Technological advances in probiotic stability in yogurt: a review

Avanços tecnológicos na estabilidade probiótica em iogurte: uma revisão

Avances tecnológicos en la estabilidad de los probióticos en el yogur: una revisión

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Abstract
Yogurt is one of the fermented dairy products widely produced and recognized around the world, in addition it is considered excellent vehicle for probiotics, which are live microorganisms that provide beneficial effects to the individual when consumed in adequate amounts. Thus, the aim of this literature review was to address the factors that affect the viability of probiotics in yogurt during the processing steps (heat treatment, homogenization, and fermentation), storage (acidification rate, pH, carbohydrate fraction, organic acids, oxygen, temperature, time, water activity and moisture content), consumption (gastric juice and bile salts) and shelflife (addition of other ingredients and packaging). However, to preserve the probiotics stability in yogurt and improve the quality and shelf life of products, several new technologies such as microencapsulation, ohmic heating, ultrasound, the addition of prebiotics, and advances in the use of packaging in production with an emphasis on improving the viability, are used and allow secure the minimum recommended level of at probiotics least 10^9 CFU per gram of product when consumed to have a beneficial effect on health and, moreover, they guarantee the growth and probiotics protection without influencing the flavor, from the production stage until the delivery of these in the gastrointestinal tract. Therefore, it is recognized from this research the need to optimize new technologies in the food environment, seeking to improve consumer products with increasingly favorable purposes for health.
Keywords: Probiotics; Yoghurt; Emerging technology; Functional foods.

Resumo
O iogurte é um dos produtos lácteos fermentados amplamente produzido e reconhecido ao redor do mundo, além disso é considerado excelente veículo para probióticos, que são microrganismos vivos que conferem efeitos benéficos ao indivíduo quando consumidos em quantidades adequadas. Assim, o objetivo dessa revisão bibliográfica foi abordar os fatores que afetam a viabilidade de probióticos no iogurte durante as etapas de processamento (tratamento térmico,

homogeneização e fermentação), armazenamento (taxa de acidificação, pH, fração de carboidratos, ácidos orgânicos, oxigênio, temperatura, tempo, atividade da água e a taxa de umidade), consumo (suco gástrico e sais biliares) e vida de prateleira (adição de outros ingredientes e embalagem). No entanto, a fim de preservar a estabilidade de probióticos no iogurte e melhorar a qualidade e o prazo de validade de produtos, várias novas tecnologias como a microencapsulação, aquecimento ôhmico, ultrassom, adição de prebióticos e avanços no uso de embalagem na produção com ênfase na melhoria da viabilidade, são utilizadas e possibilitam garantir o nível mínimo recomendado de probióticos de pelo menos 10^9 UFC por grama de produtos quando consumidos para ter um efeito benéfico à saúde, e ainda, garantem o crescimento e a proteção de probióticos sem influenciar o sabor, desde a etapa de produção até a entrega desses no trato gastrointestinal. Portanto, é reconhecido a partir desta pesquisa a necessidade da otimização de novas tecnologias ao setor alimentício, buscando aprimorar os produtos aos consumidores com fins cada vez mais favoráveis para a saúde.
Palavras-chave: Probióticos; Iogurte; Tecnologia emergente; Alimentos funcionais.

Resumen
El yogur es uno de los productos lácteos fermentados ampliamente producidos y reconocidos en todo el mundo, además de ser considerado un excelente vehículo para los probióticos, que son microorganismos vivos que brindan efectos beneficiosos al individuo cuando se consumen en cantidades adecuadas. Así, el objetivo de esta revisión de la literatura fue abordar los factores que afectan la viabilidad de los probióticos en el yogur durante las etapas de procesamiento (tratamiento térmico, homogeneización y fermentación), almacenamiento (tasa de acidificación, pH, fracción de carbohidratos, ácidos orgánicos, oxígeno, temperatura), tiempo, actividad de agua y contenido de humedad), consumo (jugo gástrico y sales biliares) y vida útil (adición de otros ingredientes y envasado). Sin embargo, con el fin de preservar la estabilidad de los probióticos en el yogur y mejorar la calidad y vida útil de los productos, varias nuevas tecnologías como la microencapsulación, calentamiento óhmico, ultrasonidos, adición de prebióticos y avances en el uso de envases en la producción con énfasis en la mejora. viabilidad, se utilizan y permiten garantizar el nivel mínimo recomendado de probióticos de al menos 10^9 UFC por gramo de productos cuando se consumen para tener un efecto beneficioso sobre la salud, y también asegurar el crecimiento y protección de los probióticos sin influir en el sabor, desde la etapa de producción hasta la entrega de estos en el tracto gastrointestinal. Por ello, se reconoce a partir de esta investigación la necesidad de optimizar las nuevas tecnologías en el entorno alimentario, buscando mejorar los productos de consumo con finalidades cada vez más favorables para la salud.
Palabras clave: Probióticos; Yogur; Tecnología emergente; Alimentos funcionales.

1. Introduction

Dairy products are one of the main trends in the food industry, in addition to be widely consumed and recognized as health promoters (Yilmaz-Ersan, Ozcan, & Akpinar-Bayizit, 2020), they also represent one of the largest segments of the functional food market (Akin & Ozcan, 2017).

