Journal of Functional Foods 87 (2021) 104821

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Probiotic bacteria and plant-based matrices: An association with improved health-promoting features

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ARTICLE INFO

Keywords:
Health-promoting plant properties
Prebiotic molecules
Food carrier for probiotics
Plant-based matrices
Fibers
Phenolic compounds

ABSTRACT

The performance of probiotic bacterial strains is influenced by the carrier food and its functional components which while buffering the probiotic through the gastro-intestinal tract, contribute to an efficient implantation of bacterial cells and regulate probiotic features. Particularly, plant-based matrices are eligible substrate for hosting and delivering microbial populations because of their richness in nutrients, fibers, vitamins, minerals and dietary bioactive phytochemicals. The available data indicate that the intrinsic health-promoting properties of diverse plant-based matrices can be successfully exploited and improved developing effective association with probiotics, whose beneficial activity could be in turn improved and modulated by components of the plant-based carrier. In this review, the health-promoting properties of solid plant-based matrices (particularly artichokes, table olives, apple and cabbage) and their association with probiotic bacteria are also described indicating the role of the food matrix in sustaining probiotic cells during product processing, digestive process, gut implantation, and finally in exerting beneficial effects.

1. Introduction

Probiotics have been defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014) and several probiotic strains are currently commercially available in milk-based preparations, fermented products or in dehydrated formula. Even if the association between milk-based products and probiotic bacteria still dominate the current market of probiotic products, there is an increasing demand of consumers and market for novel non-dairy probiotic foods. In particular, this new interest is towards products “free-from” lactose and cholesterol and is based on a changed habit limiting the animal-derived product consumption. A recent analysis (a quantitative and qualitative assessment made by leading industry experts) on the global market of plant-based probiotic products foresees for the next years a worldwide strong growth in the pharmaceutical and food sector of such products due to the consumer and producer interest. As a demonstration, the plant-based probiotic market is followed by the main players (https://www.factmr.com/report/3384/plant-based-probiotic-market). Gupta and Abu-Ghannam (2012), even if recognized the well-established beneficial effects of milk-based probiotic products, outlined the concerns related to lactose intolerance, presence of allergenic proteins and cholesterol content of such products, which are at the basis of the increased interest of the scientific world as well as consumers and market in plant-based probiotic foods. On the other hand, the preference towards plant-based probiotic products is linked to the increasing interest in a whole plant-based diet as alternative to consuming meat and dairy products. Health implications, animal welfare and environment concerns motivate the plant-based diet preference (Faber, Castellanos-Feijoó, Van de Sompel, Davydova, & Perez-Cueto, 2020; AHDB, 2018). However, taste and texture issues may limit the consumption of some plant-based products, particularly those based on soy, pea and oat proteins (http://lup.lub.lu.se/student-papers/record/8986432). Therefore, the demanding challenge for research and food industry is to find technological solutions for developing non-dairy probiotic products with good sensorial features and high consumer’s acceptability, providing the consumers with non-conventional probiotic foods, which could be more and more part of everyday diets of all age groups (Gupta & Abu-Ghannam, 2012). It should be considered that, since probiotics only transiently colonize the intestinal tract, large populations need to be daily ingested to provide health benefits (Hill et al., 2014). In fact, probiotic survival during gastro-intestinal (GI) digestion and gut colonization suitability are strain-related abilities, and the efficacy of probiotic bacteria is influenced by the carrier food and its components,

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https://doi.org/10.1016/j.jff.2021.104821
Received 19 July 2021; Received in revised form 19 October 2021; Accepted 24 October 2021

