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Enrichment of bread with encapsulated probiotics as a functional product containing bioactive compounds: Principles, outcomes, and challenges

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ABSTRACT

Bread is a well-known and popular product among people around the world. The processing stages involved, from flour milling to baking, can result in the degradation or loss of certain nutritional and bioactive compounds. Enrichment of bread with probiotics, despite their health-promoting properties and the production of a new product with high competitiveness at the commercial level, has challenges in terms of technology and production. For this study, articles were selected by order of preference in Web of Science, Scopus, Scimago, PubMed, Science Direct, Functional Food Science Publisher, Google Scholar and Google from 2000 to 2025 using a combination of the following keywords: "Enrichment", "Fortification", "Probiotics", "Prebiotics", "Synbiotics", "Encapsulation", "Bakery products", "Bread", "Cereals", "Baking", "Survival", "Viability", and "Bioactive compounds". Accordingly, the challenges for incorporating probiotics into synbiotic breads, types of probiotics and functional synbiotics in bakery products, new and common methods for encapsulation of probiotics for enrichment of different synbiotic breads, application of encapsulated probiotics in enrichment based on the types of breads, and thermal stability of probiotics during baking are discussed. Breads containing encapsulated probiotics produced on a commercial level and the therapeutic properties of these breads are also investigated.

Abbreviations list

CFU	colony forming units
CTD	colon-targeted delivery
FTIR	Fourier-transform infrared spectroscopy
GABA	γ-amino butyric acid
GIT	gastrointestinal tract
GMB	gut microbiota
OA	overall acceptability
PRE	prebiotic
PRO	probiotic
PRO_{CAP}	encapsulated probiotics
SD	sourdough
SEM	scanning electron microscopy
SGI	simulated gastrointestinal
Syn	synbiotic
Syn-BR	synbiotic bread
T2D	type II diabetic
TC/HDL-C	total to high-density lipoprotein cholesterol

1. Introduction

Cereals and their products are considered as a great source of micronutrients (vitamins E and B, and minerals) and macronutrients (fibers, proteins, lipids, and carbohydrates) (McKevith 2004; Beikzadeh et al., 2016). Most cereal grains are milled to flour, which are then mixed

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with water and other additives to form a dough for baking. Therefore, some type of mechanical (grinding, milling) and thermal (baking) treatments are usually performed during the process of converting flour into dough and bread (Alldrick 2017; Beikzadeh, Shojaee-Aliabadi et al. 2019). In this process, some treatments are important to make the bread palatable and increase its digestibility, but they may lead to a reduction of some bioactive compounds (bioactives) such as some vitamins, minerals, and prebiotic components and consequently a reduction in the nutritional value of the product. As a result, it seems necessary to return the lost components to the formulation during the fortification process (Beikzadeh, Peighambardoust et al. 2018; Kamali Rousta, Bodbodak et al. 2021).

Furthermore, bread is a well-known and staple food product throughout the world, which is affordable, commonly eaten daily, highly popular in terms of taste and aroma, and has high diversity potential, representing a carrier of choice for other health-promoting bioactives (Betoret and Rosell 2020). Therefore, bread can be enriched with bioactives that can improve health and reduce various diseases without changing or reducing the organoleptic characteristics of the product (Alashi, Taiwo et al. 2018). Accordingly, there is an increasing demand from the bakery industry to adopt encapsulation technologies in order to meet both purposes of fortification and enrichment. In fact, production of food products, especially enriched bread, requires complex properties from food ingredients that are often impossible to achieve without encapsulation, e.g., delayed release, thermal stability, and appropriate sensory characteristics (Shamil, Nautiyal and Omar 2023). Encapsulation leads to particles with milli, micro, and nanometer diameters, in which one component is surrounded by another material (Alemzadeh, Hajiabbas et al. 2020, Mirza Alizadeh et al., 2022). Encapsulation of bioactives using different wall materials as a carrier is a great approach that can protect bioactives from destruction in adverse *in situ/in vivo/in vitro* conditions and environments (such as food matrix, different processing stages, and intestinal fluid and/or gastric acid). Furthermore, it leads to enhanced solubility, controlled release, high stability, and improved bioavailability and biocompatibility of products. The most important bioactives for encapsulation can include probiotic bacteria (PRO), carotenoids, phenolic compounds, protein-pigment materials, nucleic acids, vitamins, essential oils, proteins/peptides, enzymes, and fatty acids (Rostamabadi, Assadpour et al. 2020).

PRO are known as "living organisms that if ingested in adequate quantity, confer health benefits in the host"(FAO 2006). Prebiotics are non-digestible compounds that contribute to the well-being of their host by favorably activating or selectively stimulating the growth of a number of indigenous non-pathogenic bacteria. Synbiotics are a combination of prebiotics and PRO that act synergistically (Seyedain-Ardabili, Sharifan and Ghiassi Tarzi 2016). Incorporating PRO into bread due to the high temperatures applied during baking is challenging, which can lead to a significant reduction in PRO viability (Zhang, Chen et al. 2018). However, there is a high and emerging research interest in PRO-enriched bakery products and the production of functional products (Umaña, Bauer-Estrada et al. 2024). It is reported that the annual trade in PRO-enriched foods is one of the largest worldwide, while there are no recorded statistics on the trade in PRO-containing bread. Furthermore, according to a study, the global necessity for the bread market with an annual growth rate of approximately 3.6 % is over $200 billion (Sadeghi, Ebrahimi et al. 2024).

Various studies have been conducted on the use of PRO in bread enrichment. In this regard, whole wheat and white bread was enriched with *Bacillus coagulans* GBI-30 6086 spores. The results showed that adding PRO had no effect on the properties of final product (pH, water activity, color, texture, specific volume, and moisture). Besides, fermentation and mixing phases did not change the number of PRO in manufactured bread. The greatest decrease in the number of PRO occurred in the crust during the baking phase (Almada-Érix, Almada et al. 2022). In another study, cream bread was produced with *Lactobacillus acidophilus* and alginate 2 % + maltodextrin 1 % + xanthan gum (XG) 0.1 % carriers created the best PRO protection (Thang, Hang et al. 2019). Also, it was reported that *Lactobacillus casei* was more resistant to high temperature compared to L. *acidophilus* LA-5. Adding chitosan to hi-maize resistant starch and calcium alginate as carriers significantly increased the viability of PRO (Seyedain-Ardabili, Sharifan and Ghiassi Tarzi 2016). Similarly, bread was enriched with *Bacillus subtilis* and *Lacticaseibacillus rhamnosus* (Côté, Dion et al. 2013). When sourdoughs were incorporated with *Saccharomyces boulardii, Lactiplantibacillus plantarum*, L. *rhamnosus*, and *B. coagulans*, there was an increase in the amount of arabinoxylans in bread and an improvement in the solubility of sourdough (SD) in water (Koj and Pejcz 2023). In another work, *Bifidobacterium bifidum* KD6 added to bread which enhanced amounts of released magnesium and zinc while decreased the released calcium and iron from bread (Nalepa, Siemianowska and Skibniewska 2012).