Yogurt is one of the fermented milk products best known for being a functional food and probiotics, therefore, overtaking a great market success level overall compared to other probiotic products (Parvarei, Fazeli, Mortazavian, Nezhad, Mortazavi, Golabchifar & Khorshidian, 2021), its nutritional composition is rich in vitamins, minerals and proteins of high biological value, being available in various textures and flavors (Fabersani et al., 2018).

For thousands of years, yogurt has been regularly present in the diet of various societies/communities and it is derived from the turkish word 'yogurmak' which refers to thickening or coagulating (Behera & Panda, 2020), and currently, there is growing interest in the food industry to continuously develop innovative dairy products containing probiotic microorganisms with potential for human health (Dimitrellou, Kandylis, Lević, Petrović, Ivanović, Nedović, & Kourkoutas, 2019).

The yogurt production occurs from the coagulation and gelling of milk, which occurs through the action of the bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp*, which ferment lactose, producing organic acids such as lactic acid and bioactive peptides contributing to the unique flavor and texture and characteristic of yogurt (Yerlikaya, Saygili & Akpinar, 2021).

The yogurt is considered an excellent vehicle for probiotic microorganisms and can be classified into two groups, which are standard culture yogurt and probiotic yogurt, therefore probiotic cultures in yogurt must remain alive in adequate numbers from the time of manufacture to consumption (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018). According to

Global Market Insights (2020), the size of the global market for probiotics exceeded $2 billion globally in 2018 and it is estimated to grow by more than 7.3% between 2019 and 2026.

The word "probiotic" comes from the Greek language "pro" and "bios", meaning "for all life", in other words, each substance or organism that prolongs life (Asgari, Pourjavadi, Licht, Boisen, & Ajalloueian, 2020). According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (2002), probiotics are "live microorganisms that provide health benefits to the host when administered in adequate amounts". Therefore, in recent years new definitions have been added to probiotic terminologies, such as 'para-probiotics' (dead/inactivated cells of probiotics) and 'post-biotics' (healthy probiotic metabolites) (Zendeboodi, Khorshidian, Mortazavian, & da Cruz, 2020).

The main probiotic microorganisms are generally lactic acid bacteria (LAB) belonging to the species *Lactobacillus, bifidobacterium, Propionibacterium, Peptostreptococci, pediococcus, Leuconostoc, Enterococcus, Streptococcus, Bacillus, bacteroides, Akkermansia, Saccharomyces* (Zhorzashidian & da Mor, 2020), being characterized as “generally recognized as safe” (GRAS) and they are also dominant inhabitants of the human gut microbiota (Vlasova, Kandasamy, Chattha, Rajashekara, & Saif, 2016).

The composition and activity of specific bacteria residing in the colon can be influenced by the activity of probiotics favoring the intestinal microbiota, therefore the regular intake of probiotic yogurt generates great potential in improving gastrointestinal health, decreasing the incidence of inflammatory bowel diseases, diseases autoimmune, preventing certain types of cancer and regulating factors that can lead the individual to develop obesity and type 2 diabetes (Rezazadeh, Alipour, Jafarabadi, Behrooz, & Gargari, 2021).

Furthermore, the current study of Gouda, Adbelruhman, Alenezi, and Mégarbane (2021), reported that yogurt contains several bioactive peptides benefits, including antiviral effects, antioxidants, anti-inflammatory, antithrombotic, and preventive chest infections which consequently influence the presentation and result of COVID-19.

In order to further a bioactive impact on the host, it is considered that, in most cases, a minimum number of live probiotic cells must be present in the product when consumed (above 10^6 CFU g^{-1} ou mL^{-1}) (Terpou et al., 2019b), and some regulatory agencies require that one billion cells per serving (10^9 CFU g^{-1}) be present in the probiotic food product even on the expiration date (CFIA, 2019).

The viability of probiotics is challenging, it can be lost during the manufacturing process, transport, or during product storage, due to various environmental factors such as temperature, oxygen, humidity, pH, and the presence of other cultures (Asgari, Pourjavadi, Licht, Boisen, & Ajalloueian, 2020). And even if the ingestion of probiotics may be adequate, the deactivation potential of orally administered probiotic bacteria should be examined, as their viability and functionality can be affected during passage through the gastrointestinal tract (Frakolaki, Giannou, Kekos, & Tzia, 2020).

To obtain positive therapeutic effects on the health of the host, the effectiveness of probiotics must be achieved and optimized through technological innovations and adequate yogurt processing (Champagne, da Cruz, & Daga, 2018), with the maintenance of cells viable probiotic, is considered a key step in the production of functional food (Terpou et al., 2019b).

The microencapsulation (Silva et al, 2019; Frakolaki, Giannou, Kekos, & Tzia, 2020a), the ohmic heating (Pereira et al, 2018) the low - frequency and high-intensity ultrasound (Bhargava, Mor, Kumar, & Sharanagat, 2020), the use of probiotics (Delgado-Fernández, Corzo, Olano, Hernández-Hernández, & Moreno, 2019) and the use of packaging (Terpou et al., 2019b) among others, are among the emerging technologies used as strategies of control to guarantee the quality of the probiotic yogurt without affecting the rheological, organoleptic and shelf life of the product.