which while protecting the probiotic through the GI tract, contribute to an efficient implantation of bacterial cells and regulate probiotic attributes (Flach, van der Waal, van den Nieuwboer, Claassen, & Larsen, 2018; Ranadheera, Baines, & Adams, 2010). In this regard, vegetable matrices are eligible for hosting and delivering microbial populations and particularly probiotic strains which are also able to increase their intrinsic health-promoting and functional properties. In fact, the functional attributes of plant-based matrices, their structure and their suitability to fermentation make them appropriate for carrying probiotic strains that would take advantages from the characteristics of plant-based matrices and, by exploiting prebiotic and bioactive molecules, take benefit for their survival during product processing and shelf life as well as in the digestive process and gut colonization. The functional properties of plant-based matrices depend on their richness in nutrients, fibers, vitamins, minerals and dietary bioactive phytochemicals and some of those diverse components have also an important role in the interactions with gut microorganisms (Flach, van der Waal, van den Nieuwboer, Claassen, & Larsen, 2018). In particular, the fiber content (dietary fibers) is involved, directly as well as for its effect on the gut microbiota (Holscher, 2017; Simpson & Campbell, 2015), in a number of recognized health-promoting effects of plant-based foods. Phenolic compounds of the vegetable matrices have been also associated to plant health-promoting activities; moreover, their potential prebiotic activity as well as of their process-derived bioactive molecules have been recently recognized (Alves-Santos, Araujo Sugizaki, Lima, & Veloso Naves, 2020; Debelo, Li, & Ferruzzi, 2020).

In this review, we describe relevant examples of how the health-promoting properties of plant-based matrices and of probiotic bacterial strains can be combined to obtain innovative functional probiotic products which are currently regulated by the EC Regulation N. 1924/2006 issued by the European Parliament and Council of The European Union (2006) aimed to provide consumers with scientifically-based health claims.

2. Plant-based matrices used in combination with probiotics and their health-promoting properties

The recent review by Min, Bunt, Mason and Hussain (2019) offers an effective view on probiotic food products of non-dairy origin – cereal-, fruit-, vegetable-, soy- and meat-based – highlighting the role of the matrix to obtain performing combinations with probiotic strains and confirming that survival and stability in such matrices are highly strain dependent. However, most of the reviewed probiotic food products have juice or smoothie texture and an abundant recent literature has already described plant-based probiotic beverages (Montemurro, Pontonio, Coda, & Rizzello, 2021; Munekata et al., 2020; Shori, 2016; Kandylis, Pissaridi, Bekatorou, Kanellaki, & Koutinas, 2016; Panda, Kellershohn, & Russell, 2021; Guergoletto et al., 2019; Martins et al., 2019), while the present paper is focused on solid matrices of the fruit and vegetable category (Min, Bunt, Mason, & Hussain, 2019), which have been until now poorly addressed.