Present review is mainly focused on the principles, outcomes, and challenges associated with the fortification/enrichment of bread with PRO bacteria, in particular, production of synbiotic bread. We likewise discuss different types of PRO used in bread enrichment, the most common encapsulation methods, as well as the various types of fortified bread with encapsulated PRO (PRO_{CAP}), along with thermal stability PRO_{CAP} during baking process of bread. This review concludes with remarks on the challenges and prospects for commercialized breads containing PRO_{CAP}.

2. Synbiotic bread: challenges for incorporating functional ingredients

Bread is one of the most popular foods for fortification with a wide range of ingredients including plant- or dairy-based components, minerals, biological and synthetic improvers. This staple food has several types such as yeasty or SD fermented, flat or loaf, and single or mixed flour formulation in all over the world with different or special baking methods like heating oven, steamed bread and/or processed using other novel technologies e.g., microwaves and infrared heating or their combinations (Aryashad et al., 2023; Pahlavani et al., 2024). Among fortified breads, PRO/synbiotic (Syn) added types have promising techno-functional and health-promoting potentials that can be considered as future staple foods. The Syn bread (Syn-BR) is one of the most interesting bakery products due to its emerging capabilities. Combined application of PRO cultures and prebiotic (PRE) ingredients has undeniable effects on both gut microbiota (GMB) modulation and immune system regulation thanks to the direct or indirect effects of these functional supplemented materials (Yazici, Ozoglu et al. 2023; Amiri, Hosseini et al. 2024; Sadeghi, Ebrahimi et al. 2024; Abedinia, Zambelli and Hosseini 2025).

PRO are viable microorganisms with positive effects on human health when consumed in proper population (about $10^6 10^8$ colony forming units (CFU)/mL or g of the product) and adequate survival under gastrointestinal tract (GIT) conditions. Along with other notable capabilities, these beneficial microorganisms able to reduce harmful components and risk of biohazards that are also important key phenomena in human health and well-being. Syn term refers to the combined application of PRO and PRE. PRE ingredients are non-digestible components that enhance PRO activity and viability. Considering health-promoting capabilities of PRE components, their application in bread as staple food is an interesting opportunity to improve human health especially in their combination with PRO. It is possible to add PRE ingredients as a part of bread formulation and/or as a part of wall materials used in encapsulation/coating systems. It is reported that *Lactobacillaceae* and *Bifidobacteriaceae* are the predominant PRO used in potential Syn-BR formulations. However, *Bacillus coagulans*, a heat-resistant bacterium, and *Saccharomyces boulardii*, a PRO yeast, have also promising applications in this field. Besides, inulin is the most commonly used PRE in Syn-BR formulations (Sadeghi, Ebrahimi et al. 2022; Yazici, Ozoglu et al. 2023).

As shown in Fig. 1, the journey of PRO and PRE incorporation into

Fig. 1. The involved parameters and stresses affecting probiotic survival in synbiotic bread during its journey to the final destination, as well as the key product processing and quality features. GIT: gastrointestinal tract, PRO: probiotics.

the baked goods is interesting and important for their efficient delivery into the colon as the final destination. These functional components must be able to pass harsh conditions and mechanical stresses of the dough making process. Then, they should withstand the osmotic, low pH and nutrient stresses during SD fermentation or yeasty proofing. Subsequently, thermal stresses of the baking step as the most important obstacle during this journey affect viability and activity of PRO. Next, considering the conditions of the storage period, several factors including *aw*, temperature, relative humidity and atmosphere packaging affect survival of PRO. After consumption of the product and under GIT conditions, PRO must tolerate mineral ions, mucin and mastication of the mouth, low pH of the stomach, bile salts and alkaline pH of the intestine, and their digestive enzymes, especially harsh conditions of the upper GIT transit before arrival into the colon (Sadeghi et al., 2023a; Sadeghi, Ebrahimi et al., 2024).

PRO and PRE provide numerous health benefits, and their incorporation into the bakery products enhances technological properties of the product. Research in this area primarily focuses on some aspects of the product, but comprehensive studies encompassing all characteristics remain limited. Due to the challenges of baking process, SD fermentation and encapsulation are promising strategies for improving PRO viability and nutritional benefits in Syn-BR. Accordingly, efficient protection of PRO during their journey is a fundamental part of designing a PRO/Syn bread. Encapsulation is a promising way to achieve this goal. There are different encapsulation approaches with a variety of wall materials that have their own opportunities or challenges in terms of bread matrix and/or bread processing chain, as well as logic of encapsulation. Considering the importance of viability, targeted delivery and controlled release of PRO, it is necessary to design a specific carrier for each purpose (Longoria-García, Cruz-Hernández et al. 2018; Sadeghi, Ebrahimi et al. 2024).

Generally, the diets enriched with PRE or Syn contribute to the improved lipid and glucose metabolism in type II diabetic (T2D) patients (Mahboobi, Rahimi and Jafarnejad 2018). For instance, impact of Syn-BR consumption (containing *Lactobacillus sporogenes* and inulin) on lipid profiles in T2D patients led to the significantly lowered serum triglycerides, ratio of total cholesterol to high-density lipoprotein cholesterol (TC/HDL-C) and very low-density lipoprotein cholesterol while increasing HDL-C levels (Shakeri, Hadaegh et al. 2014). Moreover, consumption of the same Syn-BR among diabetic patients had beneficial effects on insulin metabolism and reduced serum insulin levels compared to the PRO and control breads (Tajadadi-Ebrahimi, Bahmani et al. 2014). Also, Syn-BR consumption reduced insulin levels and glycated hemoglobin in T2D patients, supporting potential metabolic benefits. Besides, the combination of lactic acid and Syn-BR further improved antioxidant enzymes activity (Ghafouri, Zarrati et al. 2019; Ghafouri, Heshmati et al. 2022; İncili, Razavi et al. 2025).