Based on the above considerations, the aim of this review was to address the main latest technologies related to the maintenance of probiotics proposed to be used in the production of yogurt to ensure that the probiotic bacterias retain their

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survival and stability in effective doses in the stages of production, storage, transport and until the expiration date /or consumption.

2. Methodology

A literature review was carried out in the Science Direct and Google Academic databases, as a search strategy, the following descriptors were used: probiotics; yogurt; emerging technology; functional foods. Publications from the period between 2016 to the year 2021 were included. Thus, being a qualitative methodological search in a literature review (Pereira, Shitshuka, Pereira, & Shitsuka, 2018).

3. Results and Discussion

3.1 Factors that affect the probiotics survival in yogurt

Yogurt is a fermented milk-based product that increasingly receives the addition of probiotic bacteria to improve its quality and nutritional value, increasing its beneficial impacts on health (Aryana & Olson, 2017). This effect of probiotics is due to dairy fermentation, which plays a role in aiding milk preservation by generating lactic acid, antimicrobial compounds, desirable flavor compounds, and other metabolites (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018).

Yogurt fermentation is a chemical process, in which probiotic bacteria participate, producing lactic acid as the main end product of lactose metabolism, hydrolyzing milk proteins into individual peptides and amino acids, and degrading milk lipids into free fatty acids, hence the concentration of lactic acid, pH rate, temperature and fermentation time can affect the viability of probiotics during fermentation (Das, Choudhary, & Thompson-Witrick, 2019; Yang et al., 2021), and generate difference and change in sensory properties, organoleptic and rheological (Peng, Koubaa, Bals, & Vorobiev, 2020).

An approximate amount of 100 g of probiotic foods per day should be consumed to provide 10^9 viable cells to benefit the gut and promote health effects, so it is crucial to have the minimum recommended level of probiotics of at least 10^7 CFU g^{-1} or mL^{-1} at the time of consumption (Călinoiu, Vodnar, & Precup, 2016). Among the most used probiotics in the production of yogurt,) has been highlighted the use of *Lactobacillus* (Strain L. casei ATCC 393) (Bosnea, Kopsahelis, Kokkali, Terpou, & Kanellaki, 2017b), *Lactobacillus acidophilus, and Bifidobacterium animalis ssp* (Fazilah, Ariff, Khayat, Rios-Solis & Halim, 2018), *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Turgut, & Cakmakci, 2018), *Lactobacillus helveticusR0052* (Moineau-Jean, Champagne, Roy, Raymond & LaPointe, 2019).

The probiotic viability asserts the ability of a cell to grow and subsequently generate a colony of cells under defined conditions and it is generally considered a prerequisite for product functionality concerning consumer health-promoting properties, therefore, constitutes an industrial challenge, as many factors can influence the effectiveness of probiotics in food products during processing and storage (Terpou et al., 2019b).

Given the several technological challenges of the industry in the production of cell preparations and in the management of variables to produce probiotic foods (Wilkinson, 2018), the type of bacteria or probiotic strains to be used in fermented dairy products is determined according to various selection criteria, the which include: safety, origin, pathogenicity and infectivity properties, virulence and technological factors, genetic stability, desired viability during processing and storage, good sensory and structural properties and large-scale production, functional criteria, and resistance to acidic enzymes, biliary and pancreatic and adhesion to the mucosal surface (Dinkçi, Akdeniz, & Akalin, 2019).

Milk components used in yogurt production, such as whey proteins, caseins, enzymes, and fats, influence the survival levels of probiotic bacteria and affect their viability during fermentation; in addition, it is noted that the more bioactive compounds the milk has, the higher counts of probiotic bacteria, the yogurt perseveres until the end of its shelf life (Akalin, Unal, & Dinkci, 2018).

The probiotic viability of yogurt is changed due to the addition of prebiotics such as pectin, and other ingredients such as flaxseed mucilage, quinoa extract, which depending on can act as a carbon source for the growth of probiotic bacteria and can alter the acidity and antioxidant potential of yogurt (Khubber, Chaturvedi, Thakur, Sharma, & Yadav, 2020).

According to Bosnea, Kopsahelis, Kokkali, Terpou, AND Kanellaki (2017b), the addition of fruits like apples and dried raisins help in maintaining the viability of probiotics in yogurt during storage. However, Kumar & Kumar (2016) showed that the addition of fruits such as apricot, raspberry, plum, and jam leads to a reduction in the pH and increase in the acidity of the probiotic yogurt successively during storage for up to 15 days, and this can affect the probiotic bacteria that decrease, but even so, they keep values of 10^6 CFU g^{-1} until the end of the storage period. Similarly, Turgut & Cakmakci (2018) observed the same effect with the addition of strawberry to yogurt, with a decrease in *L. acidophilus* viability during the period of storage at refrigerated temperatures, and this may be because this fruit provides fermentable substrates for the growth of yeasts and fungi.

