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Fermentation of plant-based milk substitutes: state of the art and future research opportunities

Trabajo de final de Grado

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ABSTRACT

In recent years, the interest in plant-based beverages has increased and, consequently, so has their demand worldwide. These comprise between 60% and 70% of the functional food market, and provide benefits for the health of the consumer, having a fairly positive connotation since it comes from a non-animal source. Furthermore, plant-based beverages are considered a good food matrix for incorporation of probiotics, due to their advantages such as nutritional composition and high pH, making it possible for probiotics to survive and multiply.

Therefore, the aim of this work is to review the main conditions applied to produce plant-based milk substitute's beverages and the strategies to improve their final quality. Factors such as the pH and acidity of the medium, the antioxidant activity, the fermentation state media, the use of mixed microorganisms and their origin have been considered. In addition, the effect of fermentation on physic-chemical, nutritional, technological and sensorial properties have been analyzed.

Published works in the last ten years show the fermentation of plant-based beverages enhances nutritional quality by improving digestibility and mineral availability, eliminating anti-nutritional factors and generating bioactive compounds such as vitamins. The metabolism of probiotics in fruit matrices improves the bioaccessibility of phenolic compounds, which have shown a prebiotic effect stimulating the growth of probiotics. Also, preservative capacity, technological properties and sensorial acceptability might be increased. In this sense, fermentation causes an increase in viscosity, which provides structure to the beverage, and produces metabolites that affect aroma, flavour and texture, improving its acceptability. Non-thermal technologies can also be applied to further increase the stability and the final quality of fermented beverages, both in terms of nutrition, sensory and beneficial effects on health. These technologies may be the key to the success of plant-based probiotic foods.

Keywords: Probiotic, plant-based beverages, fermentation, quality, non-thermal technologies, nutrients

Bebidas vegetales fermentadas: estado del arte y oportunidades futuras de investigación

RESUMEN

En los últimos años el interés por las bebidas de origen vegetal ha aumentado y, en consecuencia, también lo ha hecho su demanda a nivel mundial. Comprenden entre el 60% y el 70% del mercado de alimentos funcionales, y no solo proporcionan beneficios para la salud del consumidor, sino que también tienen una connotación positiva ya que provienen de una fuente de alimentación no animal. Además, las bebidas de origen vegetal se consideran una buena matriz alimentaria para la incorporación de probióticos, debido a sus ventajas como la composición nutricional y el elevado pH, permitiendo que los probióticos sobrevivan y se multipliquen.

Por tanto, el objetivo de este trabajo es revisar las principales condiciones que se aplican para la producción de bebidas sucedáneas de origen vegetal y las estrategias para mejorar su calidad final. Se han considerado factores como el pH y acidez del medio, la actividad antioxidante, el estado de fermentación del medio, el uso de microorganismos mixtos y su origen. Además, se ha analizado el efecto de la fermentación sobre las propiedades físico-químicas, nutricionales, tecnológicas y sensoriales.

Los trabajos publicados en los últimos diez años muestran que la fermentación de bebidas de origen vegetal mejora la calidad nutricional al mejorar la digestibilidad y la disponibilidad de minerales, eliminando los factores anti-nutricionales y produciendo componentes bioactivos como las vitaminas. El metabolismo de los probióticos en las matrices de frutas mejora la bioaccesibilidad de los compuestos fenólicos, que muestran un efecto prebiótico estimulando el crecimiento de los mismos. Además, se podrían mejorar la capacidad de conservación, las propiedades tecnológicas y la aceptabilidad sensorial. En este sentido, la fermentación provoca un aumento de la viscosidad, lo que le da estructura a la bebida y produce metabolitos que afectan el aroma, el sabor y la textura, aumentando su aceptabilidad. También se pueden aplicar tecnologías no térmicas para aumentar aún más la estabilidad y la calidad final de las bebidas fermentadas, tanto en términos de efectos nutricionales, como sensoriales y beneficiosos para la salud. Estas tecnologías pueden ser la clave del éxito de las bebidas vegetales con probióticos.

Palabras clave: Probiótico, bebidas de origen vegetal, fermentación, calidad, tecnologías no térmicas, nutrientes

Begudes vegetals fermentades: estat del art i oportunitats futures de investigació

RESUM

En els últims anys l'interès per les begudes d'origen vegetal ha augmentat i, en conseqüència, també ho ha fet la seua demanda a nivell mundial. Comprenen entre el 60% i el 70% del mercat d'aliments funcionals, i no sols proporcionen beneficis per a la salut del consumidor, sinó que també tenen una connotació positiva ja que provenen d'una font d'alimentació no animal. A més, les begudes d'origen vegetal es consideren una bona matriu alimentària per a la incorporació de probiòtics, a causa dels seus avantatges com la composició nutricional i l'elevat pH, permetent que els probiòtics sobrevisquen i es multipliquen.

Per tant, l'objectiu d'aquest treball és revisar les principals condicions que s'apliquen per a la producció de begudes succedànies d'origen vegetal i les estratègies per millorar la seva qualitat final. S'han considerat factors com el pH i l'acidesa del medi, l'activitat antioxidant, l'estat de fermentació del mig, l'ús de microorganismes mixtes i el seu origen. A més, s'ha analitzat l'efecte de la fermentació sobre les propietats fisicoquímiques, nutricionals, tecnològiques i sensorials.

Els treballs publicats en els últims deu anys mostren que la fermentació de begudes d'origen vegetal millora la qualitat nutricional al millorar la digestibilitat i la disponibilitat de minerals, eliminant els factors anti-nutricionals i produint components bioactius com les vitamines. El metabolisme dels probiòtics en les matrius de fruites millora la bioaccesibilitat dels compostos fenòlics, que mostren un efecte prebiòtic estimulant el creixement d'aquests. A més, es podrien millorar la capacitat de conservació, les propietats tecnològiques i l'acceptabilitat sensorial. En aquest sentit, la fermentació provoca un augment de la viscositat, que li dona estructura a la beguda i produeix metabòlits que afecten l'aroma, el sabor i la textura, augmentant la seua acceptabilitat. També, es poden aplicar tecnologies no tèrmiques per millorar encara més l'estabilitat i la qualitat final de les begudes fermentades, tant en termes d'efectes nutricionals, com sensorials i beneficiosos per a la salut. Aquestes tecnologies poden ser la clau de l'èxit de les begudes vegetals amb probiòtics.

Paraules clau: Probiòtic, begudes d'origen vegetal, fermentació, qualitat, tecnologies no tèrmiques, nutrients

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INTRODUCTION

The probiotic foods market is growing very fast due to an increased consumer awareness about the impact of food on health. Today, probiotic products comprise between 60% and 70% of the total functional food market. The global market for probiotic foods and beverages is on the rise, it was around 24.8 billion euro in 2011, over 31.1 billion euro in 2015 and, it was predicted to reach around 43 billion euro by 2020 (Aspri et al., 2020). At the moment, the consumption of probiotic foods is higher in Asia, Australia and Eastern Europe. However, sales are growing day by day in the American continent, occupying the first places of consumption in the United States of America, Brazil, Canada and Mexico (Castillo-Escandón et al., 2019).

