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Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages

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Highlights

- Fermentation of fruits and fruit-by products add value and/or preserve raw matrices
- Lactic acid fermentation improves the functional properties of fruit beverages
- Nanotechnology is an innovative tool for the design of novel fermented beverages
- Consumers demand guides food markets towards new functional non-dairy beverages
- New technologies allow fruits by-products´ health-beneficial compounds recovery

Abstract

Current awareness about the benefits of a balanced diet supports ongoing trends in humans towards a healthier diet. This review provides an overview of fruits and fruit-by products as sources of bioactive compounds and their extraction techniques, and the use of lactic acid fermentation of fruit juices to increase their functionality. Fruit matrices emerge as a technological alternative to be fermented by autochthonous or allochthonous lactic acid bacteria (LAB such as *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, and other *Lactobacillus* species), and also as probiotic vehicles. During fermentation, microbial enzymes act on several fruit phytochemicals producing new derived compounds with impact on the aroma and the functionality of the fermented drinks. Moreover, fermentation significantly reduces the sugar content improving their nutritional value and extending the shelf-life of fruit-based beverages. The generation of new probiotic beverages as alternatives to consumers with intolerance to lactose or with vegan or vegetarian diets is promising for the worldwide functional food market. An updated overview on the current knowledge of the use of fruit matrices to be fermented by LAB and the interaction between strains and the fruit phytochemical compounds to generate new functional foods as well as their future perspectives in association with the application of nanotechnology techniques are presented in this review.

Keywords: fruits, fruit by-products, bioactive compounds, lactic acid fermentation, fermented beverages, nanotechnology.

1. Introduction. Fruits and their health benefits

Diets have become more similar worldwide and are mainly characterized by highly processed foods, cheap calories, and overconsumption. Unbalanced diets are one of the main causes of malnutrition and the proliferation of several chronic non-communicable diseases (NCDs) in most occidental countries. Thus, the World Health Organization (WHO) promotes a healthy nutrition based on a daily intake of at least 400 g or five servings of varied fruits and vegetables (2:3, respectively) selection, which would help to reduce the risk of developing NCDs and guarantee sufficient daily intake of dietary fiber (https: //www.who.int). However, the estimated intake of fruits and vegetables is variable worldwide (100-450 g/day) and a large proportion of people do not achieve the suggested values (Gómez et al., 2019; Guilbert, 2003; Miller et al., 2016; MSN, 2013; Nishida, Uauy, Kumanyika, & Shetty, 2004; Sachdeva, Sachdev, & Sachdeva, 2013). In 2015, only 55% of global population achieved the average fruit and vegetable availability above the minimum advised by the WHO (FAO, 2018; Mason-D'Croz et al., 2019; Wallace et al., 2019), which reported that their deficient intake has been one of the 10 main mortality risk factors as 3.9 million deaths have been attributed to deficient consumption of fruits and vegetables in 2017 worldwide (https: //www.who.int).

Fruits are source of carbohydrates, acids, minerals, polyphenols, water-soluble vitamins (vitamin C and B-complex vitamins), provitamin A, amino acids, aromatic compounds, carotenoids, fibers, phytosterols, and other bioactive substances in the human diet. The water content of fruits ranges from 70 to 90%. While fruits possess poor amounts of lipids in their pulp and peel, these compounds are mainly present in their seeds, which are not commonly consumed. The protein content of fruits is variable and scarce (Bates, Morris, & Crandall, 2001) (https://www.who.int/).

Fruits and vegetables are typically consumed fresh or minimally processed either canned, dried, or as juices, pastas, salads, sauces, and soup preparations. Fruit juice, which intake is very common in the occidental society, is described as the extractable liquid from the cells or tissues of the fruits. The fermentable but not fermented juice is the obtained by a mechanical process from ripe and healthy fruits and preserved by physical means; it may be turbid or clear and added or not with sugars (Bates et al., 2001; Bevilacqua et al., 2011; Di Cagno, Coda, De Angelis, & Gobbetti, 2013).

Fruit intake contributes to reduce the risk of diseases mainly due to the presence of bioactive compounds preventing chronic pathologies, cancer, premature mortality, and coronary heart disease, and reducing the risk of strokes. Fruit juices possess low sodium and potassium content, thus helping to maintain the normal blood pressure while its lack of fat is beneficial for the cardiovascular system. Some reports indicate that fruit juices would play an important role in slowing the progress of Alzheimer's disease and the development of cancer (Aune et al., 2017; Cutler et al., 2008; Dai, Borenstein, Wu, Jackson, & Larson, 2006). In recent decades, the recognition of the nutritional value of these drinks led to an increase consumption, although the daily intake is still relatively low (www.who.int/;www.fao.org/) (Di Cagno et al., 2013; E. F. Garcia et al., 2016).

Due to their properties, vegetables and fruits, and vegetable/fruit by-products are a good source to extract bioactive compounds for the production of nutraceuticals and functional foods. Functional foods are defined as those “industrially processed or natural foods that when regularly consumed within a diverse diet at efficacious levels have potentially positive effects on health beyond basic nutrition”. Functional foods possess added-value such as increased content of health-promoting compounds, reduced undesirable components, and/or the addition of new ingredients with technological properties (Jiménez-Moreno, Esparza, Bimbela, Gandía, & Ancín-Azpilicueta, 2020; Peng et al., 2020). In fact, fruits and vegetables by themselves can be considered functional foods as they contain significant amounts of bioactive compounds preventing some damaging physiological activities including metabolic and cardiovascular diseases (Peng et al., 2020). Functional foods may be classified as (i) foods with naturally present bioactive substances (e.g. dietary fibre), (ii) foods added with bioactive substances (e.g. probiotics), and (iii) derived food ingredients introduced to conventional foods (e.g. prebiotics). According to the WHO, probiotics are “live microorganisms that, when consumed in an adequate amount, confer a health benefits on the host” while prebiotics are “non-viable food components that confer health benefit(s) on the host associated with modulation of the microbiota”. Lactic acid bacteria (LAB) are considered the main probiotic microorganisms while prebiotics include compounds such as oligosaccharides, resistant starch, inulin, lactulose, pyrodextrins, sugar alcohols, levans, and lactosucrose, widely present in plants material (Mishra, Behera, Kar, & Ray, 2018; Peng et al., 2020). Other compounds or foods used in health promotion are nutraceuticals, which are defined as a food or part of a food that provides health benefits and may be used for the prevention and/or treatment of a disease. Nutraceutical products include beverages, isolated nutrients, dietary supplements, herbal products, and processed foods (Mishra et al., 2018, Bartkiene et al., 2020). According to the affore mentioned, raw or fermented fruits and vegetables can be source of numerous health benefits.

This review deals with fruit fermentation by LAB as an interesting alternative for preservation and added value to fruits and fruit by-products. The presence of bioactive compounds in fruits and their extraction methods are presented. In addition, the current trends in the fermented beverage industry, which include probiotic fruit beverages and nanotechnology applications for the design of improved and novel functional fruit-based drinks are discussed.

1.1. Increasing fruit intake: challenges that need to be overcome

There exist different barrier types determining fruit intake in populations, such as: i) economic, due to the high price of some fruits in relation to their high nutritional value and low affordability; ii) policies, such as the need for improvements in the school nutritional food education policies and programs, the poor production incentives, deficient regulations for the production and distribution of healthy foods, and the need of changes in the consumer behaviour; and, finally iii) physical, such as the low availability of fresh fruits in some areas, their short shelf-life, high levels of waste and seasonality, their conservation and preparation (Gerritsen et al., 2019; Mason-D'Croz et al., 2019; MSN, 2016; Pérez-Ferrer et al., 2019; Sachdeva et al., 2013; Wallace et al., 2019). Many of these barriers could be overcome by investments in research and development to generate new and enhanced processing, storage, and distribution technologies, and also by the establishment of proper regulations (Mason-D'Croz et al., 2019).

Fruits have short shelf-life due to their high susceptibility to microbial spoilage and contamination by pathogens; each fruit type is a unique niche in terms of chemical composition, nutrient availability, competitive host microbiota, and natural antagonist compounds, having a characteristic dominant microbiota (usual cell counts range from 5 to 7 log CFU/g) (Di Cagno et al., 2013; E. F. Garcia et al., 2016; L. G. Ruiz Rodríguez et al., 2019). While the high levels of carbohydrates and water activity of fruits are optimal for microbial growth, their low pH values (2.0-4.5) make them susceptible to deterioration by fungi and yeasts but not by human pathogens. Thus, acid- and low pH-tolerant microorganisms such as fungi and LAB are part of the autochthonous microbiota of fruits. On the other hand, fruits are exposed to numerous potential deteriorating microorganisms during the production process (Bevilacqua, Corbo, & Sinigaglia, 2012; Lawlor, Schuman, Simpson, & Taormina, 2009; Patil & Kamble, 2011; Wareing & Davenport, 2005). Pasteurization, cooking, and addition of chemical preservatives are the main technological options to guarantee the safety of fruit products; however, these processes may lead to undesirable physicochemical and nutritional changes. To reduce these drawbacks, some novel technologies have been applied such as high hydrostatic pressure processing, pulsed electric fields, and ionizing radiation, new packaging systems, and the use of natural antimicrobial preservatives (Di Cagno et al., 2013; C. Garcia, Guérin, Souidi, & Remize, 2020).

