

Potential functional bakery products as delivery systems for prebiotics and probiotics health enhancers

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Revised: 5 December 2017 / Accepted: 7 December 2017 / Published online: 5 February 2018
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Abstract Several health benefits have been associated to probiotics and prebiotics, most of these are involved in the regulation of the host's gut microbiome. Their incorporation to diverse food products has been done to develop potential functional foods. In the case of bakery products, their incorporation has been seen to improve several technological parameters such as volume, specific volume, texture along with sensorial parameters such as flavor and aroma. Scientific literature in this topic has been divided in three main research branches: nutrition, physical quality and sensory analyzes, however, studies rarely cover all of them. Due to the harsh thermal stress during baking, sourdough technology along with microencapsulation of probiotics, has been studied as an alternative to enhance its nutritional values and increase cell viability, though in few occasions. The potential functional baked goods have maintained acceptable physical characteristics and sensorial acceptability, while in some cases an improvement is seen due to the effect of probiotics and prebiotics. The results obtained from several studies done, have shown the viability of developing functional bakery products by applying prebiotics or probiotics. This could be used as an encouragement for more research to be done in this topic.

Keywords Probiotics · Prebiotics · Potential functional bakery product · Quality parameter

Introduction

In recent years, society has become more aware of the existing relationship between food consumption and personal health. As a response, food scientists and industry have been working on the design and development of not only more nutritious food, but also food that provide an extra health benefit to its consumer, these are known as functional foods (Siró et al. 2008). Besides their health properties, probiotics and prebiotics provide technological enhancements in characteristics of bakery products such as volume, spread ratio, specific volume and sensory properties such as flavor and aroma (Handa et al. 2012; Zannini et al. 2012).

The objective of this article is to present the role that probiotics and prebiotics play in the design and development of functional bakery products that have been made during recent years. However, it must be considered that for a successful product, it must meet certain physical quality parameters as well a good sensorial acceptance. Figure 1 cover the most known probiotic and prebiotic health benefits (Boeckner et al. 2001; Faghfoori et al. 2015; Mensink et al. 2015).

Functional foods

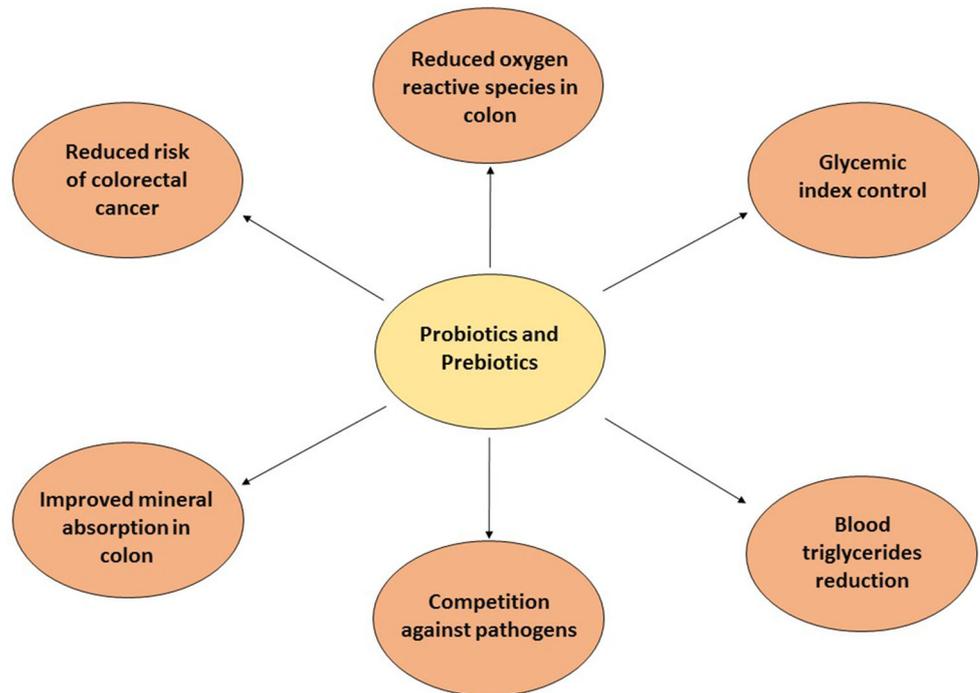
Functional foods have been defined several times, being the first time in 1980 in japan as “food products with special constituents that possess advantageous physiological effects” (Siró et al. 2008), however there is not a consensus in the definition accorded between authorities in the field.

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Fig. 1 Most known prebiotics and probiotics health enhancement benefits



Since it is a marketed product this definition not only concerns scientific principles, but legislation as well. This key factor should define what could be sold legally as a functional food product and causes the consensus of a definition to be harder. Definitions have gone from broader terms into others much more specific.

Four major types of functional foods exist, which are: fortified products, enriched products, altered products and enhanced commodities. Fortified products are foodstuffs that have additional nutrients in their composition, enriched products are developed by adding additional nutrients or components that are not found normally in that food source. An altered product is one in which non-beneficial components have been removed, reduced, or replaced with another component with beneficial effects. Enhanced commodities are products in which one of the components has been naturally enhanced through special conditions (Siró et al. 2008).