Storage, in turn, can generate changes in the acidification rate, pH, in the fraction of carbohydrates, organic acids, oxygen, considering the temperature, time, water activity, and humidity rate (Moineau-Jean, Champagne, Roy, Raymond, & LaPointe, 2019). Therefore, these changes affect the sensory properties of yogurt such as aroma, texture, and flavor, and influence the effectiveness of probiotic bacteria during its shelf life (Moineau-Jean et al., 2020).

Oxygen can reach dairy probiotics in many stages during the drafting process thus affecting the viability of probiotics may cause toxicity which is particularly critical in the case of *bifidobacteria*, which are anaerobic rigorous, then the physical composition of packaging also offers a source oxygen diffusion products decreasing the survivability capacity of these bacteria (Ladero & Sánchez, 2017).

A trend toward lower pH (< pH 3.0) in dairy products containing probiotic cultures is generally observed as a result of increased acid production lowering viable probiotic cell counts (Terpou, Bekatorou, Kanellaki, Koutinas, & Nigam, 2017). According to Turgut and Cakmakci (2018), the storage of yogurt can decrease (about 2 logs) usually the number of probiotic bacteria, and the pH level lower than 4.6 is harmful since during the storage time the interaction between starter organisms and probiotic bacteria are considered a factor influencing their viability. In the same segment, Terpou, Papadaki, Bosnea, Kanellaki, and Kopsahelis (2019a) mentioned that storage by freezing can damage bacteria probiotics due to the formation of ice crystals, in addition, the survival of the probiotics can be further reduced during thawing, where cells are exposed to osmotic stresses.

Bacteria in the initial culture, according to their type, activity, and level at the time of inoculation of the bacteria, can induce the effect of probiotic bacteria during yogurt fermentation, reducing fermentation time ensuring greater probiotic viability (Yerlikaya, Saygili, & Akpinar, 2021; Dinkçi, Akdeniz, & Akalin, 2019). But these activities in yogurt during the fermentation process and the refrigerated storage period can sometimes threaten the survival of probiotic bacteria by producing the hydrogen peroxide that suppresses probiotic growth and negatively impacts its viability (Meybodi, Mortazavian, Arab, & Nematollahi, 2020).

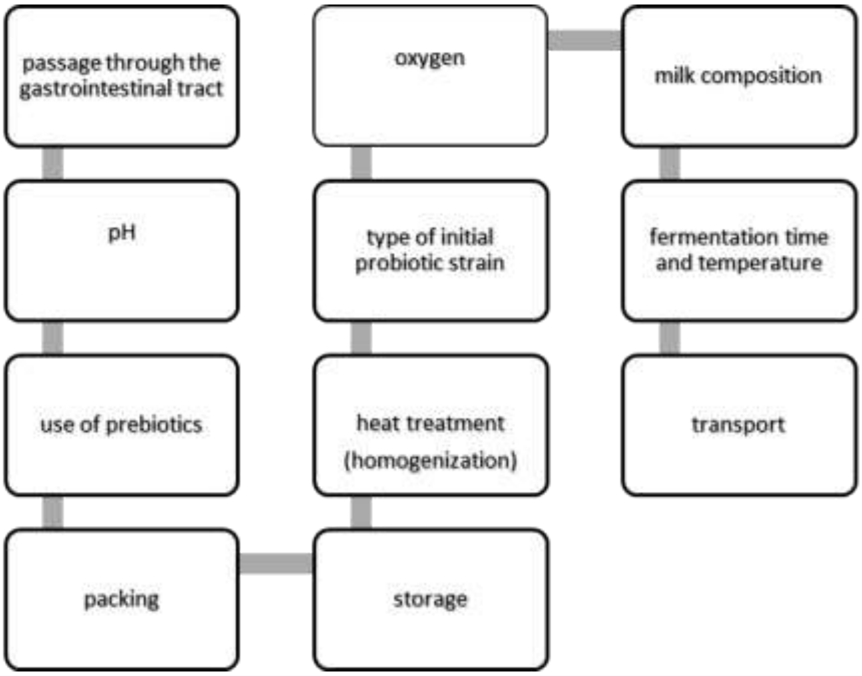
Taking into account that most probiotic bacteria can not grow at temperatures below 15 to 20 °C or above 45 to 50 °C, inadequate temperatures used during homogenization or fermentation or even in dairy storage can affect the growth of bacteria (Călinoiu, Vodnar, & Precup, 2016). Heat treatment with high temperatures in milk significantly influences the viability of probiotics in yogurt according to the quality and substrates present in the fermented milk, so yogurts have a shorter shelf life due to their sensitivity to the low pH caused by the substrates that become a limiting factor for the growth of probiotics (Zamberlin & Samaržija, 2017).

The heat treatment and the milk homogenization process during the production of yogurt are processing conditions which influence the viable count of probiotics in the production of yogurt, therefore, when applied correctly cause an increase of probiotics that can be explained by the removal of bacteria competitive in milk, decreased oxygen load, inactivation of harmful probiotic bacteriophages, and production of more free amino acids and small peptides necessary for the growth and survival of probiotics during refrigerated storage, but otherwise, any unfavorable condition can cause undesirable effects (Meybodi, Mortazavian, Arab, & Nematollahi, 2020).

