The future of functional food: emerging technologies application on prebiotics, probiotics and postbiotics

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Abstract

This review was the first to gather literature about the effect of emerging technologies on probiotic, prebiotic, and postbiotic products. Applying emerging technologies to probiotic products can increase probiotic survival and improve probiotic properties (cholesterol attachment, adhesion to Caco-2 cells, increase ACE inhibitory, antioxidant, and antimicrobial activities, and decrease systolic blood pressure). Furthermore, it can optimize the fermentation process, produce or maintain compounds of interest (bacteriocin, oligosaccharides, peptides, phenolic compounds, flavonoids), improve bioactivity (vitamin, aglycones, calcium) and sensory characteristics. Applying emerging technologies to prebiotic products did not result in prebiotic degradation. Still, it contributed with higher concentrations of bioactive compounds (citric and ascorbic acids, anthocyanin, polyphenols, flavonoids) and health properties (antioxidant activity and inhibition of ACE, α-amylase, and α-glucosidase). Emerging technologies may also be applied to obtain postbiotics with increased health effects. In this way, current studies suggest that emerging food processing technologies enhance the efficiency of probiotics and prebiotics in food. The information provided may help food industries to choose a more suitable technology to process their products and provide a basis for the most used process parameters.

Furthermore, the current gaps are discussed. Emerging technologies may be used to process food products resulting in increased probiotic functionality, prebiotic stability, and higher concentrations of bioactive compounds. In addition, they can be used to obtain postbiotic products with improved health effects compared to the conventional heat treatment.

Keywords: eco-friendly processing; innovative food processing; food safety; functional food; beneficial microbes; new food products.

1. Introduction

A striking feature of this century is the increasing demand for healthier food products, which means minimally processed foods with low or reduced calories and with the presence of functional ingredients (Nowosad et al., 2021). This fact is mainly related to changing consumer dietary habits looking for nutritional benefits and wellbeing associated with food. In this way, they demand foods to reduce the risks of developing chronic diseases like cardiovascular disorders, obesity, diabetes, and cancers (McClements & Grossmann, 2021).

Functional foods are known as foods that provide health, physical and mental wellbeing beyond essential nutrition. Thus, functional food is considered an efficient and cheaper method to maintain health and decrease expenses with medicines (Granato et al., 2020). Probiotics and prebiotics are amongst the most studied functional components (Longoria-García et al., 2018; Pandey et al., 2015) in studies with obese people (Vallianou et al., 2020).

"Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). At the same time, prebiotics are substrates mainly used by host microorganisms, which confer a health aid (Gibson et al., 2017). In this way, food products containing both functional ingredients are denominated synbiotic products, providing improved benefits mainly to the intestinal activity, with advantages over using either probiotics or prebiotics alone (Balthazar et al., 2017). Food products containing these ingredients are now more present on markets shelves as consumers demand them (González-Herrera et al., 2021).

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Paraprobiotic and postbiotic are considered another way to deliver probiotics health benefits via foods ingestion (Almada et al., 2021a; Barros et al., 2020a; Parvarei et al., 2021). Paraprobiotics are inactivated or non-viable microbial cells, while postbiotics are products or metabolic byproducts secreted by live bacteria or released after bacterial lysis. Postbiotics offer physiological benefits to the host by providing additional bioactivity (anticarcinogenic, anti-inflammatory, anti-oxidative properties, phytochemicals, and others), reducing the risk of many life-threatening illnesses (Peng et al., 2020).

Scientific evidence must prove the functional activity of the products, which will further allow health claims. Therefore, many *in vitro* and *in vivo* assays have been carried out, depending on each country's specific regulations (Díaz et al., 2020). For example, in the case of probiotics, *in vitro* tests (e.g., adhesion to intestinal cells mucosa and epithelial cells, bile salt hydrolysis action, acid tolerance, cell membrane hydrophobicity, auto-aggregation, co-aggregation, and antimicrobial activity) ensure the ability of the probiotics to reach the target organ and exert the adequate activity (Kerry et al., 2018). Besides, the *in vivo* tests ensure that *in vitro* benefits observed for probiotic, prebiotic, and postbiotic products are confirmed in a host organism, such as animals or humans (Granato et al., 2020). This observation is necessary to prove the bioactivity of a specific functional food since the functional ingredients alone may behave differently than when added to a food product.

Despite the benefits of functional foods, these products have a great challenge related to sensory properties and practicality. Furthermore, the heat treatment conventionally applied to food products may also alter the sensory aspects and decrease the content of the bioactive compounds of the food products (Augusto, 2020). Therefore, to meet the current trends for food consumption, combining health and pleasure, some food processing emerging technologies are being studied to overcome these challenges (Guimarães et al., 2018a,b; Guimarães et al., 2019a,b).

Emerging technologies are new technologies that have already been developed or developed in the following years, with the ability to alter the social, business, and environment (Misra et al., 2017). These emerging technologies are food-processing techniques that use non-thermal or optimized thermal treatments on foods, aiming to avoid nutrient losses, maintain bioactive compounds during processing and storage, and ensure food safety.

Currently, there are many emerging technologies studied for food application (Priyadarshini & Rajauria, 2019), even though still demanding substantial research to prove their practical viability. The most studied emerging technologies for the processing of food products comprise high-intensity ultrasound (Guimarães et al., 2019a,b), high pressure (Khouryieh, 2021), pulsed electric field (McAuley et al., 2016), pulsed light technology (Abida et al., 2014), supercritical carbon dioxide technology (Silva et al., 2019a), cold plasma technology (Coutinho et al., 2018), ohmic heating (Cappato et al., 2017), and microwave processing (Martins et al., 2019).

Some studies have indicated that emerging technologies for food processing resulted in food products with improved functional activities (Guimarães et al., 2018a,b). Research showing those results have been carried out with ultrasound (Lye et al., 2012), high pressure (Tabanelli et al., 2013), pulsed electric field (Lye et al., 2011), pulsed light (Jeong et al., 2018), cold plasma (Ribeiro et al., 2021), ohmic heating (Silva et al., 2021), and supercritical carbon dioxide technology (Silva et al., 2019b). Therefore, this review provides the most current knowledge concerning emerging technologies applied to probiotic bacteria strains and probiotic, prebiotic, and postbiotic food products.

2. Probiotics, prebiotics, and postbiotics: An overview

Probiotics can be delivered through food or medicine/nutraceutical capsules. Probiotics added to foods must survive during storage and passage through the gastrointestinal tract in adequate amounts to colonize the large intestine and provide health benefits (Hill et al., 2014). The beneficial effects related to probiotic intake by humans are many, such as reduction of the risk of diabetes (Wang et al., 2020), cardiovascular diseases (Vasquez et al., 2020), Crohn's disease (Lichtenstein et al., 2016), ulcerative colitis (Sandes et al., 2020), gastrointestinal disorders (Naseer et al., 2020), urogenital disease (Akgül & Karakan, 2018); liver diseases (Behrouz et al., 2020), and different types of cancer (Sieow et al., 2021). Furthermore, they can improve lactose intolerance (Masoumi et al., 2021), infection, allergies, immune system (Velez et al., 2021), respiratory infections (Robinson, 2018); oral health (Selvarajan et al., 2020), dermatitis (Kim et al., 2020), viral infections (Lopez-Santamarina et al., 2021), physical activity (Jäger et al., 2020), among others.

One of the essential criteria for potential probiotic selection comprises their adhesion to epithelial and intestinal mucosa cells. This adhesion to intestinal wall cells prevents probiotics from intestinal mobility and allows temporary colonization, increasing immune modulation and competitive exclusion of pathogenic microorganisms (Khaneghah et al., 2020). In addition, a potential probiotic is selected based on intrinsic factors such as bile salt hydrolysis action, acid tolerance, cell membrane hydrophobicity, auto-aggregation, co-aggregation, and antimicrobial activity. Beyond those, extrinsic characteristics are also associated with the bacterial strain growth and selection: time, temperature, and pH of particular food during fermentation and storage, and water activity (Fiocco et al., 2020).

Potential probiotic evaluation is comprehended by *in vitro* assessment to verify probiotic resistance to gastric juice and enteric enzymes (Almada-Érix et al., 2021a). Furthermore, the tolerance of probiotics in the product during digestion is critical for selecting the most suitable food matrix and contributes to probiotic survival overcoming gastrointestinal barriers, and efficiency on intestinal colonization (Almada-Érix et al., 2021b; Soares et al., 2019a).

Several strategies have been assessed to protect probiotics through digestion, such as prebiotic co-administration, encapsulation, and selection of suitable food matrices. The adequate concentration of probiotics in food is at least 6 - 7 log CFU/g, and the daily intake to promote health benefits is approximately 8 - 9 log CFU/g. Those concentrations are high to compensate for the possible losses through the digestion pathway, as well prebiotic added to the food product may diminish the probiotic losses through gastrointestinal digestion (Balthazar et al., 2019).

The *lactobacilli* group, together with *Bifidobacterium*, are the most familiar genera used as probiotics for human consumption. Furthermore, other genera, such as *Bacillus*, may also present strains with probiotic properties (Pereira et al., 2018; Soares et al., 2019b). Dairy food is the most popular vehicle to deliver probiotics to humans due to the easy adaptation of these microorganisms to the dairy matrix (Dunand et al., 2019) and overall high acceptance by consumers (Champagne et al., 2018). Nevertheless, other kinds of food products such as bakery products, chocolates, pasta, cereal bars, meat, fruit, and fish could also successfully deliver probiotics (Terpou et al., 2019).