Besides, the number of vegans and vegetarians increases year after year. According to the Vegan Society, the demand for food without meat grew by 987% in 2017 in the United Kingdom. In Brazil, 30 million people do not eat meat, which represents 14% of all country inhabitants. Still, 55% of Brazilian population would like to eat more “vegan products”, 49% believe these products have the same quality of animal products, and 60% do not consume more vegan products because their prices are considered higher than the animal-based ones (Ibope, 2018). At the same time, over the years, the global market for plant-based beverages has become a multi-billion-dollar business and will correspond to 26 billion USD by 2023 (Tangyu et al., 2019). In Europe, the sales of soy beverage and other non-dairy milks are increasing by over 20% per year; Spain being the EU country in which the non-dairy drinks market grew the most. Oilseed and nut milk substitutes, almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), coconut (*Cocos nucifera*), hazelnut (*Corylus*), peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), soy (*Glycine* max), tiger nut (*Cyperus esculentus*), oat (*Avena sativa*), rice (*Oryza sativa*), hemp (*Cannabis sativa*), and walnut (*Juglans*) are preferred by vegans and people who suffer from an allergy to cow’s milk (Bernat et al., 2014a). Plant-based milk substitutes are one of the food groups that are irreplaceable in the vegan food industry because plant-based milk substitutes are used as an essential ingredient in many vegan food products such as plant-based yogurt, cheese, kefir, butter, ice cream, etc.

Vegetable beverages present some advantages to incorporate probiotic, due to their nutritional composition (protein, fat, sugar and fiber) and high pH, which positively impact the survival of these microorganisms. Also, they are favourable matrices to prepare non-dairy frozen desserts (Pimentel et al., 2021).

Probiotics are microorganisms that, when ingested in adequate amounts, confer benefits to the host (Hill et al., 2014). These microorganisms have been associated with several beneficial effects, mainly related to gastrointestinal health (Floch, 2018) and the immune system (Kristensen et al., 2016) as well as diabetes

(Razmpoosh et al., 2019), obesity (Ejtahed et al., 2019), hypercholesterolemia (Sangwan & Singh, 2018), cancer (Dasari, Kathera, Janardhan, Kumar, & Viswanath, 2017), among others. On the other hand, prebiotics have been described as indigestible food ingredients whose selective fermentation results in desired alterations in the activity and/or composition of the gut microbiota (promoting growth) and eventually conferring health benefits to the host; while, the food-based synergistic mixture of probiotics and prebiotics is termed as synbiotics and are designed to improve the survival and the colonization of the ingested microorganisms to the intestinal tract (Kehinde et al., 2020).

Probiotic fermentation of plant-based beverages enhances nutritional quality by improving digestibility, eliminating anti-nutritional factors and production of nutritional factors such as vitamins. Since the lack of protein content and low bioavailability of minerals and vitamins are the main limitations in plant-based beverages, strategies such as fermentation, addition of enzymes, fortification with essential nutrients and mixing two or more vegetable drinks have been suggested to achieve a product with high nutritive value equivalent to cow's milk (Rasika et al., 2021).

Probiotics can be consumed in the form of nutraceuticals (pharmaceutical alternative with physiological benefits), added to foods as part of a fermentation process or by supplementation (Aspri, Papademas, & Tsaltas, 2020; Behera & Panda, 2020; Tangyu et al., 2019). When probiotics are added to vegetal beverages, it is necessary to carefully verify the compatibility of the ingredients with the probiotic culture, select the probiotic strain, and evaluate the processing steps. For the moment, the main challenge for the effectiveness of a probiotic food product is to maintain the viability of the probiotic strain since the health effect of probiotic food products depend upon the number of viable cells present at the moment of consumption (Aspri et al., 2020). To ensure the beneficial effects on the host, a minimum concentration of live probiotic cells must reach the intestine. The bacterial minimum concentration suggested is around 8 log CFU/g or mL of food at the time of consumption (Pimentel et al., 2021).

The aim of this work is to carry out a review of the main conditions applied to produce probiotic plant-based beverages and how their final quality can be improved. Factors as relevant as the pH and acidity of the food matrix, the antioxidant activity or the fermentation state media, which will condition the fermentation process have been considered. It has also been reviewed the effect of fermentation on physic-chemical, nutritional, sensorial and technological properties of the initial plant-based beverage.

MATERIALS & METHODS

The search for scientific articles was carried out mainly through the specialized databases ScienceDirect (https://www.sciencedirect.com) and Google Scholar (https://scholar.google.es). Given the large number of articles found online about this topic, keywords like: "vegetable drinks/beverages with probiotics", "vegetable drinks treatments", "ultrasound on fermented beverages", "encapsulation on fermented beverages", "PEF on fermented beverages" and "high hydrostatic pressure on beverages", were used to carry out this filtering.

The search focused on those articles published between 2010 and 2021. The information was within our reach without having to search too much, since it is a topic that is currently on the media and the demand for this type of products does not stop growing year after year.

The cereals and legumes most used to prepare these vegetable drinks, according to the number of searches, were rice and soy; although other searches were also found on almond, quinoa, wheat, oat, barley or maize. Also, searches were found on various fruits and vegetables, sometimes as a raw material and others as an added ingredient (apple, coconut, orange, mango, passion fruit, strawberry, pineapple, carrot or tomato).

RESULTS

Fermentation in plant-based milk substitutes beverages: microorganisms and nutrients

The most employed microorganisms for the fermentation of plant-based beverages are the probiotic bacteria, more specifically the genera of *Lactobacillus* and *Bifidobacterium*; but there are so many more involved like *Streptococcus*, *Lactococcus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Ligilactobacillus*, *Limosilactobacillus* or *Pediococcus* (Pimentel et al., 2021). These comprise a broth of fermenting and lactic acid producing bacteria, which is used in the fermented plant-based beverages industry to provide specific quality characteristics to the final product and to protect it against the action of other harmful microorganisms.

The probiotic culture can be added by prior fermentation (37 ^{o}C for 1–12 h, anaerobic or aerobic incubation) of part of the extract used in the product formulation (10–30%) and later addition of this fermented beverage. Other possible ways are adding fresh probiotic culture (biomass) to the mixture; adding the lyophilized probiotic culture to the mixture and use of the total extract of the formulation in a fermented form. The selection of the form of addition is based on the probiotic survival, technological aspects, and industry processing (Aspri et al., 2020).

The fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. Depending on whether the substrate is liquid or solid it can be defined: the solid-state fermentation (SSF) that uses a solid substrate without free-flowing aqueous phase, and the liquid-state fermentation (LSF) or submerged fermentation that uses free-flowing liquid substrates as alcohol, oil or nutrient broth (Sagar Aryal et al., 2019). Published scientific works show the LSF as the most used method since it is easier to manage at industrial level. In the LSF, probiotics are directly added to the juice or beverage producing microbial metabolites like bacteriocins that can help to improve the quality of the final product and increasing their shelf-life (Costa et al., 2017). Depending on the species of bacteria the optimal temperature varies, although according to Severson (1998), the optimal temperature of a culture is related with the optimal growth temperature of the starter bacteria used. For plant-based beverages, this is usually around 37 ^{o}C and needs much longer time (16-24 h) than those fermentations of cow milk (Bernat et al., 2014a).

Yeast and fungal species are also used, although their use is less common; yeast of the genera *Saccharomyces* or *Torulaspora* and fungi of the genera *Brettanomyces*, *Kluyveromyces* or *Candida*, are the most remarkable. This two domains are being employed mainly for solid-state fermentation (Darwish, Darwish, & Ismail, 2017; Jeske et al., 2018). The solid-state fermentation occurs in the absence of free water. This application of solid-state fermentation provides a sustainable process saving drinking water and energy and reduces the generation of new wastes (Fuentes et al., 2020). Therefore, a natural or inert substrate with high porosity and the ability to absorb water is selected offering similar conditions to their natural habitat and favouring their growth. In addition, due to the reduced water activity, the probability of contamination by others yeast or fungi is low. Fungi are the best adapted to grow in these environments; moreover, they have the ability to use polysaccharides as carbon source (Kehinde et al., 2020). They are commonly, used in the preparation of bakery products or in the elaboration of Sake.