1.2. Bioactive compounds present in fruits and their extraction methods

The current lifestyle and bad eating habits have a negative impact on human health and made consumers becoming more health conscious and aware of the possible beneficial effects of functional foods. Among them, the development and consumption of functional beverages has increased in recent years mainly due to the conscious importance of maintaining health and the ever-demanding working schedules (Routray & Orsat, 2019). In this sense, food science researchers have investigated the scientific evidence of biologically active components from plant sources to improve the physical and mental well-being of consumers.

"Bioactive compounds" are essential and non-essential compounds (e.g., vitamins or polyphenols) occurring in nature that are part of the food chain and can have a beneficial effect on human health (Biesalski et al., 2009). Chemical characterization of bioactive compounds in foods has been a major area of research emphasis in recent years. Although the fresh pulp of the majority of fruits and vegetables is commonly consumed, significant amounts of phytochemicals and essential nutrients are present in by-products such as seeds, husks, and other components (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). Fruit and vegetable by-products such as bagasse, peels, trimmings, stems, shells, bran, and seeds account for more than 50% of fresh fruits and sometimes have a nutritional or functional content higher than the final product (Ayala-Zavala et al., 2011) displaying in addition, an impact on environmental, economic, and social sectors. Food development applying food wastes or by-products from different agro-industries is a great alternative to use secondary food products. Agricultural and food industry by-products could be revalorized due to their low price, high existing amounts, and because they are sources of numerous useful biomolecules and precursors of bioactive components (Vodnar et al., 2017).

To date, five steps to recover valuable compounds from food by-products have been described: macroscopic matrix pretreatment, molecule separation, molecule extraction, purification and product (i.e. nutraceutical such as dietary fiber) formation (Galanakis, 2013).

Extraction is the essential step of value-added polyphenols recovery from vegetable and fruit by-products. Currently, the development of effective extraction techniques has received special attention due to the increase in energy prices, CO_2 emissions, among other environmental problems (Sharma, Mahato, Cho, & Lee, 2017). Conventional and novel extraction technologies have been applied for the extraction of bioactive compounds from fruit by-products sources in some Latin American countries (Table 1). Conventional solvent extraction is the most widespread applied technique at industrial scale, which includes different phases such as solid liquid extraction (e.g. Soxhlet) using organic solvents, maceration, and hydrodestillation (Barba, Zhu, Koubaa, Sant'Ana, & Orlien, 2016); however, these methods can cause degradation of thermolabile compounds. To overcome this problem, extraction techniques including ultrasound-assisted, pulsed electric field assisted, enzyme-assisted, microwave assisted, supercritical fluid, and pressurized liquid extraction are of interest to the food industry (L. Wen, Zhang, Sun, Sivagnanam, & Tiwari, 2019). Nowadays, other different innovative technologies are being used to extract valuable compounds from fruit waste and by-products (Figure 1).

i) Supercritical fluid extraction**:** Supercritical fluids, which can easily diffuse through solid materials due to the change in their density through the modification of their pressure and/or temperature, are used. Since the density is related to the solubility, by altering the extraction pressure, the resistance of the solvent to the fluid can be modified facilitating the extraction and reducing the process time (Da Silva, Rocha-Santos, & Duarte, 2016). The main factors affecting this process are the type of solvent (CO_2 is mainly used), temperature, pressure, flow rate, time and co-solvent concentration (ethanol/water). Recently, this method has been applied in tomato skin (Pellicanò et al., 2019) and tiger nuts (*Cyperus esculentus*) (Roselló-Soto et al., 2019)

ii) Ultrasound assisted extraction: Ultrasound has been defined as the frequency that exceeds 20 kHz, which is the threshold for human auditory detection. The output source of the ultrasound is usually a vibrating body, which makes the surrounding medium vibrate; the ultrasound waves transfer then energy to other neighboring particles. In ultrasound extraction the cavitation, thermal, and mechanical effects lead to the destruction of the cell wall, a reduction in particle size, and an increase in the reaction rate through mass transfer of the cell wall without causing changes in the structure and function of the extracts (C. Wen et al., 2018). The main physical parameters playing vital roles in ultrasound process include power, frequency and amplitude, pH, extraction time, extraction temperature, liquid-solid ratio, and particle size. Ultrasound has been used for the extraction of bioactive compounds in different plant by-products such *Artocarpus heterophyllus* fruit peel (Moorthy et al., 2017), tomato waste (Sengar, Rawson, Muthiah, & Kalakandan, 2020), olive waste (Z. Wang, Wang, Zhang, & Li, 2017), pomegranate (*Punica granatum L.*) peel (Sharayei, Azarpazhooh, Zomorodi, & Ramaswamy, 2019), among others.

iii) Pulsed electric field (PEF): electroporation has been proposed as the main mechanism of extraction of bioactive compounds in plants. During electroporation, a cell is exposed to high intensity electric field pulses while the lipid bilayer and proteins of cell membranes are temporarily destabilized (L.-G. Yan, He, & Xi, 2017). The effectiveness of

PEF treatment strictly depends on the electric field intensity, pulsed wave shape, solvent selection, ratio of raw material to solvent, pulse duration, and treatment temperature. PEF has been used for the extraction of bioactive compounds in cocoa bean shell and coffee silverskin (Barbosa-Pereira, Guglielmetti, & Zeppa, 2018), red prickly pear peels (Koubaa et al., 2016), mango peels (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2016), and thinned peach by-products (Redondo, Venturini, Luengo, Raso, & Arias, 2018).

iv) Enzyme-assisted extraction: Enzymes such as cellulase, α-amylase, β-glucosidase, xylanase, β-glucanase, pectinase, and other related enzymes are used to improve the extraction process by hydrolyzing the matrix of the plant cell wall, mainly from the formation of the enzyme-substrate complex during which bonds in the substrate molecules break into the final products (Marathe, Jadhav, Bankar, Dubey, & Singhal, 2019). The enzyme-substrate complex is affected by the size of the plant material, enzyme concentration, reaction time, temperature, pH, and solid-liquid ratio (Marić et al., 2018). Enzyme-assisted extraction has been reported in industrial tomato waste for lycopene extraction (Catalkaya & Kahveci, 2019).

v) Microwave assisted extraction: microwaves comprise frequencies between 300 MHz to 300 GHz; in microwave assisted extraction, the small amount of moisture present in a plant cell is heated causing an evaporation creating enormous pressure on the cell wall that further weakens and breaks it; thus, exuding the phytoconstituents to the outside (Bagade & Patil, 2019). Extraction yields depend on the temperature, microwave-power, properties of solvent (nature and volume), time of extraction, and the matrix (plant) properties. However, the dielectric susceptibility of the solvent and the matrix can affect the use of microwaves, although the degradation of thermolabile components of the plant matrix can be controlled with the use of solvents such as hexane (Wahle, Brown, Rotondo, & Heys, 2010). Microwave assisted extraction has been used in mango seed kernel (Torres-León, Rojas, Serna-Cock, Belmares-Cerda, & Aguilar, 2017), *Opuntia ficus-indica* peel (Ciriminna et al., 2019), and carob pods (Quiles-Carrillo, Mellinas, Garrigós, Balart, & Torres-Giner, 2019).

vi) High pressure solid-liquid extraction: The method is based on a suction effect, generated by a compression of extracting solvent on solids at a pressure of about 8–9 bars for a determinate time, and followed by an immediate decompression at the atmospheric pressure. Rapid release of extracting liquid from the inside of a solid matrix, due to pressure gradient, transports mechanically the extractable compounds contained in the solid matrix towards the outside (Naviglio, 2003). Applying this methodology, Naviglio, Caruso, Iannece, Aragòn, and Santini (2008) recovered 2.8 mg of lycopene/kg of tomato waste after 4 hours of extraction using tap water as extraction liquid at a 0.7-0.9 MPa pressure. Furthermore, the method is easily scalable to become an industrial application for the production of trans-lycopene with a very high degree of purity (≥ 98%) in a quasi-crystalline form obtained from tomato by-products with 14% yield (Naviglio, Pizzolongo, Ferrara, Aragon, & Santini, 2008).

vii) Fermentation technology: by this means, bioactive compounds are obtained as secondary metabolites produced by microorganisms either by submerged fermentation -based on the cultivation of microorganisms in a liquid medium- or by solid-state fermentation -microbial growth and product formation in solid particles in the absence of water- (Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016). The success of solid phase fermentations depends on the use of appropriate microorganisms and the type of the solid support. In solid-state fermentations, low-cost agricultural and agro-industrial residues have been used as substrates such as grapefruit (Larios-Cruz et al., 2019), barley industry by-products (Bartkiene et al., 2020), and fig (*Ficus carica* L.) by-products (Buenrostro-Figueroa et al., 2017). Strains of *Trichoderma harzianum*, *Bjerkandera adusta*, *Streptomyces clavuligerus*, *Aspergillus ochraceus*, *Penicillium chrysogenum*, *Bacillus subtillis* have been used (Martins et al., 2011).