Many compounds may be categorized as prebiotic. Most of the studies use non-digestible carbohydrates, such as fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), mannanoligosaccharide (MOS), and xylooligosaccharide (XOS). However, other compounds may also show prebiotic properties, such as human milk oligosaccharides (HMO), conjugated linoleic acid (CLA), polyphenolic compounds, and polyunsaturated fatty acids (PUFA) (Gibson et al., 2017). Most prebiotics requires a dose above 3 g per day to provide health benefits. However, the adequate dosage may vary according to the associated metabolic effects and microbial ecosystem (Gibson et al., 2017).

The terms paraprobiotic and postbiotic have emerged to imply that bacterial viability is vital to provide probiotic health benefits to the host. Nevertheless, dead cells and the metabolites synthesized in the food matrix may also benefit consumers (Almada et al., 2016). In addition, bioactive compounds such as antioxidant and, antimicrobial peptides (Balthazar et al., 2019; Khan et al., 2019; Yadav & Shukla, 2019), some fatty acids (Balthazar et al., 2016; Sperry et al., 2018), organic acids, complex agents (Rajakovich & Balskus, 2019), and exopolysaccharides (Mbye et al., 2020) are also beneficial for human health. Although these definitions have been established, a recent publication consensus joined paraprobiotics and postbiotics in the same term. In this way, postbiotics might be defined as preparing inanimate microorganisms or bioactive soluble factors (pili, cell wall compounds, and metabolic byproducts) produced by food grade microorganisms during growth in food matrix or fermentation in complex microbial culture (known as cell free supernatant) that confer a health benefit on the host (Moradi et a., 2020). Furthermore, information such as microorganism's species, the inactivation procedure, and the proof of safety and health benefits are needed to characterize postbiotic products (Salminen et al., 2021).

Due to food safety reasons, many studies have searched cell free supernatant as postbiotics (Moradi et a., 2020). Those researches has shown in vitro conditions, postbiotics exhibiting many antimicrobial activities againt pathogenic microorganisms spoilage ones, in cheese (Garnier et al., 2019), meat and fish (Hamad et al., 2017; Moradi et al., 2019; Ramezani et al., 2019), vegetables (Lee et al., 2016), bread (Shehata et al., 2019) and fruit juices (Tenea & Barrigas, 2018). Those features are highly interstering for food industry, as well the use of postbiotics also as potential antibiofilm agents (Koohestani et al., 2018; Sharahi et al., 2019).

Postbiotics influence signaling pathways in the body, manipulate metabolism and the composition of intestinal microflora. The use of natural and synthetic sources of postbotics in the prevention and therapy of cancer were studied. Moreover, its application is an efficient complementary strategy to combat cancer, being effective the use as postbiotic cell free supernatants, butyrate, lactic acid, hydrogen sulfide, and β-glucans (Vrzáčková, Ruml, & Zelenka, 2021).

3. Emerging technologies: A brief overview

Many novel techniques can be used to overcome the limitations of traditional thermal processes while ensuring safe, healthy, and minimally processed foods (Barba et al., 2017). Besides, emerging technologies may present critical advantages, such as being more environmentally friendly and sustainable techniques with lower energy requirements and lesser water is required for processing than traditional preservation methods. Given this, emerging technologies have been studied and are increasingly gaining industrial interest to replace, at least partially, the conventional and well-established preservation processes (Knorr et al., 2011). The emerging technologies processing types can be classified as thermal or non-thermal food processing, as the threshold 55 °C (Guimarães et al, 2018c). Figure 1 shows the types of processing by emerging technologies as thermal and non-thermal addressed in this review with application on probiotic strain and or prebiotic, probiotic and postbiotic food products.

3.1 Ultrasound technology

The sound waves above the human audition threshold (> 16–20 kHz) are ultrasound waves. The ultrasound can be divided into two different approaches based on the difference in frequency ranges: low and high-intensity ultrasound. Low-intensity ultrasound is characterized by frequencies higher than 100 kHz and intensities below 1 W/cm^2 (Soltani Firouz et al., 2019). In contrast, high-intensity ultrasound (HIUS) is characterized by frequencies between 20 and 500 kHz and intensities higher than 1 W/cm^2 (Guimarães et al., 2019b). The propagation of sound waves at low treatment intensities and amplitudes induced acoustic streaming. In contrast, high intensities and amplitudes result in local pressures below the vapor pressure of the liquid, leading to the constant growth of gas bubbles in the medium. The liquid bubbles oscillate and expand in size when they can no longer absorb enough energy, causing bubbles to collapse, known as cavitation, which results in mechanical, chemical, and thermal effects. Cavitation and associated phenomena are responsible for most of the impact of HIUS in food processing (Knorr et al., 2011; Joshi et al., 2019).

HIUS can be applied in food directly to the product (ultrasound probe), characterized by the direct dissipation of the acoustic energy from the transducer (devices formation of ultrasound waves) to the sample. This system has a horn in the transducer responsible for amplifying the signal and sending it to the sample. HIUS can also be applied indirectly to the product (ultrasound bath), which is characterized by the indirect dissipation of the acoustic energy from the transducer to the sample, using a coupling fluid (generally water) (Guimarães et al., 2019b).

HIUS can change the structure of compounds in the food, break down particles, accelerate mass transfer, or damage the wall and cytoplasmic membrane, leading to fracture and leakage of cells by surface rubbing (Guimarães et al., 2019b). At low levels of sonoporation (transient pores or cavities), HIUS may improve microbial growth because the increased cell membrane permeability allows a higher substrate transfer, efficient removal of cellular byproducts, and accelerates the supply of nutrients and oxygen to the microorganisms. However, higher sonoporation levels may result in leakage of cellular material due to the alternation and disruption of cell membrane lipid bilayer with consequent cell death (Guimarães et al., 2019a). In this way, when probiotics are added before HIUS, the process parameters should be optimized to achieve desired levels of cell permeability but without cell death.

3.2 High-pressure processing

The non-thermal emerging technology most developed is high-pressure processing (HPP), making it possible to move from the research environment into widespread industrial-scale applications (Aganovic et al., 2021). This technology was initially invented in Japan and has been increasingly exploited by the food industry since the first commercial HPP processed product was developed in 1990 (Priyadarshini & Rajauria, 2019).

HPP is a non-thermal method of preservation and sterilization of food products. It utilizes water as a medium to transmit very high pressure to the product, leading to the inactivation of certain enzymes and microorganisms, such as yeasts, molds, Gram-positive and Gram-negative bacteria (Barba et al., 2017; Mandal & Kant, 2017; Zhang et al., 2019). Besides, HPP can result in protein denaturation shelf-life extension and be applicable for all types of solid and liquid food products (Priyadarshini & Rajauria, 2019). However, the effectiveness of microbial inactivation by HPP depends on the food matrix, the resistance of the microorganism, pressure, temperature, and holding time of the HPP processing (Zhang et al., 2019). The HPP equipment requires the following systems: heat exchange, generating pressure and holding pressure, temperature measurement, treatment chamber, and process control system.

HPP is based on two basic principles: Le Chatelier's principle (food chemistry and microbiology) and the Isostatic principle (Pascal's Law) (physical properties of food). Le Chatelier's principle is that if a change in conditions is applied to a system in equilibrium, the system will try to counteract that change and restore the equilibrium. Moreover, the isostatic principle states that the pressure is transmitted instantaneously and uniformly throughout a sample, without pressure gradients, so that the size and geometry of the product are irrelevant. Usually, HPP used for food is carried out at 200 – 600 MPa at room temperature for short cycle times of less than 5 min (Aganovic et al., 2021).

The chemical properties, especially the covalent bond of molecules, stay intact, whereas the secondary, tertiary, or quaternary structure of proteins, nucleic acids, and polysaccharides may be affected. Thus, the HPP facilitates the retention of quality parameters because the smaller organic molecules responsible for colors, flavors, and nutrients have covalent bonding dominantly or exclusively, which are hardly affected by HPP (Mandal & Kant, 2017).

HPP could be used in a sub-lethal pressure homogenization of 50 MPa on probiotic cells or probiotic products, being an excellent manner to increase particular bacteria group viability, resistance to gastrointestinal barriers, and improve cell membrane hydrophobicity and auto-aggregation (Burns et al., 2015; Tabanelli at al., 2013), as discussed further in this review.

3.3 Pulsed electric field

Pulsed electric field (PEF) is an emerging non-thermal technology that utilizes short pulses of high electric fields for a small duration (micro- to milliseconds). The intensities can vary between 0.1 – 1 kV/cm, applied for reversible permeabilization for stress induction in plant cells. The range of 0.5 – 3 kV/cm is used for the irreversible permeabilization of plant and animal tissue. Finally, the range of 15 – 40 kV/cm is applied for the irreversible permeabilization of microbial cells. PEF passes through the entire product placed between electrodes inside a chamber (Knor et al., 2011; Priyadarshini & Rajauria, 2019).

PEF technology may cause lethal damage to cells or induce sub-lethal stress by transient permeabilization of cell membranes and electrophoretic movement of reactive species between cellular compartments. For example, under irreversible electroporation conditions (moderate or very high field strength) has been used to inactivate microorganisms in foods (Wang et al., 2019). The factors that affect microbial inactivation during PEF treatment depend on 1) process parameters of the equipment such as electric field strength; 2) pulse width and shape; 3) temperature and exposition time; 4) microbial factors such as type, size, concentration, and growth-stage; and 5) external factors such as pH, antimicrobials, ionic compounds, electrical conductivity, and ionic strength (Barba et al., 2017; Knorr et al., 2011).