While early studies suggest potential health advantages of Syn-BR for diabetics, further clinical trials are necessary. Moreover, textural features and staling rate of the produced bread, its overall acceptability (OA) and organoleptic properties, shelf-life and storage stability, as well as nutritional content or health-promoting capabilities are important parameters affecting the progress in development of Syn-BR world-wide. Accordingly, combined applications of PRO cultures with bakery improvers like SD, essential oils, enzymes and other additives are recommended to enhance the quality attributes of the product. Considering the importance of mold-free shelf-life and staling rate as the most important obstacles in bread marketing, storage stability and/or consumer acceptability, combined application of PRO cultures and these bakery improvers is an ideal strategy to enhance quality characteristics of the produced Syn-BR.

3. An overview of probiotics and their application in bakery products

There are different types of PRO with promising functional capabilities and emerging potential applications in a wide range of food matrices. It should be noted that, each food has its unique formulation, processing and storage situations. Bread-making process involves mechanical, thermal and biochemical stresses that affect pivotal prerequirements of PRO like their viability, adhesion and antimicrobial activities during processing and storage conditions of the product. Usually, PRO can be used as adjunct cultures in bread-making; therefore, it is necessary to enhance techno-functional properties of the product using a proper starter culture especially in terms of PRO SD breads. Some PRO are also starter cultures in the production process of these products with other applied capabilities such as antifungal activity, which enables them to be used as starter protective cultures. Although PRO cultures in bread processing are usually adjunct cultures, they have also some proper effects on technological functionalities of the product probably due to their interactions with other microorganisms and/or bioactives. These PRO cultures are especially responsible for improvement of health-oriented capabilities of the product. Moreover, some nutritional/pro-functional effects have been reported for these PRO. Accordingly, it is possible to select proper starter cultures for combined application with PRO as co-cultures and modify the bread-making process. Combination of PRO cultures with baker's yeast and/or SD starter cultures may affect negatively their techno-functional features. So, it is hypothesized that the quality characteristics of the produced PRO SD or yeasty bread should be evaluated for their combined applications. Besides, possible diverse effects of PRE ingredients increase the complexity of the process in terms of Syn-BR (Sadeghi et al., 2022, 2023a; Ziaee Rizi et al., 2024).

Dairy-based PRO foods are widely available, but they pose challenges like high fat content, allergenicity, lactose intolerance and refrigeration needs. To address these obstacles, non-dairy PRO alternatives, like bakery products, have been explored. There are some approaches for application of PRO in bread processing. Use of thermophilic PRO bacteria like *Bacillus* sp., PRO-added edible films, 3D printed products containing PRO, and PRO_{CAP} are common methods for incorporating PRO in bread-making process. Considering multiple stresses during bread-making and storage conditions, among the available strategies, encapsulation has received considerable attentions. By this method, it is possible to functionalize PRO cultures along with their protection from adverse conditions of processing, storage and or GIT transit. Along with protective effects of encapsulation on survivability of PRO during their journey into the colon as the final destination, encapsulation has crucial effects on controlled release and/or colon-targeted delivery (CTD) of PRO. Moreover, it is possible to add other functional ingredients through coating materials into the final product. This opportunity enables us to produce functional breads with enhanced quality and extended shelf-life, as depicted in Fig. 2 (Sadeghi, Ebrahimi et al. 2024; Zhang, Sadeghi et al. 2024). Potential strategies for incorporating PRO in baked goods including edible films, spore-forming bacteria, and post-baking PRO addition have been reviewed by Mani-López, Ramírez-Corona and López-Malo (2023). Moreover, Arepally, Reddy et al. (2022) reviewed encapsulation techniques e.g., emulsion, extrusion, freeze-drying, spray drying and spray chilling to enhance PRO viability in bakery products (Table 1).

In this regard, the positive effect of baobab pulp powder on *Lactobacillus rhamnosus* GG viability during post-baking in white pan bread and gastrointestinal passage was verified (Adedeji, Okehie and Ezekiel 2022). These additives did not significantly impact Syn-BR volume, moisture or nutritional composition. Baobab pulp powder also enhanced the product quality and exhibited superior texture and elasticity compared to the control sample, demonstrating its PRE potential. In the same vein, a bilayer emulsion film comprising blueberry anthocyanin extract + gellan gum (as the inner layer) and baobab seed oil + gelatin (as the outer layer) was developed to enhance survival of *Bifidobacterium longum* CICC 6068 during storage of the manufactured bread. Scanning electron microscopy (SEM) and Fourier-transform infrared (FTIR) spectroscopy confirmed successful PRO entrapment, and the bilayer film maintained high bacterial viability. The film's potential was demonstrated in steamed bread applications, offering a novel PRO delivery system (Yang, Li et al. 2023). Additionally, konjac glucomannan-based edible films with L. *casei* 01 and chicory-derived inulin extended PRO stability in bread coatings compared to the control (Pruksarojanakul, Prakitchaiwattana et al. 2020).

4. Application of probiotics in dough and its effects on the nutritional quality of the bread

Among various food matrices, bread presents a unique opportunity for fortification with PRO, especially through encapsulation techniques that protect the viability of these microorganisms during processing and storage. Incorporating PRO_{CAP} has been found to positively influence bread quality and sensory attributes without substantial differences in consumer acceptance, and as a result, leading to longer shelf-life (Pejcz 2024). The development of PRO-enriched bread aligns with growing consumer interest in functional foods that offer health benefits, such as reduced triacylglycerol levels and improved gut health (Mani-López, Ramírez-Corona and López-Malo 2023). Several studies on bread fortification with PRO_{CAP} are summarized in Table 2, which will be discussed in the following sections.