2. Fermentation of fruits using lactic acid bacteria as alternative of preservation and added value

Facing the need to preserve fruits and juices while minimizing the alteration of their properties, lactic acid fermentation emerged as an alternative for bio-preservation since it has been one of the oldest techniques to extend the shelf-life of perishable foods (Swain, Anandharaj, Ray, & Parveen Rani, 2014). Currently, there is a sustained increase in the demand for non-dairy beverages of high functional value, fresh, nutritive, healthy, and appetizing foods and drinks. Additionally, popular trends towards vegetarianism and veganism and the prevalence of lactose intolerance and allergy to cow´s milk proteins are in accelerated development. In this context, single-fruit, blend smoothies, or fruit juices that can be fermented by LAB constitute a promising alternative to supply the mentioned needs and to promote fruit consumption (Nazhand et al., 2020; L. G. Ruiz Rodríguez, 2018; Szutowska, 2020). In addition, the scientific interest in the design of lactic acid fermented juices and the study of their functional properties have been increased in the last years.

Lactic acid fermentation is a simple and valuable technology, low-cost, and sustainable process to maintain and/or improve the nutritional and sensory properties of raw materials, and to extend the shelf-life of fruits and vegetables under sanitary safety conditions (Di Cagno et al., 2013). Lactic acid fermentation-derived fermented foods have been produced for thousands of years due to their healthy feature, being accepted by consumers without restriction. Food fermentation processes have several advantages since: i) preserve and improve food safety, mainly as a result of the formation of organic acids (lactic, acetic, formic and propionic acids, etc.), ethanol, antimicrobial compounds; ii) improve their nutritional value; and iii) retain their organoleptic quality. Biopreservation by lactic acid fermentation occurs mainly through the synthesis of a wide variety of antagonistic metabolites such as organic acids, carbon dioxide, ethanol, diacetyl and hydrogen peroxide, antifungal compounds (fatty acids, phenyl-lactic acid), and bacteriocins (Bourdichon et al., 2012; Buckenhüskes, 1993; Di Cagno et al., 2013).

Fruit fermentation may be carried out "spontaneously" by the autochthonous lactic acid microbiota present in the raw material, i.e. *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Weissella* spp., *Fructobacillus* spp., and *Enterococcus* spp. under favorable conditions of anaerobiosis, water activity, salt concentration, and temperature. However, controlled fermentations using starter cultures containing LAB, e.g. *Lactiplantibacillus plantarum* COMB. NOV., *Lacticaseibacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus acidophilus*, confer consistency, reliability, control, and reproducibility in the process providing standardized, safe, and constant quality final products (Buckenhüskes, 1993; Di Cagno et al., 2013; L. Ruiz Rodríguez, Bleckwedel, Eugenia Ortiz, Pescuma, & Mozzi, 2017; Swain et al., 2014; Szutowska, 2020). Nevertheless, the use of starter cultures in the elaboration of fermented fruits and vegetables is still a developing area, unlike other fermented foods with matrices of animal origin such as cheeses and sausages (Di Cagno et al., 2016; Filannino, Di Cagno, & Gobbetti, 2018; C. Garcia et al., 2020; Szutowska, 2020).

Industrial starter cultures should meet certain requirements such as few nutritional needs, rapid growth and fast acidification, or the ability to ferment diverse carbohydrate substrates (Szutowska, 2020). However, in plant-based fermented foods the main requirement for starter cultures is environmental adaptation to the stress conditions present in plant matrices. Fruits are good matrices for lactic acid fermentation due to their high content of carbohydrates, minerals, vitamins, and dietary fibers. In contrast, the main environmental factors affecting the growth and acidification of LAB in fruits include the concentration of fermentable carbohydrates, the extremely acidic environment and buffering capacity, the presence of non-digestible nutrients (fiber, inulin, fructooligosaccharides, etc.), anti-nutritional factors and inhibitory compounds (tannins and phenolic compounds). High number of starter culture cells (8.0-9.0 log CFU/mL) guarantees the hygiene of the product and the eventual probiotic properties of the LAB used. The adaptation of LAB to different fruit ecosystems is species- and strain-dependent; despite the importance of this process, the adaptation metabolism and the response to these ecosystems have been scarcely studied comparing to other fermented foods (Di Cagno et al., 2013; Filannino, Cardinali, et al., 2014; Szutowska, 2020).

The use of selected autochthonous starter cultures in the elaboration of fermented foods guarantees better yields compared to the use of commercial or allochthonous strains or spontaneous fermentation processes, enhancing the nutritional, sensory, and rheological properties of the products and ensuring a long shelf-life. The use of autochthonous starter cultures in fruit fermentation would allow preserving the natural color, firmness, antioxidant activity, and other health-promoting compounds. This effect would be consequence of the organic acid profile (synthesis of lactic and acetic acids) modification and the metabolism of free amino acids. All these modifications may have direct (pH) or indirect (redox potential) effects on enzymes responsible for endogenous browning, and on the oxidative and sensory properties (color, flavor, and aroma) of plant matrices. On the other hand, the maintenance of high cell viability during stationary growth phase in the matrix environmental conditions is a requirement that ensures the long shelf-life of fermented products, especially those containing functional starter cultures. Thus, to carry out successful controlled vegetable and fruit fermentations, the selection of LAB to be used as starter cultures should be based mainly on pro-technological, sensory, and/or nutritional criteria (Di Cagno, Filannino, & Gobbetti, 2015). Furthermore, LAB belonging to specific niches could present distinctive metabolic traits as a result of environment adaptations (Endo, 2012; Filannino et al., 2018; Siezen & Bachmann, 2008). The use of autochthonous starter cultures in plant-based matrices have the following advantages: i) rapid acidification, ii) high cell growth; iii) inhibition of harmful microorganisms; iv) antioxidant power; v) sensory properties, vi) high viscosity of the juice; vii) high consumption of fermentable carbohydrates; and viii) longer survival (Azam et al., 2017; Di Cagno et al., 2015; Di Cagno et al., 2011; Di Cagno et al., 2009; C. Garcia et al., 2020). Among pro-technological desirable traits, LAB starter cultures for plant-based products should be adapted to the fruit/vegetable matrix to rapidly grow and acidify the media even at low values of pH and temperature, and should also tolerate and/or metabolize phenolic compounds. Additionally, fruit/vegetable starter cultures must have the ability to effectively ferment diverse carbohydrate substrates, increasing nutrient density, enhancing the bioavailability of nutrients, and guaranteeing a successful and complete fermentation process. It is also desirable that LAB are able to synthesize antimicrobial compounds increasing safety and shelf-life extension by foodborne pathogens and spoilage microorganism elimination. Regarding sensory criteria, the presence of aroma compounds in fermented fruit/vegetable juices has a crucial impact on the sensory quality and consumer acceptance of the final product. Thus, selected LAB are expected to synthezise aroma compounds or their precursors, such as acetic acid, esters, ketones, alcohols, and terpenes, allowing to obtain fermented juices with satisfactory flavors and odors and reduced off-flavours like undesirable aldehydes. Concerning functional metabolic traits is preferable the selection of LAB capable of producing exopolisaccharides (EPS). These compounds improve the rheology of fermented juices mainly due to their high viscosity, affecting positively the perception of the final product. Furthermore, certain EPS present some functional properties such as immunomodulatory, anticancer, antioxidant, and antibacterial activities, and may enhance intestinal colonization by probiotics. Additionally, LAB may increase the antioxidant activity of the matrix, and be able to produce or release bioactive compounds such as peptides, vitamins, amino acids, and phenolics. Thus, fermented juices as source of B-group vitamins (e.g. riboflavin or cyanocobalamin), ascorbic acid, and phenolic derivatives such as health-beneficial ellagic acid may be attractive functional drinks (Ayed, M'hir, & Hamdi, 2020; Di Cagno et al., 2015; C. Garcia et al., 2020; Szutowska, 2020).

To date, the suitability of various fruits as raw materials for the preparation of fermented fruit juices as well as the use of starter cultures have been evaluated (Di Cagno et al., 2010; Di Cagno, Filannino, & Gobbetti, 2017; Fessard et al., 2017; Verón, Di Risio, Isla, & Torres, 2017). Fruit-based beverages and purees made through controlled lactic acid fermentation are relatively new products that respond to consumers' demand for alternatives to dairy products and minimally processed and/or functional foods; additionally, fruit juices may be considered new type carriers for probiotic bacteria. The consumption of fermented fruits with LAB could improve human nutrition by the balanced intake of vitamins, minerals, and carbohydrates, and prevent diseases depending on the probiotic properties of the starter culture. In addition, some fermented fruits contain colored pigments such as flavonoids, lycopene, anthocyanin, β-carotene, and glucosinolates that act as antioxidant compounds in the body helping to eliminate harmful free radicals involved in degenerative diseases such as cancer, arthritis, and aging (Di Cagno et al., 2015; C. Garcia et al., 2020; Swain et al., 2014; Vitali et al., 2012).