Several authority agencies have given their definition in hopes to be able to regulate functional foods. Such is the example of the International Food Information Council (IFIC) from USA, which states that functional foods are “Foods that may provide health benefits beyond basic nutrition”, the Food and Nutrition Board state “Functional foods as one that encompasses potential healthful products, including any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrients it contains” and the European Commission Concerted Action Group of Functional Food Science (FUFOSE) states “Food which could be regarded as

functional as being one that has been satisfactorily demonstrated to beneficially affect one or more functions in the body, beyond adequate nutritional effects, in a way which is either relevant to either and improved state of health and wellbeing and/or a reduction of risk” (Kaur and Das 2011). These definitions show the wide difference between definitions that exists and the difficulty of getting into a consensus.

For practical purpose, all references to functional foods in this review article will be per IFIC’s definition. However, most studies discussed don’t include in vivo assays to demonstrate a functional effect. Therefore, the bakery products will be considered as potential functional.

Up to this date there are a wide variety of potential functional products under commercial names such as Yakult[®], Actimel[®], Activia[®], Snackfibra[®], ProViva[®], among others. This products are, mostly, yoghurts and other dairy based products with the addition of probiotics (Siró et al. 2008; Kaur and Das 2011).

However, studies have been conducted for the development of different kinds of functional products such as ice-cream (Ahmadi et al. 2014), fruit juices (Fontes et al. 2015; Kumar et al. 2015), cheese (Mäkeläinen et al. 2010), chocolate (Erdem et al. 2014) as well as bakery products (Collar and Angioloni 2014; Marpalle et al. 2014).

When taking into account baked goods, it must be considered that several compounds are used in the production of bakery products, which could have an impact on their potential functionality. The ingredients, such as the flour, fat, and salt as well as the additives such as emulsifiers,

preservatives, and oxidizing agents are used. The processing aids such as lipases, proteases and xylanases are also used (Cauvain and Young 2007). The different kinds of functional breads and cookies were developed, considering the previously mentioned compounds (Handa et al. 2012; Shakeri et al. 2014; Soukoulis et al. 2014).

Functional properties of alternative flours

Few flours from alternative sources have been studied to determine their prebiotic potential or properties. Yacon (*Smallanthus sonchifolius*) flour has shown high prebiotic properties in guinea pigs due to its high fructooligosaccharides and chlorogenic acid content (Campos et al. 2012). Green dwarf banana (*Musa* sp. AAA) flour prevented intestinal inflammation in murine model's colitis due to its high content of resistant starch (RS) (Scarminio et al. 2012). Most of the research done in the development of potential functional bakery products, are developed by including probiotics and prebiotics to show an enhanced health benefit to its consumer.

As part of the research done to evaluate prebiotic potential for flours from alternative sources, several functional physical properties are measured to establish a base of knowledge of the physical behavior of the flour of interest. These physical properties end up playing an important role in key physical parameters that determine what kind of baked product can be made from it, as well as playing a role in sensorial analyzes results. Table 1 include some of these parameters, nonetheless it is important to note that individual studies rarely cover all of them and it depend of which kind of baked good is developed (Esteller et al. 2006; Cevoli et al. 2015).

There is a marked tendency in research of bakery products, which involves the design and development of new products made from non-traditionally flour sources and/or the use of composite flours. Composite flours are a mix of wheat and a non-traditional flour source (Chandra

et al. 2014). The novelty of this approach relies in the use of alternative flour sources added to wheat flour; this could be for different reasons with some of them being: modifying its physical properties, cost reduction, harnessing an abundant crop or raw material that otherwise would go to waste (Awolu et al. 2015), while providing the potential functionality. When composite flours are developed, dough's and bread's physical characteristics are modified, and should be evaluated for a successful design of a functional bakery product. These physical properties also serve as a predictor of the quality of the product that could be obtained and can be used to compare against commercially successful ones. This comparison can be done by evaluating quantitatively a parameter through a reproducible methodology, which would help define the suitability of that flour for use in bakery products.

The most common properties measured are: water absorption capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC), emulsion capacity (EC) and emulsion stability (ES) (Falade and Okafor 2014; Joshi et al. 2015). The knowledge of this properties help predict the behavior of the flour and the dough when developing the product, for instance, a flour with a high WAC will require the addition of more water to achieve the same consistency of a flour with a lower WAC. These properties play a key role in understanding the mechanical properties which could serve as predictors for the quality of products.

Depending on the interest of each research group as well as the potential functionality of the alternative flour, they might evaluate different mechanical properties. In a study made to evaluate the optimal percentage of substitution of a wheat-taro composite flour used to made biscuits, alveographic properties such as extensibility, rupture pressure, strength and elasticity index were measured to evaluate the fitness of that dough to be escalated into an industrial process. Taro has shown to play a role in the reduction of high blood pressure, showing a potential functionality. It was concluded that up to 10% taro substitution could be used and have optimal properties for development of baked goods (Himeda et al. 2012).

Functional properties from flours from alternative sources have been researched along with their physical and chemical composition parameters. Banana pseudostem was processed into flour; its swelling power, solubility profile along with WAC and OAC were measured. These parameters resulted higher than expected and greater than other high fiber food products such as oat bran, rice bran, soy flour and wheat bran, which suggests this flour would provide the benefits associated with high fiber consumption. Researchers concluded that banana pseudostem is suitable to be used as a functional ingredient due to the high fiber content as well as the WAC and OAC observed (Aziz et al. 2011).