When food is subjected to the PEF with high-intensity pulses (15 – 40 kV/cm per μs), several events, such as resistance heating, electrolysis, and disruption of cell membranes, can occur, contributing to microorganism inactivation. The damage caused by PEF on the cell membrane is called electroporation, in which the cell develops pores that may be permanent or temporary membrane permeabilization, depending on the intensity and treatment conditions, affecting its functioning and leading to cell death. The electroporation mechanism of PEF has been used in various applications for liquid or semi-solid food products. For example, it has successfully been applied to process and preserve fruit juices, milk, and other food matrices. Besides, it can be used in bioprocessing, such as increasing the yield of bioactive compounds in food products. However, PEF processing is unsuitable for solid food products with no air bubbles and very low electric conductivity (Barba et al., 2015; Priyadarshini & Rajauria, 2019). The alterations in the process for mild parameters may allow keeping the probiotic culture added before PEF processing alive and induce bioactive compounds to increase in a food product by reversible membrane permeabilization.

PEF technology is cost-effective, energy-efficient, and can easily be implemented into the production lines, such as successfully employed for various fruit juices. However, more studies are needed to improve large-scale industrial operations (Priyadarshini & Rajauria, 2019).

3.4 Pulsed light

The pulsed light technology is based on the application of high-power pulses of electromagnetic radiation, which can be of different wavelengths, including infrared (700 - 1000 nm), visible light (400 - 700 nm), and ultraviolet (100 - 400 nm) (Gómez-López et al., 2012; Mahendran et al., 2019). These light spectrum (100 – 1000 nm) usually have insufficient penetration capacity due to their low frequency and high wavelength. However, the ultraviolet (UV) spectrum has the highest penetration rates among these types of light, which is the most used for microbial inactivation. Therefore, despite the slightly higher UV light capacity to penetrate materials, it is still considered that the pulsed light technology acts at a surface level of food and packaging material.

In general, pulsed light involves the application of short-time light pulses with an intense broad spectrum. Concerning the mechanism of microbial inactivation, the microbial DNA absorbs UV light, causing physicochemical changes in its structure, then hindering the replication and gene transcription that may lead to cell death (Mahendran et al., 2019). Therefore, most studies on applying pulsed light in foods are related to preserving foods and packaging materials decontamination.

3.5 Cold plasma technology

The cold plasma (CP) emerging non-thermal treatment for food products safety and quality is a very recent technology. Plasma is the fourth state of matter, and CP can be induced through an electric discharge in a gas at room temperature and atmospheric pressure or reduced pressure (vacuum). This ionizing gas consists of free electrons, ions, and neutral particles, as well as reactive species (such as superoxide, hydroxyl radicals, nitric oxide, ozone, and others) in constant interaction, with enough electrical energy to break covalent bonds and induce numerous chemical reactions (Liao et al., 2017). The compounds produced by CP have been widely reported to have a critical role in microbial inactivation. In addition, they have been shown to interact with food components such as proteins, lipids, water, carbohydrates, and phenolic compounds (Priyadarshini & Rajauria, 2019).

There are three mechanisms triggered by the plasma and contribute to cell death: reactive species etching in cell surfaces formed during plasma generation; volatilization of compounds and intrinsic photodesorption of UV photons; and destruction of genetic material (Coutinho et al., 2018). After the exposition of plasma, the microorganisms are bombarded by radicals (OH and NO), which are absorbed onto the bacterial surface and form volatile compounds (CO_2 and H_2O) that provoke rupture on the surface resulting in cell death (Xu et al., 2021). Furthermore, CP can cause pores that increase membrane permeability, altering the cell transmembrane potential and regulating intracellular pH, with acidification provoked by the humid air plasma. Moreover, it can contribute to releasing the inner fluid cell, allowing entry of the active species, damaging DNA, proteins, and other internal cell components (Phan et al., 2017).

For the proper functioning of CP, essential parameters should be considered: gas composition, gas flow, electrical input (voltage, frequency, power), mode of plasma exposure (exposure can be direct or indirect/remote), treatment time, and relative humidity. In addition, the product parameters are also vital such as the type (liquid or solid), initial concentration of microorganisms, and food composition (Misra & Jo, 2017).

Plasma processing is considered environmentally safe and can fulfill all ecological standards once the active species disappear after turning off the plasma power. However, CP has some disadvantages, such as the volume and size of the desired food product to process, due to microbial inactivation occurring on the food surface. Reactive plasma species have little penetration power, and as such, this is a crucial point to be considering when choosing a product to be processed. In addition, the economic cost associated with using the new technology, mainly energy and general production, can be considered to the applicability of CP technology in the food industry (Coutinho et al., 2018).

3.6 Supercritical carbon dioxide

Supercritical fluids are substances that present pressure and temperature above their critical values, changing the physicochemical property of fluids, improving viscosity, diffusivity, density, dielectric constant, and solvating properties, which make supercritical fluid excellent solvents (Knez et al., 2019). This technology has been widely used to extract and fractionate complex matrices in laboratories such as food, which contain various biochemical compounds in different amounts to make the food matrix stable. However, many other applications have been recently studied, mainly for food processing purposes, such as microbial and enzyme inactivation and microencapsulation (Amaral et al., 2017; Silva et al., 2020).

Many fluids can be used in their supercritical state. Still, the most used and advantageous is carbon dioxide (CO_2), which is efficient for a wide range of food. CO_2 is a standard gas found in the atmosphere, it is safe to be used, recyclable, thus supercritical carbon dioxide technology (SC-CO_2) is considered the most eco-friendly technology (Matos et al., 2018). Therefore, besides extracting bioactive compounds from several matrices, SC-CO_2 can be used to preserve food products by inactivating spoilage and pathogenic microorganisms.

$SC-CO_2$ can inactivate microorganisms, depending on the processing temperature, pressure, and CO_2 concentration. $SC-CO_2$ is a non-thermal technology using moderate temperature (31.1°C) and pressure (7.38 MPa). The inactivation of microorganisms occurs when the CO_2 causes oxygen displacement, lowering the pH to inhibit microbial growth (Amaral et al., 2017).

3.7 Ohmic heating

Ohmic heating (OH) is a thermal processing method wherein an electric current passes through food, producing heat due to the electrical resistance of food components. It is also known as Joule heating, electro-conductive heating, or electro-heating (Tinoco et al., 2020). The OH converts electrical energy into thermal energy when the electric current passes by the conductive material in food. The resistance of electricity passing agitates atoms and charges particles, causing an increase in temperature. The technology has achieved some industrial applications, including the pasteurization of liquid eggs and the processing of fruit products. OH has shown good potential for blanching, evaporation, dehydration, fermentation, extraction, sterilization, and pasteurization of food products (Cappato et al., 2017; Kaur & Singh, 2016; Koubaa et al., 2019; Makroo et al., 2020; Varghese et al., 2014).

To process a wide range of liquid food products, OH has been recommended because of the abundant water and polar components such as minerals and proteins. The critical prerequisite for OH application is that material must be electrically conductive, considering water and ionic content (Parmar et al., 2018). The OH technique has no penetration depth limitation compared to microwave and radiofrequency heating. However, the electrodes in OH should contact the food containing enough liquid to modulate energy. Thus, in contrast to conventional thermal processing, OH uniformly heats the entire mass of the product, resulting in high-quality products with almost no deterioration of their nutrients (Cappato et al., 2017).

The electric field generated by OH in food products affects moisture content starch gelatinization by application of different frequencies, and different voltages can inactivate foodborne contamination microorganisms and pathogens depending on the processing duration (Makroo et al., 2020). Kim and Kang (2015), Rodrigues et al. (2017), and Pereira et al. (2020) showed the inactivation kinetics of foodborne pathogens, such as *E. coli*, *Listeria monocytogenes*, *S. aureus*, and *Salmonella*, under sub-lethal pasteurization temperatures (65°C) in comparison conventional heating method of pasteurization and concluded that OH was more efficient because faster and homogenous heating in milk and dairy products occurred. Moreover, many studies reported that OH does not affect bioactive compounds, which seem to be preserved during the process (Makroo et al., 2020; Pires et al., 2021; Rocha et al., 2020). Since the mechanism of bio-compounds degradation is related to the time and temperature of food exposition to heat treatment, the food processing occurs in a shorter time when applied OH technology than conventional heating pasteurization (Salari & Jafari, 2020). Thus, the preservation of functional compounds in food products opens a field to study the development of available functional ingredients, such as bioactive peptides and phenolics, and probiotic addition that could be elaborated using OH.

4. Emerging technologies applied to probiotic, prebiotic, and postbiotic products

Probiotics, prebiotics, and postbiotics may be included before or after applying the emerging technologies (Fig. 2). The step of addition is essential in the observed results, mainly for probiotics that must be alive in the desired product throughout the shelf-life period (Barros et al., 2021b). The prebiotic ingredients are usually added before the heat treatment and, consequently, used before applying emerging technologies. In the case of probiotic products, these cultures are generally added after the heat treatment due to their heat sensitivity, as well added after emerging technology application. However, there is no evidence that heat treatment interferes with postbiotic compounds availability. Then, postbiotic products are usually developed by probiotic addition to the food product prior to heat processing, which will inactivate those cells (Pérez-Sánchez et al., 2020).