2.1. Lactic acid bacteria and phenolic compounds

Phenolic compounds, present at high concentrations in vegetables and fruits, are a wide group of bioactive molecules produced as secondary metabolites by plants. More than one hundred molecules of phenolic compounds can be found in edible plants, sharing a similar structure characterized by the substitution of a benzene ring in the molecule by at least one hydroxyl group. Phenolics can be found as single forms (phenolic acids) or bound to carbohydrates forming complex molecules as glycosides. The large family of polyphenols is known for their antioxidant, anticarcinogenic, antimutagenic, and hypoglucemic activity (Andriantsitohaina et al., 2012; Coban et al., 2012; Huang, Cai, & Zhang, 2009). In addition, polyphenols are also associated with anti-platelet activity, a risk factor for cardiovascular disease, by affecting different pathways such as the activation, adhesion, degranulation, and aggregation (Ed Nignpense, Chinkwo, Blanchard, & Santhakumar, 2020). Besides their relevant functionality for human health, phenolics have a great diversity of technological applications contributing also to color, flavor, and astringency of foods.

Microorganisms and especially LAB are susceptible to phenolic compounds despite their presence as natural plant microbiota. Within the adaptations of plant-associated LAB, the presence of several enzymes capable of degrading polyphenols toward less toxic derived compounds is remarkable. Thus, phenolic bioconversion includes decarboxylation, reduction, de-esterification, and deglycosylation reactions (Lee & Paik, 2017; Rodríguez et al., 2009).

Within microbial enzymes, β-glucosidases, phenolic acid reductases and decarboxylases (PAD), and tannases have been associated with the main phenolic metabolism. LAB can degrade and modify these secondary metabolites during fermentation. Plant-based foods are the most important source of polyphenols for human diet but some of them have low availability at intestinal level because of their high complexity. Microbial degradation to produce simpler phenolic compounds is of interest as they may be absorbed at duodenum, especially their aglycones or conjugated glycoside forms. The modification of phenolic compounds during fermentation clearly improves the bioavailability and/or functionality of the new derived products (Lee & Paik, 2017; Pontonio et al., 2019). In terms of their healthy value, the use of fruits and vegetables as food matrices to develop fermented functional foods is strongly encouraged.

Although a broad diversity of LAB genus and species have been isolated from vegetables, fruits, leaves, and flowers (L. G. Ruiz Rodríguez et al., 2019; Yu, Leveau, & Marco, 2020); *L. plantarum* is one of the most widely species used to ferment plant origin matrices, for which the metabolism of phenolic compounds has been widely reported. The specific pathway of polyphenols that produces bacterial inhibition is still unclear, but changes in the membrane fatty acid composition in *L. plantarum* were determined (Rozès & Peres, 1998). The presence of caffeic and ferulic acids induced an increase in the concentration of myristic (C14:0), palmitoleic (C16:1) and stearic (C18:0) acids into the cytoplasmic membrane of this species. However; after addition of tannins, an increment of lactic acid and a decrease in vaccenic acid (C18:1 t11) was determined. Filannino, Gobbetti, De Angelis, and Di Cagno (2014) reported on degradation of phenolics as an advantaged process to preserve the energetic balance of heterofermentative LAB.

The capacity to tolerate the presence of phenolic compounds seems to be species-dependent. The antimicrobial effect of wine phenolics on *L. plantarum* growth was reported by Landete, Rodriguez, De Las Rivas, and Munoz (2007); *p*-coumaric and ferulic acids produced the strongest inhibition among ten compounds assayed. In contrast, *p*-coumaric acid inhibited the growth of *Lentilactobacillus hilgardii* whereas caffeic and ferulic acids stimulated it (Campos, Couto, & Hogg, 2003). On the other side, catechins and epicatechins do not have any bacteriostatic effect on *L. hilgardii* (Figueiredo, Campos, de Freitas, Hogg, & Couto, 2008). *Furfurilactobacillus rossiae* was mostly affected by the presence of hydoxycinnamic acids in MRS broth, whereas *Latilactobacillus curvatus* was the most tolerant species (Filannino, Cardinali, et al., 2014).

2.1.1. LAB enzymes involved in phenolic metabolism

Tannases: LAB can degrade hydrolysable tannins by tannase activity, enzyme which belongs to the superfamily of esterase-hydrolyzing cell walls of plants liberating phenolic compounds bound to cellulose matrix. Tannase or tannin acyl hydrolase (EC 3.1.1.20) is a useful tool employed for some wines and juices clarification at industrial level. Due to their complexity, gallotannins and ellagitannins are not absorbed by humans and their conversion to simpler forms to increase their bioavailability is required. It has been reported that *L. plantarum* efficiently degraded tannin acid at percentages as high as 95% (Rodríguez, de las Rivas, Gómez-Cordovés, & Muñoz, 2008). Indeed, *L. plantarum* is able to break down hydrolysable tannins by an extracellular tannase (Rodriguez, de las Rivas, Gomez-Cordoves, & Munoz, 2008). Moreover, at least two tannase enzymes TanBLp and TanALp were isolated and characterized from *L. plantarum*, (Iwamoto, Tsuruta, Nishitaini, & Osawa, 2008; Jiménez, Esteban-Torres, Mancheño, de las Rivas, & Muñoz, 2014). Recently, a tannase from Miang, a naturally fermented Thai tea containing high phenolic content, was isolated and characterized from

Lactiplantibacillus pentosus (Kanpiengjai, Unban, Nguyen, Haltrich, & Khanongnuch, 2019).

Reductases and decarboxylases: several phenolic acids are first target of decarboxylase enzymes producing vinyl derivatives, considered as flavouring compounds; secondly, ethyl phenols will be produced by the action of a reductase enzyme. Rodríguez, Landete, de las Rivas, and Muñoz (2008) reported that six phenolic acids namely caffeic, ferulic, *p*-coumaric and *m*-coumaric, gallic and protocathecuic acids were substrates by reductase or decarboxylase enzymes producing compounds with impact in aroma. Several LAB species can metabolize these compounds through the action of reductases and decarboxylases (Figure 2). In a first step, hydroxycinnamic acids are converted into reduced forms i.e. caffeic to dihydrocaffeic, ferulic to dihydroferulic, and *p*-coumaric to phloretic acids (Filannino, Gobbetti, et al., 2014). These derived forms have stronger antioxidant properties than their corresponding precursors (Baeza et al., 2017; Silva et al., 2000). Strains as *Limosilactobacillus fermentum* and *Leuc. mesenteroides* were unable to reduce hydroxycynnamic acids but were capable of converting them into vinyl-derivatives by decarboxylation instead (Filannino, Gobbetti, et al., 2014). The reduction of some phenolics by LAB seems to take place with an energetic advantage by NAD^+ regeneration, especially in heterofermentative LAB (Filannino, Gobbetti, et al., 2014).

β-glucosidases: the metabolism of complex polyphenols can involve more than one enzyme. *β*-glucosidases hydrolyze the glycosidic bond from glucosides; the released carbohydrates are further used as energy source. This enzyme was found in strains of *L. plantarum*, *L. fermentum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lacticaseibacillus casei* and *Bifidobacterium* sp. (Michlmayr & Kneifel, 2014; Spano et al., 2005). Oleuropein, a phenolic glucoside found in high concentration in olive fruits, could be hydrolyzed by some LAB barboring *β*-glucosidase (Ciafardini, Marsilio, Lanza, & Pozzi, 1994; Marsilio, Lanza, & Pozzi, 1996).

Ginsenoside, a phenolic glucoside compound found in ginseng (*Panax ginseng*) can also be hydrolyzed by LAB, producing deglycosilated ginsenosides and several minor ginsenosides with high biological properties (Lee & Paik, 2017). Among LAB, strains of *F. rossiae* and *Leuc. mesenteroides* showed the capacity to hydrolyze ginsenosides (Huq, Kim, Min, Bae, & Yang, 2014; Quan et al., 2011).

2.1.2. Changes in phenolic profile during fruit fermentation

The bioconversion of phenolic compounds is strain-dependent and it has been proposed as a detoxification or energetic balance mechanism, for which some specific pathways and enzymes involved are still unclear. During the last few years, LAB were used as starter cultures to ferment vegetables and fruits to improve their antioxidant activity by phenolic metabolism. Certainly, phenolic changes during fermentation depend on several factors such as the microbial strain, matrix, pH, temperature, and time of fermentation. As consequence, some authors have determined an increment (Yuxuan Liu et al., 2019; Mantzourani et al., 2019; Y. Yan et al., 2019) or a significant decrease (Li et al., 2019; Z.-P. Zhang, Ma, He, Lu, & Ren, 2018) in the total phenolic content after fermentation of fruits juices.