To date, plant-based beverages fermentation mainly uses mono-cultures of microbes, such as lactic acid bacteria, bacilli and yeasts, for this purpose. More recently, new concepts have proposed mixed-culture fermentations with two or more microbial species. These approaches promise synergistic effects to enhance the fermentation process and improve the quality of the final product (Aspri et al., 2020).

The probiotics used can be provided by the raw material. Naturally, cereals, legumes, and nuts host microorganisms that promote spontaneous fermentation giving raise a wide variety of traditional fermented products like Nigerian cereal-based foods fermented by *Lactobacillus plantarum* YO175 (Adesulu et al., 2018) or homemade highland barley wines in Tibet fermented by *Lactobacillus reuteri* WHH 1689 (Chen S. et al., 2018). This type of fermentation results in a shorter shelf life and varying quality (Rasika et al., 2021). Plant-based traditional beverages, mostly made by spontaneous fermentation, is what happens when you leave the inoculation moment up to whatever organisms happen to be in the air or on the fruit that you are fermenting. This delivers unexpected flavours that range from ripe raspberries to fresh cane sugar, to tart lemon or to the juiciest of melons. It is an ancient method of fermentation and just as ancient are the drinks that were made with this method, drinks such as: rice beer locally known as Apong in Assam (India) fermented by *Bacillus velezensis* D14*,* Koji used in the production of rice wine (China) fermented by *Lactobacillus plantarum* 430 (Rasika et al., 2021) or *Levilactobacillus brevis* KU15151 isolated from traditional Korean food (octopus jeotgal and radish kimchi) and used to ferment black gamju (Pimentel et al., 2021).

On the other hand, probiotics can be isolated from sources with a similar composition to the juice or beverage being fermented; other grains like oatmeal, soybean or rice, or traditional foods as Kimchi (Pimentel et al., 2021). After that, probiotics can be added to the plant-based beverage for fermentation or to the naturally fermented beverage to improve its quality like traditional Chinese sauerkraut fermented by *Lactiplantibacillus plantarum* NCU 116 (Li et al., 2016).

Nevertheless, most of the used probiotics are isolated from dairy sources like yogurt, vegetable beverages, chocolate or plant-based substitutes as Koji, traditionally prepared rice beer, naturally fermented tofu or Tchapalo beverage (usually lactic acid bacteria, is isolated) (Rasika et al., 2021). For example, according to Solange (2020) the study of lactic acid bacteria diversity in Tchapalo helps to develop starter cultures to produce safe beverages, finding mesophilic and thermophilic lactic acid bacteria in the different steps of the processing (spontaneous lactic fermentation followed by alcoholic fermentation). These are species like *L. fermentum* (predominant), *L. plantarum*, *P. acidilactici*, *E. faecium* and *E. gallinarum*, which potentially are able to produce bacteriocins to inhibit the activity of harmful strains/pathogens. In most cases, these microorganisms can growth in MRS agar (Man-Rogosa-Sharpe) with a carbon source and subsequently the activated cells, previously separated from the broth, are incorporated as an ingredient into the new product (milk or cereals/grains) (Dallagnol et al., 2012). Anyway, for industrial application, it is important to consider the study of resistance to phage attack, the ability to be produced on a large scale and the absence of metabolites that can impair the sensory characteristics of the vehicle (Castillo-Escandón et al., 2019).

Probiotics were commonly recognized to be derived from yoghurt or other dairy-based products (Aspri et al., 2020). However, it should be noted that those probiotics isolated from similar sources to the desired product have several advantages over those of dairy origin. Among these advantages the most important are: the greater resistance of the probiotic to adverse conditions in the substrate or in the intestinal tract (gastrointestinal conditions) and the improvement of the growth of these microorganisms on the substrate to be treated, having a higher growth rate, due to the contribution of nutrients that favour this increase (Kandylis et al., 2016). Strains isolated from dairy products may not have favourable conditions to grow, multiply and remain viable in plant-based beverages due less availability of nutrients, presence of anti-nutritional factors, unfavourable pH and lack of buffering capacity (Rasika et al., 2021).

Soy, rice and coconut beverages are the major carrier matrices used in probiotic plant-based food development (Rasika et al., 2021). Moreover, mixture of grains contributes to supply the required amounts of carbohydrates, starch and protein for growth of bacteria (Maselli & Hekmat, 2016). For example, *Lactobacillus casei Zhang*, originally isolated from a traditional homemade koumiss in Inner Mongolia (China) grows faster in soy beverage than in milk at inoculation rates ranging from $2{\cdot}10^6$ to $2{\cdot}10^7$ CFU/g. It was explained, because a major part of the identified proteins in soy drink are associated with transport and metabolism of carbohydrates, nucleotides and amino acids (Wang et al., 2013). Okara, the residue generated during manufacture of soy beverage, is used for preparation of ingredients containing probiotics like *L. casei* that grew with the highest rate in the medium containing okara due to its high moisture content (70-80%); with the slowest rate observed with extruded-expelled meals (Fuentes et al., 2020).

The addition of nutrients to plant-based beverage may increase its functionality, since the addition of certain nutrients provides with beneficial effects both to the fermentation process and to the quality of the final product. Some beneficial effects are to provide consistence during storage, to improve viability of probiotics or to fortify with prebiotics (Aspri et al., 2020).

Phenolics are organic compounds whose molecular structures contain at least one phenol group, an aromatic ring attached to a hydroxyl group (OH-), as a functional group. Many are classified as secondary metabolites of plants, namely biosynthesized products in plants that have the biological characteristic of being secondary products of their metabolism. It was believed that the presence of phenolic compounds in cereals, nuts or legumes would inhibit probiotics growth. However, some studies have shown a prebiotic effect of phenolic acids stimulating the growth and metabolism of probiotic bacteria (Luciano et al., 2018; Morais, Borges, dos Santos Lima, Martín-Belloso, & Magnani, 2019).

The addition of fruit juices, like passion fruit juice incorporated with *Bifidobacterium animalis ssp.*, increases sugar content which can stimulate the growth of probiotics. Most of probiotics are able to use fruit sugars as sustenance for their growth and multiplication (Pimentel et al., 2021). Simple sugars can act as a supplement of nitrogen source and as a result, the concentration of lactic acid with its consequent acidity will be increased in the final probiotic drink (Chavan et al., 2018). Waxy corn starch provides consistency during cold storage of fermented products and improve the viscosity characteristics and texture. Related to that, microorganisms, like *Lactobacillus acidophilus* and *Bifidobacterium spp*., produce some sort of enzymes responsible for the disruption of the molecules of starch (non-soluble) making them soluble and able to be used for fermentation process (Costa et al., 2017).