4.1 Application of emerging technologies on probiotic strains

Emerging technology used on probiotic strain could be interesting for industrial applications. Emerging technologies applied to probiotic strains *in vitro* studies are reported in Table 1. It is essential to note that the effect of each emerging technology must be evaluated according to the metabolic of each probiotic strain. In this sense, a previous step of optimization of intrinsic parameters must be considered in preliminary experiments. Indeed, emerging technologies may improve probiotic resistance to the simulated gastrointestinal tract and improve probiotic properties as the adhesion on the gastrointestinal tract.

For example, HPP technology modulated the functional and biological properties of probiotic lactobacilli bacteria in phosphate-buffered saline solution under 50 MPa. The study provided information that HPP could modulate cell membrane hydrophobicity and auto-aggregation without modifying cell viability and decarboxylase activity. However, the effect on gastrointestinal resistance was strain-dependent. For example, after 30 days of refrigerated storage, the highest cell viability loss was observed for *Lactobacillus acidophilus* Dru, independently of the application of HPP. However, after the same period, the resistance of *Lacticaseibacillus paracasei* A13 to simulated gastric digestion was significantly increased in HPP-treated cells (Tabanelli et al., 2013).

On the other side, HIUS and PEF may change the probiotic cell membrane properties, resulting in enhanced hydrophobicity and cholesterol incorporation. In this way, it may increase the probiotic ability to intestinal cell adhesion and reduce the cholesterol levels in the host bloodstream. For example, PEF was used to enhance the lactobacilli ability to remove cholesterol *in vitro* by electroporation on the membrane (Lye et al., 2011). Among different electric field strengths during different periods, they observed that 7.5 kV/cm for 4 ms increased probiotic viability from 0.89 to 1.96 log CFU/mL upon fermentation at 37 °C for 20 h. This event was attributed to the reversible and transient formation of pores and clumped defragmentation cells. Moreover, cholesterol assimilation was increased 127.2% for most cells electroporated at 7.5 kV/cm field strength for 3.5 ms. In addition, cholesterol saturation was observed in different membrane bilayer regions such as upper phospholipids, apolar tails, and polar heads (Lye et al., 2011).

Moreover, US provided that lactobacilli cholesterol removal ability enhanced after ultrasound treatment, using different intensities (W) and time duration (min) to treat lactobacilli cells, which showed viability decrease upon high intensities at a more extended period. A reversible effect on membrane properties was observed, as the treated lactobacilli cells could regain viability upon fermentation. The HIUS also increased lactobacilli cells ability to remove cholesterol from the medium via cholesterol assimilation and incorporation into the cellular membrane, attributed to increased membrane permeability (Lye et al., 2012). Thus, those studies demonstrated that lactobacilli ability to remove cholesterol from media could be increased by applying the PEF technique or ultrasound as bloodstream cholesterol-lowering adjuncts. Perhaps a synergic application could also be an exciting approach to optimize processing and enhance the benefits. However, no research on this topic was not yet performed.

PEF also increased the vitamin bioactivity via probiotic fermentation *in vitro*. Ewe et al. (2012a) studied the inheritance of electroporation caused by PEF strength of 7.5 kV/cm for 3.5 ms on *Limosilactobacillus fermentum* BT 8219 through three subsequent subcultures in biotin-supplemented soymilk substrate, based on their growth, isoflavone bioconversion activity, and probiotic properties. A significantly higher growth ($p < 0.05$) than the control during fermentation in biotin-soymilk due to enhanced intracellular and extracellular β-glucosidase specific activity leading to increased bioconversion of isoflavone glycosides to aglycones. Inheritance did not affect growth characteristics, enzyme, and isoflavone bioconversion activity. However, electroporation affected probiotic properties, reducing tolerance towards acid (pH 2) and bile, lowering inhibitory activities against selected pathogens, and reducing adhesion ability. It was suggested that electroporation could increase soymilk biotin bioactivity via fermentation with probiotic *L. fermentum* BT 8219 when developing novel functional foods (Ewe et al., 2012a). Nevertheless, the reduction of gastrointestinal survival and anti-pathogenic abilities must be carefully studied and solved prior to developing new probiotic food with vitamins enhancement.

In addition to probiotic gastrointestinal resistance enhancement as previously discussed concerning ultrasound application, Racioppo et al. (2017) studied the effect of HIUS on probiotic strains, namely *Lactiplantibacillus plantarum*, *Limosilactobacillus reuteri* DSM 20016, *Bifidobacterium longum* Bb46, and *B. infantis* Bb02. The authors observed an increase in hydrophobicity, lower post-acidification and growth at different temperatures (37 or 45 °C), pH (4 or 9), or in the presence of 7% NaCl after HIUS-attenuation on probiotic under acid conditions (pH 2 and 2.5, added or not with 0.3% of bile salt). The combination of HIUS parameters applied were 40, 60, and 80% of 130 W power for 2, 4, and 6 min with 2 s pulses. The authors found an optimized combination to avoid post-acidification and maintain probiotic viability with 60% power for 6 min. The study confirmed that sonication might control post-acidification in MRS broth (Racioppo et al., 2017). Also, it was observed that post-acidification did not occur at 4 °C, suggesting that refrigerated storage of attenuated strains helps maintain low post-acidification levels. Moreover, the HIUS did not affect viability at 45 °C or pH 9. However, there was a decrease in microbial growth at pH 4. There was an increase of hydrophobicity and adhesion to Caco-2 cells after HIUS-exposure for *Limosilactobacillus reuteri*, which according to the authors (Racioppo et al., 2017), could be the background for future research to improve or modulate the adhesion of this microorganism. In this way, this study observed an essential effect of post-acidification control and improved probiotic properties with HIUS. It is an attractive solution for the post-acidification problem of fermented dairy products, being interesting to use on development of new innovative fermented dairy products with probiotic properties.

In other studies concerning electricity application to improve probiotics abilities, the electroporation phenomenon could increase the bioactivity of some nutrients such as vitamins and reduce fermentation time (Loghavi et al., 2007, 2008). These authors reported the effect of different frequencies from moderate electric field strength (1 V/cm) applied to *L. acidophilus* growth during suboptimum fermentation temperature (30°C). They observed kinetics growth alteration and increasing bacteriocin production, which indicates that sinusoidal waves at 45 and 60 Hz were responsible for reducing the lag phase. However, no reduction in lag phase was observed for a 60 Hz waveform containing high-frequency harmonics. Instead, significantly increasing bacteriocin production during 5 h of fermentation was also observed (Loghavi et al., 2007, 2008). Bacteriocins are peptides synthesized in ribosomes metabolites produced by a specific probiotic strain in the gastrointestinal tract, displaying antimicrobial activity against another kind of bacteria (Peng et al., 2020). Those results are encouraging from the economic point of view due to shorter fermentation time and modifying the set conditions to increase bacteriocin production. As the experiments were performed in de Man, Rogosa, and Sharp (MRS) medium (Loghavi et al., 2007, 2008), further studies on food products must be carried out to confirm the *in vitro* results.

SC-CO_2 technology has been used to extract polar lipids from probiotic bacteria, which constitute part of the bioactive molecules of the bacteria and may be used for therapeutic purposes. Thus, it is possible during the SC-CO_2 probiotic products processing extract lipids from probiotic cells. The lipids extraction from this technology may also help the encapsulation of probiotics since it can effectively extract polar lipids that spontaneously form liposomal structures (microcapsules) without using solvents (Silva et al., 2020). However, all those studies were performed in specific controlled media *in vitro* studies. Thus, it is essential to evaluate if the cited benefits and others are available when applied in food products.

Figure 3 shows a general scheme indicating the improvement of use emerging technology on probiotic strain and benefits for industrial application as processing optimization, use of clean and renewal energy source and supply of food safety.

4.2. Applications of emerging technologies in probiotic food

Despite the scarce studies aiming at applying emerging technologies on probiotic strains, the potential practical applications of these technologies in probiotic food are an evolving field. Emerging technologies were used on probiotic fortified food products to verify the positive effect of these technologies on microorganisms to improve health benefits (Table 2), such as optimizing and stimulating probiotic fermentation and increasing bioactive compounds in the food matrix. However, those improvements were achieved differently by applying different emerging technologies separately or combined, which is essential.

The application of emerging technologies may also improve the fermentation ability of probiotic cultures and enhance the nutritional profile of the products. Nguyen et al. (2009) used HIUS to study the effects of fermentative activity by bifidobacteria strains during milk fermentation. Ultrasound parameters used were approximately 100 W wave amplitude at 20 kHz frequency for 7, 15, and 30 min. They verified that probiotic viability was the same as the product without ultrasound application. However, intracellular β-galactosidase was released from ruptured cells, and milk lactose was hydrolyzed by remaining bacteria, enhancing their growth during fermentation. Also, transgalactosylation induced short-chain oligosaccharides (polymerization degree = 3) formation in fermented milk with lower lactose content. Later, Nguyen et al. (2012) demonstrated that HIUS could accelerate lactose hydrolysis and transgalactosylation of bifidobacteria in milk and opened up a possibility to balance the ratio of acetate to lactate, total acetate and propionate to lactate in sonicated fermented milk by Bb -12 and Bb -46, respectively. As a result, the fermented milk was low in lactose, high in oligosaccharides, and showed less undesirable flavor from acetic and propionic acids. The use of HIUS on fermented milk with bifidobacteria was advantageous because HIUS was responsible for diminishing lactose in milk by releasing β-galactosidase, which was hydrolyzed and turned to sub-products easier available for the remaining probiotic to grow. Also, oligosaccharides content in fermented milk and flavor was improved, making this fermented milk beverage better from the functional and sensory point of view.