Pomegranate (*Punica granatum*), a “superfruit” rich in ellagintannis, has been used for the elaboration of functional beverages due to its high antioxidant activity in humans (Matthaiou et al., 2014). Ellagitannins belongs to a subclass of hydrolysable tannins, which have one or more hexahydroxydiphenoyl (HHDP) molecule in a glucopyranose core that can be degraded by tannase enzyme during lactic acid fermentation. The hydrolysis of ellagintannins leads to simpler phenolic forms as gallic, ferulic, quinic, caffeic, and ellagic acids with great antioxidant properties (Ascacio-Valdés et al., 2011). During pomegrante juice (PJ) fermentation, Filannino et al. (2013) reported ellagic acid release by the action of a *L. plantarum* tannase. Other study determined changes in the phenolic profile of fermented PJ by the strain *L. plantarum* PU1 showing an increase up to 60% of ellagitannin derived-compounds such as hexahydroxydiphenolyl-hexoside, ellagic acid deoxy-hexoside, and ellagic acid hexoside (Pontonio et al., 2019). Interestingly, the fermented PJ exhibited higher antioxidant activity than the unfermented juice (Pontonio et al., 2019).

The improvement of functional properties of fermented cornealian cherry (*Cornus mas* L.) juice using immobilized cells of *L. plantarum* ATCC 14917 showed higher phenolic content after fermentation than with free bacterial cells (165 and 214 mg GAE/100 mL, respectively), and after four weeks of cold storage (195 and 24 mg GAE/ 100 mL, respectively) (Mantzourani et al., 2018).

Time of fermentation can also influence the content of phenolic compounds as it was observed in a mixed vegetable-fruit beverage fermented by two strains of *L. plantarum.* In this study, total phenolic content increased up to 8 days and then decreased until 14 days of fermentation (Yang et al., 2018) with the concomitant drop in the antioxidant activity.

A mixed fermentation of blueberry pomace with the probiotic strains *L. plantarum-1* and *L. rhamnosus* GG showed a 3-fold increase in phenolic content comparing to the unfermented sample, with the consequent enhancement of the antioxidant activity (Y. Yan et al., 2019).

Mulberry (*Morus nigra*) fruit is rich in polyphenols, particularly in anthocyanins and flavonnols. The inclusion of *L. plantarum*, *L. acidophilus,* and *Lacticaseibacillus paracasei* as single starter cultures clearly affected the phenolic profile of fermented mulberry juice (Kwaw et al., 2018). After 36 h of incubation at 37 °C, total phenolic, anthocyanin and flavonnol content increased in the fermented beverages mainly with the *L. plantarum* strain*.* Thus, phenolic acids such as gallic, chlorogenic, protocatechuic, caffeic, ferulic and quinic acids among others, significantly enhanced after fermentation; in addition, the anthocyanin and flavonol profiles varied according to the strain. All changes in the phenolic content were correlated with a high antioxidant activity in fermented mulberry juices (Kwaw et al., 2018). Besides the antioxidant activity, some phenolics as chlorogenic and ellagic acids also inhibit the platelet aggregation (Ed Nignpense et al., 2020).

The capacity to metabolize phenolic compounds by *L. plantarum* ATCC14917 grown in apple juice for 72 h was recently reported by Li et al. (2019). These authors noticed that a significant decrease in total phenolic and total flavonoid compounds occur along fermentation while the antioxidant activity increased over time. The polyphenols drop was attributed to simple phenolic conversion and to depolymerisation of high molecular weight phenolics in apple juice. Indeed, quinic acid, quercetin-3-O-galactoside, quercetin-3-O-glucoside, and phlorizin were metabolized and consequently 5-O-caffeoylquinic acid, quercetin, and phloretin increased during fermentation; all these products showed stronger antioxidant activity than their respective precursors (Li et al., 2019).

Free phenolic compounds from plant cell walls can be released by the action of hydrolytic enzymes. Fermentation of goji juice by different strains of *L. plantarum*, *L. rhamnosus, Limosilactobacillus reuteri, Bacillus licheniformis,* and *B. velezensis* increased the TPC after incubation at 37 °C for 24 h (Yuxuan Liu et al., 2019). After fermentation, the conjugated forms of *p*-hydroxybenzoic and chlorogenic acids were not detected while only the free forms of both phenolic acids were noticed instead, which significantly increased through bacterial metabolism. The concentration of free forms of ferulic and caffeic acids were also higher in fermented juices than in raw goji juice.

Regardless the mechanism involved in phenolic degradation during plant fermentation, LAB successfully produce bioactive compounds of interest for humans.

2.2. Formation of flavor compounds in fermented fruit juices

Lactic acid fermentation of fruit juices can modify the juice aroma profile: some compounds may increase, some can diminish, while others may remain unchanged during fermentation.

According to their chemical composition, aroma compounds from fruit juices can be classified into: volatile, non-volatile, and acidic (Figure 3). Among the volatile compounds, alcohols, acids, ketones, hydrocarbons, aldehydes, and esters are found. Non-volatile compounds may include amino acids such as L-serine, L-proline, L-glutamic acid, and L-aspartic. Finally, citric, malic and lactic acids can be found among the acidic group.

When fermenting tomato juice using *L. plantarum* and *L. casei* strains, alcohols were the most abundant (49-52%) volatile compounds, which were increased by fermentation with both strains. In addition, the fatty acid concentration in the fermented juice by *L. casei* increased due to the formation of ammonium acetate (10%). Changes in the acetic acid concentration (from 3 to 27%) when *L. plantarum* was used were observed. Ketone concentrations increased from 0.5 to 15% (*L. casei*) and up to 12% (*L. plantarum*) after fermentation being 6-methyl-5-heptan-2-one the main detected compound. On the other hand, volatile hydrocarbons diminished significantly from values of 15 to 4% (*L. casei*) and to 2% (*L. plantarum*) in the fermented juice. All hexadecans with exception of a few compounds were increased in both fermented juices. In contrast, aldehydes decreased almost completely by the fermentation effect with both lactobacilli although the formation of undecanal was detected. Among aroma compounds, esters are known to confer fruity and floral flavor to fermented juices, which are formed during the fermentation process. A slight decrease in the total ester concentration was noticed due to the disappearance of ethyl acetate; however, new compounds were formed when the *L. casei* strain was used. Differences observed between both lactobacilli could be due to the variety and activity of their enzymes. Regarding alcohols, the formation of a new group of aromatic compounds was detected. Besides esters and alcohols, other compounds contributed to the antioxidant and antimicrobial properties of the tomato juice (Yiyun Liu et al., 2018).

Other authors studied the aroma compounds in mixtures of fermented fruit and vegetable (apple, carrot, tomato, cucumber, and haw) juices by different lactobacilli; the authors could identify a total of 14 compounds as determinant markers of the aroma and flavor of the samples by mass spectrometry coupled to gas chromatography (GC-MS) (Cui et al., 2019). Statistical analysis (ACP and PLS-DA) identified three types of compounds that significantly affected the profile of volatile and non-volatile compounds in fermented fruits and vegetables. Thus, for example, one group included strains of *L. casei* and *L. rhamnosus* as major contributors to umami flavor. Another group that included strains of *L. plantarum* and *L. acidophilus* contributed primarily to the acidic taste, while *L. fermentum* strongly affected the production of volatile compounds. Importantly, different strains of *Lactobacillus* may play a different role in modifying aroma- and flavor-related compounds.

In another study, the effect of lactic fermentation of elderberry juice (Ricci et al., 2018) was analyzed using 15 strains of *L. plantarum*, *L. rhamnosus,* and *L. casei* isolated from different types of matrices. The volatile compound profile was evaluated by gas chromatography (HS-SPME / GC-MS) after 48 h fermentation and a shelf-life of 12 days at 4 °C. The aromatic compound profile of all fermented juices was characterized by the presence of 82 volatile compounds belonging to different classes: alcohols, terpenes and norisoprenoids, organic acids, ketones, and esters. The juice of elderberry fermented with *L. plantarum* strains showed an increase in total volatile compounds after 48 h while juices fermented with *L. rhamnosus* and *L. casei* exhibited an even greater increase after shelf-life. The highest concentration of volatile compounds was found in the juice fermented with a dairy-origin *L. plantarum* strain. Ketones, mainly acetoin and diacetyl, were increased in all fermented juices after fermentation and shelf-life. Acetic and isovaleric acids were the most abundant organic acids found. Hexanol, 3-hexen-1-ol, and 2-hexen-1-ol alcohols increased during fermentation with bacteria of dairy origin. The most representative esters, ethyl acetate, methyl isovaleriate, isoamyl isovaleriate, and methyl salicylate were correlated with the fruit notes. Among terpenes and norisoprenoids, *β*-damascenone was the main compound with a typical elder note. Finally, by applying multivariate statistical analyses, the characteristic volatile profile of the samples was correlated with the different species and strains of lactobacilli used in this work.

The effect of fermentation on the volatile compound profile of fermented melon and chesnut juices using HS-SPME/GC-MS was studied using a *L. casei* strain. A reduction of ethyl butanoate, 2-ethyl methylbutyrate, and ethyl hexanoate in melon juice, and ethyl acetate, ethyl 2-methyl butanoate, ethyl crotonate, ethyl isovalerate, benzaldehyde, and ethyl hexanoate in chestnut juice was observed. The stable measurements of these compounds and the formation of 3-methyl-2-butenyl in melon juice could be used as markers of volatile compounds during fermentation (de Godoy Alves Filho et al., 2017).