The intrinsic health-promoting and functional characteristics of plant-based matrices depend on their bioactive components and among them dietary fibers play a relevant role due to their effects on the gut microbiota. “Dietary fibers” is a wide term including compounds with different physicochemical characteristics whose diverse definitions have been reviewed and discussed by Jones (2014), mainly concerning the definition by the CODEX Alimentarius Commission (CAC) that is: “Dietary fibers means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories………”. The CAC differentiates within this definition the “edible carbohydrate polymers naturally occurring in the food as consumed” from synthetic carbohydrate polymers and from carbohydrate polymers, which have been obtained from food raw material. A suitable classification of dietary fibers is also based on their viscosity, potential fermentation and water solubility (Bozzetto et al., 2018). In fact, dietary fibers are traditionally differentiated into soluble and insoluble fibers. The insoluble fiber consists of cellulose, some hemicelluloses and lignin, whereas soluble fiber includes fructans (fructooligosaccharides, inulin), beta-glucans, pectin, gum, mucilage, and some hemicellulose (Soliman, 2019). Due to their different chemical composition and physicochemical properties, soluble and insoluble fibers show diverse physiological effects on the human intestine (Müller, Canfora, & Blaak, 2018). In fact, soluble viscous fibers cause an increase of the food transit time with a delay of the gastric emptying (Müller, Canfora, & Blaak, 2018; Soliman, 2019). On the other hand, insoluble fiber decreases the transit time and increases fecal bulk, thus contributing to alleviate constipation (Müller, Canfora, & Blaak, 2018; Soliman, 2019). Moreover, while both those categories of fibers are not digested or adsorbed in the small human intestine due to the lack of suitable human enzymes, the soluble fiber is much more easily fermentable to short-chain fatty acids (SCFA) by colonic bacteria in the large intestine (Sivaprakasam, Prasad, & Singh, 2016). In fact, part of the fiber can be fermented by the gut microbiota whose composition and metabolic activity can be in turn modified depending on the amount and the quality of the available fiber components. Actually, the health-promoting properties of the fiber components depend in part on their suitability as “prebiotics” which have been defined by Bindels, Delzenne, Cani and Walter (2015) as “nodigestible compounds that, through their metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host”. The effects of fiber compound intake on the composition and metabolic activity of the gut microbiota as well as on the resulting beneficial effects on health have been objects of several reviews (Bozzetto et al., 2018; Müller, Canfora, & Blaak, 2018; Holscher, 2017; Simpson & Campbell, 2015; Soliman, 2019) which also indicate the main role of the end products derived by gut bacterial fermentation of fiber. It is generally accepted that the fiber intake, by stimulating resident microflora to produce SCFA (Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016) is associated to a number of beneficial and protective effects including a significant decrease of the blood pressure, a trend of total and LDL cholesterol decrease, an overall reduction in the risk of heart diseases, a protective effect against the metabolic syndrome as well as against the cancer of the colorectal intestinal region. In particular, this effect has been related to the ability of the insoluble fiber to link harmful compounds, including carcinogens, thus preventing their absorption and facilitating their elimination. Furthermore, resident bacteria by producing organic acids and bioactive molecules in the colon through the fiber metabolism, provide a range of potential benefits to the intestinal tract and beyond. Particularly, SCFA - of which the major acids in human adults are found as acetate, propionate, and butyrate, mainly deriving from the fiber degradation - represent the main source of energy for the epithelial cells of the colon playing an undisputed role in fuelling colonocytes, the main barrier that regulates the mechanisms of nutrient absorption and the passage of toxic molecules and microorganisms. SCFA may also limit the onset of inflammatory processes by acting as signaling molecules reducing production of pro-inflammatory cytokines (Tsai et al., 2019). Furthermore, SCFA when entering in portal circulation, slow the upper GI passage of food, finally contributing in maintaining the health status of the whole GI system (Topping & Clifton, 2001; LeBlanc et al., 2017; Tsai et al., 2019).

Among the other components of the plant-based aliments, polyphenols have also been associated to health-promoting activities. The polyphenols in native form might be more active in exerting biological properties but the modifications caused by gut microbiota deeply influence the possible health effects (Banerjee & Dhar, 2019). In fact, the wide collection of genes belonging to the resident microbiome influences the host ability to metabolize nutrients that would pass unmodified the GI tract. In this regard, the potential prebiotic activity of polyphenols has been recently recognized (Alves-Santos, Araujo Sugizaki, Lima, & Veloso Naves, 2020) because of their ability to stimulate the growth of bacterial genera (such as *Bifidobacterium, Lactobacillus, Faecalibacterium, Akkermansia* and *Roseburia*) which are considered as target of other prebiotic compounds and associated to beneficial health effects.

However, one of the more relevant critical point in “functionalize” vegetable matrices is to meet regulation requirements while obtaining products with high level of consumer satisfactions (health-orientated as well as taste-orientated). Galgano, Condelli, Caruso, Colangelo and Favati (2015) report an exhaustive list of technological and sensory effects determined by the addition of probiotic lactic acid bacteria (LAB) in fruit and vegetable-based products. Furthermore, the loss of microbial cell viability during processing or storage represents the main challenge to warrant more than 1 billion of live cells per portion during the product shelf-life, the minimal cell content representing one of the required features to claim a food product as probiotic (Hill et al., 2014). Technological tools can be applied to improve microbial cell survival (i.e. encapsulation, freeze drying etc.), even if the positive health image for consumers of fermented foods led to select fermentation as the preferable way to produce probiotic products (Gupta & Abu-Ghannam, 2012). In this regard, it is relevant that beyond the nutrient composition, the micro-architecture characterizing fruit and vegetable surfaces represent a suitable niche for hosting microbial cells, where they are protected from environmental and processing stresses, as well as from those caused by enzymes and chemicals that attempt cell integrity during the digestive process. Aguilera (2019) defines the food matrix as a part of the microstructure of foods that interacts with a constituent (e.g., a nutrient) or with an element linked to the food, for example microorganisms. Plant tissues, composed by an intricate internal microstructure of cells, pores and intercellular spaces, may play a role in microorganism’s adhesion and may act as carrier for probiotic cells (Peres, Peres, Hernández-Mendoza, & Malcata, 2012). In general, the steps occurring in the processing of vegetables, such as peeling and cutting may promote the release of cellular content creating ideal conditions for microbial growth.