Table 1 List of main physical and sensorial quality parameters measured in bakery products

Baked goods physical quality parameters	Baked goods sensorial quality parameters
Color	Cell uniformity
Loaf volume	Adhesiveness
Specific volume	Chewiness
Density	Crumbliness
Firmness	Dryness
Heterogeneity in cell distribution	Taste intensity
Rollability	Aftertaste
Three-point bending test	Aroma intensity

Functional properties of flours as well as chemical composition can be modified by pretreatments, thermal ones being the most studied so far. These treatments have the ability to allow starch pre-gelatinization, cause protein denaturation, Maillard's reaction enzymes inactivation, among others (Martínez et al. 2014), modifying the potential functionality an alternative flour has. Depending on the type of flour researchers are studying, they might consider applying a pretreatment per their specific needs. Further studies done on the research of some of the functional properties of alternative flours are shown in Table 2.

Potential functional probiotic bakery products

The design of potential functional bakery products including probiotics in their formulation, require a different approach due to the high temperatures in which they are baked. Since most probiotic microorganisms would be eliminated through the baking process, an alternative to provide a benefit to its consumers is sourdough technology. Sourdough technology provide the benefits of probiotics in human health by supplying bioactive compounds derived from dough's fermentation. Therefore, delivering the benefits of probiotics into consumer's health without the actual delivery of live probiotic cells. It is important to note that most research done in this topic, cover more the nutritional value rather than its physical parameters.

Sourdough is defined as a mixture of wheat or rye flour and water that is fermented by lactic acid bacteria, with or without yeasts (Gaggiano et al. 2007). Several benefits have been found using this technology (Zannini et al. 2012):

1. Wider range of aroma, flavor, and texture.
2. Increased shelf-life by a higher content of organic acids.
3. Enrichment with compounds originated from either biotransformation such as: proteins, essential amino acids, essential short chain fatty acids (SCFA) or biosynthesis for example, of vitamins.

Since sourdough fermentation can be done in both aerobic and anaerobic conditions, this has an influence of the outcome of its chemical composition as well as its structure in baked goods. A fermentation in both aerobic and anaerobic conditions using *Lactobacillus casei* N87 and a commercial yeast culture was conducted. The effect of acidification was evaluated, as well as the final biomass available in each sourdough. After 24 h, *L. casei* N87 in aerobic conditions showed a lower pH value (3.6 ± 0.11) than its anaerobic counterpart (3.7 ± 0.002). Out of the original $8.1 \log \text{ cfu/g}$ inoculated cells, both conditions showed an increase in biomass. However, the aerobic

condition showed a greater biomass increase, to a total cell count of $10.0 \pm 0.21 \log \text{ cfu/g}$ against the $9.3 \pm 0.29 \log \text{ cfu/g}$ of the anaerobic condition. These results indicate that aerobic conditions could lead to higher performance in sourdough elaboration by obtaining higher probiotic biomass, as well as a higher amount of bioactive compounds (Reale et al. 2016).

Studies in potential functional probiotic bakery products

Several studies have been done regarding the use of sourdough technology for the development of potential functional probiotic baked goods.

One of them included *Bifidobacterium pseudocatelunatum* ATCC 27919 in the development of a potential functional bread with enhanced nutritional values. Phytic acid is considered an antinutritional component causing negative effects in mineral absorption in humans and animals. Lactic and acetic acid lower the rate of starch digestion and the gastric emptying rate, respectively. *B. pseudocatelunatum* ATCC 27919, has shown to produce organic acids such as lactic and acetic, as well as phytases. Therefore, the main objective of this study was to determine the amount of organic acids that were present, the rate of phytic acid hydrolysis as well as the physical quality characteristics of the different substitution breads along with the control.

It was concluded that *B. pseudocatelunatum* can be used for production of a potential functional bread through sourdough technology with increased amount of nutritional value through organic acids and lower phytic acid. Higher amounts of lactic and acetic acids allow a lower glycemic index, two main reasons accomplish this. The first one is a lower starch digestion rate and the second one is lower bread consumption. However, the most important find of this study was decreased amount of phytic acid, reducing the amount of antinutritional components and potentially allowing improved mineral absorption. No significant difference was found in quality parameters of bread such as crumb color and crumb structure with the only exception of specific volume (decrease from 2.46 to 2.22 mL/g) and firmness (increase from 2.61 to 3.18 N). While further studies are needed in order to conclude if the lactate and acetate produced would have an effect on human health, it was found an increase of 1.83 ± 0.87 to 10.55 ± 0.54 and 2.51 ± 0.05 to $23.53 \pm 0.24 \mu\text{mol/g}$, respectively. This increase could lead to improved starch digestibility (Sanz-Penella et al. 2012), providing an additional health benefit to its consumer.