Incorporation of micronutrients like peptides or amino acids is a good strategy to improve the viability of the probiotic microorganisms (Aspri et al., 2020). Peptides and amino acids serve as a substrate for the growth of probiotics and, in addition, some peptides have an antimicrobial function; *L. plantarum* strain can degrade quinoa peptides and release amino acids and antifungal compounds, thus eliminating the competition with other species and allowing probiotics to multiply greatly (Dallagnol et al., 2012). Various pulse crops could potentially be used as alternative protein sources in the formulation of milk alternatives and related products, including pea, faba bean, lentil, lupin, chickpea and common bean. Pulse protein ingredients must possess good functional properties, such as solubility and emulsifying properties. Already, high-protein pea protein-based milk substitutes have begun to be commercially available (Mintel Group Ltd., 2018). Food additives as emulsifiers (lecithin), stabilizers (hydrocolloids or carrageenan), sweeteners, natural (sucrose, fructose or glucose syrups from corn, rice or wheat) or synthetic (acesulfame K, aspartame or sucralose) and flavouring agents (cocoa, soluble coffee, vanilla or cinnamon), are usually added before the homogenization step; the amounts of these additives range from 0.4-2.5% in emulsifiers, 0.025-0.3% in stabilizers, 5-8% in sweeteners and 0.5-3% in flavouring agents (Erra, 2012; Pereyra & Mutilangi, 2012; Triantafyllou, 2002; Marti, Martinez, Miralles, & Perez, 2010). These compounds are added to facilitate the optimal dispersion of ingredients and to ensure safety and quality of the product (Bernat et al., 2014a).

In the case that the extracts to be fermented do not have enough vitamins to guarantee the survival of the probiotic microorganisms until the end of storage, it would be necessary to fortify it with vitamins such as folic acid, niacin or riboflavin, to reduce the risk of the probiotic cell's death (Costa et al., 2017). If the food matrix contains fructans (inulin) various benefits are reported. They improve the efficiency of fermentation and promote the growth and survival of the probiotic, since they serve as a carbon source. Adding prebiotics or soluble fibers such as fructooligosaccharides (FOS), it is an alternative to protect probiotics from adverse environment of gastric juices, helping to preserve the viability of probiotics. These can also act as plasticizers, reducing the viscosity of the product (Castillo-Escandón et al., 2019). According to

Gibson and Roberfroid (1995) among the prebiotics, fructooligosaccharides (inulin or oligofructose) have shown convincing evidence of a health promoting effect *in vitro* and *in vivo*.

Effect of fermentation on the physical-chemical, technological and sensory properties of vegetable drinks

Plant-based milk alternatives are often nutritionally unbalanced, and their flavour profiles limit their acceptance. Milk normally has quite a bland flavour and a light colour, which means that any off-notes or off-colours are easily detected by consumers. This is one of the major challenges for many plant-based products that come with characteristic flavours or pigments, such as almond, coconut, flaxseed, legume, or oat sources (Jeske, Zannini, & Arendt, 2018). Milk also has a relatively low viscosity and creamy mouthfeel that can be hard to simulate.

Addition of probiotics as an ingredient or involving fermentation can improve final quality of plant-based beverages. The benefits are greater when fermentation occurs and are mainly associated with the production of secondary metabolites. Thus, probiotic fermentation is a good option to improve the nutritional value, aroma and taste, texture, and stability as well as microbial safety. Also, in some cases digestibility is increased and anti-nutritional factors eliminated by fermentation, improving the absorption of minerals and vitamins (Rasika et al., 2021).

Next, a review is made of the main changes that occur with fermentation.

Acidity, pH and shelf-life

The pH changes as the beverage manufacturing process progresses. Depending on the substrate employed the higher pH value can be explained by the buffer capacity of proteins (Lopes et al., 2020). On the other hand, pH will increase with the use of germinated grains, instead of non-germinated ones (Chavan et al., 2018). Nevertheless, pH can decrease faster in substrates with a lower pH buffering capacity from proteins or when Lactobacillus is employed as fermenting culture (Wang et al., 2013). In this case, higher product acidity can protect the product from the development of spoilage microorganisms, increasing shelf life without changing the final product's characteristics (Costa et al., 2017). Moreover, plant-based beverages may contain opportunistic pathogens expressing thermal resistance, which counts can be fallen below the detection limits after fermentation with specific strains which has been attributed to nutrient competition or antimicrobial substances excretion (Paz et al., 2020).

However, probiotic viability will decrease as the pH decreases and the time of exposure increases (Lopes et al., 2020). Tamime and Robinson (2000) stablished that to obtain an optimal formulation, the fermentation process must reach a pH of 4.83 ± 0.03. Fuentes et al. (2020) showed that a decrease in pH to values around 4.0 does not really exert a negative influence on the survival of the probiotic (protective effect). Nevertheless, in some cases as the almond drink, this pH value can cause the formation of a 3D network of protein aggregates decreasing the quality of the final product (Garcés et al., 2019).

Strategies to improve viability of probiotic microorganisms include the selection of acid and bile resistant strains, use of oxygen impermeable containers, two-step fermentation, incorporation of micronutrients and microencapsulation (Aspri et al., 2020).

Dourado Costa (2017) evaluated the physicochemical, microbiological and sensory characteristics of a probiotic fermented drink made of mixed extract of soy and rice by-products with added waxy corn starch during cold storage (5 °C for 28 days). The pH values found during the storage period met the optimal pH range for beverages based on the lactic acid fermentation of oats (3.6–4.9). This range allows lactic acid bacteria, including probiotics to grow normally with no damage to the product. The results obtained also showed that the product was microbiologically stable during the 28 days of cold storage.

Nutritional and antioxidant properties

In the case of plant-based beverages, fermentation improved the antioxidant activity of raw material due to the release of phenols or smalls peptides, which also occurs during storage (Pimentel et al., 2021). The antioxidant activity increased significantly ($P<0.05$) in vegetable probiotic drinks with mixture of germinated grains; being the germinated mixture more suitable for the growth of probiotics as such mixtures may supply required amounts of carbohydrates, starch and protein (growth factors) (Chavan et al., 2018). At the same time, the addition of probiotics resulted in an increase in bioactive compounds and antioxidant activity. The consumption of probiotic beverages (soy-milk) improved antioxidant status in patients with type II diabetes (Pimentel et al., 2021). According to a study carried out by Hariri (2015) with two groups of patients with type II diabetes, the intervention group consuming the probiotic soy drink (200 mL/day) showed a significant decrease of promoter methylation in proximal and distal MLH1 promoter, while plasma concentration of 8-OHdG (8-hydroxy-20-deoxyguanosine) decreased compared to the control group that consumed conventional soy drink. This means the consumption of soy beverage containing *Lactiplantibacillus plantarum* A7 resulted in anti-oxidative properties and decrease the risk of mismatch base pair in DNA among patients with type II diabetes.

Overall, the metabolism of probiotics in fruit derived matrices can enhance the bioaccessibility of phenolic compounds and increase their functionality (Morais et al., 2019). In red pitaya pulp the effects of the incorporation of *Lactobacillus acidophilus LA-05* or *Bifidobacterium animalis ssp*. *Lactis BB-12* in the content and bioaccessibility of phenolics were studied. Fermentation with probiotics decreased ($P<0.05$) the content of phenolic acids, however both strains increased the presence of phenolics in the non-dialyzed fraction. So, the bioaccessible fraction of red pitaya pulp fermented by *L. acidophilus LA-05* or *B. lactis BB-12* showed higher antioxidant activity than the non-fermented one. The bioaccessibility of phenolic compounds in non-fermented red pitaya pulp ranged from 4.33 - 80.34%, while in red pitaya pulp fermented by *L. acidophilus LA-05* it varied from 14.01 - 85.22%. These findings indicate the fermentation of red pitaya by probiotics as an alternative to increase the bioaccessibility of specific phenolics (Morais et al., 2019).

