream was subjected to fat, total solids content, protein, coliforms and melting quality determinations. *L. acidophilus* viable count was done at 0, 7 and 15 days. The synbiotic ice cream presented a faster melting compared to the control. No significant difference was observed in flavour, body and texture; however, there was a significant difference in overall acceptation, synbiotic ice cream got lower scores than the control. Also, a higher growth of *L. acidophilus* in ice cream with inulin was observed during 15 days of storage at − 18 to − 23 °C. The freezing process decreased the microorganisms concentration between 0.66 and 0.77 logarithmic cycles. During storage a reduction of 0.45 logarithmic cycles (from 8.83 to 8.38LogCFU/mL) was observed.

In this research the objective of adding inulin was only to increase the viability of the microorganism, without considering its prebiotic potential; besides, the amount of inulin used (0.3 g) does not meet the necessary requirements to confer positive effects on consumer health (7–10 g/day, 2 g/day minimum).

An experiment with two types of synbiotic ice creams was conducted by Homayouni et al. (2008) an ice cream with free microorganisms and an ice cream with encapsulated microorganisms. Next formulations were used: (1) free *L. casei* (9LogCFU/g)—1% resistant starch, (2) free *B. lactis* (9LogCFU/g)—1% resistant starch, (3) encapsulated *L. casei* (9LogCFU)—1% resistant starch, (4) encapsulated *B. lactis* (9LogCFU/g)—1% resistant starch, and (5) control with 1% resistant starch as prebiotic and without microorganisms. Free probiotics were added as freshly-grown liquid culture, and encapsulated probiotics were added in alginate beads. The production method was as follow: mixing of the ingredients, heating, homogenization, pasteurization, cooling, ripening, microorganisms addition and freezing at − 20 °C. After one week, the different formulations were subjected to pH, titratable acidity, dry matter, fat content, overrun, microstructure and sensory evaluation analyses. Viable count was done at time 0 and every 30 days for 6 months. In both cases (*L. casei* and *B. lactis*) the microorganisms concentration at time 0 (before freezing) is higher for the free than for the encapsulated ones. Free probiotics have a concentration a bit above 10LogCFU/g, and encapsulated probiotics a bit below 10LogCFU/g. Approximately after one month the concentration were almost the same, and from that moment on, the free microorganisms concentration was drastically reduced up to approximately 7LogCFU/g, whilst encapsulated microorganisms concentration reached approximately 8.5 CFU/g for *L. casei* and more than 9 CFU/g for *B. lactis.* The prebiotic effect is not investigated.

The purpose of prebiotics addition in this type of synbiotic foods, in general is to use them as a substrate for fermentation, thus keeping or increasing the probiotic concentration, without carrying out in vitro or in vivo tests in order to ensure the prebiotic effect of the selected bioactive component; except in a very few cases where the prebiotic potential is suggested, although without performing the correspondent experimentations. In most of the cases the probiotic is added as freshly-grown liquid culture, without considering giving a protection to the probiotic as an important factor (for example, the different microencapsulation technologies), since the food matrix is considered as appropriated and enough to maintain the viability of microorganisms. Further in most of the investigations is not indicated how the food production processes affect the probiotic, since the first report of viable count is when the food product is already finished.

In the case of ice cream, the mechanic stress, as it is a whipped product, oxygen is incorporated in large quantities, hence the oxygen toxicity is one of the main causes of cellular death for lactic acid bacteria. The decrease in viable count as a result of the freezing process, is due to the cold damage that cells suffer because of the ice crystals formation, as well as the injuries that they suffer by the freezing equipment blades, leading them to death eventually. The bacteria survival to unfavourable conditions, is dependent on the microorganism specie. It is suggested that the bigger the cell, the bigger the mechanical damage, hence a smaller cell is less susceptible (Homayouni et al. 2008).

Another general objective of prebiotics addition is for the technological functionality that they confer to the food in which they are added. In most of the research the necessary concentration to confer prebiotic benefits to health is not considered, although in these investigations the prebiotic qualities of the ingredients are highlighted. The addition of prebiotics has an effect on the instrumental texture of the ice cream, this could be because of the changes in the freezing point due to a higher solute concentration. In most of the investigations of the processes in which probiotics are added, logarithmic reduction is not described, it is only mentioned on a few cases.