On a different study targeting a specific desired population, sourdough technology has been used in the development of a bread that targets blood pressure reduction, through the production of a higher amount of γ -

Table 2 Recent studies done of functional properties of alternative flour sources

Source	Scientific name(s)	Properties measured				References
		WAC	OAC	FC	ES	
African breadfruit	<i>Treculia africana</i> Decne	√	√	√	–	Giami et al. (2000)
		2.1 g/g	1.9 g/g	107 ml		
		√	–	√	±*	
Sweet detar	<i>Detarium microcarpum</i>	180–174%		11.3–2%	29–6.9%	Badifu and Akubor (2001)
		√	√	√	×	
		5.1 g/g	0.73 g/g	8.75 cm	3.5%	
Soybean different varieties	<i>Glycine max</i>	√	√	×	√	Heywood et al. (2002)
		3.7–4.2 g/g	1.9–2.2 g/g	1.98–2.57 ml foam/mL of N ₂ x min	18.3–23.9 min	
Cowpea	<i>Vigna unguiculata</i>	√	√	±	±	Akubor (2003)
		71%	112%	20.8%	14.1%	
Plantain	<i>Musca acuminata</i>	√	√	×	×	
		286%	143%	1.9%	2.8%	
–	<i>Pleurotus tuberregium sclerotia</i>	√	√	√	√	Alobo (2003)
		337%	30.2%	24.8%	18.1%	
Papaya kernel	<i>Carica papaya</i> L.	√	√	√	√	Alobo (2004)
		1.98 g/g	1.29 g/g	50%	58%	
Taro	<i>Colocasia esculenta</i> cv foue	√	–	√	–	Amon et al. (2014)
		198%		7.07%		
Taro	<i>Colocasia esculenta</i>	√	√	√	–	Falade and Okafor (2014)
		44–61%	22–27.5%	13.43–18.23%		
Arrowleaf elephant ear	<i>Xanthosoma sagittifolium</i>	√	√	√	–	
		32–69%	24–27.5%	7.19–14.30%		

*: might be suitable for industrial applications based on pH and sodium chloride

√/: suitable for use in food products

±: mildly suitable for use in food products

×: unsuitable for use in food products

aminobutyric acid (GABA) and angiotensin I converting enzyme (ACE) inhibitory peptides. This was targeted through sourdough fermentation using *L. brevis* CECT 8183 with the addition of a commercial protease. GABA production using sourdough technology was found to be increased sevenfold than control bread (4.99 ± 0.07 to 5860.93 ± 176.59 mg/100 g dry matter). These results suggest that the consumption of 100 g per day of that bread would be enough to display health benefits to the consumer (Peñas et al. 2015).

As well as providing an additional health benefit, other studies have evaluated the potential of sourdough technology increasing the product’s shelf-life. Three Different *Lactobacillus* species were used in a study to evaluate antifungal carboxylic acids production in wheat sourdough. *L. amylovorus* DSM19280, *L. brevis* R2Δ and *L. reuteri*

R29 were the strains used. Several acids were found in the sourdoughs, being the most abundant 3-phenyllactic acid, 4-hydroxyphenyllactic acid and 2-hydroxyisocaproic acid ranging from 0.1 and 360 mg/kg. After 48 h fermentation, it was found that *L. reuteri* R29 had the greater amount of acids mentioned before (2-hydroxyisocaproic acid and 3-phenyllactic acid) 360 and 194 mg/kg respectively, however, breads baked with sourdough fermented with *L. amylovorus* DSM19280 showed an increase in its maximum average shelf life of 6 days without showing any sign of fungi spoilage. However, it must be considered that authors did used artificial acidified dough (1.4% w/w with acetic and lactic acid in 4:1 proportion) mixed with the fermented one. Authors conclude that there is a synergistic effect between the compounds produced by *L. amylovorus* DSM19280 and the artificially added ones that show an

increase in its average shelf life (Axel et al. 2016). However, further research could be done studying the functionality of the previously mentioned organic acids, as well as sensory acceptance.

During baking, acrylamide is formed caused by Maillard's reactions coming from the carbonyl groups of reducing sugars and amino group of free L-asparagine. With the aim of reducing the amount of acrylamide production in baked goods, probiotics were used through sourdough technology in two traditional Iranian flat breads; sangak and bread roll. *L. casei-casei* DSM 20011 and *L. reuteri* DSM20016 were used in this study which quantified the amount of acrylamide as well as the sensory acceptability through 10 trained panelist evaluators using a 0 (unacceptable) to 5 (ideal) scale. It was found that fermentation with *L. reuteri* DSM20016 decreased the amount of acrylamide present in both sangak flat bread (135.06 ± 3.3 to 104.33 ± 2.9 $\mu\text{g}/\text{kg}$) and bread roll (239.1 ± 1.9 to 219.1 ± 5.3 $\mu\text{g}/\text{kg}$) while increasing its general acceptability in sangak flat bread (4.10 ± 0.5 to 4.50 ± 0.0) and bread roll (4.00 ± 0.0 to 4.70 ± 0.5) (Dastmalchi et al. 2016). These results suggest that sourdough fermentations not only provide an increased number of bioactive compounds, but also could make baked goods safer to consume.

Sourdough technology can also be used as a method for improving the baking quality of flours of alternative sources such as date seed, which is considered agricultural waste but possess several components of nutritional value such as tannins, RS, and selenium. Such is the case in a study which used *L. plantarum* and *L. brevis* along with the yeast *Saccharomyces cerevisiae*, on their own and mixed together to evaluate its effect in sensory and physical parameters. Bread formulations consisted in 10 g sourdough date seed per 100 g wheat bread to develop traditional "Barbari" Iranian flat bread. The highest specific volume, which is a key quality parameter in bread acceptability ($4.65 \text{ cm}^3/\text{g}$), was obtained in the bread developed with sourdough in co-culture of *L. brevis* and *S. cerevisiae*. Overall sensory acceptance ranged from 3.48 to over 4.35, obtaining the highest score the sourdough fermentation with *L. brevis* and *S. cerevisiae*. Still, all sourdough breads presented a higher sensory score than that of the control bread (3.48) (Habibi Najafi et al. 2016). Even if date seed flour RS content is higher than wheat, functionality tests could be done to assess the potential functionality.