Pectinases synthesized and secreted during microbial fermentation reported to increase protein content in cereal-based beverages. Moreover, certain microbial strains synthesize vitamins during fermentation including vitamin K and B-group vitamins (e.g. folate, vitamin B12, riboflavin, thiamine, niacin), thus increasing the nutritional value of the final product (Bernat et al., 2014b). Also, in some cases, nutritional value is improved because digestibility of proteins is increased, and anti-nutritional factors eliminated (Rasika et al., 2021). Some studies show phytase enzyme, which is found in lactic acid bacteria and yeasts, is responsible for the hydrolysis of phytate (Rekha & Vijayalakshmi, 2010) increasing bioavailability of minerals such as Fe or Zn. Additionally, short-chain fatty acids and organic acids originated during fermentation contribute to enhance solubility of minerals and vitamins and improve their absorption (Aydar et al., 2020). It is evident that fermentation helps to enhanced mineral availability and digestibility of nutrients. For rice, Ghosh (2015) observed free minerals accumulation during fermentation; Ca (0.78 ppm on 2nd day), Fe (0.33 ppm on 3rd day), Mg (4.87 ppm on 2nd day), Mn (0.45 ppm on 1st day) and Na (0.86 ppm on 2nd day), and these were higher than the unfermented material. To highlight that *L. acidophilus* enriched the fermented juices with minerals, decreasing the fermentation time and affecting positively to the viability of probiotic bacteria (Aspri et al., 2020).

Anti-nutrients are natural or synthetic compounds that interfere with nutrient absorption. The phytic acid and saponins are important anti-nutrients in some cereals. Phytates like phytic acid (organic acid containing phosphorus) and saponins form insoluble complexes with cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} and decrease their bioavailability. However, certain lactic acid bacteria and yeasts produce phytases that catalyse the hydrolysis of phytates into myoinositol and phosphate leading to increased mineral bioavailability. For instance, fermentation could greatly reduce phytate levels in wheat, oat and rye; thanks to the action of microorganisms such as *S. cereviasiae* and *S. Boulardii* (Rasika et al., 2021). A mix culture of *L. acidophilus* and *L. plantarum* is more effective than fermentation by the individual strains in eliminating phytic acid in cowpea (Sanni et al., 1999). Tangyu (2019) also reports that mix culture fermentation was effective in eliminating or reducing the contents of phytic acid, trypsin inhibitors, saponins and tannins present in cowpea and finger millet that increased bioavailability, extractability and digestibility of minerals.

The consumption of soy beverage is limited due to the presence of non-digestible oligosaccharides, such as raffinose and stachyose; enzymes present in the small intestine do not digest these oligosaccharides, as they lack in α-galactosidase activity (Rasika et al., 2021). In this way, the probiotic *Lactobacillus casei Zhang* can metabolize ribose, glucose, mannose, fructose, sucrose, maltose, and sorbitol, but not (or barely use) arabinose, xylose, melibiose, raffinose, and lactose (anti-nutritional factors) (Wu et al., 2009a). However, probiotics with α-galactosidase activity were able to hydrolyze non-digestible oligosaccharides, such as raffinose and stachyose. By these means it is possible to reduce non-digestible oligosaccharides content in fermented soy drink by 29% - 34%. In other cases, fermentation results in a complete elimination of raffinose and partial elimination of stachyose (Rasika et al., 2021).

Technological properties

Vegetable drinks have, in general, a low viscosity. So, this parameter must be increased during production because consumers do not accept a product with this characteristic. Low viscosity promotes the phase separation of the unstable fat globules caused by flocculation and coagulation phenomena in a short period of time (Bernat et al., 2014a). Some fruits and vegetables, such as oranges and tomatoes, contain natural stabilizing pectin, and during homogenization they release that pectin into the juice, increasing viscosity and stability. In plant-based beverages viscosity can be provided by starch and other polysaccharides after thermal treatments, considering the type of product (Bernat et al., 2014a). For example, waxy starch can bind to water improving the viscosity characteristics, decreasing texture throughout the storage period, related to the progressive loss of drink viscosity (Costa et al., 2017).

The fermentation process causes an increase in the viscosity values of the vegetable extract by forming a weak gel structure. On the one hand, the synthesis of exopolysaccharides produced by *Lactobacillus*, might contribute to the gel formation and to increase the viscosity values. Fermentation with exopolysaccharides producing bacteria strains showed a less phase separation in comparison with other strains not producing exopolysaccharides (Bernat et al., 2014a). Moreover, the addition of some nutrients such as inulin would increase viscosity of the final product, having a synergic effect on probiotic survival during processing and storage (Bernat et al., 2014b). High viscosities were achieved by incorporating either papaya or mango pulp (20%) in a probiotic rice beverage (Rasika et al., 2021).

Viscosity and firmness in yoghurts from plant-based beverages can also be improved because exopolysaccharides secreted by specific strains. For instance, *Bifidobacterium aesculapii* reported to grow well in soy beverage while producing considerable amounts of exopolysaccharides (5−131 μg/mL) leading to high product viscosity and firmness values (Patrignani et al., 2017).

In the same way, in symbiotic beverages, as storage time increases, an increase in viscosity is observed (Castillo-Escandón et al., 2019).

Sensory properties

Regarding the sensory properties, the probiotic fermentation of vegetable juices can produce metabolites which affect the aroma, flavour, texture and viscosity of the fermented product (Castillo-Escandón et al., 2019). Overall, several research showed that probiotic fermentation results in better sensory acceptability compared to unfermented products. In this way, the raspberry syrup was used as a sweetener in quinoa seeds beverage for development of fermented beverage with an increased sensory acceptability (Karovičová et al., 2020). Also, the acceptance of functional probiotic products based on fermented buckwheat was greatly satisfactory (Matejčeková et al., 2017). The formation of flavour compounds, such as acids, alcohols, aldehydes, esters, ketones, pyrazines, and others, is usually detected in fermented beverages. Their concentrations are affected by microbial cultures, the type of substrate and the presence or not of other prebiotics, affecting taste and smell of the final product (Xu et al., 2020).

In this way, Freire et al (2017) developed a new fermented beverage from maize and rice. Fifty-five volatile compounds including ethyl acetate were quantified. Ethyl acetate presence was most associated with the fermented beverages from maize. This volatile compound has a pineapple odor note, and its ester of short-chain fatty acids, were also detected in the beverages and, generally, provide fruity notes. Sensory acceptability was higher in fermented beverage from maize probably due to this flavor. Chavan (2018) also showed that fermentation with *L. acidophilus* starter culture improved the overall acceptability of soy, almond and coconut beverages although they did not explain why.

Table 1 includes a summary of the aspects considered in the nore relevant references.

Table 1. Main aspects considered in fermented plant-based beverages.