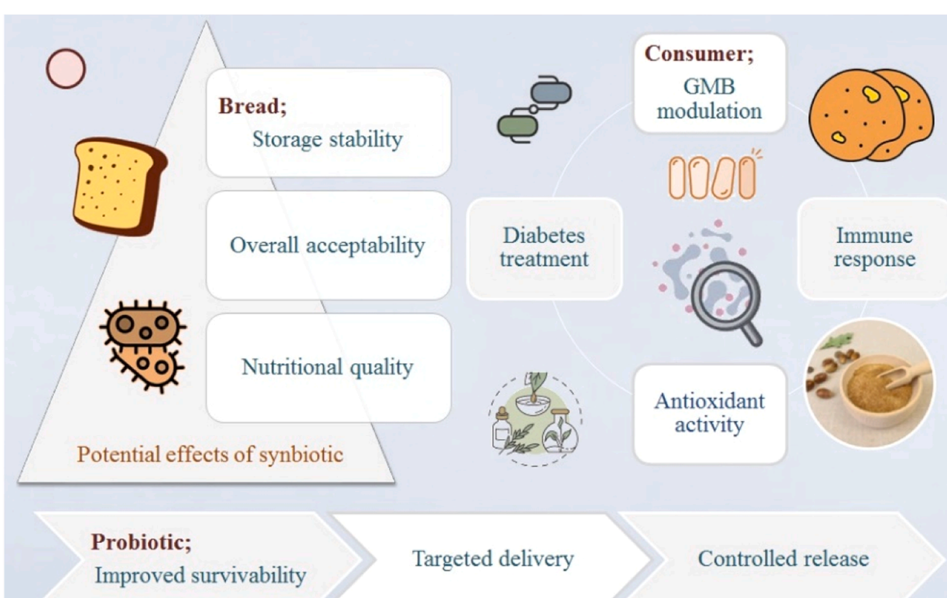

Fig. 2. Potential effects of synbiotic addition on properties of the produced bread, probiotic capabilities, and consumers' health/well-being. GMB: gut microbiota.

Table 1
Some of the most common encapsulation methods for probiotic protection in bread matrices.

Method	Principles	Considerations	Ref.
Electrospinning	A high electric field is applied to a fluid, either a solution or a melt, as it exits the die tip, which serves as an electrode. This causes the droplet to deform and eventually eject a charged jet toward the opposing electrode, resulting in the formation of continuous fibers	It offers the advantage of producing ultra-thin fibers or capsules, often just a few nanometers in size, with a high surface area. Additionally, its simple process and scalability make it a highly appealing technique for various applications	Martín, Lara-Villoslada et al. (2015)
Emulsion	This process consists of two phases: a small amount of cell polymer solution as the dispersed phase and a larger quantity of oil as the continuous phase. An emulsion is formed by dispersing these immiscible liquids with a stabilizer that prefers the continuous phase, achieved through homogenization	A solidifying agent introduced into the homogenized mixture to crosslink the water-soluble polymer, rendering it insoluble and forming dispersed phase droplets. The resulting microbeads then collected via filtration or centrifugation. Their size is influenced by factors such as the water-to-oil ratio, surfactant level, viscosity and concentration of the emulsion, emulsifier type and agitation speed	Camelo-Silva, Verruck et al. (2022)
Extrusion	A hydrocolloid solution with microbial cells is extruded through a syringe needle at high pressure into a crosslinking solution, causing gelation and bead formation after a short period	Bead size affects PRO protection, with larger beads offering better survival. Factors influencing bead size include the distance from the nozzle to the hardening solution, hardening time and nozzle diameter, as well as temperature, viscosity and concentration of the hydrocolloid solution	Bamidele and Emmambux (2021)
Fluid bed	A Wurster-based fluidized bed system is used to spray and dry the microbial suspension on inert carriers. This process offers lower cost and temperature control, but is challenging to master and time-consuming. Prior to drying, PRO cultures must be encapsulated in a supporting material	Fluid bead encapsulation is easily scalable, making it a popular commercial technology for PRO. Several companies have developed products using this method, and it can also be adapted for multilayer coatings	Agriopoulou, Tarapoulouzi et al. (2023)
Freeze drying	Under vacuum, the solvent in the solution freezes, concentrating the unfrozen portion and causing chemical and osmotic stress. During primary drying, the frozen solvent is sublimed at low temperatures, and the unfrozen portion is removed via desorption in 2nd drying	While this approach is used commercially for its gentler effects on PRO, leading to higher survival rates, it is expensive due to its high maintenance, low production rate, operational and energy costs	Rezvankhah, Emam-Djomeh and Askari (2020)
Spray chilling	In spray chilling, cool air is used to solidify droplets by cooling the atomization spray, while spray drying uses hot air to evaporate the solvent. The solidification in spray chilling depends on the cooling chamber's temperature and material properties such as surface tension, concentration, viscosity, and with the temperature kept below the gelling/melting point	Since spray chilling operates at low temperatures, it is suitable for encapsulating heat-sensitive products like PRO. This process typically uses hydrophobic materials like oils, fats and triglycerides as encapsulants, making the beads water-insoluble and protecting PRO cells. Lipid coatings offer protection, enhancing organism viability during storage and enabling controlled release in the GIT	Arepally, Reddy et al. (2022)
Spray drying	A liquid slurry is formed by dispersing the core material into a polymeric solution to create an emulsion, which is homogenized and atomized into the drying chamber through a pressure nozzle. The high inlet temperatures cause rapid surface evaporation, while the dried particles are exposed to mild temperatures, reducing thermal degradation of the PRO	Operating at lower inlet temperatures reduces water evaporation, leading to aggregated particles, while higher temperatures can damage PRO cells, reducing their survival. However, cell viability is more influenced by the outlet temperature	Huang, Vignolles et al. (2017)

GIT: gastrointestinal tract, PRO: probiotics.

4.1. Wheat breads

Encapsulation of L. *plantarum* was explored using a spray-drying technique, employing maltodextrin and sweet whey as encapsulating agents. The study investigated PRO survival under various conditions (heat treatment, storage, and SGI), as shown in Fig. 3a (Umaña, Bauer-Estrada et al. 2024). The viability of L. *plantarum* was 7.6 Log CFU/g after baking for 180 °C and 15 day-storage. Also, encapsulation of L. *plantarum* maintained a survival in bread of 6.1 Log CFU/g after

Table 2
Examples of different breads fortified with encapsulated probiotics.