On a novel approach, probiotics have also been used in food technology in the design of an edible film covering pan bread for potential functional baked goods. Using film forming solutions based on sodium alginate and/or whey protein concentrate, hydrogels that contained *L. rhamnosus* GG E-96666VTT were developed. Hydrogels were developed by dispersing dry sodium alginate 1% w/w or a blend

0.5% sodium alginate and 2.0% w/w whey protein concentrate in distilled water under agitation at 25 °C for 1 h. After the addition of glycerol acting as plasticizer at 50% polysaccharides total solids, the solutions were heated at 80 °C for 30 min. Film forming solutions were cooled down at 25 °C. Inoculation with *L. rhamnosus* was done by suspending 6 pellets in 80 mL of film forming solution and degassed by using a vacuum pump for 5 min. The probiotic edible film was applied by uniformly brushing bread's crust.

Cell's viability was determined by suspending 1 mL probiotic film forming solution in PBS as a control and 1 g of bread's crust for sample evaluation. Serial dilutions were made and plated on MRS agar, stored at 37 °C in anaerobic conditions. Enumeration was performed by triplicate standard plating methodology. Results were expressed in log CFU/g.

Several quality parameters were evaluated such as color, moisture, and water activity. The probiotic edible film had significant effect lowering the bread's luminosity (L^*). Its L^* was decreased from 68.9 ± 0.2 down to 64.0 ± 0.2 , depending of the time of film utilized. A major concern during this study was cell viability. Bread samples were microbiologically tested 2 h after baking and up to 7 days in storage. Cell viability decreased during the first 24 h, during the second to the fifth day the microorganism entered a latent phase. Finally, on the last 2 days (6th and 7th), an increase on cell viability was observed depending on the film forming material used. It was found that, depending of these materials, after 7 days of storage, breads had 6–8.4 log cfu/g of probiotic viable cells in bread's crust. After an in vitro gastric digestion simulation, it was found that a single 30–40 g bread slice was enough to deliver up to 6.91 log cfu/portion, meeting WHO recommended viable cell counts for probiotic bacteria (Soukoulis et al. 2014). This study provided valuable information that could lead into the development of novel methodologies in which viable probiotic cells are delivered through potential functional bakery products. To the best of our knowledge and the day of writing, this is the only study done addressing this research area.

Microencapsulation of Probiotics

A general definition of microencapsulation is the following: "Entrapment of a compound or a system inside a dispersed material for its immobilization, protection, controlled release, structuration and functionalization". In the context of the present article, the microencapsulation of probiotics is another approach to increase cell viability in foodstuffs and deliver their health benefits by protecting them from adverse conditions such as high temperatures, shear stress and gastric transit. Several microencapsulation

techniques are available, the most common will be briefly mentioned (Poncelet 2006):

1. Prilling: Method based on forming droplets from a needle, creating small droplets/microcapsules with low size dispersions. Generally, energy is required to reduce the droplet size.
2. Spraying: Flowing liquid through an air/liquid nozzle, causing it to explode into fine droplets.
3. Emulsification: The liquid containing the ingredient to be encapsulated can be dispersed in an immiscible liquid to form an emulsion. For microencapsulation, one favors systems allowing dispersion at low shear to avoid denaturation of the active ingredients (for example biological cells).

On few occasions this approach has been exploited in the development of potential baked goods. Using a coacervation of whey protein isolate and arabic gum, *L. paracasei* E6 and *L. paraplantarum* B1 were microencapsulated and treated under different heat stress conditions. Microencapsulated probiotics showed a much higher survival rate under stress conditions than free probiotics; comparisons showed a survival rate of 73% against < 19% in a simulated gastric environment. However, the thermal stress treatment reached 80 °C (Bosnea et al. 2014), while temperatures in bakery products usually are 200 °C during 20–30 min. While this approach showed an important increase in cell viability, the temperatures in which bakery goods are baked should be considered if the main goal of the study is developing a functional baked good. Similarly, on a different study, *L. acidophilus* LA1 was microencapsulated using sodium alginate and starch. It was found that the microorganism could survive well enough in simulated gastric conditions (pH 1.0, 1.5 and 2.0), with high salts concentrations (1.0, 1.5 and 2.0%) and up to 90 °C (Sabikhi et al. 2010).

While these last two studies showed that microencapsulation increases cell viability, further studies on the application on baked goods would provide more information about the feasibility of this technology in the development of potential functional baked products. While the thermal stress treatments went up to 80 and 90 °C, respectively in the last two studies, temperatures in bakery goods can reach up to 200 °C and up to a period of 20 min, meaning further research needs to be done. Also, the inner temperature of the baked product should be considered to determine the higher temperature's effect on the microencapsulated probiotics and thus evaluating cell viability after baking.