Emerging technologies may also improve the evaluated food products bioactive compounds content and health effects. Ruan et al. (2020) studied the impact of US bath to assist the liquid state fermentation of *Bacillus subtilis* on fermented soybean meal. The authors verified increasing *in vitro* ACE inhibitory activity and peptides content, biomass over 25%, and peptides yield, purity, and conversion rate improvement. Moreover, fermented soybean meal decreased systolic blood pressure (SBP) by approximately 20.7 mmHg ($p < 0.01$) in rats at 3 h post-administration of 3.0 mg/kg of body weight dose. The optimal ultrasound condition for microorganism incubation for 36.7°C was 0.08 W/mL for 1h. This study might provide the basis for technological support toward better understanding and choosing ultrasonic conditions to produce antihypertensive peptides in the fermentation industry.

Huang et al. (2019) described ultrasonic schemes to increase peptide content in fermented skim milk enriched with *L. paracasei*. After incubating skim milk for 9 h, they applied ultrasonic treatment with the following parameters: 3 min, 100 W/L power, 28 kHz frequency, and 100 s/10 s on-off pulses. The authors demonstrated a 64.23% peptide increase due to these ultrasonic treatments. Thus, HIUS applied after milk fermentation could enhance the peptide yield present in the product.

Wang et al. (2021) applied HIUS assisted by UV light on fermented mango juice with *Lactiplantibacillus plantarum* Lp-115 strain and evaluated the products during 30-days of cold storage (4 °C). They observed improvement in physicochemical properties and more stable and accessible bioactive compounds. In addition, the HIUS/UV fermented mango juice enhanced the probiotic bacteria strain viability, which was maintained over 7 log CFU/mL during the entire shelf-life. The parameters of emerging technology used were 20 kHz and 600 W for 10 min (HIUS application) under UV-8 W lamps with 254 nm wavelength. Thus, ultraviolet-assisted ultrasonic pretreatment in mango juice fermentation was validated, improving the storage stability of fermented mango juice and prolonging its shelf life during refrigerated temperature storage.

PEF is an exciting technique for vitamin and minerals bioavailability enhancement due to electroporation of probiotic membrane cell formation in mild electric field strength. Ewe et al. (2012b) showed that electroporation could be used to increase aglycones bioactivity in biotin-soymilk fermented with many strains of lactobacilli. They used PEF to alter bacteria membrane fluidity, causing lipid peroxidation and enhancing β-glucosidase activity. The electric potential applied across cell membrane induced pores formation, followed by increased membrane permeability. This membrane permeability was reversible because of lactobacilli cells growth (> 9 log CFU/mL) after fermentation in biotin soymilk. Better results were observed in electric field strength of 7.5 kV/cm for 3.5 ms. Thus, PEF improved the viability of lactobacilli strains and aglycones bioactivity.

Nonetheless, Pankiewicz et al. (2020) applied PEF on the probiotic strain *Lacticaseibacillus rhamnosus* B442 to study calcium ion attachment to bacteria membrane in enriched probiotic ice cream. They verified that PEF increased surface calcium-ion binding efficiency and incorporated elements into cellular structures. The highest accumulation of Ca^{2+} ions in cells was achieved when the PEF was applied at a 3.0 kV/cm electric field strength and a calcium concentration of 200 µg/mL of medium. PEF improved Ca^{2+} attachment to the probiotic bacteria membrane, as well Ca^{2+} could be considered more available to the host in ice cream which probiotic bacteria were subjected to PEF because soluble calcium (Ca^{2+}) is almost ready to be absorbed by the human small intestine and colon (Jiang et al., 2020).

Recently, Sotelo et al. (2018) used PEF to process cherry containing *Lactobacillus acidophilus* ATCC1643 probiotic strain at a constant pulse frequency of 100 Hz and a regular pulse width of 20 µs with different electric field strengths between 0.3 and 2.5 kV/cm. These authors successfully demonstrated significant polyphenol increase as the electric field intensities decreased and probiotic growth by low or moderate PEF stimulation.

Jeong et al. (2018) investigated the light effect on blueberry fruit fermentation by probiotics strains of *Bacillus amyloliquefaciens* and *Levilactobacillus brevis*. They used green, red, blue, and white LED illumination, sunlight, and dark environments to verify total phenolic and flavonoids contents, antibacterial, antioxidant, and cytotoxic activities on fermented fruit extracts. Their experiment demonstrated that white and green LED and sunlight improved bacterial growth with higher total phenolic and flavonoids content and significant antibacterial and antioxidant activities. This study indicated that different light sources could naturally increase bioactive compounds with antioxidant and antibacterial activities in fermented blueberry fruit by probiotic strains, demonstrating potential applications in medical, health, and cosmetic fields.

Emerging technologies may also be used to control post acidification of food products *in vitro*. For example, Bevilacqua et al. (2016) used ultrasound technology to modulate probiotic metabolism and attenuate post acidification. HIUS-attenuated wild strain *L. plantarum* and commercial strains *L. casei* LC01 and *B. animalis* subsp. *lactis* Bb12 were inoculated in a commercial rice drink. They verified that HIUS-attenuation did not affect the microorganisms' viability or sensory acceptance of the rice beverage. Further, HIUS-attenuation could prevent rice-drink post-acidification if it undergoes a short thermal abuse (4 h at 25 or 37 °C then at 4 °C). Thus, the authors concluded that HIUS-attenuation could be interesting to avoid probiotics post-acidification throughout storage in rice drinks without affecting the sensory perception of the products.

Emerging technologies may alter the probiotic properties of food products. For example, Burns et al. (2015) produced a probiotic Caciotta cheese using sub-lethal HPP homogenization (50 MPa) to study the bioactive compounds increased by probiotic strain. They verified that adding *L. paracasei* A13 previously submitted to HPP maintained high strain viability (approximately 9.2 log CFU/g) for 14 days. Also, *L. paracasei* presented enhanced gastric resistance. However, cytokine IL-10 producing capacity was lost during the HPP procedure, and IgA production was not modified. Nevertheless, the sub-lethal HPP application during food processing is promising because high viable and resistant probiotics through the gastrointestinal barrier were obtained, ensuring that *L. paracasei* A13 arrives adequately to the intestine and provides probiotic benefits to the host.

Post-contamination control is also a compelling topic in emerging technologies to produce functional dairy foods. Ohmic heating (0-8 V/cm) was used to process milk and produce probiotic fermented milk containing *L. acidophilus* (Silva et al., 2021). The product was submitted to post-fermentation contamination with *Listeria monocytogenes* (9 log CFU/mL). The utilization of OH reduced the *L. monocytogenes* counts, and the probiotic culture survived in the product and simulated gastrointestinal conditions. Furthermore, the OH-treated product had a higher content of bioactive compounds and improved sensory acceptance (Silva et al., 2021).

Many emerging technologies enhanced the viability of probiotic properties, improving bioactive compounds, vitamins and mineral availability in the food matrix, as shown in a general scheme of use emerging technology on probiotic food processing and benefits for industrial application as processing optimization, use of clean and renewal energy source and supply of food safety in Figure 3. Thus, these facts could be interesting for the food industry, improving probiotic traits on products as bioactive properties (peptides, phenolic compounds, flavonoids, and aglycones) and availability (vitamins and minerals), indeed raising it.

Finally, it is essential to comment on the limited studies related to emerging technologies covering new generation probiotics, which are commensal organisms identified as potential bacteria with probiotic effects on the host (O'toole et al., 2017). As such, these bacteria could be added to food products as delivery matrices (Saarela, 2019). As in future studies, they will be part of the wide range of probiotic food products available on the market, and there will be studies using emerging technologies to improve the probiotic properties of new generation probiotics to support their industrial application in the food sector.

Shortly, this topic will be relevant and eminent in the probiotic field and will deserve more attention and research.

4.3. Applications of emerging technologies in prebiotic foods

Products containing prebiotics should keep the prebiotic ingredients at suitable concentrations to provide health effects. In this way, it is essential to evaluate the prebiotic stability after emerging technologies processing. In Table 3, it is presented the main effects and benefits of emerging technologies applied to prebiotic foods.

For example, Alves Filho et al. (2016) assessed the FOS stability to emerging technologies, such as ultrasound (600 and 1200 W/L, 5 min), high pressure (450 MPa, 300 s), and cold plasma (15-60 s, 70 kV, direct or indirect). The emerging technologies did not induce any significant change in the FOS concentrations. However, the behavior could differ depending on the prebiotic ingredient added to a food product. Thus, more research in this aspect should be performed.

In another study, Ribeiro et al. (2021) evaluated the effect of cold plasma (0-15 min) on the properties of whey dairy beverages supplemented with XOS (1.5%). The cold plasma or pasteurization application resulted in products with lower color intensity, reduced consistency, and no apparent viscosity and sensory characteristics changes compared to the untreated product. No impact on XOS concentration was observed, demonstrating that cold plasma maintained the XOS quantities in the products. Furthermore, the cold plasma-treated products showed higher concentrations of bioactive compounds (antioxidant activity and inhibition of ACE, α-amylase, and α-glucosidase) than the pasteurized product. This study demonstrated the essential advantages of using cold plasma to process prebiotic whey beverages.