For a more active and viable activity, it is necessary that probiotic bacteria can proliferate and colonize the wall of the gastrointestinal tract to carry out their health-positive activities (Zendeboodi, Khorshidian, Mortazavian, & da Cruz, 2020). Generally, the efficiency of probiotic bacteria added to yogurt is affected at the time of consumption, by the low pH of the gastric fluid, bile salts, digestive enzymes, and competitive bowel conditions during its passage through the gastrointestinal tract (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018). According to Halim et al. (2017), the survival of probiotics decreases in proportion to the time when they were exposed to gastric juices at pH = 1.55 and simulated bile salt solution.

Thus, different factors are effective in the viability of probiotic bacteria in yogurt at different stages of production, storage, and consumption, thus, probiotic foods require high technological demands to maintain the viability of probiotics at all stages to ensure consumer health benefits as shown in Figure 1 below.

Figure 1. Factors that affect the survival of probiotics in yogurt**.**



Source: Authors (2021).

3.2 Technologies used to provide greater stability of probiotic bacteria

To prevent the intrinsic and extrinsic factors that influence the survival of probiotics in yogurt production during processing and under storage conditions, several technologies are provided as alternative solutions (Halim et al., 2017). Scientifics studies recently concentrated themselves on different innovative techniques to increase the viability of probiotic cells to increase the shelf life of products (Champagne, the Cross & Dagger, 2018).

3.2.1 Microencapsulation

Microencapsulation is an important technology to support cell viability during production, storage, and consumption, to ensure the survival of probiotic cells successfully influencing the protection or targeting of the distribution of probiotics in the production of yogurt (de Prisco & Mauriello, 2016). The technique is defined as a physical-chemical or mechanical process to trap a substance, in a suitable material, to produce spherical particles with a thin but strong semi-permeable membrane, with diameters from a few nanometers to a few millimeters, being these parameters and the conditions during the process must be taken into account to protect the bacteria and simultaneously avoid negative effects on the sensory characteristics of the products (Frakolaki, Katsouli, Giannou, & Tzia, 2020b).

Microencapsulation facilitates the processing, with proper handling, as well as the administration of probiotics, so this technology is suitable and of great interest for applications in probiotic bacteria in food matrices (Da Silva et al., 2019; Frakolaki, Giannou, Kekos, & Tzia, 2020a). Recently, the use of polymers such as pectin, alginate, carrageenan, chitosan, starch, whey, gelatin, and lipids in the microencapsulation of bacteria has been proposed as a protective coating material capable of forming a barrier with positive effects on the protection of probiotic cells during the storage condition and the gastrointestinal tract environment (Sarao & Arora, 2016). This effect was proved by a study by Di mitrellou, Kandylis, Lević, Petrović, Ivanović, Nedović, & Kourkoutas (2019), where the micro-encapsulation of the probiotic *Lactobacillus casei* ATCC 393 in alginates using the extrusion technique, led to significantly higher viability compared to free cells in simulated gastrointestinal conditions as well as after storage for 28 days in fermented milk (7.13 log CFU g^{-1}), therefore, the aroma was improved for typical compounds generally produced by in *L. casei* fermented dairy products, without affecting the physicochemical and sensory characteristics of the final product.

In the studies carried out by Jouki, Khazaei, Rezaei, and Taghavian-Saeid (2021), the production of powdered symbiotic yogurt through the use of microencapsulation technology associated with lyophilization and the use of cryoprotectants together with the probiotic *L. plantarum*. and sorbitol, glycerol, and sodium-skimmed milk alginate, revealed that it was possible to maintain yogurt as a probiotic product stored for 10 weeks at 25 °C, without the need to maintain the cold chain, and after this process the product in reconstitution proved dissolves easily in water, creating rheological properties and appearance similar to yogurt, so this technology ensures the increased to *L. plantarum* survival rate during storage time and permits the production of functional dairy products with long shelf life and desirable quality properties.

Lactobacillus acidophilus was encapsulated by complex conservation followed by crosslinking by transglutaminase, later the microcapsules were dried by lyophilization, microencapsulation was effective and provided greater protection and viability of probiotics about thermal resistance, exposure to the simulated gastrointestinal tract and regarding storage for 60 days (Da Silva et al., 2019).

Afzaal et al. (2019) evaluated the effect of microencapsulation on the stability of probiotic bacteria in yogurt under simulated conditions, probiotic bacteria were encapsulated with sodium alginate and carrageenan, unencapsulated (free) cells exhibited poor survival after 28 days of storage in simulated gastric solution (encapsulated bacteria decreased only 3 logs, while for free cells, reduction of 7 logs is recorded), the same result was obtained by Atraki and Azizkhan (2021) who concluded that the viability rate of *Lactobacilli* and *Bifidobacteria* strains in an acidic environment and simulated gastrointestinal condition significantly increased through microencapsulation compared to free cells.