The foods that are under fridge conditions, in which an effect on the probiotics survival or even their growth is clearly observed, could be due to the effect of the hydroxyl groups substitution on the membrane of the microorganisms for its integrity protection, rather than a dynamic interaction between prebiotics and probiotics, as fridge temperatures are not ideal for the growth of these type of microorganisms; so that, further investigation is needed.

Cheeses

Semi-hard cheese

Langa et al. (2019) developed a synbiotic semi-hard cheese. Three different formulations were used: (1) Cheese with *L. paracasei* (7.4LogCFU/mL), (2) Cheese with fructooligosaccharides (1%), and (3) Cheese with *L. paracasei* (7.4LogCFU/mL) and fructooligosaccharides (1%). Besides there was a control cheese without probiotic nor prebiotic.

Fructolooligosaccharides and the cultures were added to the pasteurized whole milk, then the rennet was added, the curd were cut, the whey was drained, then the product was distributed in moulds, pressed, packaged and ripened.

Fructooligosaccharides meet their function in the synbiotic cheese, as they promote the growth of *L. paracasei* in an in vitro colonic model, nevertheless, the prebiotic content is not enough to exert a prebiotic potential to the consumer. The probiotic concentration is 7LogCFU/g, hence this requirement is meet for the potential to give a health benefit to the consumer.

Soft white cheese

Klobukowski et al. (2009) conducted an experiment to evaluate calcium absorption in rats, whose diet include white cheese with the probiotic *L. plantarum* (7LogCFU/g), and the prebiotics 2.5% inulin or 2.5% maltodextrin. In this research the cheese elaboration is not mentioned, therefore, it does not mention whether the probiotic is free or protected, as well as it does not delve into the technological, physicochemical and/or sensorial effects of the bioactive compounds addition. In this research there was no significant difference between the control cheese and the *L. plantarum*-inulin cheese regarding to calcium absorption, however there was a significant difference between the control cheese and the *L. plantarum*-maltrodextrin cheese, being the last mentioned with the less calcium absorption in rats. In several investigations a stimulant effect on calcium bioavailability is observed in presence of 5–10% prebiotics, nevertheless in this study, rats were fed with 2% inulin, due to this low concentration, an effect on its bioavailability is not appreciated. It is probably that the only statistically significant increase in this study, which was the calcium apparent retention, could be due to the low prebiotic concentration used.

Cottage cheese

With the aim to evaluate the probiotics survival under in vitro simulated gastrointestinal tract conditions (Araújo et al. 2010) a cottage cheese was elaborated with

8.2LogUFC/g of *L. delbrueckii* and 5% inulin. The cheese was produced through a patented method. The inulin and the probiotic microorganism were added to the cheese by mans of a dressing. It was analysed every 5 days during 20 days storage for pH, water activity, titratable acidity, fat, humidity, total chloride and nitrogen. Also, sensory analyses were carried out after 3 and 15 days since the cheese elaboration, evaluating flavour, texture and overall acceptance.

There were not observed significant differences related to probiotic viability through 20 days storage; in terms of physicochemical properties there were not significant differences respect to the control. For sensory evaluation, the synbiotic cheese did not presented significant differences in flavour, texture, and overall acceptance respect to the control, thus addition of probiotics and prebiotics did not cause sensory changes after 15 days of storage.

In this research, a certain protection given to the probiotic is highlighted, since the viable count does not decrease during the storage period, attributing it to the acid matrix provided by the cheese, without considering a possible protective effect of the inulin. Regarding the probiotic resistance in the presence of bile salts, it is also attributed to its adaptation to acidic medium due to the cheese storage, without considering a possible interaction with inulin. This cottage cheese complies with the recommended doses per portion for probiotics and prebiotics, considering a consumption of 50 g.

In this investigation the aim of the added inulin was to decrease the fat content in the cheese, and not to increase the probiotic viability, either by fermentation or protection.

Fresh cream cheese

Buriti et al. (2007) investigated the influence of *L. paracasei* and *Streptococcus thermophilus* addition on fructan content at the beginning and at the end of 21 days storage at 4 °C of fresh cream cheese with inulin added. The cheese formulation was *S. thermophilus* (10LogUFC/g)—*L. paracasei* (9LogUFC/g)—8% inulin. The control cheese had only *S. thermophilus* as starter culture. The microorganisms were added in lyophilized form. Part of drained cheese whey was used to dissolve inulin. The cheese was stored at 4 °C for 21 days. The physicochemical analyses that were carried out on the cheese were minerals, fat, protein, fructans, dry matter, pH, water activity and humidity.