On a similar study which combined both edible film and microencapsulation technology, *L. acidophilus* was microencapsulated and sprayed into breads before baking. Part-baked breads were sprayed with the film and fully

baked at 180 °C during 16 min on a preheated (220 °C) convection oven. Three levels of coatings were created, from an initial concentration of 4.83E+08 UFC/bread, after 24 h there was found 1.70E+06, 1.15E+06 and 1.22E+06 UFC/bread respectively. These results show that even after baking, viable probiotic cells can be found in bread's crust. Its L^* decreased in function of the number of coatings applied, ranging from a 61.7 down to a 56.4, which indicates a browning effect. Nevertheless, sensory evaluation through a training panel showed that there was no significant difference in neither of their evaluated parameters (Altamirano-Fortoul et al. 2012).

This study suggests that probiotic edible films can be a successful approach into the development of potential functional bakery products that include microencapsulated viable cells.

To our knowledge and up to this date, no other study has related microencapsulation to probiotic survival rate during the cooking process in bakery products. This is presented as an area of opportunity that can be exploited and could bring important breakthroughs in this field. This way, the development of baked goods that not only can provide the benefits of sourdough technology, but also the health benefits of live probiotic bacteria entering the consumer system could be possible. For reference, Table 3 shows other research done in probiotic microencapsulation in other foodstuffs.

Prebiotic bakery products

A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host's health (Praznik et al. 2015). Most prebiotics are plant polysaccharides, being inulin and RS the most studied so far.

RS refer to the portion of starch that can resist digestion by human pancreatic amylase in the small intestine within 120 min of being consumed, thus reaching the colon where microbiota will ferment it. The most important sources of RS are legumes, cereal grains and flours (Fuentes-Zaragoza et al. 2011).

Regarding inulin, it has been used as a low calorie sweetener, fat replacer and texture improver by increasing viscosity (Röbke et al. 2011; Crispín-Isidro et al. 2015; Mensink et al. 2015). Inulin's caloric value is 1.5 kcal/g or 6.3 kJ/g, 25–35% of a fully absorbed fructose molecule, which represent only a fraction of other sweeteners used, providing a nutritional advantage if used to replace fat and added sugars (Roberfroid 1999).

Table 3 Recent studies done of encapsulated probiotics in food products

Food product	Probiotic microorganism(s)	Encapsulation material	Particle size	Max time stability measured	Survival rate in the food product	References
Yogurt	<i>L. acidophilus</i> <i>Bifidobacterium</i> spp.	Alginate	0.5–1 mm	8 weeks	±	Sultana et al. (2000)
		Glycerol				
		Hi-Maize starch				
	<i>L. acidophilus</i> <i>L. casei</i> <i>L. rhamnosus</i> <i>B. spp.</i>	Alginate	–	6 months	✓	Capela et al. (2006)
		Alginate	1–3 mm	7 weeks	✓	Kailasapathy (2006)
		Hi-Maize Starch				
	<i>L. acidophilus</i> <i>B. lactis</i> <i>B. bifidum</i> <i>L. casei</i>	Alginate	–	4 weeks	✓	Krasaekoopt et al. (2006)
		Chitosan				
		Alginate	–	15 days	✓	El-Dieb et al. (2012)
<i>B. bifidum</i> <i>L. rhamnosus</i>	Alginate	0.3 mm alginate	15 days	✓	Ashwani and Dinesh (2016)	
	Carrageenan	0.5–1 mm carrageenan				
Frozen-yogurt	<i>Bifidobacterium</i> BB-12	Reconstituted skim milk Inulin	–	90 days	✓	Pinto et al. (2012)
Ice cream	<i>L. casei</i> and <i>B. lactis</i>	Alginate Hi-Maize resistant starch	17.80 ± 3.55 µm	180 days	✓	Homayouni et al. (2008)
Cheese	<i>L. acidophilus</i> <i>B. bifidum</i> <i>B. longum</i>	Alginate	0.2–0.3 mm	90 days	✓	Özer et al. (2009)
		κ-Carrageenan				
		Alginate Palmitolated alginate	500 µm	30 days	✓	Amine et al. (2014)
Orange and peach juice	<i>L. paracasei</i>	Alginate Chitosan Dextran sulphate	20–120 µm	50 days	✓	Rodrigues et al. (2012)
Chocolate soufflé	<i>L. reuteri</i>	Alginate Chitosan	3 µm	–	✓	Malmö et al. (2013)
Doogh	<i>L. plantarum</i>	Alginate	–	22 days	✓	Hashemi et al. (2015)
Noodles	<i>L. plantarum</i>	Fructooligosaccharides whey protein isolate denatured whey protein isolate	10.58–89.71 µm	60 days	✓	Rajam et al. (2015)

✓: increase in cell viability after food processing

Studies in potential functional prebiotic bakery products

RS may be formed during the baking process or during the storage of the product. This type of starch known as resistant starch type 3 (RS3), can be formed due to starch chains re-associating as double helices and forming tightly packed structures stabilized by hydrogen bonds or retrogradation. A recent study evaluated the most relevant

factors affecting RS formation in breadmaking, adding different amounts of spelt flour (*Triticum aestivum* spp. *spelta*) and water along with different baking times and temperatures. RS increased from 1.18 ± 0.02/100 g all the way to 1.39 ± 0.07/100 g; water to flour ratio was found to be the reason for this increase. Lower temperature and longer baking time (120 °C/4 h) instead of a higher temperature and shorter baking time (150 °C/3 h) resulted in a higher amount of RS being formed (1.46 ± 0.12/100 g

against a $1.36 \pm 0.09/100$ g) (Amaral et al. 2016). These results suggest that RS formation can be controlled for the development of a prebiotic product based only on product formulation and processing conditions, which could result in potential functional prebiotic bread. However, the processing conditions in this study would hardly be economically viable as well as further studies are needed to assess if the increase of RS would provide a health benefit.