The most relevant plant-based matrices suitable as vectors for delivering probiotics are those described in the following paragraphs, in Fig. 1 and in Tables 1 and 2.

2.1. *Artichokes*

2.1.1. *Health-promoting properties of artichokes*

Artichoke is an important source of health-promoting compounds including, in particular, high levels of the prebiotic inulin and a number of polyphenols (Lattanzio, Kroon, Linsalata, & Cardinali, 2009). Inulin is a fructan polysaccharide, accumulated in plants as a carbohydrate reserve and classified as soluble fiber, in which a variable-length chain of D-fructose, linked by ß-(2 → 1) glycosidic bonds, is typically terminated with a single glucose ring. The degree of polymerization is variable, depending on genetic and environmental factors, and affects the inulin water-solubility and fermentation; in particular, artichoke inulin includes also long-chain molecules with a length up to 200. Also the percentage of inulin content in the edible portion of artichoke is variable depending on the cultivar, and percentages ranging from 18.9% to 36.2%, on a dry matter basis, have been detected in different artichoke cultivars. The health-promoting effects of inulin have been extensively studied and recently reviewed (Shoaib et al., 2016; Ahmed & Rashid, 2019). In particular, the following effects have been also reported, even if in some cases they have been observed as results of the combined use of inulin with other factors (such as probiotics or other prebiotics): improvement of blood lipid profile with reduction of triglyceridemia, a prebiotic effect (particularly supporting the growth of *Lactobacillus* and *Bifidobacterium* strains), assistance in relieving constipation, reduction of intestine inflammation (in combination with probiotic strain), enhanced mineral absorption.

Concerning phenolic compounds in artichoke heads, they are mainly caffeoylquinic acid derivatives, and the 5-O-caffeoylquinic acid (chlorogenic acid) is the most abundant of them. The wide range of those compounds also includes relevant amounts (greater than 300 mg/100 g dry weight) of 1,5-O-dicaffeoylquinic acid, 3,4-O-dicaffeoylquinic acid and 3,5-O-dicaffeoylquinic acid; whereas, a minor percentage of the total phenolic content is constituted by flavones (such as apigenin and luteolin) and anthocyanidins, which, nevertheless, are considered