Almeida et al. (2015) evaluated the impact of cold plasma (70 kV, 15-60 s) and ozone (0.057-0.230 mg/O3 mL of juice) on the properties of prebiotic oligosaccharides produced by dextransucrase enzyme addition (0.05 UI/ mL) to orange juice. Both emerging technologies resulted in partial degradation of oligosaccharides with a high degree of polymerization after processing, generating oligosaccharides with a low degree of polymerization. However, the concentration of prebiotic was kept higher than necessary to classify the product as prebiotic. At the same time, Almeida et al. (2017) evaluated the impact of cold plasma (70 kV, 15-60 s) and HPP (450 MPa, 5 min) on the properties of prebiotic (FOS) orange juice. HPP promoted a higher FOS degradation. Both treatments increased the citric and ascorbic acid content. Moreover, Gomes et al. (2017) studied the effect of HPP (450 MPa, 5 min) and US (600 and 1200 W/L, 5 min) on the properties of prebiotic cranberry juice. The initial prebiotic concentrations and organic acid in the juices were kept after emerging technologies processing. Furthermore, the juices presented higher anthocyanin concentrations (24%) when processed by both technologies. Finally, Silva et al. (2019b) evaluated the effect of SC-CO_2 (10-20 MPa, 35 ^{o}C, 10 min, 67% CO_2 volume ratio) on the characteristics of prebiotic (inulin) apple juice. The SC-CO_2-treated products kept the compounds associated with the functional properties of the product, its antioxidant activity, and the natural components of the juices (sugars). The inulin profile was not changed, while the heat-treated juice showed a breakdown of the chain in compounds of smaller size. These results demonstrated the prebiotic stability to diverse emerging technologies and the maintenance of higher concentrations of bioactive compounds in the products compared to conventional heating.

Guimarães et al. (2019b) evaluated the impact of HIUS (0-600 W) on the properties of prebiotic (inulin) soursop whey beverages. The authors reported that HIUS improved the nutritional profile of the products (higher phenolic compounds content, antioxidant activity, and antihypertensive activity). However, a decrease in some minerals and ascorbic acid was observed. At the same time, Rodríguez et al. (2017) applied indirect cold plasma (10-50 mL/min for 5-15 min) on cashew apple juices. The cold plasma-treated juices showed higher vitamin C, polyphenol, and flavonoid contents, resulting in higher antioxidant activity. However, overexposure to cold plasma impacted negatively on these parameters. It is essential to highlight that these studies did not evaluate the prebiotic stability after applying emerging technologies.

In this way, optimizing the process parameters does not change prebiotic concentrations (Alves Filho et al., 2016; Ribeiro et al., 2021); however, it increase the positive impact on the food properties such as antioxidant activity, polyphenols, and flavonoids (Rodríguez et al., 2017).

The positive impacts of emerging technologies applied on prebiotic food, according to literature, is summarized in Figure 4.

4.4. Applications of emerging technologies for postbiotic food products for clinical trials

Emerging technologies may be applied to obtain postbiotics with increased health effects (Table 4). The clinical trial is the most critical step involved in the development of functional foods and is compulsory in several regulatory agencies.

Brandão et al. (2021) inactivated probiotic cultures (*Lacticaseibacillus casei*) using HIUS (20 Hz for 40 min). They evaluated its ingestion on the health parameters and intestinal microbiota of rats fed a high-fat diet. As well, live probiotic cultures were also considered. The authors observed that both live and ultrasound inactivated probiotics could prevent the increase in cholesterol levels and insulin resistance and modulated the intestinal microbiota of the animals. The postbiotic product could also attenuate the blood pressure. The results corroborate that metabolite compounds released in the food matrix from probiotic bacteria themselves have beneficial effects on consumers; also, the study opens up opportunities for the industry to obtain postbiotics with increased health properties by using emerging technologies.

Almada et al. (2021b) investigated the effect of postbiotic administration on the intestinal microbiota and biochemical parameters of Wistar male rats. The authors inactivated *L. casei* cells by HIUS and *Bifidobacterium animalis* cells by heat, high and low pH, HIUS, SC-CO_2, and irradiation. They observed that the properties investigated were dependent on the technology used. SC-CO_2, for example, increased the creatinine and albumin levels and decreased the total and HDL cholesterol. In addition, the intestinal microbiota had subtle changes, indicating maintenance regardless of the inactivation method.

Barros et al. (2021a) used ohmic heating (4, 8, and 12 V/cm at 60 Hz) as a method of obtaining postbiotics (*L. acidophilus*, *L. casei* and *B. animalis).* The authors observed that OH was an adequate technology for producing postbiotics, with 8 V/cm and 95 ^{o}C/ 7 min resulting in lower damage to the probiotic cell membrane integrity than the conventional heat treatment. In another study from the same group, a whey grape juice with postbiotic (*L. casei*) obtained with the conditions of the previous research was administered to healthy individuals, and the effect on postprandial glycemia was evaluated. The effect was compared to that of probiotic whey drink (Barros et al., 2020a). The administration of both postbiotic and probiotic whey drinks increased the incremental glucose due to their sugar content. However, no effect on glucose additional percentage, maximum glucose value, and peak blood glucose time was observed, demonstrating the reduced glycemic response of the probiotic and postbiotic products. Furthermore, the postbiotic product prevented the maximum glucose increase. These studies showed that postbiotic obtained with OH might have health effects similar or superior to the probiotic products.

Overall, more studies in different animal and human clinical trials are needed using postbiotics produced by emerging technologies, with adequate sampling size and defined clinical markers to elucidate the advantage of emerging technologies considering a functional perspective.

5. Conclusions and future perspectives

This review was the first to gather literature about the effect of emerging technologies on probiotic bacteria strains; probiotic, prebiotic, and postbiotic products. In a general view, applying emerging technologies in probiotic and prebiotic products may increase the probiotic survival and resistance to the products, and digestive conditions improve its probiotic properties and maintain or increase the bioactive compounds. Furthermore, it could be used to reduce the fermentation length and improve the sensory characteristics of the products, which is an essential feature for the food industry. Prebiotic ingredients were stable to most emerging technologies, while the breakdown of the chains is commonly observed after conventional heating treatments.

The studies concerning emerging technology applied to probiotic strains showed positive results on probiotic properties, such as improving cholesterol attachment to the probiotic cell membrane by using different techniques (PEF and HIUS), rising probiotic adhesion to Caco-2 after HIUS application, optimization of probiotic fermentation, and bacteriocin production at the moderate electric field, increasing probiotic growth and vitamin bioactivity when PEF was applied, and enhancement of probiotic viability and gastrointestinal resistance using HPP. However, most of the studies were conducted in vitro, and the health effects of the products subjected to emerging technologies need to be evaluated in *vivo* tests.

Differently, postbiotics studies *in vivo* are already found in literature, which might be due to background knowledge on emerging technology effects on inactivating bacteria and sub-lethal processing on probiotic strain studies. This fact possibly makes more accessible studies *in vivo* with postbiotic products processed by emerging technologies. However, this new approach using emerging technologies to obtain postbiotics with increased health effects is scarce.

Today, emerging technologies still faced some barriers for implementation in the food sector due to more expensive investments in machinery implementation, which could increase the final price of the food products. Also, in developing countries, the fuel energy source is cheaper in food production, which discourages industries from investing in emerging technologies. However, humanity faces a new era, where science shows to be even more important to answer our diseases and environmental problems. Thus, emerging technologies can use renewable energy sources, supported by consumers' pressure towards environmental-friendly technologies. The food industry sector will manage, adapt and invest more in knowledge to elaborate healthy food products with less environmental impact.

Declaration of interest

The authors declare no conflicts of interest.

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Figures captions

Figure 1. Thermal and non-thermal processing emerging technology applied to probiotic strain and or prebiotic, probiotic and postbiotic food products.

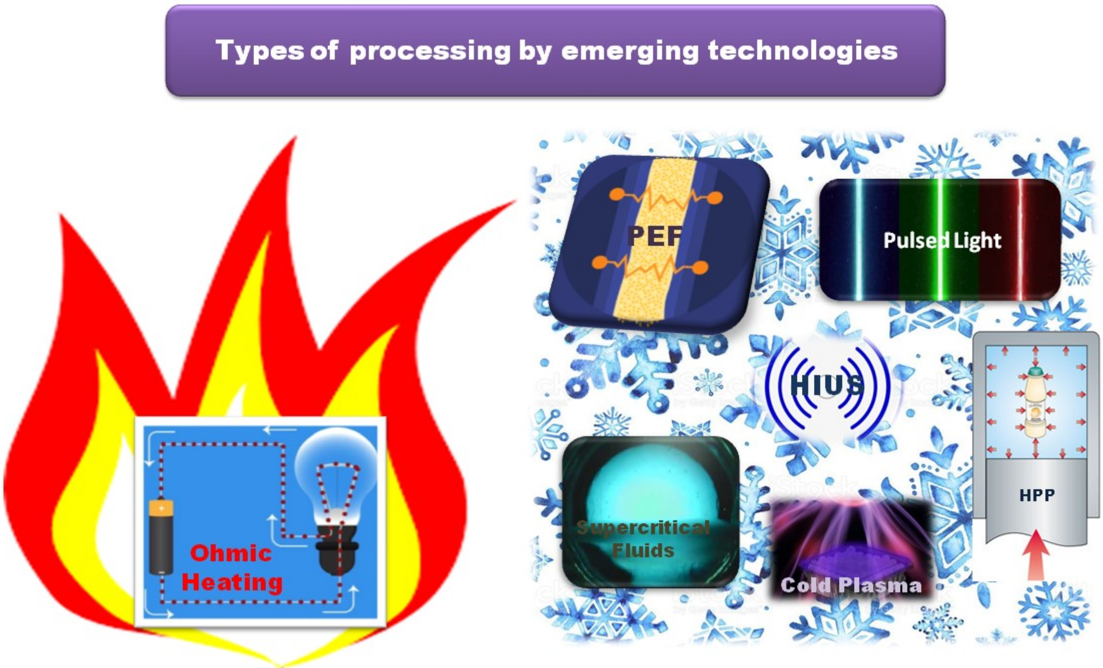

Figure 1.