Most prebiotic bakery product research and development is more focused in the effect of the physical quality parameters of the products, while paying less attention into the nutritional aspect.

Wheat's bran prebiotic potential has been gaining attention and being studied along with its use in potential prebiotic bread. Some of the health benefits associated to it, include benefits in cardiovascular diseases, as well as prevention of colon cancer, obesity, and gastrointestinal diseases. In a study, the effect of different levels of bran addition ranging from 5 up to 30% in sensory and mechanical properties in durum wheat breads was evaluated. Using 10 sensory trained specialists, the different levels of bran addition were evaluated to the sensory threshold (score = 5) using a 1 (extremely unpleasant) to 9 (extremely pleasant) hedonic scale. Up to 30% bran addition was just below the sensory threshold (4.25 ± 0.29); researchers selected this addition level to be used in the following studies which involved the enhancement of mechanical properties with the addition of different gums (agar agar, modified food starch-Capsul[®], guar seed flour, tapioca starch). Breads with 2% modified food starch-Capsul[®] and guar seed flour presented improved mechanical properties such as the force needed to break (F_{break}), which increased from 0.009 ± 0.01 up to 0.15 ± 0.01 and 0.12 ± 0.02 N respectively. Sensory evaluations were carried out in samples with these two gums and saw an increase in overall acceptability, the final acceptability were 6.67 ± 0.29 and 7.38 ± 0.25 respectively (Previtali et al. 2016). These results suggest that higher levels of bran addition could be used to give a functionality while still getting acceptable sensory results.

One of the functional attributes given to inulin, is the enhancement of mineral absorption in colon. Therefore, in a study, calcium carbonate and inulin were added to flours and baked goods on different levels. An evaluation on rheological properties was performed, it was found an increase in hardness (1.25–3 N), adhesiveness (5–10 N s) and consistency (10–25 N) of the dough, important parameters in bread quality control. These results are explained by the authors by calcium and inulin both acting as dough strengtheners through interactions. This would not only provide of the health benefits of inulin, but also could further enhance its consumer's health by providing with calcium.

In the same study, flour's WAC was measured and found it was decreased and therefore, the amount of water needed due to inulin's high water retention capacity was lowered (Salinas et al. 2011). This result supports the claims that inulin can be used as a texture modifier, which presents an important alternative when non-traditional flours are used due to the lack of gluten and when dough present poor consistency.

Similarly, a bread quality study was done using inulin, calcium lactate (CaLa_2) and calcium citrate (Ca_3CI_2). Several parameters such as fermentation time (t_f), specific volume (V_s) and browning index of crust (BI) were evaluated to obtain a formula optimization. Inulin values ranged from 0 up to 13%. T_f was reduced significantly by the addition of 1% inulin and calcium citrate from 75 up to 47 min. V_s and BI increased along the addition of higher amounts of inulin. Authors conclude that fortification with inulin and Ca_3CI_2 lead to higher quality breads than that with CaLa_2 (Salinas et al. 2016). However, sensory evaluations would be ideal for further support of this conclusion.

Still with inulin, on a similar study inulin and fructooligosaccharides (FOS) were used as fat and sugar replacements. The effects they had on physical quality parameters of quick breads (scones) were evaluated. These parameters included crust and crumb color (75.9 ± 4.6 to 136.1 ± 1.7 and 39.4 ± 1 to 44.3 ± 0.7 , respectively), crust and crumb hardness (1.7 ± 0.2 to 4.9 ± 0.5 and 5.4 ± 0.2 to 9.4 ± 0.9 N, respectively) and loaf volume (91.6 to 101.5 ± 3.3 mL), traits affecting consumer acceptance. A better nutritional value was also observed since the optimized formula led to up to 10% of sugar and fat replacement along with the prebiotic benefits these breads bring to their consumer's health. However, the study does not cover sensorial analyzes which must be taken into account in order for a product to be launched successfully (Röble et al. 2011).

Prebiotics have shown that applied correctly, not only can match commercial products but also even have better results. A descriptive quantitative analysis (QDA) was performed on orange cakes with inulin added in 9 and 8.3/100 g respectively as a functional component. Parameters such as crust brownness, hardness and stickiness increased when comparing with the control cake due to the addition of inulin. Crust brownness saw an increase from 4.5 ± 1.2 to 6.7 ± 1.4 and 6.6 ± 1.4 , hardness from 3.9 ± 2.3 to 5.6 ± 2 and 5.3 ± 2.2 and stickiness from 4.0 ± 1.9 to 6.2 ± 1.3 and 5.5 ± 1.6 all respectively from control to 9 g inulin/100 g cake and 8.3 g inulin/100 g cake. These parameters are all related to consumer acceptance of a product, depending on the kind of baked product is desirable. These potential functional cakes were compared against three commercially available orange cakes. Results showed that there was no difference in the acceptance

between the functional cakes but they were better received when compared to the commercial cakes in sensory attributes such as appearance, aroma, texture, flavor and overall acceptability (Volpini-Rapina et al. 2012).