Bread type	Probiotic strain	Wall material	Encapsulation technique	Results	Ref
Bread	*L. paracasei*	Calcium alginate and chitosan	Freeze-drying	• EE: 97.97 and 96.71 % • High L. *paracasei* survival; 89.51 % and 96.90 % in wet and dried microbeads, respectively • Effective fermentation • Improved bread quality	(Zadeike, Gaizauskaite et al. 2024)
Soft bread	*L. plantarum*	Maltodextrin and whey	Spray drying	• Survived bacteria (7.6 Log CFU/g) in baking at 180 °C and 15 days storage	(Umaña, Bauer-Estrada et al. 2024)
Gluten-free sorghum bread	*L. plantarum, L. acidophilus*	Tragacanth gum	Freeze-drying	• Improved viability of bacteria by 2 Log CFU/g	(Ghasemi, Nouri and Mohammadi Nafchi 2024)
Bread	*L. acidophilus LA-5*	Alginate/fish gelatin	Emulsion method	• Increased viability of bacteria by 2.49 and 3.07 Log CFU/g during baking and storage • Reduced staling rate	(Hadidi, Majidiyan et al. 2021)
Bread	*L. acidophilus*	Xanthan/alginate Chitosan/gellan	Fluidized bed granulation	• Acid resistance: high viability with 1 % alginate • Heat resistance: high viability with 0.5 chitosan • High survival on fresh bread and 24 h after baking	(Mirzamani, Bassiri and Tavakolipour 2021)
Bread	*B. lactis*	Hydroxypropyl cellulose/ sodium alginate and microcrystalline cellulose	Freeze-drying	• Increased resistance to extremely high temperatures and low pH	(Penhasi, Reuveni and Baluashvili 2021)
White pan bread	*L. rhamnosus GG*	Sodium alginate/hi-maize resistant starch/chitosan		• Improved viability in baking and simulated gastrointestinal conditions	(Ezekiel, Okehie and Adedeji 2020)
Cream bread	*L. acidophilus*	Alginate/maltodextrin/ xanthan gum	Emulsion method	• EE: 92.9 % in AMX and 92.37 % in AX • Best prevention with alginate, maltodextrin, xanthan gum	(Thang, Hang et al. 2019)

EE: encapsulation efficiency; AMX: 2 % Alginate + 1 % maltodextrin + 0.1 % xanthan gum; AX: 2 % Alginate + 0.1 % xanthan gum.

digestion, which facilitated the release of viable bacteria within the small intestine, enabling them to exert their beneficial effects on human health. Bacterial viability varied between the bread crust and crumb, likely influenced by differences in oxygen availability and moisture content. In another study, L. *acidophilus* LA-5 was encapsulated using a two-stage approach (Hadidi, Majidiyan et al. 2021). Initially, the bacteria were encapsulated within a sodium alginate matrix, followed by a secondary coating of fish gelatin (0.5, 1.5, and 3 %). The survival rate of L. *acidophilus* LA-5 within these encapsulated matrices and the technological properties of bread were evaluated throughout baking and subsequent storage for 7 days (Fig. 3b). Encapsulation of L. *acidophilus* exhibited a substantial increase in viability, reaching 2.49 and 3.07 Log CFU/g during baking and storage, respectively. Notably, encapsulated L. *acidophilus* with 1.5 % and 3 % fish gelatin maintained a high viability of 10^6 CFU/g after 4 days of storage, indicating its potential for use in PRO bread formulations. The study highlights significant opportunities to enhance survival of PRO bacteria, specifically L. *acidophilus,* during bread-making and storage by encapsulating them.

In another study, Mirzamani, Bassiri and Tavakolipour (2021) applied sophisticated encapsulation technique (Wurster fluidized bed) to enhance the survival of L. *acidophilus* in bread baking. They evaluated XG and alginate as the first coating and chitosan and gellan as the second coating at 0.5, 1 and 1.5 % w/v. Microcapsules coated with 1 % alginate demonstrated the highest relative survival under simulated gastric conditions; also, microcapsules containing 1 % XG exhibited a significantly higher encapsulation efficiency (EE). Encapsulation using a wall matrix containing 1 % alginate significantly improved the acid resistance of L. *acidophilus*. Encapsulation with 0.5 % chitosan enhanced the heat stability of L. *acidophilus* after heating for 90 °C and 15 day-storage. The PRO counts declined when the heating time was extended to 30 min. The use of 1 % chitosan increased the survival of fresh bread and 24 h after baking. As another example, Penhasi, Reuveni and Baluashvili (2021) developed a robust three-layered encapsulation system to protect *Bifidobacterium lactis*, against the extreme heat of baking (180 °C for 40 min) and the acidic environment of the stomach (pH 1.2 for up to 1 h). The three-layered encapsulated *B. lactis* cells exhibited significantly enhanced resistance to bread baking, and exposure to the highly acidic environment of simulated gastric fluid.

Ezekiel et al. (2020) evaluated the survival of L. *rhamnosus* GG (LGG) encapsulated either with sodium alginate alone or in combination with cassava starch, hi-maize resistant starch, and chitosan in white pan bread under different baking conditions (180 °C/30 min, 220 °C/20 min, and 250 °C/15 min) and SGI conditions. The highest viability of LGG was observed in the combined group compared to LGG unencapsulated. LGG encapsulated within sodium alginate, hi-maize resistant starch, and chitosan exhibited the highest viability after exposure to SGI fluids. Thang, Hang et al. (2019) fortified cream bread with *L. acidophilus* coated with alginate (A), alginate + maltodextrin (AM), alginate + XG (AX), and AX + maltodextrin (AMX); they observed the highest viability of *L. acidophilus* (reduction by 3.64 Log CFU/g bread) for AMX during baking and SGI conditions (during storage for 8 days). Similarly, encapsulation of *L. acidophilus* and *B. coagulans* (1, 1.5 and 2 %) was done with a 5 % starch, and its resultant incorporation into bread (Hosseininezhad and Abedfar 2018). In PRO bread, specific volume decreased and porosity increased within 48 h, while hardness increased slightly compared to control after 24 h.