Because of the inulin addition, the synbiotic cheese presented less minerals, fat and protein content than the control. Changes in pH, water activity and humidity between day 1 and 21 of storage were similar in both cheeses; humidity content did not present significant difference between days 1 and 21 for each cheese; the observed pH reduction during storage is an expected process due to the continuous lactic acid production and other organic acids by the starter culture and probiotics. In terms of viable count, the synbiotic cheese at day 1 presented 7.12LogCFU/g, increasing this concentration to 7.39LogCFU/g at day 21 of *L. paracasei*. It is noteworthy that the presence of inulin does not have an effect on the growth or maintaining of *S. thermophilus* or *L. paracasei*, because of the fructan content in the cheese did not significantly change since day 1 to day 21 of storage; besides, the cheese was kept al 4 °C, and the optimum temperature for *L. paracasei* growth is 37 °C.

In this product, both the concentration of probiotics and prebiotics, are those recommended to provide benefits in consumer health.

Petit-suisse cheese

The study carried out by Cardarelli et al. (2007) had the principal aim to evaluate the prebiotic potential in synbiotic petit-suisse cheese in an in vitro fermentation model. The formulations were as follows: (1) 3% *S. thermophilus* as starter culture—3% *L. acidophilus* (9LogCFU/g)—2% *B. lactis* (9LogCFU/g)—10% prebiotic mixture (fructooligosaccharides—inulin—eucalyptus honey, in equal proportions), and (2) 3% *L. acidophilus* (9LogCFU/g)—2% *B. lactis* (9LogCFU/g)—10% prebiotic mixture. The control cheese only had the starter culture *S. thermophilus.* The cheese base was produced from skim milk, heated, and the microorganisms were added in lyophilized form, rennet addition, draining, homogenization, and fridge storage were done. The cheese was subjected to in vitro digestion probes, to fermentation of the digested samples by using faecal samples from two healthy volunteers, to bacterial enumeration by fluorescence in situ hybridisation, to short chain fatty acids, and to total carbohydrates analyses.

The synbiotic cheese presented a faster fermentation than the control and the cheese with only starter culture, showing a higher value in the measure of the prebiotic effect (MPE) of 0.84, comparing it with a value of 0.6 of the cheese that also contained starter culture, and with a value of 0.25 of the control cheeses (only with starter culture, without probiotics and prebiotics). The synbiotic cheese presented a higher modified prebiotic index (PI_m) of 1.82, whilst the control presented 0.59; besides, the synbiotic cheese presented a higher growth in the bifidobacterial, lactobacilli and eubacteria groups, and less growth in the detrimental clostridia and bacteroides groups, thus showing selectivity for beneficial bacteria for consumer health. The presence of prebiotics and probiotics in this cheese effectively promoted the increase in maximum growth rate of bifidobacterial and lactobacilli compared to other cheeses.

The main objective in this investigation was to determine the functional capabilities of the petit-suisse cheese, no further investigation on physicochemical and sensorial analyses was done.

Fermented skim milk

Amongst dairy products there is a synbiotic fermented skim milk (Oliveira et al. 2011). The formulations were as follow: (1) *S. thermophilus* (6LogCFU/mL)—*L. bulgaricus* (6LogCFU/mL)—*L. acidophilus* (6LogCFU/mL)—*L. rhamnosus* (6LogCFU/mL)—*B. lactis* (6LogCFU/mL)—4% inulin; (2) *S. thermophilus* (6LogCFU/mL)—*L. bulgaricus* (6LogCFU/mL)—4% inulin; (3) *S. thermophilus* (6LogCFU/mL)—*L. acidophilus* (6LogCFU/mL)—4% inulin; (4) *S. thermophilus* (6LogCFU/mL)—*L. rhamnosus* (6LogCFU/mL)—4% inulin; (5) *S. thermophilus* (6LogCFU/mL)—*B. lactis* (6LogCFU/mL)—4% inulin.

The fermented milk was prepared from powdered skim milk and adding inulin. Milks were heated, cooled and inoculated with commercial lyophilized microorganisms activated 15 min prior to fermentation. Firmness and microbiological analyses were done at day 1 and 21 after its elaboration.