3. Current trends in the fermented beverage industry

Nowadays, drink consumers worldwide demand nutritive and healthy beverages, with pleasant flavor, low-caloric intake, and low content of dairy derivatives. This behavior explains why the increase in the consumption of fermented products increased by 46% only in the United States with the concomitant 6% reduction in yogurt consumption (Sloan, 2019).

The current trend in the consumption of fermented foods leads to a variety of products that can be grouped into 4 from a marketing point of view: products derived from non-dairy kefir, fermented juices, fermented soft drinks, and non-dairy yogurt-like drinks. The increase in the consumption of fermented beverages is associated with consumers´ knowledge about chronic diseases (NCD) caused by the high content of sodium and sugars in ultra-processed foods. Additionally, consumers are aware that more than 70% of the body's immunity is generated in the colonic microbiota.

The consumption patterns of fermented beverages depend on the region of the world, while in Asian countries the consumption of fermented juices and soy-based products is common, in Europe is associated with fermented soft drinks and juices, in Latin America drinkable yogurts are preferred, and in the United States kefir-derived products are increasing. However, many of the fermented products are only consumed locally, failing to have an impact at the international level (Bader-Ul-Ain, Abbas, Saeed, Khalid, & Suleria, 2019). From the health point of view, fermented beverages can be classified into drinks with live cultures such as probiotics, and beverages with microorganisms that have evidence of some beneficial, specific effects on health (Marrero, Martínez-Rodríguez, Pérez, & Moya, 2019).

A stratified search to understand the tendency of the world market of fermented beverages (Stefanowski & Weiss, 2003) was done using keywords and boolean operators (Melini, Melini, Luziatelli, Ficca, & Ruzzi, 2019). The obtained information explaining the principal topics about this market is shown in Figure 4. The potential consumer trends were divided into nine different topics about new fermented milk products (Figure 4a) and their correlation with potential nutritional benefits focused on moderate consumption, in addition to presenting trends in the development of new products depending on the type of consumer. Trends on cereal-based fermentations (Figure 4b) are directed towards a greater development of products using cereal derivatives or cereal by-products; in addition, a tendency to develop functional fermented cereal-based products compared to the fermented milk chart is noticed.

In Figure 4c describing fermented beverages and organism well-being, a lesser trend in information regarding the benefits obtained from consuming fermented beverages is observed, even though younger consumers are conscious of the importance of food product health benefits. Much more information related to drinks for lactose-intolerant drinks is shown in Figure 4d; the topic has been divided into 6 subclasses due to constant problems that a large part of the population has and more information related to food nutrition, including clinical studies, is provided indicating that many fermented dairy products are trying to decrease the lactose content.

Regarding fermented beverages rich in antioxidant compounds (Figure 4e), 6 subtopics in which the relationship among fruits, plants, and the antioxidant activity that these types of products should have are distinguished, considering the good quality and the nutritional aspect of these formulations. Figure 4f describes the healthy components of fermented beverages clustered into 3 subtopics, which include an analysis about how the components of fermented beverages can provide a health benefit, even considering the genetic modifications of some of the raw materials used indicating that long-term effects of genetic modifications on people's nutrition are not yet known. Figure 4g shows fermented enriched drinks comprising 6 subtopics, which are associated with food nutrition, fermented milk, their effect on inflammation problems, in addition to the importance of agriculture for these drinks. They can be enriched with healthy products using i.e. legumes in their formulation. Finally, the fermented drinks and their health benefits are shown in Fig. 4h with fewer subtopics since they only contemplate an overview on the health benefits obtained by the intake of fermented beverages.

In a general context, the consumption of fermented beverages has had a significant boom in the last years, associated with the consumption culture of biotransformed products from these fermentation processes, coupled with the fact that a drink is easy and fast to consume and their benefits can be reflected in a shorter time compared to food intake. Also, it is important to highlight that if a balance between the consumption of fermented beverages and healthy foods is noticed, the bacterial populations present in the colonic microbiota will be able to generate more lasting health benefits.

Despite the trend in the consumption of fermented beverages and the scientific evidence regarding their health benefits to the body, there is no adequate legislation currently in Latin American countries to suggest a recommended daily consumption. Countries such as Kenya, South Africa, Australia, India, Sri Lanka, Oman, Qatar, and Bulgaria have made recommendations in their dietary guidelines to encourage the consumption of this type of products. In countries with a significant presence of indigenous population, the consumption of fermented beverages is more associated with a cosmogony and cultural heritage where the beneficial effects on health are associated with this ethnobotanical knowledge, while in more industrialized countries their consumption is more related to the search for better gastrointestinal health (Melini et al., 2019). In Mexico for instance, where a large part of the population is indigenous, only the microbiological safety of food products is emphasized (Good Manufacturing Practices). In this regard, official standards on product labeling for food and non-alcoholic beverages with modifications in their composition exist. In addition, nutritional specifications and standards for milk and dairy products such as probiotic beverages have been recently implemented (Castillo et al., 2019). In Brazil, laws and regulations that consider fermented beverages as functional foods exist since 2008 and they allow regulating their consumption according to the scientifically reported benefits (Prado et al., 2008). In countries such as the United States, legislation on fermented beverages is based on the regulations of the Codex Alimentarius, with the main objective to reduce possible intoxication by allergens present in beverages (Mainente et al., 2017).

3.1. Probiotics in fermented fruit juices

Nutritional science is focused on studying and formulating foods that have a health benefit beyond the ones intrinsic of the raw materials. This new concept of nutrition arises from the need to emphasize on prevention instead of medication, due to the high costs of health care (Nguyen et al., 2019). The food market has been increasing its attention towards functional foods, which is the most innovative and expanding food field (Mantzourani et al., 2018). As mentioned before, functional foods cluster those containing bioactive compounds, prebiotics, and probiotics. Although probiotics are consumed mainly in dairy products, these bacteria can be included in vegetable, fruit, and grain fermentations. The consumption of dairy products is limited due to the high amount of lactose intolerant individuals or those who need low cholesterol diets. On the other hand, the increased number of vegans also restricts the intake of dairy products (Fernandes Pereira & Rodrigues, 2018). Consequently, the formulation of functional fermented fruit drinks or the use of fruits as probiotic carriers is an attractive alternative for vegans, lactose intolerant and milk allergic individuals (Mantzourani et al., 2018).

The health benefits associated to probiotic consumption are mainly the reduction of the cholesterol levels (Istrati, Pricop, Profir, & Vizireanu, 2018; Swain et al., 2014; Sybesma & Hugenholz, 2004), immune system modulation, increase in mineral absorption, decrease in constipation, and anti-cancer and anti-hypertensive effects.

However, the main characteristic of probiotics is their ability to inhibit pathogens and to modulate intestinal microbes by means of competitive exclusion by mucosal adhesion, bacteriocin production, and hydrogen peroxide and acid release, which reduce intestinal pH inhibiting the growth of acid sensitive pathogens (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). The only source of probiotic strains commercially available are those isolated from human gut. However, fruits have chemical and physical characteristics similar to those found in the gastrointestinal tract given by the fruit high acidity and concentration of anti-nutritional factors and fibers as well as other nutrients resistant to digestive enzymes. Moreover, autochthonous bacteria must adhere to the fruit matrix and compete with pathogenic and spoilage microorganisms (Di Cagno et al., 2013).

Starter cultures commonly used for fruit and vegetable fermentation are not autochthonous and come from distant niches, mainly from animal sources. However, these microorganisms showed low survival during storage. Fruit spontaneous fermentations may allow isolating microorganisms which have adaptive advantages that would guarantee a prolonged shelf-life and ensure nutritional, sensorial, and rheological improved characteristics. Selected microorganisms must be able to consume the sugars present in fruits and tolerate the fruit environment such as low amino acids and protein content, presence of phenolic compounds, and acidic pH. Moreover, the characteristics of each fruit such as their content and spectrum of fermentable sugars and nitrogen (protein) concentration will affect the probiotics growth (Nguyen et al, 2019). Indeed, isolation of authoctonous bacteria from the type of fruit to be fermented is recommended to achieve a successful fermentation, as those isolates have specific traits acquired during niche adaptation (Garcia et al, 2020). Nevertheless, some features have to be considered apart from the fermentative capacity, such as the increase in safety by eliminating pathogens and the inability to produce biogenic amines. Moreover, survival and host colonization is a requisite to consider the selected LAB as probiotics.