Microorganism			Incorporation way			Origin		Nutrient enrichment	Main effect	Reference
Bacteria	**Yeast**	**Fungi**	**Solid fermentation**	**Liquid fermentation**	**Encapsulated bacteria addition**	**Lactic origin**	**Others**			
Lactobacilli acidophilus	-	-	-	Drink mixtures of un/germinated cereals were added to 100 mL of milk	-	-	-	4 g of sugar and 0.07g of cardamom added to the drink mixture. Supplement of nitrogen source	Sensory evaluation was performed to found differences between germinated & ungerminated drink mixture	Chavan et al. 2018
Bifidobacterium genus, species of Lactobacillus, Lacticaseibacillus, Lactiplantibacillus, Ligilactobacillus, Limosilactobacillus	*Saccharomyces cerevisiae*	-	-	-	Added as a supplement. Encapsulation with sodium alginate provides protection to cells, improving the survival	-	Can be isolated from other grains: oatmeal, soybean or rice. Isolated from vegan products: Kimchi. LAB isolated from fermented cocoa juice	Species of lactic acid bacteria (LAB) were found capable of growing well without any nutrient supplementation on carrot juice	Maintain the sensory acceptance of the probiotic. Resulting in better colour parameters	Pimentel et al. 2021
L. acidophilus & Bifidobacterium spp	-	-	-	Lactic fermentation of soymilk to produce "yogurt"	-	Culture of lactic acid bacteria	-	Waxy corn starch added during cold storage. Saccharose (10/100 g) and guar gum (0.5/100 g) were added to the mixed extract. Folic acid (B9)	Sensory characteristics being modificated throughout the storage period. Starch can bind water improving viscosity	Costa et al. 2017
Lactobacillus plantarum 229v and other species of *Lactobacillus* & *Bifidobacterium*	*Saccharomyces cerevisiae*	*Kluyveromyces marxianus, Candida tropicalis, C.glabrata, Geotrichum penicillatum*	-	Fermentation of the juice with the probiotic. Bacteriocins are produced and help to improve the product	-	Probiotics were recognized to be derived from yogurt and other dairy-based	-	Incorporation of peptides and amino-acids to improve viability. Fortification by prebiotics like dietary fiber or	Fermented cereals can increase the availability of minerals (Ca, P, Fe) due to the action of phytases	Aspri et al. 2020

				quality		products		cellulose		
Lactobacillus plantarum CRL 778	-	-	-	Growth of the microorganism in MRS broth. Cells separated from the broth are incorporated into the quinoa	-	-	-	-	Fermentation by LAB contributes to enhancing the storage periods and the release of antifungal compounds	Dallagnol et al. 2012
Bifidobacteria, Lactobacillus & Streptococcus genera	-	-	-	From nut and cereal fermented vegetable products (milks)	Micro-encapsulation as a mean of promoting the survival through the gastrointestinal tract	-	Probiotics obtained by confocal laser scanning microscopy of fermented oat milk	Food additives such as emulsifiers, stabilizers, sweeteners and flavoring agents are introduced	Fermentation can reduce or eliminate the presence of anti-nutrients like phytic acid or saponins	Bernat et al. 2014a
Lactobacillus reuteri & Streptococcus thermophilus	-	-	-	Almond milk fermentation. Growth in MRS broth and incorporated later in milk	-	Activated from their frozen forms by transferring them to their selective broths	-	Addition of glucose (0.75 g/100 mL), fructose (0.75 g/100 mL) and inulin (2g/100mL)	Fermentation increases the viscosity values, forming a weak gel. Improves the bioavailability of peptides and immune response	Bernat et al. 2014b
Lactobacillus casei Zhang	-	-	-	Growth of *Lb. casei Zhang* in soy milk, an aqueous extract of soybean	-	-	Isolated from a traditional homemade koumiss in Inner Mongolia (China)	Soy milk constitutes sufficient nutrients to support growth of lactic acid bacteria	Fermentation helps to improve the quality by removing the unfavorable flavor.	Wang et al. 2013
Species of *Lactobacillus, Bifidobacterium, Lactococcus & Streptococcus*	*Saccharomyces cerevisiae, S. boulardii*	-	-	Naturally, cereals, nuts and legumes harbor microorganisms that promote spontaneous fermentation. In	Micro-encapsulation in calcium-alginate hydrogel to improve viability during storage and	-	Isolated from plant-based substitutes: Tchapalo beverage, homemade highland barley	To improve the quality, stabilizers like carrageenan or locust bean gum have to be used	Fermentation improves nutritional value, aroma & taste, texture & stability. Microbial safety.	Rasika et al. 2021

				traditional beverages	fermentation		wines or Koji		Eliminates anti-nutritional factors	
Lactobacillus plantarum CIDCA	-	-	Okara used for preparation of ingredients containing probiotics.	-	Encapsulation applied to improve probiotic resistance and to extend shelf life (based on polysaccharides)	-	Okara, solid residue generated during manufacture of soy milk	Addition of cheese whey (0.32/1g)	Improve nutritional, physical and flavour properties of food matrix. There's better nutrient accessibility	Fuentes et al. 2020
Lactobacillus plantarum ZSM-002	-	*Brettanomyces custersii* ZSM-001	Indica rice flour used to prepare fermented rice tablets	-	-	-	Isolated from fermented rice slurry	Mango pulp added to improve sensory acceptability. Calcium chloride (0.01g/mL) and sucrose	Organoleptic properties improved due to formation of flavor precursors during fermentation	Xu et al. 2020
Lactobacillus acidophilus La-05	-	-	-	-	Micro-encapsulation by external ionic gelation technique in alginate (30g/L) and chitosan coating	-	The inoculum of the strain was obtained after preparing suspensions in sterile saline solution	Salt addition may compromise the cell viability (NaCl affected by reduction of Aw)	-	Lopes et al. 2020
Lactobacillus plantarum CCMA 0743 & *Lb. acidophilus* LACA 4	*Torulaspora delbrueckii* CCMA 0235	-	Cereals used as substrates to produce traditional fermented foods	-	-	-	Isolated from cassava fermentations from the beverages cauim and tarubá (positive for amylase activity)	Addition of fructooligosaccharide (FOS). Prebiotic addition (20 & 50 g/L) influenced the carbohydrates concentrations (starch 72.4 g/L)	FOS was important to maintain viable the probiotic population (≥107 CFU/mL)	Freire et al. 2017
Lactobacillus fermentum KKL1	-	-	-	Fermentation of haria (rice based	-	-	Isolated from an Indian rice based fermented	-	Accumulation of free minerals increased during fermentation (Ca,	Ghosh et al. 2015

				beverage)			beverage		Fe, Mg, Mn, Na). Processing improves functional composition, therapeutic potentialities	
Species of *Lactobacillus y Bifidobacterium & Bacillus coagulans*	Species of *Saccharomyces & no-Saccharomyces*	-	-	Two strategies: a) the direct addition of unfermented probiotics to the juice. b) the fermentation of the juice with the probiotics	Micro-encapsulate bacteria, to protect them from the acidic environment	Isolated from dairy products like chocolate or coconut milk	-	Addition of fructooligosaccharide to the juice helps to preserve viability. Addition of prebiotics or soluble fibers like inulin. Addition of whey proteins	Fermenting juices with probiotics can produce metabolites that affect the aroma, flavor, texture and viscosity of the food	Castillo-Escandón et al. 2019
Lactobacillus delbrueckii spp. Bulgaricus & Streptococcus thermophilus	-	-	-	Probiotic fermented product based on almond milk	-	Lyofast culture Y430-A (starter lactic culture)	-	Addition of simple carbohydrates (sugars). In the food matrix 0.75g/mL of glucose was added. Use stabilizers like pectin, gelatin and starch	The gels formed were stable and confirm the pseudoplastic nature of the fermented product	Garcés et al. 2019

Non-thermal technologies to improve quality in plant-based beverages

Some research works showed technological, nutritional, and sensorial deficiencies in plant-based beverages. The two most common sensorial problems observed were: the "beany" or "painty" off-flavor due to lipoxygenase activity, and a chalky mouthfeel caused by insoluble large particles (Kwok and Niranjan, 1995, Durand et al., 2003). Coupled with this, low viscosity and stability are technological problems that decrease consumer acceptance.