The results of this research might support that inulin addition does increase firmness for all formulations (14% average) since firmness is total solid content dependent in this product, besides the interactions between saccharides from inulin and milk proteins. Nevertheless, it is also supported that *S. thermophilus* is the main responsible for the provided firmness, however it is observed a firmness increment when *S. thermophilus* is in co-culture with probiotic microorganisms. The firmness increment due to microorganisms is because of the exopolysaccharides production from glucose, galactose and other monosaccharides. Another important aspect to be considered is the time the product spent in cold storage had influence on firmness (13% average).

In terms of viability, the *S. thermophilus* concentration was not affected by the type of microorganism in co-culture or in cocktail, inulin addition as well as the time in cold storage were the factors that had an effect on increasing or maintaining of the different microorganisms concentration; however, all formulations meet the recommended concentration to have a beneficial effect on health. With this research was demonstrated the selectivity of certain microorganisms for inulin, *B. lactis* in particular, whose concentration was notably increased from 7.5 to 9.1LogCFU/mL in both storage conditions (1 day or 7 days at 4 °C).

In this case, inulin is added in order to improve the technological properties of the fermented milk. There is no evidence of the prebiotic effect that this food may have.

Infant formula

Another study carried out by Pérez-Conesa et al. (2006) the effect of a synbiotic infant formula on calcium, magnesium and phosphorus bioavailability in weanling rats, and its effect on physiological and nutritional parameters was investigated. The formulations used were the following: (1) 7.74LogCFU/g of *B. bifidum* and *B. longum*—1.2% galactooligosaccharides, (2) 7.74LogCFU/g of *B. bifidum* and *B. longum* —5% galactooligosaccharides, and (3) 7.74LogCFU/g of *B. bifidum* and *B. longum*—10% galactooligosaccharides.

The bacteria used in this study were in lyophilized form, for their incorporation into the milk formula they were added to maltodextrin. The tests that were carried out on the food were moisture content, crude protein, fat, total dietary fibre and ash content. The absorption and retention analyses of the minerals in the rats were carried out in three periods: from 8 to 10 days, from 18 to 20 days and from 28 to 30 days. The rats consumed on average 8LogCFU/g of probiotics per day, and of prebiotic depending on the formulation 0.07 to 0.13 g/day in the presence of 1.2% galactooligosaccharides, from 0.26 to 0.51 g/day of 5% galactooligosaccharides and from 0.59 to 1.07 g/day of 10% galactooligosaccharides. It was demonstrated that the synbiotic infant formula did stimulate the bioavailability of calcium, phosphorus and magnesium in weanling rats, specially the formulations that contained 5 and 10% of prebiotic. It was also observed that the younger the animals, the more bioavailability these minerals presented.

It is not indicated how long had passed since the synbiotic formula was elaborated until its consumption by the rats. Also, it can be observed that as time passes, the rats presented a better tolerance to ingest more prebiotics, since they were fed ad libitum (Table 1).

Other no dairy products

Bread buns

Synbiotic bread buns were developed by Pruksarojanakul et al. (2020) by means of an edible synbiotic film. The probiotic used was *L. casei,* and the prebiotics used were konjac glucomannan and inulin. The bread was traditionally made, then the film was applied by brushing. Finally, the bread with its edible cover was dried at 60 °C for