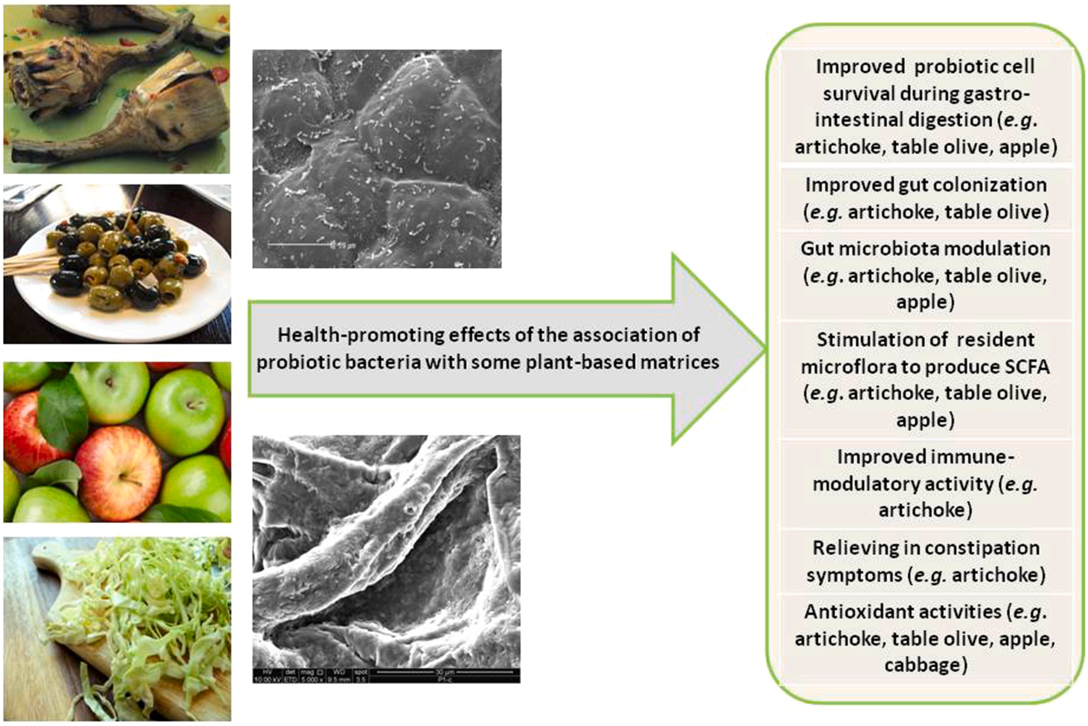

Fig. 1. Main plant-based matrices associated with probiotic bacteria and their relevant health-promoting properties.

Table 1
Examples of application of probiotic bacteria in solid plant-based matrices.