Microencapsulation of probiotic strains by high-pressure homogenization maintains high probiotic viability during refrigerated yogurt storage, meeting, at the end of storage, the rules required for functional foods (at least 7 logs/ UFC g^{-1} products), and avoiding the hyper-acidification in products and modulating the release of volatile molecules and exopolysaccharides (Patrignani et al., 2017).

According to Bosnia, Moschakis, Nigam, and Biliaderis (2017a), the viability of lactobacilli cells encapsulated by complex coacervation or incorporated in alginate gel microspheres, after 48 hours of fermentation increased even at simulated pH 2.0, and had greater survival after heating and in simulated gastric juice for 3 hours, about a reduction in free bacteria that reduced over time and when exposed to a stressful environment, noting that this encapsulation technique provides additional protection for probiotics against harmful conditions.

The microencapsulation technology encompasses several techniques, and each one of them has its characteristics and the same purpose of action on probiotic bacteria, among which the use of the extrusion technique stands out mainly; coacervation technique; spray drying; ultrasonic vacuum dryer; lyophilization; the ultrasonic drying; lyophilization and spray cooling spray (Frakolaki, Giannou, Kekos, & Tzia, 2020a).

3.2.2 Ohmic heating (OH)

Ohmic heating (OH) also known as Joule heating is an alternative heat treatment, considered to have less impact on biologically active compounds in food products compared to traditional thermal processes (Alkanan, Altemimi, Al-Hilphy, Watson & Pratap-Singh, 2021), also OH is a technique proposed as an alternative power of time and energy savings to conventional methods, therefore, the ohmic treatment should modify the according to the properties of the food material to ensure the quality of food without affecting the texture (Gavahian, Tiwari, Chu, Ting, & Farahnaky, 2019).

Among the new technologies in dairy processing, ohmic heating (OH) stands out in the development of new products in the industry such as probiotic yogurts (Pereira, Teixeira, Vicente, Cappato, Ferreira, Rocha & Cruz., 2018). The OH technology consists of a heating process in which the electric current passes directly through the food, promoting instantaneous and homogeneous heating through the conversion of electric energy into thermal energy, resulting in shorter and more effective heating time and temperature to the conventional process leading to greater retention of nutrients and sensory attributes (Cappato, Ferreira, Guimaraes, Portela, Costa, Freitas, Cunha, Oliveira, Mercali & Marzack., 2017). The OH is capable of increasing the rate of growth and activity of probiotic bacteria, by acting on the adjustment of activity enzyme thus better walk the interaction between enzymes and substrate, therefore it favors the fermentation by promoves increased permeability cell and of the ions diffusion and molecules across the cell membrane, resulting in better nutrient absorption (Pereira, Teixeira, Vicente, Cappato, Ferreira, Rocha & Cruz., 2018).

According to Silva et al. (2021) ohmic heating (0, 4, 6, and 8 V / cm; CONV, OH, 90-95°C / 5 min) can provide satisfactory results in cell survival at the gastrointestinal tract level in probiotic fermented milk, in addition, improve the generation of bioactive compounds increase the antioxidant capacity of the product and resulting in fermented milk with higher health benefits, especially when used electric field intensities medium or high, and this study also showed that the OH is an interesting alternative to minimize the effects of contamination with potential pathogens (*L . monocytogenes*) after fermentation can then secure the OH functional and safety goals in the manufacture probiotic fermented milk.

According to Barros, Pires, Guimaraes, Abboud, Almada, Pimentel, Santanna, De-Melo, Duarte and Silva (2021) OH by generating stone less cell damage can maintain the probiotic functions and preserve its properties, and this effect is dependent on the applied electric field and the evaluated probiotic strains, therefore, analyzes performed with *Lactobacillus acidophilus LA 05*, *Lacticaseibacillus casei 08* and *Bifidobacterium animalis Bb 12* with OH in different magnitudes of electric field (4, 8 and 12 V / cm at 60 Hz), resulted in greater preservation of these probiotic strains and OH proved to be an adequate technology for the efficient production of paraprobiotic products.

Even so, the understanding of the effect of OH on the properties of dairy products and especially on probiotics is not well established and presents itself as one of the great challenges for the elaboration of probiotic dairy products and requires investigation before industrial adaptation (Kim, Jo, & Kang, 2017; Cappato et al., 2018).

3.2.3 Low frequency and high-intensity ultrasound

A promising new technology reported in the literature to improve the viability of probiotics is low-frequency, high-intensity ultrasound, which has great potential for a wide variety of applications in dairy processing (Bhargava, Mor, Kumar, & Sharanagat, 2020).

The type of ultrasound treatment is very important and has a great impact on yogurt production (Carrillo-Lopez et al., 2021). The energy supplied by an ultrasound generator is emitted through an ultrasonic bath or a probe-based system and generates intense sound waves that propagate through a medium and create regions of compression and rarefaction (Peng, Koubaa, Bals, & Vorobiev, 2020); this action provides high potency, which is enough to generate cavitation, so it is capable of producing mechanical, chemical and biochemical effects in liquids through the production and subsequent collapse of cavitation bubbles (Akdeniz & Akalın, 2019).