It has also been mentioned that the antinutrient content is another of the problems of vegetable drinks. Lectins (abundant in membranes of plant and animals cells) available in soy, peanuts and others beans significantly affect glucose absorption in the intestine. Saponins present in soy, oats, peas and beans intervene in protein absorption through the formation of insoluble saponin-protein complexes which are resistant to digestion (Rasika et al., 2021). Plant phenols (including tannins and saponins) impart bitter, acrid or astringent tastes depending on their molecular weights (Drewnowski and GomezCarneros, 2000), and this coming from legume-based products (undesirable smell to beany and earthy). Protease inhibitors (anti-proteases) in vegan beverages interfere with protein and starch digestion by inactivating the digestion enzymes (breakdown of proteins) (Rasika et al., 2021). Various polyphenols cause inactivation of thiamine leading to malabsorption; digestion disorder caused by difficulty of assimilating, absorbing or digesting nutrients in food throughout the gastrointestinal tract.

To overcome the nutritional and sensory limitations, commercial plant-based beverages (based on nuts, seeds or beans) are supplemented with sweeteners, artificial flavours, protein, amino-acids, minerals and vitamins. Moreover, thermal pre-processing is applied to reduce anti-nutrients such as protease inhibitors (Jiang et al., 2013; Yuan et al., 2008), to decrease and mask off-flavour and improve mouthfeel and colour (Dakwa et al., 2005; Kim et al., 1986). However, when talking about treatments, thermal treatment destroys heat sensitive minerals and could lead to poor solubility and extraction (Rasika et al., 2021).

Non-thermal technologies (NTT) such as high hydrostatic pressure, pulsed electric field, ultrasound and high homogenization pressure are being used from some years ago to improve safety and quality of some beverages like fruit juices or beer (Mesa et al., 2020). Since NTT contribute to microorganisms and enzymes inactivation, the thermal treatment required for conservation can be reduced and some advantages in nutritional and sensorial characteristics are derived. Moreover, high homogenization pressure is used to reduce particle size improving physico-chemical stability of fat in water emulsions or cloud stability in pulped fruit juices. Although to a lesser extent, these technologies are also applied to plant-based beverages in order to increase physical stability and inactivating microorganisms and enzymes (Rasika et al., 2021). NTT are recent processing methods with many expectations for the future.

As examples, using Ultra High-Pressure Homogenization allows for a longer shelf life of the product, since greater physical stability is achieved, due to a reduction in the size of the fat globule that prevents coalescence (Thiebaud et al., 2003). This technology could also contribute to the development of an excellent product with desirable sensory attributes. Ultra-High-Pressure Homogenizated soy-yogurts displayed greater firmness and higher water holding capacity (Bernat et al., 2014a). Pulsed electric field (PEF) is suitable technological option for pasteurization, able to preserve valued bioactive compounds in beverages. Pulsed electric field processing on cactus juice fostered a higher retention of polyphenolic bioactive compounds and antioxidant activity, also reported to enhanced bioaccessibility of bioactive compounds (Gabrić et al., 2017). Ultrasonic treatment contributes to enhancing stability and rise the quality of fermented beverages. Ultrasonic processing on yogurt presented a lower pH decrease rate and a lower lag phase duration, the viscosity was also affected having the final yogurt coagulum higher viscosity value (Pinto et al., 2021).

In the case of fermented beverages, we have already seen how fermentation helps to correct many of the defects responsible for the lack of quality in non-fermented beverages. However, non-thermal technologies can also be applied to further increase the final quality, both in terms of nutrition, sensory and beneficial effects on health. So far, few research papers have been published in this area, although many references are found in the case of fermented fruit juices.

In this way, ultrasound treatment on lactic acid bacteria has been suggested as an effective strategy to enhance metabolic activity, remarkable product stability and reduce processing time enhancing quality, improving fermentative processes, releasing antioxidant components which provide health-promoting effects (Pinto et al., 2021; Abesinghe et al., 2018). Pulsed electrical field minimize changes in physicochemical (changes in colour or sweetness) and nutritional properties (reduction of the sugar content) with retention of higher amounts of health-related phytochemicals and enhance the total of anthocyanins and carotenoids (Carbonell-Capella et al., 2016; Gabrić et al., 2017). High hydrostatic pressure offers advantages in terms of processing time, quality and energy. Moreover, it makes a low impact on nutritional components and helps in decontamination of aflatoxins.

Encapsulation is another technology that can be used to increase probiotic viability and therefore to improve the quality of fermented plant-based beverages. Encapsulation of microbial cells would increase their ability to survive under adverse conditions such as low pH, low oxygen, extreme temperatures in subsequent processing steps production, storage and gastrointestinal digestion (Aspri et al., 2020; Pimentel et al., 2021). The main function of encapsulation is to protect the microbial cells by incorporating a protective coating that resists the adverse conditions of pH or temperature. The use of biopolymers such as sodium alginate showed good results (Rasika et al., 2021). To exemplify this, in the case of the incorporation of chitosan alginate coating resulted in a less porous and more stable structure. It could be associated with the interactions between oppositely charged polymers of chitosan and alginate, resulting in a stabilized microparticle (Shi, Alves, & Mano, 2008, Jiang et al., 2014) due to the ability to cover the porous structure. Chitosan coating can decrease the alginate permeability, resulting in a more compact and denser microcapsule (Vaziri, Alemzadeh, & Vossoughi, 2018), increasing the protection of the probiotic culture (Lopes et al., 2020). Holkem (2016) showed probiotic cultures did not suffer damage during the process of encapsulation, demonstrating that external ionic gelation in alginate technique is adequate to produce microcapsules. Microencapsulation also prevents the strains from interacting with the matrix, reducing their impact on the sensory properties of the product.

CONCLUSION

The use of plant-based beverages as a vehicle for probiotics is a promising field for the food industry that can benefit the common consumer, as well as specific groups that have allergies or prefer vegan consumption.

Although some of the beneficial effects that fermentation has on the physical-chemical, technological and sensory properties are described, a lot of research is needed to improve the properties of the final product and to develop successful products. It is necessary to better understand the changes that probiotics suffers when subjected to different conditions associated with the production process to increase their survival and their resistance to gastrointestinal tract. It is also important to improve knowledge about the most suitable raw materials and how to apply technologies such as microencapsulation, high hydrostatic pressure, pulsed electric field or ultrasound, to protect probiotics, to guarantee their stability and to assure a final product with an improved quality.

Attention to these needs by research will contribute positively to the success of non-dairy probiotic foods, a very attractive field for the food industry, as well as for consumers with special needs or looking for sustainable and healthy alternatives.

REFERENCES

Maria Aspri, Photis Papademas and Dimitrios Tsaltas (2020). *Review on Non-Dairy Probiotics and Their Use in Non-Dairy Based Products.*

Elif Feyza Aydar, Sena Tutuncu, Beraat Ozcelik (2020). *Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects*.