Table 1 Dairy synbiotic products

Food	Probiotics	Probiotic form added	Prebiotic	Tests in final product	Author
Yoghurt	*Lactobacillus helveticus* None (sterilized product)	Not specified	Polydextrose 1.7%	None (irritable bowel syndrome patients)	Bahrudin et al. (2020)
Yoghurt	*Lactobacillus casei* *9–10LogCFU/g*	Freshly-grown liquid culture, alginate beads and alginate beads coencapsulated with chitosan	Lactitol ≤ 1.5%	Viable count, texture and syneresis	Li et al. (2020)
Yoghurt-based drink	*Bifidobacterium bifidum* Concentration not specified	Lyophilized	Arabic gum 0.25%, 0.5% and 1%, ginger extract 0.25% and 0.5%	Titratable acidity, pH, apparent viscosity, phase separation, probiotic viability and sensory analyses	Haji-Ghafarloo et al. (2020)
Chocolate ice cream	*Lactobacillus acidophilus* Concentration not specified	Freeze-dried encapasulated	Inulin 6.7%	Quantitative descriptive analysis and affective test	Peres et al. (2018)
Semi-hard cheese	*Lactobacillus paracasei* 7.4LogCFU/g	Fresh-grown in milk culture	Fructooligosaccharides 1%	pH, organic acids and volatile compounds content, colour, water activity and dry matter content and sensory evaluation	Langa et al. (2019)
Ice cream	*Lactobacillus casei* *Lactobacillus rhamnosus* 6LogCFU/g 7LogCFU/g	Freshly-grown liquid culture	Inulin 3% and 6%	Acidity, pH, total solids content, fat, protein, firmness, deformation of maximum load, microbiological and sensory analyses	Di Criscio et al. (2010)
Strawberry ice cream	*Lactobacillus acidophilus* 0.06% 0.13%	Freshly-grown liquid culture	Yacon flour 1.5% and 3%	Acidity, pH, viable count and acceptability of sensory attributes	Parussolo et al. (2017)
Ice cream	*Lactobacillus acidophilus* 0.3%	Lyophilized	Inulin 0.4%	Viable count, fat, total solids content, protein, coliforms, melting quality, flavour, body, texture and overall acceptation	Pandiyan et al. (2012)
Ice cream	*Lactobacillus casei* *Bifidobacterium lactis* 9LogCFU/g	Freshly-grown liquid culture Alginate beads	Resistant starch 1%	Titratable acidity, pH, dry matter, fat content, overrun, microstructure, sensory evaluation analyses and viable count	Homayouni et al. (2008)
Soft white cheese	*Lactobacillus plantarum* 7LogCFU/g	Not specified	Inulin Maltodextrin 2.5%	None (calcium absorption in rats)	Klobukowski et al. (2009)
Cottage cheese	*Lactobacillus delbrueckii* 8.2LogCFU/g	Free, re-suspended pellet	Inulin 5%	Fat, probiotic viability and sensory analyses	Araújo et al. (2010)
Fresh cream cheese	*Lactobacillus paracasei* 9LogCFU/g	Lyophilized	Inulin 8%	Minerals, fat, protein, fructans, dry matter, pH, water activity, humidity and viable count	Buriti et al. (2007)

Table 1 continued

Food	Probiotics	Probiotic form added	Prebiotic	Tests in final product	Author
Petit-suisse cheese	*Lactobacillus acidophilus* *Bifidobacterium lactis* 9LogCFU/g	Lyophilized	Fructooligosaccharides Inulin Eucalyptus honey 10%	In vitro digestion, fermentation of the digested samples by using faecal samples from two healthy volunteers, bacterial enumeration by fluorescence i*n situ* hybridisation, short chain fatty acids, total carbohydrates analyses, prebiotic effect, modified prebiotic index and sensorial analyses	Cardarelli et al. (2007)
Fermented skim milk	*Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Bifidobacterium lactis* 6LogCFU/mL	Lyophilized	Inulin 4%	Viability, firmness and microbiological analyses	Oliveira et al. (2011)
Infant formula	*Bifidobacterium bifidum* *Bifidobacterium longum* 7.74LogCFU/g	Lyophilized	Galactooligosaccharides1.2%, 5% and 10%	Viable count, moisture content, crude protein, fat, total dietary fibre and ash content	Pérez-Conesa et al. (2006)

25 min. In the final product, there is a *L. casei* concentration of 9–10LogCFU/portion, the portion is one bread (50 g). As the prebiotics concentration is not specified, it can not be assessed whether the product could be considered as synbiotic.

Chocolate

Waghmode et al. (2020) carried out an investigation with the objective of formulating a synbiotic chocolate adding a probiotic strain. There were used flax seeds as the prebiotic component, and *Leuconostoc mesenteroides* as the probiotic component. Flax seeds and *L. mesenteroides* were incubated together for 4 days; then the freshly—grown culture was added to melted chocolate, molded, and stored at 4 °C and 30 °C. The final product was tested for survival every 15 day, and antioxidant activity.

The concentration of both components in the final product is not mentioned, since it is stated that the microorganism was incubated with 9 g of flax seed, but it does not state de quantity or grams of the culture added to 125 g of chocolate. In addition, the microorganism concentration is just mentioned at the beginning of the fermentation. Subsequently, the prebiotic and probiotic concentration in the final product can not be inferred. Not only there is a remarkable percentage of survival since the first day through out the storage, but also the microorganism colony forming units per gram are not mentioned at any time. Because of the above exposed, it can not be assessed if the chocolate meets the required probiotic concentration in order to have a potential synbiotic effect.