Ultrasound is a technology that can be used to increase quality, improve processing effectiveness and efficiency, and provide food safety while extending product shelf life through enzymatic modification, homogenization, pasteurization, reduction in shelf life yogurt fermentation and improvement of yogurt rheological properties (Carrillo-Lopez et al., 2021).

An increase in the activity of probiotic cultures with the use of ultrasound results in greater probiotic viability, due to faster acid development and, consequently, reduced fermentation time, which generates less processing time, minimizing ingredient requirements and additives and reducing the resources needed, improving sensory properties and nutritional value of products (Guimarães et al., 2019).

The low-intensity ultrasound can improve the fermentation of *Lactobacillus casei subsp. casei ATTC 39392*, inferring an increase in lactic acid production, cell reproduction, and substrate consumption by *Lactobacillus casei subsp. casei ATTC 39392*, in addition to increasing the levels of growth, such as specific growth rate and duration of the logarithmic phase (Dahroud et al., 2016). In this way, ultrasound can be used properly in the fermentation industry leading to lower production costs.

According to Ojha, Mason, O'Donnell, Kerry, and Tiwari (2017), low-frequency ultrasound applications in the stimulation of probiotics such as *Bifidobacterium sp*, resulted in accelerated hydrolysis of lactose and transgalactosylation of bifidobacteria in milk, reducing fermentation time for up to 30 minutes depending on the probiotic strain, consequently, an increase in cell growth and probiotic viability is noted. This effect was also investigated by Akdeniz and Akalın (2019), who highlighted the advantages of high-intensity ultrasound in yogurt technology, acting in prioritizing the fermentation and storage of honey, improving the viability of probiotics.

In cases where the viability of the probiotic is not necessary, ultrasound can be used for the generation of paraprobiotic (or phantom probiotic) and post-biotics, where the inactivation of probiotic bacteria in these cases is interesting, as there is less interaction with the matrix food (Ojha, Mason, O'Donnell, Kerry, & Tiwari, 2017; Guimarães et al., 2019).

However, additional scientifics studies is needed to efficiently control the ultrasonic treatment process, due to a lack of knowledge, understanding, and reluctance to abandon traditional practices, which prevents the implementation and commercialization of ultrasound at an industrial level (Bhargava, Mor, Kumar, & Sharanagat, 2020; Huang et al, 2017).

3.2.4 Use of prebiotics

Currently, scientific studies on the effect of prebiotics on the acidification rate and viability of probiotic bacteria during the manufacture and refrigerated storage of yogurts are being discussed (Delgado-Fernández, Corzo, Olano, Hernández-Hernández, & Moreno, 2019). Due to their technological characteristics, some prebiotics can lead to improvements in the quality of products regarding sensory properties, texture, and physicochemical characteristics, in the addition of fibers and the partial replacement of sugars and fats (Xavier-Santos, Bedani, Perego, Converti, & Saad, 2019).

Prebiotics falls into a category of functional foods and can be defined as non-digestible food ingredients classified as soluble fiber, which beneficially affect their host by selectively stimulating the growth and activity of bacteria in the colon, thus improving the health of the host, In yogurt, the prebiotic acts as a substrate for the growth of probiotic bacteria and, consequently, improves gastrointestinal functions and the immune system (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018).

Different types of prebiotics, such as galactooligosaccharides (GOS), inulin, fructooligosaccharides (FOS), resistant starch and lactulose have been used in the manufacture of yogurts and other fermented milks (Delgado-Fernández, Hernández-Hernández, Olano, Moreno, & Corzo, 2020). Many studies on laboratory media or in controlled conditions, similar to how food systems showed a positive effect of prebiotics, keeping the probiotic bacteria cultivability at high levels (Speranza et al.,2019).

Prebiotics such as potato starch and inulin in yogurt favors the growth and viability of probiotic bacteria acting as a carbon source, resulting in bacterial numbers between 9 and 10 log CFU mL^{-1} throughout storage, and this effect varies according to the type of prebiotic used, with *Plantago psyllium* resulting in a lower rate of bacterial growth (Peredo, Beristain, Pascual, Azuara, & Jimenez, 2016). According to Li et al. (2019), population counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus* in yogurt supplemented with long-chain inulin increased after 14 days of storage, however, prebiotics had a positive effect on increasing the viability of Lactobacillus in probiotic yogurt during storage.

At the laboratory level the effects of prebiotic compounds (FOS, inulin, and lactulose) were evaluated are the cultivate and counting in various samples of milk product with probiotic bacteria, so during 100 days of storage at 4, 15, 30, and 45 °C, it was observed that in samples added with FOS a higher probiotic load of about 7 logs CFU ml^{-1}, even at the end of the observation period was recorded compared to samples added with lactulose and inulin, this controlled study showed prolonged viability of probiotic bacteria added with the FOS prebiotic compounds. Moreover, it was noted that the addition of a probiotic and prebiotic ingredient microorganism does not adversely affect the sensory acceptability of the product (Speranza and t al., 2019).