4.2. Sorghum breads

The effect of tragacanth gum encapsulation on the viability of L. plantarum and *L. acidophilus* during the production and storage of gluten-free sorghum bread for 3 days was evaluated by (Ghasemi, Nouri and Mohammadi Nafchi 2024). The baking process resulted in a lower viability of *L. plantarum* and *L. acidophilus* by ~ 3 Logs CFU/g. The composition of the encapsulating matrix plays a crucial role in the survival of PRO bacteria. The viable PRO_{CAP} counts in the tragacanth gum were 2 Log greater than the control in bread during 3 days of storage. The PRO bread with a tragacanth coating had significantly higher moisture content after baking compared to the control group. Tragacanth coating in PRO bread helped retain moisture, likely due to its water-holding capacity and ability to form hydrogen bonds with water molecules, thus reducing water loss during baking.

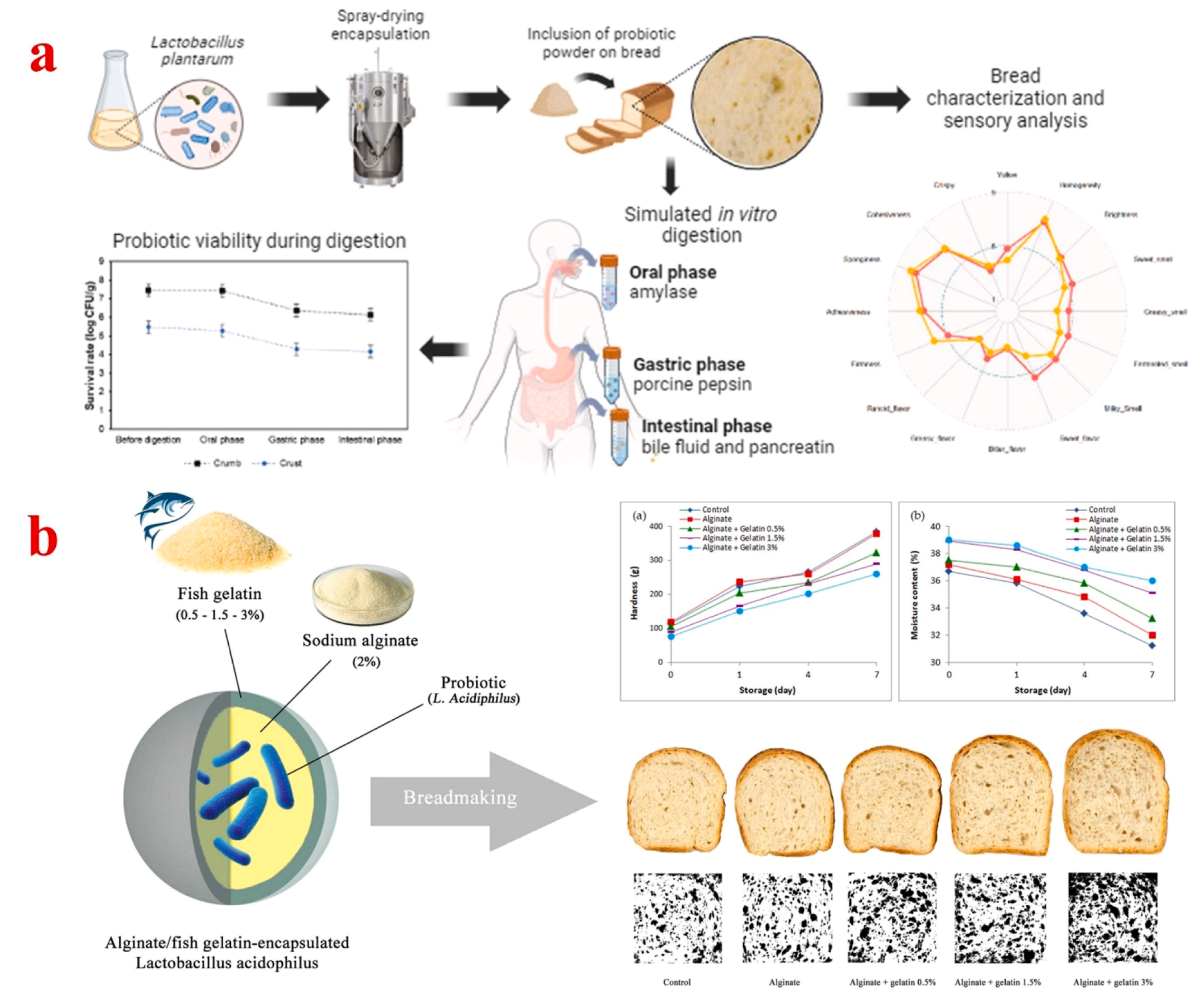

Fig. 3. a) Schematic representation of fortified bread production with: (a) spray-dried encapsulated L. *plantarum* and results of simulated *in vitro* digestion and sensory analysis (Umaña, Bauer-Estrada et al. 2024); (b) encapsulated *L. acidophilus* and the effect of encapsulation on bread properties (Hadidi, Majidiyan et al. 2021).

4.3. Rice breads

Zadeike et al. (2024) investigated the effectiveness of encapsulating acid-tolerant *Lacticaseibacillus paracasei* within an alginate-based gel for repeated SD fermentation cycles. A double-coating technique was employed to enhance the stability of L. *paracasei*; it was encapsulated in alginate, and then coated with chitosan. EE was comparable for both alginate and alginate-chitosan coatings, with values of 97.97 % and 96.71 %, respectively. L. *paracasei* showed higher survival rates of 89.51 % and 96.90 % in wet and dried double-coated microbeads, respectively. While PRO_{CAP} effectively fermented SD, double gel-coated cells demonstrated a slower rate of acidification, which may contribute to improved performance, particularly in subsequent fermentation cycles. Notably, the incorporation of freeze-dried, alginate-encapsulated bacteria significantly amended bread quality and prolonged its shelf-life. Baked products incorporating powdered alginate or alginate-chitosan encapsulated L. *paracasei* microbeads at suitable concentrations can exhibit desirable quality, sensory attributes, and a lactic acid bacteria count of approximately 10^6 CFU/g in the crumb, fulfilling the standard criteria for PRO bakery products.