Fruit and vegetables products	Probiotic bacteria	Incorporation method	Main outcomes	References
Artichoke	*L. paracasei* IMPC2.1 (LMG-P22043)	Inoculation of brine - surface adhesion	The probiotic strain: - survived on the matrix with a load ≥ 7 log CFU/g - survived simulated gastro-intestinal digestion - transiently colonized the gut of 17 / 20 subjects - antagonized *E. coli* and *Clostridium* spp. - increased the genetic diversity of lactic population - improved symptoms of constipation.	Valerio et al. (2006, 2011, 2013);Riezzo et al. (2012)
Table olives	*L. paracasei* IMPC2.1	Inoculation of brine - surface adhesion	The probiotic strain: - colonized the olive surface dominating the natural LAB population - survived on the matrix with a load ≥ 7 log CFU/g - survived simulated gastro-intestinal digestion. - A final low-salt-probiotic product was obtained.	De Bellis et al. (2010); Valerio et al. (2006)
	L. pentosus B281, *L. plantarum* B282	Inoculation of brine - surface adhesion	- The strains were ≥ 6 log CFU/g on olive drupes. - *L. pentosus* B281 and *L. plantarum* B282 showed a high survival rate on the matrix.	Argyri et al. (2014);Blana et al. (2016)
	L. pentosus TOMC-LAB2	Inoculation of brine - surface adhesion	A favorable effect on fermentation and strain predominance was observed by: - an immediate post-brining inoculation - the use of a re-inoculation - an early processing in the season.	Rodríguez-Gómez et al. (2017)
Table olive paste	*L. plantarum* 33	Microencapsulation with sodium alginate and starch	- Encapsulation conferred additional protection to *L. plantarum* 33 (about 7 log CFU/g), when exposed to simulated gastro-intestinal conditions. - Microencapsulation did not adversely affect adhesion capacity to intestinal epithelium. - Microcapsules incorporated in olive paste did not affect physicochemical and sensory properties.	Alves et al. (2015)
Dried apple	*L. rhamnosus* ATCC7469	Vacuum impregnation - air and REV drying	- Apple samples contained ≥ 7 log CFU/g of probiotic cells. - The bacterial stability at 25 °C depended on the dehydration techniques. - Sensory properties of the dried apple slices remained above the acceptable level for 30 days at 25 °C and 180 days at 4 °C. - Apple protected probiotics during exposure to low pH of stomach.	Noorbakhsh et al. (2013)
	S. cerevisiae CECT 1347 *L. casei* spp. *rhamnosus* CECT 245	Vacuum impregnation - air-drying	- Apple samples contained about 7 log CFU/g of probiotic cells.	Betoret et al. (2003)
	L. plantarum or *L. kefir* strains	Immersion/vacuum impregnation - air-drying	- Apple cubes inoculated by immersion contained about 7 log CFU/g of probiotic strains after drying. - After 3 months at 4 °C the strains survived at around 6 log CFU/g.	Rêgo et al. (2013)
	L. paracasei IMPC2.1	Inclusion - pectin coating - dehydration	- Dehydrated apple contained ≥ 7 log CFU/g of the probiotic strain. - The strain survived simulated gastro-intestinal digestion.	Valerio et al. (2020)
Fresh-cut apple	*L. plantarum* 299 v	Osmotic dehydration	- The strain maintained the viability of 7 log CFU/g after 6 days at 4 °C. - The strain survived simulated gastro-intestinal digestion.	Emser et al. (2017)
	L. rhamnosus CECT 8361 or *B. lactis* CECT 8145	Alginate coating	- Counts of *B. lactis* and *L. rhamnosus* were ≥ 9 log CFU/g after 8 days at 5 °C. - Both probiotics in apples survived simulated gastro-intestinal digestion. - Nutritional and microbiological quality was maintained during storage. - Probiotics exerted antagonistic effects on *Ls. innocua* and *E. coli* O157:H7.	Alvarez et al. (2021)
Cabbage	*L. paracasei* IMPC2.1	Inoculation of brine - surface adhesion	- The final product contained about 8 log CFU/g of the strain. - Blanching before fermentation preserved glucosinolates. - The acidification performed by the probiotic ensured a microbiological stabilization of the product.	Sarvan et al. (2013)
Sauerkraut (Cabbage)	*L. plantarum* L4, *Lc. mesenteroides* LMG 7954	Inoculation of brine - surface adhesion	- Viable probiotic cells count in final product was ≥ 6 log CFU/g of product. - The strains were used as starter cultures for fermentation allowing a NaCl reduction from 4.0% to 2.5% (w/v).	Beganović et al. (2011)
Dried yacon root	*L. casei* LC-1	Homogenization - air-drying	- The strain survived at greater than 5 log CFU/g after 56 days of product storage. - The strain survived simulated gastro-intestinal digestion.	De Souza Leone et al. (2017)
Potato Cheese	*L. brevis* CJ25	Potato puree inoculation	- Counts of *L. brevis* in the product were ≥ 8 log CFU/g. - Addition of oregano and NaCl stimulates *L. brevis* growth in the potato cheese. - The strain exhibited high level of survival in simulated gastro-intestinal conditions.	Mosso et al. (2016)
Fresh-cut cantaloupe	*L. plantarum* B2 or *L. fermentum* PBCC11.5	Dipping in probiotic suspension - air-drying	- High viability of both probiotics strains at about 8 log CFU/g. - Riboflavin-overproducing strains increased the vitamin B2 content of fresh-cut melon. - Both probiotics showed antagonistic effect against *Ls.*	Russo et al. (2015)

(*continued on next page*)

Table 1 (*continued*)

Fruit and vegetables products	Probiotic bacteria	Incorporation method	Main outcomes	References
			monocytogenes. - Both probiotics did not affect melon visual quality, while some sensorial attributes were affected by *L. plantarum* B2.	
Fresh-cut carrot	*L. acidophilus* La-14	Sodium alginate coating	- Counts of the probiotic was ≥ 7 log CFU/g. - The coating contributed to the quality of the minimally processed carrots by conserving their moisture and minimizing acidity variation and color changes during storage.	Shigematsu et al. (2018)
Fresh or dried beetroot	L. plantarum MIUG BL3	Spraying - surface adhesion	- Dried chips: strain load was greater than 7 log CFU/g. - Fresh cubes: strain load was greater than 8 log CFU/g.	Barbu et al. (2020)

B.: Bifidobacterium; *E.: Escherichia*; *L.: Lactobacillus*; *Lc.: Leuconostoc*; *Ls.: Listeria*; *S.: Saccharomyces.*

Table 2

Main health-promoting properties and compounds of plant-based matrices suitable as carriers for probiotic strains.