Neus Bernat, Maite Cháfer, Amparo Chiralt and Chelo González-Martínez (2014a). *Vegetable milks and their fermented derivative products.*

Neus Bernat, Maite Cháfer, Amparo Chiralt and Chelo González-Martínez (2014b). *Development of a non-dairy probiotic fermented product based on almond milk and inulin.*

Valeria Castillo-Escandón, Silvia Guadalupe Fernández-Michel, María Cristina Cueto-Wong y Gabriela Ramos-Clamont Montfort (2019). *Criterios y estrategias tecnológicas para la incorporación y supervivencia de probióticos en frutas, cereales y sus derivados.*

Champagne CP, Green-Johnson J, Raymond Y, Barrette J and Buckley N. (2009). *Selection of probiotic bacteria for the fermentation of a soy beverage in combination with Streptococcus thermophilus.*

Mayuri Chavan, Yogesh Gat, Mugdha Harmalkar, Roji Waghmare (2018). *Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume.*

Kassia Kiss Firmino Dourado Costa, Manoel Soares Soares Júnior, Sarah Inês Rodrigues Rosa, Márcio Caliari, Tatiana Colombo Pimentel (2017). *Changes of probiotic fermented drink obtained from soy and rice by products during cold storage.*

Andrea Micaela Dallagnol, Micaela Pescuma, Graciela Font De Valdez & Graciela Rollán (2012). *Fermentation of quinoa and wheat slurries by Lactobacillus plantarum CRL 778: proteolytic activity.*

A. Durand, G.V. Franks, R.W. Hosken (2003). *Particle sizes and stability of UHT bovine, cereal and grain milks.*

Ana Luiza Freire, Cintia Lacerda Ramos, Rosane Freitas Schwana (2017). *Effect of symbiotic interaction between a fructooligosaccharide and probiotic on the kinetic fermentation and chemical profile of maize blended rice beverages.*

Adriana P. Castellanos Fuentes, Carolina E. Genevois, Silvia K. Flores, Marina F. de Escalada Pla (2020). *Valorisation of soy by-products as substrate for food ingredients containing L. casei through solid state fermentation.*

Domagoj Gabrić, Francisco Barba, Shahin Roohinejad, Seyed Mohammad Taghi Gharibzahedi, Milivoj Radojčin, Predrag Putnik, Danijela Bursać Kovačević (2017). *Pulsed electric fields as an alternative to thermal processingfor preservation of nutritive and physicochemical propertiesof beverages: A review.*

Laura Isabel Delgadillo Garcés (2019). *Desarrollo de un producto fermentado probiótico no lácteo a base de leche de almendras.*

Kuntal Ghosh, Mousumi Ray, Atanu Adak, Suman K. Halder, Arpan Das, Arijit Jana, Saswati Parua (Mondal), Csaba Vágvölgyi, Pradeep K. Das Mohapatra, Bikas R. Pati, Keshab C. Mondal (2015). *Role of probiotic Lactobacillus fermentum KKL1 in the preparation of a rice based fermented beverage.*

M. Hariri, R. Salehi, A. Feizi, M. Mirlohi, R. Ghiasvand, N. Habibi (2015). *A randomized, double-blind, placebo-controlled, clinical trial on probiotic soy milk and soy milk: Effects on epigenetics and oxidative stress in patients with type II diabetes.*

Bababode Adesegun Kehinde, Anil Panghal, M.K. Garg, Poorva Sharma and Navnidhi Chhikara (2020). *Chapter four: Vegetable milk as probiotic and prebiotic foods.*

K.C. Kwok, K. Niranjan (1995). *Effect of thermal processing on soymilk.*

Laênia Angélica Andrade Lopes, Rafaela de Siqueira Ferraz Carvalho, Nereide Stela Santos Magalhães, Marta Suely Madruga, Ana Julia Alves Aguiar Athayde, Isabella Araújo Portela, Carlos Eduardo Barão, Tatiana Colombo Pimentel, Marciane Magnani, Thayza Christina Montenegro Stamford (2020). *Microencapsulation of Lactobacillus acidophilus La-05 and incorporation in vegan milks: Physicochemical characteristics and survival during storage, exposure to stress conditions, and simulated gastrointestinal digestion*.

Winnie Alencar Luciano, Thais Costa Matte, Isabella Araújo Portela, Lorena Lucena de Medeiros, Marcos dos Santos Lima, Janeeyre Ferreira Maciel, Evandro Leite de Souza, Estefânia Fernandes Garcia, Marciane Magnani (2018). *Effects of Lactobacillus acidophilus LA-3 on physicochemical and sensory parameters of açaí and mango based smoothies and its survival following simulated gastrointestinal conditions.*

José Mesa, Leidy Indira Hinestroza-Córdoba, Cristina Barrera, Lucía Seguí, Ester Betoret, Noelia Betoret (2020). *High Homogenization Pressures to Improve Food Quality, Functionality and Sustainability.*

Suênia Gabriela Gonçalves Morais, Graciele da Silva Campelo Borges, Marcos dos Santos Lima, Olga Martín-Belloso, Marciane Magnani (2019*). Effects of probiotics on the content and bioaccessibility of phenolic compounds in red pitaya pulp.*

F. Patrignani, M. Modesto, Samanta Michelini, Maria Cristina Sansosti, Diana I. Serrazanetti, Linnea Qvirist, Lorenzo Siroli, Lucia Camprini, Paola Mattarelli, Rosalba Lanciotti (2017). *Technological potential of Bifidobacterium aesculapii strains for fermented soymilk production*.

Pamela Canaviri Paz, Rownoke Jannat Janny, Åsa Håkansson (2020). *Safeguarding of quinoa beverage production by fermentation with Lactobacillus plantarum DSM 9843.*

Tatiana Colombo Pimentel, Whyara Karoline Almeida da Costa, Carlos Eduardo Barao, Michele Rosset, Marciane Magnani (2021). *Vegan probiotic products: A modern tendency or the newest challenge in functional foods.*

Carlos A. Pinto, Vasco J. Lima, Renata A. Amaral, Mirian Pateiro, Jose Manuel Lorenzo and Jorge A. Saraiva (2021). *Development of fermented food products assisted by ultrasound.*

Dissanayake MD Rasika, Janak K Vidanarachchi, Ramon Silva Rocha, Celso F Balthazar, Adriano G Cruz, Anderson S Sant'Ana and Chaminda Senaka Ranadheera (2021). *Plant-based milk substitutes as emerging probiotic carriers.*

Solange Aka, Bedis Dridi, Alexandre Bolotin, Elysée Armel Yapoa, Marina Koussemon-Camara, Bassirou Bonfoh, Pierre Renault (2020). *Characterization of lactic acid bacteria isolated from a traditional Ivoirian beer process to develop starter cultures for safe sorghum-based beverages.*

Tamime AY and Robinson RK. (2000). *Yoghur: Science and Technology*

Muzi Tangyu, Jeroen Muller, Christoph J. Bolten & Christoph Wittmann (2019). *Fermentation of plant-based milk alternatives for improved flavour and nutritional value.*

Martin Vogelsang-O'Dwyer, Emanuele Zannini, Elke K. Arendt (2021). *Production of pulse protein ingredients and their application in plant-based milk alternatives.*

Jicheng Wang, Rina Wu, Wenyi Zhang, Zhihong Sun, Wenjing Zhao and Heping Zhang (2013). *Proteomic comparison of the probiotic bacterium Lactobacillus casei Zhang cultivated in milk and soy milk.*

Rina Wu, Liping Wang, Jicheng Wang, Haiping Li, Bilige Menghe, Junrui Wu, Mingruo Guo, Heping Zhang (2009a). *Isolation and preliminary probiotic selection of lactobacilli from koumiss in Inner Mongolia*.

Yan Xu, Hui Zhao, Xiaolong Yan, Siming Zhao (2020). *Preparation of a probiotic rice tablet: Sensory evaluation and antioxidant activity during gastrointestinal digestion.*