Different strategies can be applied to ensure good microbial probiotic growth and survival in fruit fermentation processes. Some non-thermal treatments, i.e. high hydrostatic pressure, supercritical carbon dioxide, high intensity electric field pulses, and high intensity hydrostatic pressure have been successfully applied for improving the fermentation process, being high hydrostatic pressure and high intensity electric field pulses the most effective ones on reducing the fermentation time and increasing the cell count yield (Knorr et al, 2003). Also, sonication showed some positive results on preventing syneresis in fruit juices with high pulp amounts. Moreover, this process was able to reduce the peroxidase and polyphenol oxidases (preventing color changes) and the juice viscosity (Vidal Fonteles et al, 2013). Furthermore, an increase in the fermentable sugar concentration, which consequently improved bacterial growth was observed (Garcia Maia Costa et al, 2013). Another well-known strategy is to increase the fruit juice pH value to 6.0-7.0 at the beginning of fermentation. Bujna, Farkas, Tran, Sao Dam, and Nguyen (2018) formulated an apricot fermented juice with a mixed culture of *B. lactis* Bb-12, *B. longum* Bb-46*, L. casei* 01, and *L. acidophilus* La-5, while Nguyen et al. (2019) reported the production of a fermented pineapple beverage with the probiotic strain *L. plantarum* 299V. Lu, Tan, Chen, and Liu (2018) formulated a fermented star fruit (*Averrhoa carambola*) drink with a *L. rhamnosus* strain, which showed proper growth and lactic acid production. Another method that could increase probiotic growth is clarification using different enzymes. Nithya and Vasudevan (2016) observed an increased growth of *L. plantarum* and *L. acidophilus* strains when clarifying papaya juice with pectinase. Furthermore, addition of prebiotics can improve probiotic growth and survival in fruit juices. Indeed, the addition of oat flour and *β*-glucan to apple juice could protect probiotics during refrigerated storage, while the inclusion of inulin to fig juice increased the growth of a *L. delbrueckii* strain, and improved the antioxidant and organoleptic properties of the beverage comparing to the control fig juice (Chaudhary, 2019). Moreover, Nguyen et al, 2019, showed that the addition of fructooligosaccharides during fermentation increased the production of lactic acid by bifidobacteria strains and improved the stability of probiotics during storage of fermented pineapple juice.

Another interesting approach is the use of immobilized microbial cells, as mentioned earlier in the work done by Mantzourani et al. (2018) with *L. plantarum* ATCC 14917, which produced higher amounts of phenolic compounds when used immobilized respect to its planktonic cells grown in cornelian cherry juice. Cell survival during storage is an important issue that also has to be considered. In this respect, microencapsulation can protect cells from refrigeration conditions and digestion (Gandomi, Abbaszadeh, Misaghi, Bokaie, & Noori, 2016). Moreover, adapting cells to fruit environment also showed to increase cell viability. Perricone, Bevilacqua, Altieri, Sinigaglia, and Corbo (2015) observed that the loss of viability of *L. reuteri* DSM 20016 found in fermented red fruit juices during storage could be attenuated if cells were previously grown in a laboratory medium containing different amounts of red fruit juices or vanillic acid (phenol stress) or acidifying to pH 5.0 (acid stress). Saarela et al. (2011), showed that survival of a *B. breve* strain in grape, pineapple, and passion fruit juices could be increased by UV mutagenesis or growth in a medium with sub-lethal pH values. On the other hand, the robustness of the selected LAB to tolerate acidification during storage has to be taken into account. Vidal Fonteles et al, 2012 showed that *L. casei* NRRL B-442 viability was not affected by the post-acidification observed during the storage period of a cantaloupe melon juice, showing final cell counts above 8.5 log cfu/ml after 45 day-storage. Besides acid resistance, the capacity of selected probiotics to grow or resist the presence of phenolic compounds in fruits has to be assessed to select the adequate strains for the desired formulation. Indeed, only a few LAB species are able to grow in raw vegetable materials where phenolic compounds are abundant (Rodriguez et al, 2009). For this reason, in order to select fruit starters culture is important to find strains able to express genes of phenolic compound-degrading enzymes. In this respect, strains of *L. plantarum* and *L. pentosus* strains are able to grow in vegetables with high phenolic compounds concentrations such as olives (Rodriguez et al 2009, Carrasco et al, 2018). It has been reported that strains belonging to these species can switch their enzymatic metabolism to rapidly adapt to the inhibitory activity of the phenolic compounds present or released during fermentation of rich-phenolic vegetables by inducing specific gene transcription in response to the presence of phenolics (Carrasco et al, 2018). On the other hand, it has been reported that the phenolic compounds present in some fruits can boost probiotic growth while inhibiting the propagation of pathogenic bacteria such as *Escherichia coli* and *Salmonella* Thyphimorium (Pacheco-Ordaz et al, 2017). Moreover, de Souza et al. (2019) reported that some phenolic compounds have a positive effect on probiotics adhesion, growth and survival when using different fruit extracts.

New technologies are focused on avoiding loss of nutritional and sensorial characteristics of fruits during processing. Recently, several studies have been conducted using tomato, mango, orange, grape-water melon, pomegranate, and plums fermented by different LAB and *Bifidobacteium* strains (Istrati et al., 2018). Several traditional fruit products produced by bacterial fermentation include addition of salt to the preparation. These include pickles from lime and lemons produced by lactic acid fermentation which are typical in India, Pakistan, and North Africa (Battcock & Azam-Ali, 1998). Also, *Tempoyak* is produced by fermentation of salted durian fruit and its acidity has been attributed to the presence of *Levilactobacillus brevis*, *Liquorilactobacillus mali*, *L. fermentum*, *Paucilactobacillus vaccinostercus,* and *Leuc. mesenteroides* in the final product (Swain et al., 2014). Moreover, green mango pickles are common in African, Asian, and South American countries. Peach pickles (Yan-taozih) are popular in China and Taiwan and the LAB responsible for their taste have been identified as strains of *Leuc. mesenteroides*, *Lact. lactis, W. cibaria*, *W. paramesenteroides*, *W. minor*, *Ent. faecalis,* and *L. brevis* (Swain et al., 2014). On the other hand, false banana (*Ensete ventricosum)* fermentation to produce *kocho* pulp is an ancient method for preserving fruits and vegetables in the South Pacific countries. Another traditional fermented functional beverage consumed mainly in Turkey and Eastern Europe is kefir. Water kefir is a homemade fermented beverage based on a sucrose solution with or without fruit addition and fermented with kefir grains, which are a consortium of yeast, acetic acid bacteria and LAB embedded in a polysaccharide matrix (Shneedorf, 2012). This drink became very popular because of its recently proved health benefits. In this respect, Ranzazzo et al (2015) formulated a kefir-like beverage using Mediterranean fruits with an increased number of desirable volatile compounds and antimicrobial activity with a good sensory profile.

Although fruit fermented products are traditional foods in some cultures, there is not yet a consensus respect of the constituent of the natural fruit microbiota and the contribution of each microorganism to the final product characteristics. New technologies have been used to analyze traditional beverage composition and microbe contribution and an increase interest on identifying and confirming the health claims of vegetable fermented products have also been analyzed. However; the first probiotic vegetable-based product was commercialized not before 1994. Indeed, fruit-based probiotic juices are in their early stage of development and need further research for characterizing their sensorial profile and health benefits (Marsh, Hill, Ross, & Cotter, 2014). To date, the commercial probiotic beverages available are GoodBelly® in the USA, and ProViva® in Sweden, for which the probiotic strain used is *L. plantarum* 299v, while Biola® in Norway and Gefilus® in Finland are produced with the probiotic strain *L. rhamnosus* GG. On the other hand, Healthy Life® in Australia contains the strains *L. plantarum* HEAL 9 and *L. paracasei* 8700:2, for which a modulating effect against viral infections has been claimed (Berggren, Ahrén, Larsson, & Önning, 2011). Xu et al. (2018) reported that some ProViva® formulations could attenuate the early insulin response due to the high concentration of phenolic compounds present in the fruits while Nobaek, Johansson, Molin, Ahrné, and Jeppsson (2000) demonstrated that a ProViva® drink based on rose-hip could relieve symptoms in patients with irritable bowel syndrome. Recently, Y. Wang et al. (2019) showed that a Changbai Mountain fruit commercial fermented beverage could regulate the intestinal microbiota in mice, increasing the bacterial proportion of the family *Prevotellacea*, *Bacteroidales* S24-7, and *Bacteroidaceae,* which are present in healthy individual guts. Y. Yan et al. (2019) showed that fermented pomace blueberry juice by *L. rhamnosus* GG and *L. plantarum*-1 could increase its antioxidant activity, cholesterol clearance rate, and health-promoting benefits by increasing the concentration of phenols and flavonoids. On the whole, these results suggest that fermented fruit juices have health benefits beyond the ones of the probiotics used by the release of functional compounds bound to the fruit matrix.

Although the benefits on consuming fruit-based probiotics have been reported and are being commercialized, new formulations have been analyzed for improving probiotic viability and drinks´ taste. ProViva® has added sugar or stevia to mask the acid taste caused by lactic fermentation (https://www.beveragedaily.com/Article/2015/05/18/The-taste-test-How-do-you-make-a-probiotic-drink-taste-good). Moreover, the inhibition of the malate metabolism of the probiotic strain *L. plantarum* 299v was studied in the ProViva® apple juice to prevent overproduction of carbon dioxide by adding lactic acid to the preparation (Göransson, 2016). Also, the impact and optimization of citrate and lactic acid production by *L. plantarum* 299v in ProViva® orange formulations was studied to inhibit the beverage carbonation at the end of the storage period (Jönson, 2016).