5. Application of probiotics in dough and its effects on the physical and textural quality of bread

Considering the advancement in bread technology and importance of bread production with special characteristics as a personalized food harboring unique capability, manufacturing of PRO/Syn bread is undeniable progress in the field. Some PRO such as *Bacillus* sp. inherently are able to tolerate thermal stress of baking. Besides, there are several effective strategies such as encapsulation to improve thermal stability of PRO. Encapsulation not only can enhance viability of PRO during stresses of bread-making process but also is able to improve their CTD and antimicrobial activity. There are some promising reports about application of PRO_{CAP} in bread processing focusing on their improved survival especially during baking step. Moreover, enhanced survival of PRO before and after baking step, as well as after consumption and during GIT transit has been documented using encapsulation strategy. Meanwhile, there is limited data about enhancing technological functionalities of the product by the wall materials used in the produced microcapsule, possibly due to the low amounts of these ingredients in bread formulation (Mani-López et al., 2023; Sadeghi et al., 2024; Zarali et al., 2024). Accordingly, development of heat-resistant materials as coating ingredients in PRO encapsulation systems, and combined application of the wall materials with positive effects on quality indices of the product can be considered as the next generation progresses in the field.

Coating materials for PRO encapsulation can be divided into different groups for logical applications in terms of efficient protection, adhesion, antimicrobial, delivery and functional activities to improve health-promoting potentials of PRO or their extra functionalities (pro-functional capabilities) like antioxidant, anti-toxigenic and/or anti-fungal activities for special applications or special target consumers. In this regard, co-encapsulation with PRE, particularly hydrocolloids, offers added benefits to the manufactured Syn-BR. Along with positive effects of PRE ingredients on PRO survival and GMB activity, their effects on techno-functional features of the product must be determined in detail. Although the amount of PRE ingredient used in formulation of Syn-BR is low, effect of these components on dough rheology and subsequent textural features of the product and its staling rate must be assessed. Considering the type of bread formulation, application of bakery improvers like hydrocolloids as water holding/binding agents can modify bread characteristics and enhances its shelf-life. Moreover, interactions between these additives with PRO or PRE should be highlighted due to the possible positive or negative effects on their functionality. Effect of these ingredients on some organoleptic properties of the product like its color is also interesting (Beikzadeh, Peyghambardoust et al. 2017; Abedinia, Alimohammadi et al. 2021; Arepally, Reddy et al. 2022; Sadeghi et al., 2023b).

The survival of PRO_{CAP} during bread baking is associated with the physical properties of the encapsulating materials, the approach used to incorporate PRO, and their exposure to moist-heat. There are also promising reports about capability of PRO_{CAP} in different wall matrices to withstand harsh conditions of bread pre- and post-baking. For example, physical entrapment of the PRO cells in the dense microstructure of reconstituted skim milk and gum Arabic matrices and their high glass transition temperatures resulted in better survival of PRO during isothermal heating at 90 °C (Zhang, Chen et al. (2018). Survival of *L. plantarum* P8 under different baking conditions and storage durations revealed that PRO viability declined from 10^9 CFU/g to $10^4 10^5$ CFU/g post-baking. Distinct differences in bacterial viability between bread crust and crumb were also observed, influenced by thermal and moisture exposure. Interestingly, stored samples showed bacterial regrowth of 23 Log cycles, correlated with increased titratable acidity and pH reduction (Zhang, Taal et al. 2018). Seyedain-Ardabili, Sharifan and Ghiassi Tarzi (2016) reported that encapsulated *L. acidophilus* LA-5 and *L. casei* 431 with calcium alginate and hi-maize resistant starch coated with chitosan/inulin did not impact PRO viability for four days storage, and its addition had no adverse effects on flavor or texture of the product.

There are also some published data about quality and textural features of PRO_{CAP} added breads. For instance, adding PRO-γ-amino butyric acid (GABA) co-encapsulated powders to dough reduced bulk density while enhancing water absorption and solubility. Structural and textural analyses revealed that freeze-dried PRO-GABA powders caused uneven protein distribution, whereas spray-dried powders improved adhesiveness, cohesiveness and viscoelasticity, leading to better dough pasting and product textural properties (Misra, Mandliya et al. 2024). It is also reported that encapsulation of PRO with sago starch and tragacanth gum decreased the dough stability time and softening degree, and increased farinograph number, dough development time and water absorption, as well as increased moisture retention in bread with decreased staling rate (Ghasemi, Nouri et al. 2022). Meanwhile, the presence of PRO_{CAP} in bread did not significantly affect key physical properties such as volume, ash, moisture and fiber content in comparison with the control (Ezekiel et al., 2020). According to these findings, considering the types and amounts of the wall materials used, as well as the PRO strain, its release and maintenance maybe completely different. Effects of these parameters on quality indices of the produced Syn-BR are also undeniable considering the complexity of the substrates used in bread formulation and its processing conditions that need to be addressed for further characterizations.

6. Commercialized breads containing encapsulated probiotics and clinical trials

Until recently, PRO in food were primarily associated with dairy products like yogurt. These products naturally contain beneficial bacteria or yeasts (Staniszewski and Kordowska-Wiater 2021). The global market for PRO foods boasts substantial annual trade, though specific sale figures for PRO-bread products remain unconfirmed. This analysis also projects that global bread consumption was 24.1 kg per person in 2023 on average (Sadeghi, Ebrahimi et al. 2024). In 2011, True Grains introduced the first line of PRO breads to the North American market, featuring breads specially formulated with PRO cultures. True Grains breads incorporate GanedenBC30®, a robust and safe PRO strain of *B. coagulans*. In Iran, the Sahar Bread Co. developed bread with *B. coagulans*, known as the Bionan. This unique PRO strain demonstrates exceptional resilience, thriving even under demanding conditions such as high temperatures, freezing, and extended storage periods. Commercialized bread containing PRO_{CAP} represents a growing functional food market segment. The encapsulation of PRO not only enhances their stability during processing and storage, it ensures consumers receive a consistent and viable dose that may improve gut health and immune function. One of the important issues in the successful commercialization of PRO bread is maintaining bacterial viability throughout processing and storage, as consumers expect both sensory appeal and health benefits from these products (Rokka and Rantamäki 2010; Sadeghi, Ebrahimi et al. 2024).