A recent study by Tiwari, Kavitake, Devi, and Halady (2021) presents the use of exopolysaccharides (EPS) as a potential source of prebiotics in the production of symbiotic yogurt, and according to the results of this study, EPS stimulate the growth of lactic acid bacteria, such as *Lactobacillus Plantarum MTCC9510* and *L. fermentum MTCC903* in yogurt, and manage to maintain the viability of probiotics in the gastrointestinal tract.

The good quality yogurt production using wheat bran as prebiotic favored the viability of probiotics, which were kept in high numbers of viable bacteria during storage at 4 °C, the yogurt remained with acceptable levels of pH and showed a more complex flavor profile, being it resulted in a symbiotic product of high nutritional value (Terpou, Bekatorou, Kanell Aki, Koutinas, & Nigam, 2017).

The influence of the type and amount of prebiotic (FOS, GOS, or lactulose), as well as the storage time, were evaluated for *S. thermophilus* and *L. delbrueckii ssp. Bulgaricus* in yogurt, lactulose in high dose (4%) significantly influenced probiotic bacteria and significantly increased bacterial cell survival up to 14 days of storage, and the total count of this microorganism at the end of storage compared to FOS and GOS showed that time, type and amount of prebiotics significantly influenced bacterial counts (Delgado-Fernández, Corzo, Olano, Hernández-Hernández, & Moreno, 2019).

Results of a study by Mudgil, Barak, Patel, and Shah (2018) showed that partially hydrolyzed guar gum, considered a potential source of prebiotics, maintained a stable pH and increased the viable count of various lactic acid bacteria that ranged from 7.24 to 7.78 log CFU ml^{-1}, with *S. thermoplhilus MD2* exhibiting increased growth, so they concluded that prebiotics help to stimulate the growth of potentially probiotic bacteria and/or balance native intestinal microflora to improve health and well-being social.

Therefore, prebiotic addition with probiotic bacteria has significant application to improve the characteristics, texture, and prolong the shelf life of yogurt, and offers the possibility of using this technology in different food products without affecting the bacteria's viability or its physical chemical characteristics (Peredo, Beristain, Pascual, Azuara, & Jimenez , 2016).

3.2.5 Packaging

To protect and influence the survival of probiotics, food packaging techniques are used to reduce oxygen permeability in dairy probiotics stored and sold on the market in plastic packaging, with thicker packaging and glass bottles for improved survival of *L. acidophilus* and *Bifidobacteria* in yogurt have been proposed due to poor or influence of oxygen in these bacteria (Terpou et al., 2019b).

Majid, Nayik, Dar, and Nanda (2018) claimed that packaging technologies emerged as a result of the consumer's desire for convenient, ready-to-eat, tasty, and light processed food products, with extended shelf life and maintained quality, within the new food packaging techniques note the use of active packaging (natural, recyclable and biodegradable packaging that prolong storage time and increase the food safety margin), intelligent packaging (linked to the advancement of time-temperature regulators, maturation monitors, biosensors and radiofrequency indicators and regulators) and bioactive packaging (which has the potential to keep bioactive substances in the desired proportions within the packaged food during storage or before consumption), and these innovative packaging technologies have contributed to improving the quality, safety, viability and bioactivity of functional food components.

Renovating packaging technologies can be a solution to replace plastics used in the food industry that have different porosity and permeability leading to continuous diffusion of oxygen in the food matrix, depending on the thickness of the container, which can impact the probiotic properties (Ladero & Sánchez, 2017).

The packaging plays an important role, preventing oxygen from affecting active probiotic cells, reducing their survivability throughout the storage period. This purpose is acquired in glass packaging, due to its low permeability to oxygen, favoring the survival of probiotic cultures, however, food manufacturers prefer the use of plastic packaging due to the high cost of glass and the dangers inherent in its use (Călinoiu, Vodnar, & Precup, 2016).

Other emerging technologies, including pulsed electric fields (PEF), high pressure processing (HPP), ultraviolet (UV) and microwave (MW) have attracted great attention for their implementation in the food industry as moderate processing technologies, which have the potential to improve various processes, such as microbial inactivation of pathogens, improvement in microbial growth and fermentation conditions, as well as modified metabolic properties of probiotics, without detrimental effect on the nutritional and organoleptic quality of foods, however the application of these technologies in industry it is quite limited due to high investment costs, as well as intensive maintenance, hinder the wide implementation of these technologies in the industry, so several studies have shown a high survival of LAB as the use of these technologies in foods such as apples, fruit juice and in vegetables, frozen potatoes or ice cream and its untested effect on yogurt (Peng, Koubaa, Bals, & Vorobiev, 2020).

4. Final Considerations

In view of the above study carried out, followed by scientific bases, it can be concluded that there is still a long way of research to be carried out about the viability of maintaining probiotics in the reference dairy product, yogurt. Yogurt is a nutritionally rich food, in addition to carrying probiotics to benefit the consumer's health, which can positively colonize the gastrointestinal tract. Currently, there is a growing search for new technologies to overcome existing factors that are harmful to their activities, however, it is worth mentioning that new studies are needed to evaluate the behavior of probiotic cells in yogurt in different application parameters, in addition to research in small and large production scales, so that one day they are applied in the daily production.

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