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Figure 2. Step where prebiotics, probiotics and postbiotics are added to food in emerging technology processing.

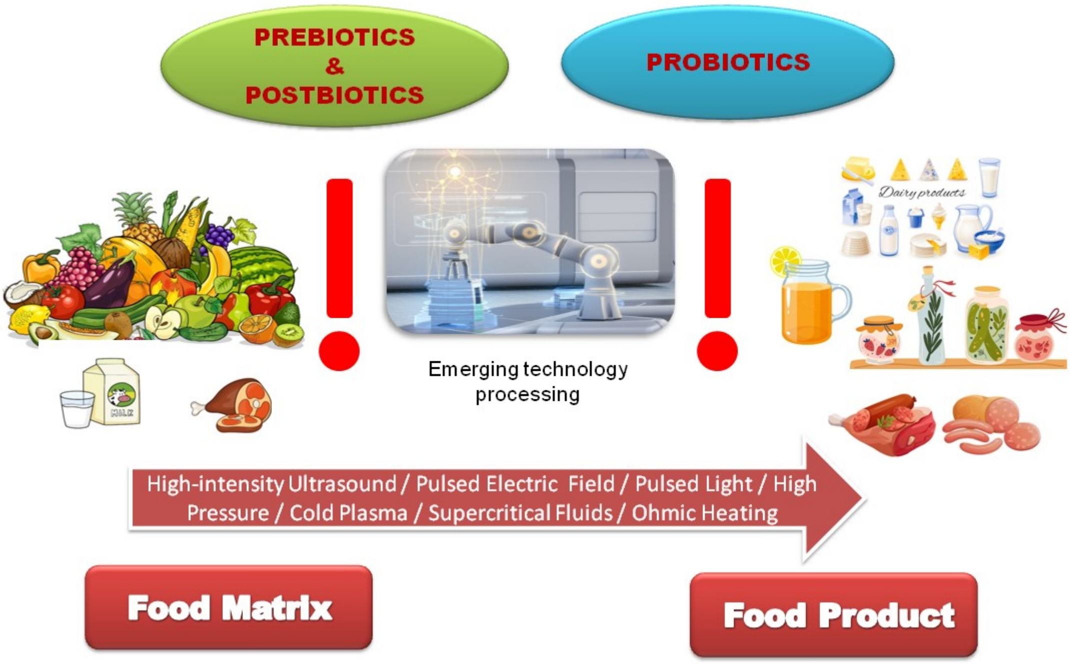

Figure 2.

Figure 3. General scheme of beneficial improvement in probiotic bacteria strains and food matrix using emerging technology.

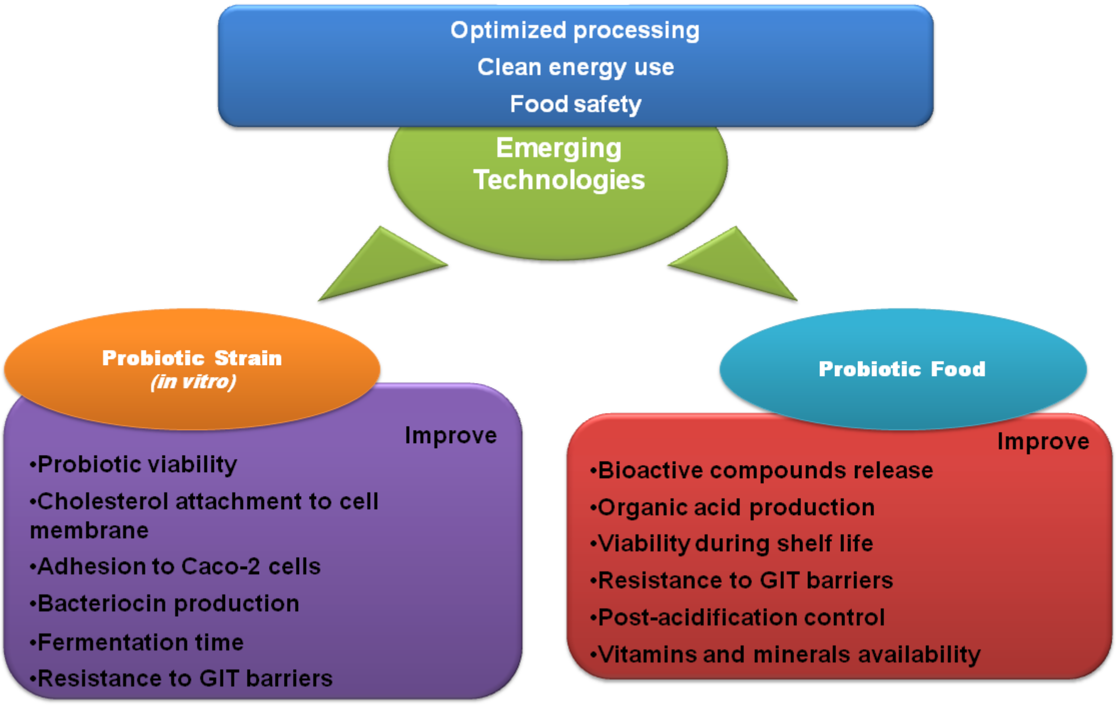

Figure 3.

Figure 4. Summary of the currently available literature on emerging technologies and their impact on prebiotic foods.

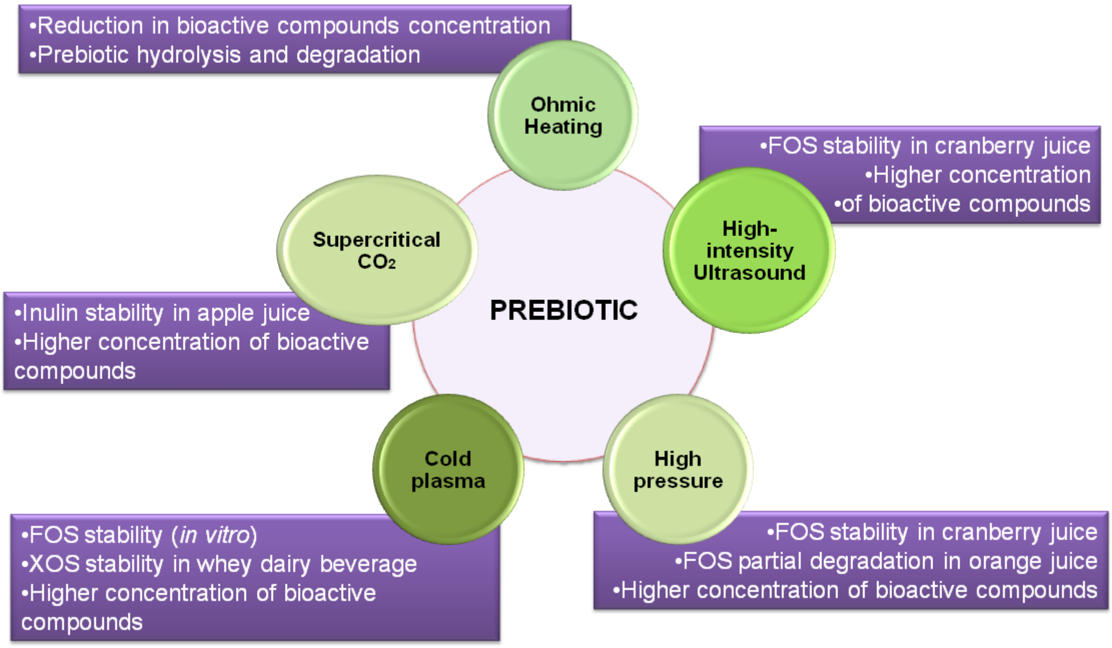

Figure 4.

Tables captions

Table 1. Applications of emerging food processing technologies on probiotics in vitro.