3.2. Strategies of assessment and design of fermented beverages

For the evaluation and design of fermented beverages, good manufacturing practices are needed. According to Marsh et al. (2014), the following criteria are of relevance: a suitable starter culture for the production of a fermented drink should contain strains capable of metabolizing the polymers present in the substrate; commonly strains of *Lactobacillus*, *Lactococcus*, *Bifidobacterium,* and of yeasts such as *Pichia kudriavzevii* (DCNa1) and *Wickerhamomyces subpelliculosus* (DFNb6) (Di Cagno et al., 2020) or symbiotic cultures of bacteria and yeast are used (SCOByk) (Jakubczyk, Kaldunska, Kochman, & Janda, 2020). Likewise, milk, soybeans, cereals, tea, whey proteins, fruit juices with high concentration of fermentable carbohydrates are among the most used substrates. Depending on the substrates used, different metabolites can be obtained: peptides (Erdogan, Ozarslan, Guzel-Seydim, & Taş, 2019), bioactive compounds such as organic acids (Mauro & Garcia, 2019), phenolic compounds (Flores-García et al., 2019), antimicrobials, trace elements (Souza et al., 2019), or aroma and flavor compounds (W. Zhang et al., 2020), etc.; their potential health effects have been mentioned earlier in this review (Prado et al., 2015; Yamahata, Toyotake, Kunieda, & Wakayama, 2020). In this context, during the manufacturing process and considering the constant growth of fermented beverages in the market, it is necessary to consider the barrier technologies that allow conserving and consuming these products. Control processes are associated with constant monitoring of pH, organic acids, and formation of volatile compounds that may affect the sensory characteristics of the final product. Additionally, the integration of other components, which are not necessary to carry out the fermentation process *per se*, would allow enriching the final products; among the compounds that stand out for their popularity, economic access, and constant supply are antioxidants, soluble fibers, vitamins or fatty acids; however, their excessive addition can affect the sensorial acceptance of the product and have a negative impact on the market (Fernandesa, Sonawaneb, & SS, 2020).

3.3. Nanotechnology associated with beverage development

During the last decade, there has been a significant impact on the advancement of nanotechnology, which has been reflected in a high increase of citation (average of 262 per year) on topics related to nanotechnology. From these publications, countries with leadership in these issues are the United States, China, South Korea, Canada, and Germany (Yeung et al., 2020). The main research topics for the application and development of nanoparticles are the controlled release of drugs, their toxicity, biocompatibility, and biodistribution in model organisms (Yeung et al., 2020). However, it is important to identify and regulate the potential toxic effect of nanometric formulations from the environmental point of view, since some international agencies such as the United States National Nanotechnology Initiative (NNI) consider the necessity to homogenize criteria, especially with the European Union, to have a correct handling of nanoparticles once their use is concluded (Souto et al., 2020).

A potential application of nanotechnology is in the transport and protection of nutraceuticals, since it is known that nutraceuticals can have a better activity compared to drugs since they can be linked to food matrices (Santini & Novellino, 2018). However, there are no homogeneous criteria at the international level that allow deciding whether or not to approve the use of any nutraceutical in food matrices, since while the United States Federal Drug Agency focuses on the nutraceutical being safe from the point of view of sanitary view (good manufacturing practices) and zero adverse effects on consumers, in other countries such as Mexico or Colombia, it is only required to make a notification that it is being used in the product (Daliu, Santini, Novellino., 2018). Although nutraceuticals can help improve people's health, in combination with conventional pharmacological therapies (Durazzo, Lucarino, Santini., 2020), the inclusion of nutraceuticals in controlled release systems such as nanoparticles can have better effects, including the release controlled and better effects such as in the treatment of diabetes (Souto et al., 2020) or even in the formulation of prebiotics, probiotics, and symbiotics (Durazzo et al., 2020).

Among the multiple applications of nanotechnology, encapsulation of bioactive compounds, enzymes, or food additives that help to protect these compounds to maintain their biological and techno-functional properties or to facilitate their inclusion in some food matrices such as beverages in sizes below 100 nm is of outmost interest (Pund, Joshi, & Patravale, 2020). In beverage development, nanoparticles obtained through various encapsulation techniques such as emulsion evaporation, complex coacervation, emulsification, and nanoprecipitation have been used to increase the functional attributes of fermented beverages (Pund et al., 2020). The addition of nanoparticles loaded with bioactive compounds have better biological effect compared to drinks lacking of them; furthermore, the addition of nanoparticles in fermentation processes can act as adjuvant for the correct development of microorganisms (Neri-Numa, Arruda, Geraldi, Júnior, & Pastore, 2020). However, the best application of nanotechnology is precisely obtaining more stable emulsions, where the obtained nanoparticles interact with components through hydrogen bridges or Van der-Waals forces, which remain stable without presenting i.e. phase separation (Saari & Chua, 2020). This fact is important from the sensory point of view since a more pleasant appearance is offered to the consumer and improved absorption of added nutritional components compared to other formulations is reached (Hu, Hu, Fleming, Lee, & Luo, 2020). Additionally, the nanometric size of encapsulated compounds can increase their bioaccessibility and bioavailability (Ubeyitogullari & Ciftci, 2019).

When carrying out a stratified search (Stefanowski & Weiss, 2003) for the application of nanotechnology in the formulation of fermented beverages (Figure 5), six subtopics were obtained; the main topics related to the use of nanotechnology in the development of fermented beverages are focused on the stabilization of the formulations, in addition to the generation of emulsions rich in bioactive compounds comparing the different types of nanocarriers (lipids, dendrimers, proteins, polymers).

Chaudhari, Pan, and Nitin (2015) evaluated the effect of adding silica nanoparticles and modified starch (5% w/v, each) on the stability of a concentrated reconstituted pear juice drink; the added compounds improved the emulsion stability up to 21 days compared to the control drink. Lycopene, a compound that has several relevant biological attributes, is difficult to absorb in the body due to its chemical characteristics; however, its bioavailability and biological activity in the body can be increased using an oil-in-water emulsion (Jain, Winuprasith, & Suphantharika, 2020). Another compound of significant biological relevance is resveratrol, which can be encapsulated in a polymeric matrix such as chitosan and γ-poly glutamic acid (γ-PGA); thus, increasing its solubility up to 153 µg/mL and maintaining its antioxidant properties even in direct exposures to UV radiation (Chung, Lee, & Lee, 2020).

Nanoparticles can also be used as carrier of microorganisms such as *P. acidolactici,* in this case the selection of the material vehicle is very important since lower cell viability values can be obtained compared to micron-sized polymer matrices (Ahmad, Gani, Hamed, & Maqsood, 2019). Some secondary metabolites can act as emulsifying agents as it has been reported for galactane exopolysaccharides produced by *W. confusa* KR780676; different flavors can be added in double emulsions (owo), thus achieving the longest prevalence in the polymer matrix (Kavitake, Balyan, Devi, & Shetty, 2019).

Conclusions and Perspectives

For a long time, highly processed foods were preferably consumed worldwide increasing the risk of suffering chronic diseases such as diabetes, cancer, coronary heart disease, obesity, and premature mortality. The interest of consumers has now changed towards alternative healthier foods. Interestingly, fermented beverages have been identified as a relevant category in the functional food market, which has shown a sustainable increase in the last years. Biotechnological challenges to design novel functional products and to exploit fruit juices and fruit by-products are dealt with in this review. Indeed, fermentation of fruit juices by LAB is a feasibly process to obtain new functional beverages, especially for Western diets. The carefully selection of LAB starter strains ensures bacterial adaptability and further multiple reactions in the matrix to improve the bioavailability of interesting compounds and the biological properties of the fermented product. In search of benefits for human health, fruit juices are promising and suitable vehicles to deliver probiotics and prebiotics, as alternative to dairy foods consumed conventionally. Beyond the nutritional value of fruits, fruit by-product processing is a valuable tool for recovering bioactive compounds in a sustainable manner, throughout implementation of innovative technologies. There is a growing interest to use fruits by-products to recover underutilized health-beneficial compounds to be potentially included in foods or used as nutraceuticals. The development of eco-friendly technologies to minimize the generation of waste during food production with the consequent environmental problems represent a concerning topic for sustainability. At the same time, efforts to formulate new beverages and added value products are still especially needed with under-utilized fruits. Finally, fruits and fruit by-products constitute excellent raw materials for the development of novel functional foods when combining their nutritional characteristics with the versatile enzymatic machinery of rationally selected LAB.

Figure legends

Figure 1. Controllable parameters in the extraction processes of bioactive compounds in plant by-products

Figure 2. Phenolic acids metabolism by lactic acid bacteria (adapted from Filannino, Gobbetti, De Angelis, and Di Cagno (2014)

Figure 3. Flavor compounds produced by LAB in fruits

Figure 4. Trends in non-alcoholic fermented beverages: a) Fermented milk; b) Cereal-based fermentations; c) Fermented beverages and the organism well-being; d) Drinks for lactose intolerant people; e) Fermented beverages rich in antioxidants; f) Healthy components of fermented beverages; g) Fermented enriched drinks; h) Fermented drinks and health benefits

Figure 5. Stratified search for the application of nanotechnology in the formulation of fermented beverages

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