Candy

Praveen et al. (2020) developed a candy with polysaccharide extracts from three different Indian seaweeds as the prebiotic compound. The formulations were as follow: (1) *L. plantarum* (> 11LogCFU/g)—∼ 30% *S. wightii* (brown seaweed), (2) *L. plantarum* (> 11LogCFU/g)—∼ 30% *E. compressa* (green seaweed), and (3) *L. plantarum* (> 11LogCFU/g)—∼ 30% *A. spicifera* (red seaweed). Simultaneously, inulin was used as the prebiotic positive control, and alginate as the negative control. The seaweed was mixed with alginate, and then the microorganisms were incorporated by raindrop in calcium chloride. The tests performed to the candies were survival and texture profile analysis. Furthermore, this is a product with a high potential to exert benefits upon consumers health not only because it meets the required concentration of both prebiotics and probiotics, but also because in this investigation these compounds are investigated in regards to their health benefits with promising results. It would be worthy to perform sensory analyses in the near future.

Soybean beverage

Battistini et al. (2018) carried out an investigation with the objective of investigating the acidification rate of soymilk produced with vegetable soybeans supplemented with inulin and fructoologosaccharides (FOS). The formulations used were: (1) *L. acidophilus, B. animalis* and *S. thermophilus* (0.02% mixture)—4% fructooligosaccharides, (2) *L. acidophilus, B. animalis* and *S. thermophilus* (0.02% mixture)—4% inulin and, (3) *L. acidophilus, B. animalis* and *S. thermophilus* (0.02% mixture)—4% fructooligosaccharides and 4% inulin. Besides, there was a control without prebiotics. After addition of prebiotics, the soymilk was pasteurized, and then the probiotics were added as a freeze-dried commercial culture. The inoculated soymilks were incubated at 37 °C until a pH of 4.7–4.8 was reached and finally stored at 5 °C for 28 days. The tests applied to this fermented beverage were pH, total solid, moisture, ash, protein, fat and carbohydrates content, stachyose and raffinose content, lactic and acetic acids and viable count.

The viable count after 28 days storage meet the requirements to consider this beverage as a probiotic food, on the other hand, the prebiotics were added only in order to investigate the effect on probiotics survival. There was no significative difference with respect to the control. Not only this beverage has a synbiotic potential, but also more tests are needed to assess it as a synbiotic functional food.

Mousse

In 2019, with the objective to improve probiotic survival to in vitro gastrointestinal stress in a mousse, Santos et al. optimized the spray drying process for the microencapsulation of *L. acidophilus* La-5, using inulin as coating agent. The mousse production process was the mixture of the ingredients, thermal treatment, cooling, probiotics addition, cooling, aeration, packaging and storage. As the aim of this investigation was to optimize a spray drying process, the probiotics and prebiotics proportion in the mousse formulation was kept constant.

This food product meets the requirements for being a synbiotic food, besides inulin, it has fructooligosaccharides, and it has prebiotic and probiotic potentials.

Andean blackberry slices

The objective of Rodríguez-Barona et al. (2012) was to develop a potentially synbiotic vacuum impregnated product of Andean blackberry slices. For this purpose, they used the following solutions: (1) fructooligosaccharides solution (0.45% in final product)—*L. casei* (9LogCFU/mL) and (2) Andean blackberry juice–fructooligosaccharides solution (0.22% in final product)—*L. casei* (9LogCFU/mL).

The blackberries were washed, cut, and subjected to impregnation solutions (microorganisms were reactivated before use and added as pellets) under vacuum pressure and stored at 6 °C for 72 h. The tests that were performed on the blackberry slices were viable count every 24 h, moisture content, fructooligosaccharides content and impregnation parameters.

The probiotics viability maintained in the slices was measured for 72 h with a reduction of less than 2 logarithmic cycles; in addition, no significant differences were found in each one of the samples viability with the different impregnation solutions.

This product complies with the required dose to show a probiotic effect, however, although authors publish it as a synbiotic food, the doses of prebiotic are well below to the recommended daily dose to exert a beneficial effect on consumer health. Although it should be noted that the managed dose of prebiotic is useful for the microorganisms viability in the blackberry slices during 72 h of storage at 6 °C.

In this research the probiotic is used in free form, without a technology providing extra protection, only the blackberry porous matrix in which the microorganisms are placed in this product. The proportions of juice and fructooligosaccharides are not mentioned.