Probiotic strain	Substrate	Method	Main effects	Beneficial effects/advantages	Reference
Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. *bulgaricus Lacticaseibacillus casei*	Sterile MRS broth containing 100 µg mL^{-1} cholesterol 0.15% (w/v) L-cysteine hydrochloride, 0.3% (w/v) ox gall and 0.1% (w/v) pancreatin	HIUS (30 kHz) 20, 60, and 100 W 1, 2 and 3 min	• Bacterial cell viability decreased upon treatment at higher intensities and longer durations; • Ultrasound treatment increased the incorporation of cholesterol into the cellular membrane.	Cholesterol-lowering ability in media by incorporation to the lactobacilli cell membrane.	(Lye et al., 2012)
Limosilactobacillus reuteri, Lactiplantibacillus plantarum, Bifidobacterium longum, *B. infantis*	MRS media adjusted to pH 4 and 9, supplemented with 7% (v/v) NaCl.	HIUS (20 kHz, 52–104 W, 2–6 min, pulse of 2 s)	• Ultrasound processing exerted a positive effect on the hydrophobicity and adhesion of *L. reuteri* to Caco-2 cells; • There was a negative effect on acid and bile resistance when using other strains.	Increase hydrophobicity and adhesion to Caco-2 cells after US exposure, improving or modulating *L. reuteri* adhesion.	(Racioppo et al., 2017)
L. paracasei A13, *L. acidophilus 08 and Dru,* *L. delbrueckii* subsp. *lactis 200*	MRS broth	HPP homogenization (50 MPa)	• The HPP effect on probiotic properties depended mainly on the strain;	Enhance the raw material quality and nutrient availability, resulting in a safer product with increased viability and gastrointestinal	(Tabanelli et al., 2013)

			• *L. paracasei* A13 increased its hydrophobicity and autoaggregation capacity after HPP treatment; • No decrease in bile resistance was observed; • HPH treated *L. paracasei* A13 showed enhanced resistance to simulated gastric digestion	fluids resistance.	
L. acidophilus, *L. bulgaricus* *L. casei*	Sterile MRS broth containing 100 µg mL^{-1} cholesterol 0.15% (w/v) L-cysteine hydrochloride, 0.3% (w/v) ox gall and 0.1% (w/v) pancreatin	PEF (7.5 kV/cm for 4 ms)	• Increased the growth of lactobacilli cells; • Electroporation increased the incorporation of cholesterol into the cell membranes.	Cholesterol-lowering ability in media by incorporation to the lactobacilli cell membrane.	(Lye et al., 2011)
Limosilactobacillus fermentum BT8219	Biotin-supplemented soymilk	PEF (7.5 kV/cm for 3.5 ms)	• Electroporation did not affect the acid tolerance of *L. fermentum* to pH 3 but decreased at pH 2; • Electroporation of parent probiotic cells also decreased the tolerance to bile acids, antimicrobial activity, and adhesion ability; however, the probiotic properties	Electroporation increases biotin-soymilk bioactivity via fermentation with probiotic *L. fermentum* BT 8219, a strategy for developing functional foods.	(Ewe et al., 2012a)

			were recovered in the following subcultures.		
L. acidophilus	MRS medium	OH (1 V/cm, 60 Hz)	- Compared to conventional fermentation, bacteriocin activity increased when the moderate electric field was applied.	Optimize fermentation time and increase bacteriocin production.	(Loghavi et al., 2007; Loghavi et al., 2008)

Table 2. Recent applications of emerging processing technologies in probiotic food products.

Food	Probiotic strain	Method	Main effects	Reference
Soybean meal	*Bacillus subtilis*	US bath (28 kHz, 0.08 W/mL)	• Ultrasound applied to assist soybean fermentation improved ACE inhibitory activity, peptides content, and *B. subtilis*.	(Ruan et al., 2020)
Fermented milk	*Bifidobacterium breve, B. infantis, B. animalis, B. lactis* (Bb-12), and *B. longum* (Bb -46)	HIUS (20 kHz, 1 W/mL for 7 – 15 min)	• The ultrasonication stimulated the fermentative activity and the growth of bifidobacteria; • Also stimulated the production of primary organic acids (e., g. lactic, acetic, and propionic acids)	(Nguyen et al., 2012)
Fermented milk	*Lactobacillus acidophilus*	OH (0, 4, 6 and 8 V/cm)	• The OH use after post-contamination product reduced the *L. monocytogenes* counts while probiotic cells survived in the product and simulated gastrointestinal conditions. • The OH-treated product had a higher content of bioactive compounds and improved sensory acceptance.	(Silva et al., 2021)
Fermented skim milk	*Lacticaseibacillus paracasei*	HIUS (28 kHz, 100 W/L for 35 min beginning at nine h of the fermentation) pulsed mode (100s on/10s off)	• Optimum peptide production by high-intensity ultrasound processing was studied; • Peptide content increased by 64.23%; • The US increased the extracellular enzyme activities	(Huang et al., 2019)
Fermented mango juice	*Lactiplantibacillus plantarum* Lp-115	HIUS (20kHz, 600 W for 10 min) pulse (5s on/ 5s off) combined to UV-8 W lamps with 254 nm wavelength	• Improved physiochemical properties • Retained bioactive compounds during storage (4°C/30days) • Improved probiotic survival GIT passage	(Wang et al., 2021)
Italian Caciotta cheese	*L. paracasei* A13	HPP homogenization(50 MPa) of Late exponential phase probiotic cells in MRS broth	• *Lb. paracasei* A13 treated by HPH and added to the cheese was more resistant to simulated gastrointestinal digestion than non-treated ones.	(Burns et al., 2015)
Rice Beverage	*L. plantarum 12* *L. casei* LC01 *B. animalis subsp. lactis* Bb *12*	HPP (50-100 MPa for 1, 2 or 3 times) and US (60-100% of 130 W once or 80% 2-3 times, during 4 min, pulse 4s)	• US-attenuation did not affect viability or sensorial acceptance of the beverage; • US-attenuation avoided the product post-acidification.	(Bevilacqua et al., 2016)

Biotin-enriched Soymilk	*Lacticaseibacillus casei, Limosilactobacillus fermentum* and *Lactobacillus gasseri*	PEF (7.5 kV/cm for 4 ms)	• PEF increased the β-glucosidase activity of the lactobacilli cells, leading to increased bioconversion of isoflavone glucosides to bioactive aglycones in biotin-soymilk	(Ewe et al., 2012b)
Ice Cream	*Lacticaseibacillus rhamnosus*	PEF (3.0 kV/cm for 10 min and 1 Hz)	• PEF increased the bioaccumulation of calcium ions; • The application of PEF increased the survival of *L. rhamnosus* during the freezing process	(Pankiewicz et al., 2020)
Red cherries	*Lactobacillus acidophilus* ATCC1643	PEF (0.3 and 2.5 kV/cm for 20 µs) and pulse frequency of 100 Hz	• Enhanced probiotic bacteria growth	(Sotelo et al.,2018)
Fermented blueberry fruit	*Lactobacillus amyloliquefaciens* and *Levilactobacillus brevis*	LED pulsed light shaking at 80 pm LED pulsed light height from sample = 30 cm.	• Fermentation by both bacterial species under white and green LED light increased bacterial viability and total phenol and flavonoid contents; • Blue and red LED light fermentation and darkness resulted in fermented samples with moderate antibacterial activity.	(Jeong et al., 2018)

Table 3. Applications of emerging processing technologies in prebiotic food products.

Food	Prebiotic	Method	Main effects	Reference
FOS	FOS	HPP (at 450 MPa for 5 min) US (600–1200 W/L for 5 min) CP (at 70 kV for 15–60 s)	• no FOS hidrolyzation by HPP, US or CP; • no compromising prebiotic concentration.	(Alves Filho et al., 2016)
Cranberry juice	FOS	HPP for 5 min (450 MPa) US for 5 min (600 and 1200 W/L)	• preserved the FOS content; • high organic acids retention (> 90 %) and increased anthocyanin (24%) after US processing followed by HPP; • no changes in color, soluble solids content, and pH.	(Gomes et al., 2017)
Orange juice	Oligosaccharides with different polymerization degrees	CP (at 70 kV for 15, 30, 45 or 60 s) Ozone (load 0.057, 0.128 or 0.230 mg/ O_3.mL)	• partial oligosaccharides degradation; • well preserved phenolic content, antioxidant capacity, pH, and color.	(Almeida et al., 2015)
Orange juice	FOS	CP (at 70 kV for 15, 30, 45 and 60 s) HPP(at 450 MPa for 5 min at 11.5 °C)	• FOS degradation after HPP; • increased content of citric and ascorbic acid.	(Almeida et al., 2017)
Whey dairy beverage	XOS	CP (15 kV for 0, 5, 10, and 15 min)	• low hydroxymethylfurfural level (1.91–2.10 μmol/L) and whey protein nitrogen index (6.09–6.66 μmol/L); • high antioxidant activity (5.31–9.30%), antihypertensive activity (14.17–22.53%),and hypoglycemic activity (α-amylase: 18.52–25.67%; α-glucosidase: 22.50–27.50%)	(Ribeiro et al., 2021)
Apple juice	Inulin	SCF (10, 15, and 20 MPa at 35 °C, 10 min, and a 67% CO_2 volume ratio)	• reduced particle size suspended in juice; • increased functional compounds; • increased sorbitol, glucose, fructose, and sucrose; • no reduction in antioxidant activity;	(Silva et al., 2019b)

			• maintained inulin polymerization degree.	

Table 4. Applications of emerging processing technologies in postbiotic food products for clinical trials.

Food	Postbiotic	Method	Main effects	Reference
High-fat diet chow	*Lacticaseibacillus casei*	US (20 kHz, 40 min)	• prevented total cholesterol and LDL increase; • controlled the insulin resistance; • modulated of the intestinal microbial, increasing beneficial bacteria and decreasing those harmful; • attenuated the blood pressure.	(Brandão et al., 2021)
Chow	*Bifidobacterium lactis*	SCF (CO$_2$ at 10 MPa, 40°C for 180 min)	• increased albumin and creatinine levels; • decreased HDL cholesterol levels.	(Almada et al., 2021b)
Whey-grape juice drink	*Lacticaseibacillus casei 01*	OH (8 V/cm, 95 C/7 min, 60 Hz)	• similar hypoglycemic activity of probiotic drink; • reduced glycemic response; • maintained the maximum glucose increase; • reducing the postprandial glycemia.	(Barros et al., 2020a)