The use of prebiotics includes more than cakes and breads. Enriched cookies with fructooligosaccharides (FOS) in 40, 60 and 80% sugar replacement basis were evaluated. The primary focus was on the sweetener activity that FOS possesses, with the aims of evaluating the most acceptable percentage on a sensorial analysis for the replacement of sucrose. Physical properties of cookies such as diameter, hardness, spread ratio and height were measured to elaborate a quality assessment in the cookies produced. Diameter increased significantly with increasing FOS substitution, it increased from 5.500 ± 0.055 up to 5.830 ± 0.020 cm. Similarly, spread ratio was higher as FOS substitution levels increased, it ranged from 4.400 and up to 5.205. Both height and hardness decreased with increasing FOS substitution. Height decreased from 1.250 ± 0.01 to 1.120 ± 0.102 cm while hardness from 7319 ± 166 to 6539 ± 128 g.

All these parameters behaved as an improvement in desirable quality attributes. Furthermore, a sensorial analysis was conducted. The overall average of acceptability for control cookies was on a 1–9 hedonic scale, was 8.2 ± 0.78 . FOS substitution cookies had lower scores, the closer being the one with up to 40% FOS substitution with 8.1 ± 0.56 , however cookies with 60% had 8.0 ± 0.66 . Since 60% FOS replacement cookies provide more total dietary fiber, it was concluded that up to a 60% replacement of sucrose with FOS can be done with high sensorial acceptance and with the ability to be considered as potential functional cookies (Handa et al. 2012).

These last two results discussed, show that potential functional baked goods such as breads, cakes and cookies can perform as good as commercial products in sensorial analyzes. However, commercial products rarely provide a functional component.

Synbiotic products

The future into the development of functional products is the combination of both prebiotics and probiotics with the idea that, in the right proportions and quantities, the health benefit to its consumer will be greater than products only including either one by itself.

Since it is a relatively new tendency in food science, there is not an extensive amount of information available. Currently, synbiotics are being developed as a therapy rather than their use in formulated food products.

However, studies have shown promising results in the health enhancement of patients with different diseases

using synbiotics. HIV infections alters gut ecology. A synbiotic therapy was developed and studied on adult patients with this disease, seeking to reestablish the natural microbiota. Significant difference was found in the decrease of bacterial DNA concentrations in plasma, a decrease in the bacterial load in feces and an increase in CD4+T lymphocytes. The study concludes that a synbiotic therapy effectively decreases microbial translocation and enhances the patient's immune system, which could lead to the delay of the initiation of an antiretroviral therapy seeking to reduce the cost of therapy against HIV (González-Hernández et al. 2012).

Synbiotic therapy enhances health by reducing pathogens due to the increase of organic acids in colon. Specifically, it has been shown that synbiotic therapy reduces pathological-gram negative bacilli as a result of an increased amount of acetic acid. This was achieved by the correlation made between the decrease in pH as well as the increase of *Bifidobacterium* sp. and *Lactobacillus* sp. against the decrease of *Enterococcus* sp. and *Pseudomonas* sp. (Hayakawa et al. 2012).

To our knowledge, there is only one study concerning the use of a synbiotic bakery product to enhance human health. The study was made to evaluate the positive effects that consuming a synbiotic bread might have on the blood lipid profiles of patients suffering from type 2 diabetes mellitus (T2DM). The synbiotic components in bread were *L. sporogenes* along with inulin. Researchers found a significant decrease in levels of triacylglycerols, very low density lipoprotein-cholesterol and the ratio between total cholesterol over high density lipoprotein cholesterol. This concludes a positive effect on patients with T2DM, however further studies are needed in order to obtain more confident results and to be able to gather more information into establishing the mechanisms involved in the reduction of lipid fractions in plasma through a synbiotic therapy (Shakeri et al. 2014). However, this study didn't consider any food science perspective or any kind of sensorial acceptance.

From the food science perspective, there are no studies concerning the design of synbiotic functional bakery products, it is all about synbiotic clinical therapy. This is an area of opportunity to be developed and taken advantage of, considering the design of a synbiotic bakery product which has high sensorial acceptance as well as good physical quality parameters could be of great value.

Conclusions and perspectives

Potential functional bakery goods can be used as delivery systems for prebiotics and probiotics without sacrificing either their physical quality or their sensorial acceptance. Consumption of probiotics, prebiotics and synbiotics are

highly related to human health. Developing functional bakery products and including them in everyday diet, provide an alternative into the preservation and improvement of human health. However, it is important to note that these products are not to be taken in place of pharmaceuticals. Their best use is being taken on a regular basis which will help improve consumer's health as acting more as a prevention rather than a medical treatment. To develop a successful functional bakery product, it not only must have a demonstrated prebiotic, probiotic or synbiotic effect, but also achieve the physical quality characteristics that are needed and have a good sensorial acceptance for it to be consumed with low to none rejection.

The use of alternative flour sources for the development of potential functional bakery products not only provides different flavors and aromas, but they can also appeal to a different consumer base. Non-traditional flour sources that happen to be gluten-free can be consumed by celiac patients which otherwise wouldn't be able to consume these products. The prebiotic, probiotic or synbiotic component from potential functional bakery products could enhance human health in patients with diseases such as colorectal cancer and T2DM, as results from studies with other functional foods suggests (Rafter et al. 2007; Ejtahed et al. 2011).

Acknowledgements This work was financed by the Mexican Consejo Nacional de Ciencia y Tecnología (CONACYT) through a graduate studies scholarship.

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