Fermented soy food

Mondragón-Bernal et al. (2017) used the Plackett & Burman experimental design applied for 12 trials and 4 central points in a total of 16 assays in order to elaborate a fermented soy food. Amongst the independent variables are: the ratio of *L. rhamnosus* and the standard mixture of probiotics (30:10/60; 20:20/60; 10:30/60 being (*L. acidophilus* + *L. paracasei*) *L. rhamnosus/B. longum*), fructooligosaccharides (0, 2 and 4% w/v) and polydextrose (0, 2 and 4% w/v) amongst other variables. The combinations were made according to the design of experiments, however, for the microorganisms ratios, in all cases a total count of 6.7LogCFU/mL was considered.

The ingredients of the fermented product were soy extract as the main ingredient in the fermentation medium, with or without sucrose, fructooligosaccharides and/or polydextrose, and silica as antifoaming agent. Viable count was made at the end of the fermentation (day 0), and on days 10, 20 and 30 of storage at 4–6 °C. The probiotics concentration after 30 days of storage remained between 8 and 11.18LogCFU/g, the higher the viability, the lower the pH, up to a value of 4, however, such a low pH leads to sensory problems. The prebiotic concentration is within the recommended dose for a prebiotic potential to occur.

Cereal mix, traditional Indian dry snack, and dry malted drink

Moumita et al. (2017) developed several synbiotic foods. First, synbiotic capsules were elaborated to add them to different foods. As prebiotic an extract from *Pleurotus ostratus* (4%) was used, it was mixed with sodium alginate beads at 2%, then, the lyophilized bacteria were added. The synbiotic capsules were lyophilized at − 5 °C. The bacteria used as probiotics were *L. bulgaricus, L. acidophilus, L. fermentum* and *L. plantarum*, which were microencapsulated separately, thus having four types of synbiotic microcapsules.

Once the synbiotic microcapsules were obtained, they were mixed in the following commercial dry foods in a 1:1 ratio: a mixture of local cereals, a traditional Indian fried food and a dry malted drink. For viable count study, in the case of the cereal mixture (which was stored at room temperature), it was done every week for three weeks after performing a simulated in vitro digestion. For the cases of traditional fried food and the powdered drink, they were stored at room temperature for two weeks, and the viability study was performed by separating the beads from the food matrices, dissolving and pouring them on MRS agar, and incubating for 48 h at 37 °C.

In the case of cereal mixture, the synbiotic beads presented a survival between 71 and 90% depending on the microorganism, after 3 weeks of storage at room temperature. There were no significant sensory differences between the foods with the synbiotic beads and the control foods (without the presence of the beads).

It should be mentioned, that it is not reported the initial microorganisms concentration in the beads, and the results are reported in LogCFU/10 mg of beads, and under this assumption, the food do have the necessary doses of both prebiotic and probiotic, so that they can exert a beneficial effect on consumer health. It is also noteworthy, that in this study the foods were not developed or elaborated, only the beads that were added to dry foods already marketed. The method of encapsulation by alginate beads was not very efficient since the authors themselves recommend a double encapsulation.

Salad dressing

Mantzouridou et al. (2012) carried out an investigation with the objective of adding inulin, taking advantage of its technological properties to stabilize a dressing emulsion, besides improving the viability of the probiotic microorganisms added to the product. Different formulations were used. Each one had *L. paracasei* (8.2LogCFU/g) and the inulin concentration varied from 1 to 6% for the different formulations. 20 experiments were done according to an unblocked full factorial central composite statistical design with five levels and three factors. The first factor was the storage time, its five levels were 0.3, 2, 4.5, 7 and 8.7 weeks; the second factor was inulin concentration, its five levels were 0.9, 2, 3.5, 5 and 6% (w/w); and the third factor was yolk concentration, its five levels were 0.9, 2, 3.5, 5 and 6% (w/w). The probiotics were added in two ways: in the aqueous phase (where they remained free) and in the oil phase (leaving the cells trapped or "protected" in the oil drops). The dressing was tested for viability in the weeks 1, 2 and 4 of refrigerated storage at 4 °C; however, these tests had to be performed after a gastrointestinal simulation, since the microorganisms could not be released from the oil drops, so there were no viable counts after the different storage times to know directly if the food complied with the minimum recommended dose to exert its probiotic effect on consumer health. After the gastrointestinal simulation, the dressing presented the minimum recommended dose, so that, it had a probiotic effect for up to a week.

The prebiotic effect is not investigated; however, it is established that incorporating inulin to the dressing apparently provides favourable conditions to support the growth of probiotic cultures and maintain their viability over a long storage period combined with gastrointestinal simulations (Table 2).