To improve components of metabolic syndrome in animal and human studies, oligofructose and/or inulin and PRO (*Bifidobacterium* and *Lactobacillus* strains) have shown beneficial effects (Scavuzzi, Henrique et al. 2014). These studies could include consuming products, especially breads, enriched with both PRO and PRE (Syn), and supplemented with PRE along with commercial PRO. Clinical trials (Table 3) in different steps can include analysis of glucose metabolism markers (serum insulin, blood glucose), inflammation (PAI-1, s-CRP, s-IL-18, s-IL-6), and levels of gut derived hormones effective in satiety and glucose homeostasis (p-GLP-1, PYY) and intestinal barrier integrity (p-GLP-2). Colonic fermentation is usually characterized by breath hydrogen (Nilsson, Johansson-Boll et al. 2016). In a crossover study, the consumption of barley kernel bread as a source of PRE was evaluated in the normal diet of 21 healthy adults in 14 days with a combination of PRO including *L. plantarum, Lactobacillus reuteri, Bifidobacterium animalis*. The barley kernel bread-PRO intervention, in comparison to barley kernel bread and white wheat bread, enhanced contents of s-PAI-1, and p-GLP-2 after the standardized breakfast. In addition, PRO bread enhanced p-GLP-1 and breath H2 (Nilsson, Johansson-Boll et al. 2016). In another study, the effects of consuming Syn-BR containing *L. sporogenes* and inulin at a dose of 120 g/day were investigated for 8 weeks on blood lipid profiles (triacylglycerol, LDL, HDL) in patients with T2D. Consumption of this bread increased HDL levels compared to PRO and control samples, but decreased LDL and triacylglycerol levels (Shakeri, Hadaegh et al. 2014).

Daily consumption of Syn-BR (*L. sporogenes* and inulin) had beneficial effects on insulin metabolism and led to a significant reduction in insulin levels in patients with T2D compared to the control sample (Tajadadi-Ebrahimi, Bahmani et al. 2014). Similarly, consuming 120 g of Syn-BR (*L. sporogenes* and inulin) for 8 weeks resulted in a significant decrease in malondialdehyde and a significant increase in plasma nitric oxide (a biomarker of oxidative stress) in patients with T2D compared to the control sample and the PRO-containing sample (Bahmani, Tajadadi-Ebrahimi et al. 2016). Also, daily consumption of a Syn-BR + lactic acid for 2 months had beneficial effects on superoxide dismutase,

Table 3
Clinical trials of synbiotic breads.

Functional ingredients		Intervention/control group						Clinical results	Ref.
PRO strain	PRE	Study design	Population	Dosage	Sample size	Duration (weeks)	Age (years)		
L. plantarum 299v, *L. reuteri* DSM 17938, and *B. animalis* DN-173 010	Oligofructose and inulin	Cross-over	Healthy subjects		21	2	23.9 0.7	↑ p-GLP-1 and serum inflammation markers	(Nilsson, Johansson-Boll et al. 2016)
L. sporogenes	Inulin	Double-blinded	Patients with type 2 diabetes	120 g/ day	26	8	35–70	↑ HDL and ↓ LDL levels	(Shakeri, Hadaegh et al. 2014)
					27			↓ Serum insulin levels	(Tajadadi-Ebrahimi, Bahmani et al. 2014)
		Randomized, Double-Blind, Placebo-Controlled Trial			27			↓ Malondialdehyde levels	(Bahmani, Tajadadi-Ebrahimi et al. 2016)
B. Coagulans	β-glucan, inulin	Double-blinded			120	8	20–60	↑ Serum superoxide dismutase and glycemic status	(Ghafouri, Zarrati et al. 2019)

serum glutathione peroxidase, inflammation, and glycemic status in patients with T2D (Ghafouri, Zarrati et al. 2019). In an animal study, the effects of bread enriched with fructans, β-cyclodextrin, and commercial PRO was examined in mice. The results showed that all diets had a hypoglycemic effect (Ocampo, Figueroa-Arriaga et al. 2023).

7. Future prospective and challenges

Despite the promising benefits, delivering viable PRO to the intestine remains a significant challenge. While lab trials have shown the successful integration of numerous PRO_{CAP} into breads, the feasibility of applying these methods to large-scale industrial production remains uncertain. Implementing encapsulation needs specialized equipment and compounds, which will undoubtedly increase production costs. This could potentially decrease the competitive benefit of low cost that usually characterizes breads. Practical application of this technology requires the development of economical and scalable methods for producing the carriers and incorporating PRO effectively into the final product. Furthermore, lab studies may overlook the potential hazards of ingredients employed to enclose bacteria. So, the application of these carriers in commercial breads necessitates a thorough assessment by food scientists to ensure both chemical safety and compliance with relevant regulations. Addressing these challenges will undoubtedly position the bakery industry as a key player in developing innovative functional foods utilizing encapsulation techniques (Kamali Rousta, Bodbodak et al. 2021; Misra, Pandey and Mishra 2021).

8. Conclusion

Bread, as a well-known and major food product in the world, has high potential for enrichment with bioactives, especially PRO and Syn, due to its high consumption, variety of flavors, and different packaging. Bread enriched with PRO can have a positive effect on its various technological aspects and quality; consuming this type of bread also has health-promoting capabilities. Producing PRO and Syn bread without encapsulation is impossible on a commercial scale due to problems in terms of thermal instability of bacteria and bioactives during baking, decomposition and destruction of these compounds during dough mixing, delayed release, and achieving a suitable sensory profile. However, there are several challenges in encapsulating PRO on an industrial scale that must be considered. For example, cost of this process, uniform distribution of PRO, non-degradation and changes of physicochemical characteristics of PRO due to the high temperature and pressure involved in some encapsulation methods can be a limiting factor in the industrial production of PRO/Syn bread. Also, the effectiveness of carriers in protecting PRO against environmental factors, pressure and temperature caused by dough mixing, baking temperature, as well as during cooling and storage conditions play a very important role. The development of encapsulation methods using organic, natural, native and biodegradable materials is very important, which, in addition to reducing environmental pollution, can be desired by the consumer as a greener product. However, the use of these compounds at the commercial level of bread production requires strict regulatory standards, extensive testing, and compliance with labeling and marketing guidelines.

CRediT authorship contribution statement

Samira Beikzadeh: Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation. **Alireza Sadeghi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Arezou Khezerlou:** Writing – original draft, Investigation, Data curation. **Elham Assadpour:** Writing – review & editing, Validation, Methodology. **Seid Mahdi Jafari:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

All authors declare that there is no conflict of interest.

Ethical Statement

There is no studies with humans/animals in this work.

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Data availability

No data was used for the research described in the article.

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