Conclusion

In this review in synbiotic foods, just the 63% meet the necessary requirements to confer both benefits, the probiotics and the prebiotics potential on human health. The main reason is that in the other 37%, the prebiotics are added just taking care about technological properties or to benefit the added probiotics, but it is not considered the minimum prebiotic quantity in order to confer a health benefit on the consumer. The reviewed articles show that the most used carrier matrices are the dairy products,

Table 2 Non diary synbiotic products

Food	Probiotics	Probiotic form added	Prebiotic	Tests in final product	Author
Bread buns	*Lactobacillus casei*	Freshly—grown liquid culture in an edible film	Konjac glucomannan and Inulin Concentration not specified	Viable count, water activity and colour	Pruksarojanakul et al. (2020)
Chocolate	*Leuconostoc mesenteroides* Concentration not specified	Freshly—grown liquid culture	Flax seeds (*Linum usitatissimum* L.) Concentration not specified	Survival and antioxidant activity	Waghmode et al. (2020)
Candy	*Lactobacillus plantarum* > 11LogCFU/mL	Alginate beads	Polysaccharide extract of seaweed ∼ 30%	Survival during storage and texture profile analysis	Praveen et al. (2020)
Mousse	*Lactobacillus acidophilus* 9LogCFU/g	Freeze—dried	Frucooligosaccharides 6% Inulin 4%	Viable count and i*n vitro* gastrointestinal simulation	Santos et al. (2019)
Soybean beverage	*Lactobacillus acidophilus* *Bifidobacterium animalis* 0.02% Mixture	Freeze—dried	Fructooligosaccharides 4% Inulin 4% FOS and inulin 4% and 4% respectively	pH, total solid, moisture, ash, protein, fat and carbohydrates content, stachyose and raffinose content, lactic and acetic acids and viable count	Battistini et al. (2018)
Andean blackberry slices	*Lactobacillus casei* 9LogCFU/mL	Free, re—suspended pellet	Fructooligosaccharides 0.45%	Viable count, moisture content, fructooligosaccharides content and impregnation parameters	Rodríguez-Barona et al. (2012)
Fermented soy food	*Lactobacillus acidophilus* *Lactobacillus paracasei* *Lactobacillus rhamnosus* *Bifidobacterium longum* 6.7LogCFU/mL in different proportions	Not specified	Fructooligosaccharides Polydextrose 0%, 2% and 4%	Viable count	Mondragón-Bernal et al. (2017)
Cereal mix	*Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus fermentum* *Lactobacillus plantarum* Concentration not specified	Lyophilized bacteria encapsulated in alginate beads	*Pleurotus ostratus* 4%	Sensory attributes, simulated in vitro digestion and viable count	Moumita et al. (2017)
Traditional Indian dry snack	*Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus fermentum* *Lactobacillus plantarum* Concentration not specified	Lyophilized bacteria encapsulated in alginate beads	*Pleurotus ostratus* 4%	Sensory attributes and viable count (just to the beads)	Moumita et al. (2017)

Table 2 continued

Food	Probiotics	Probiotic form added	Prebiotic	Tests in final product	Author
Dry malted drink	*Lactobacillus bulgaricus*	Lyophilized bacteria encapsulated in alginate beads	*Pleurotus ostratus* 4%	Sensory attributes and viable count (just to the beads)	Moumita et al. (2017)
Salad dressing	*Lactobacillus acidophilus* *Lactobacillus fermentum* *Lactobacillus plantarum* Concentration not specified *Lactobacillus paracasei* 8.2LogCFU/g	Freshly—grown liquid cultures (aqueous and oil phases)	Inulin 0.9%, 2%, 3.5%, 5% and 6%	Simulated gastric digestion, survival during storage	Mantzouridou et al. (2012)

probably because of their acidity and pH are the most suitable for probiotics. The majority of synbiotics are found in liquid or semiliquid matrices, and just a few are found in solid matrices. Also, this review allows to observe that the probiotics concentration used ranges between 6LogCFU/g or mL to 9LogCFU/g or mL, and the concentration of prebiotics is more variable from 0.4 to 10%. Probiotics alter the physicochemical characteristics of food truly little, whilst prebiotics show a clear alteration of such characteristics. The overall results are positive, nevertheless further investigations are required on prebiotic effect of this synbiotic food, since in general terms, the objective of its use is for technological purposes.

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