Plant-based matrix	Main health-promoting properties	Main health-promoting compounds	Suitability as a carrier for probiotic strains
Artichoke	- anti-oxidant activity - anti-inflammatory activity - anti-thrombotic and anti-atherosclerotic activities - choleretic activity - improved blood microcirculation	- polyphenols - high levels of the prebiotic inulin	- artichokes supported the growth of the probiotic strain and carried more than 8 log CFU/g of product - improvement of the probiotic survival during gastro-intestinal digestion - probiotic cells were recovered from stool samples
Table olives	- anti-oxidant activity - anti-inflammatory activity - protection against the risk of cardiovascular diseases	- polyphenols (hydroxytyrosol and its derivatives) - mono-unsatured oleic acid - selenium	- the probiotic strain successfully colonized the olive surface with more than 7 log CFU/g of product - improvement of the probiotic survival during gastro-intestinal digestion - probiotic cells were recovered from stool samples
Apple	- bifidogenic effect - reduced cholesterol and triglyceride concentrations - modulation of fecal microbial compositions	- polyphenols - fiber (pectin)	- the probiotic strain covered the apple surface and penetrated in intercellular spaces of parenchymal tissue - dried apple samples carried more than 7 log CFU of probiotic cells /g of product - improvement of the probiotic survival during gastro-intestinal digestion
Cabbage	- protective effect against the colon rectal cancer - anti-oxidant activity	- glucosinolates - polyphenols - carotenoids	- the probiotic strain colonized the vegetable surface and the final product contained about 8 log CFU/g of product

responsible for health-promoting properties. In fact, artichoke flavonoids luteolin and cynaroside were considered responsible for antithrombotic and anti-atherosclerotic activities as they increased the expression of the endothelial nitric-oxide synthase (eNOS) and the resulting vasodilator response, while the cynarin and chlorogenic acid did not show this effect (Li, Xia, Brausch, Yao, & Forstermann, 2004). In this regard, Rossoni, Grande, Galli and Visioli (2005) also demonstrated that luteolin and apigenin improved aortic relaxation of isolated rat aortic rings and that an artichoke extract restored the appropriate vasomotor function in aged rats. Moreover, Xia et al. (2014) reported that cynarin and cyanidin reduced the expression of the inducible nitric-oxide synthase (iNOS) to which a detrimental vascular effect is attributed because it may generate large amounts of NO leading to vascular dysfunction. The dual role of an artichoke phenolic extract (APE), which increased the expression eNOS in endothelial cells but decreased the expression of iNOS in macrophages, was also confirmed in a more recent study (D'Antuono et al., 2018b). The authors also showed that APE stimulated gene expression of Superoxide Dismutase and of genes encoding for tight junction proteins in lymphatic endothelial cells; they concluded that the phenolic extract improved blood microcirculation, showed an anti-inflammatory activity, induced reinforcement of the tight junctions and protection against oxidative damage in lymphatic vessels. Moreover, artichoke and their phenolic compounds have been considered as source of health-promoting activities such as antioxidant activity and protection of low density lipoprotein (LDL) against oxidation (Garbetta et al., 2014; D'Antuono, Garbetta, Linsalata, Minervini, & Cardinali, 2015) and choleretic activity (Gebhardt, 2001; Saénz Rodriguez, García Giménez, & de la Puerta Vázquez, 2002).

2.1.2. *Suitability of artichokes as a carrier of probiotics*

Processing of vegetables in brine using probiotic LAB strains may represent a way to develop fermented foods with a functional appeal. In particular, it was demonstrated that artichokes subjected to a mild cooking step to limit enzymatic activities and soaked in a brine solution inoculated with a probiotic strain are suitable for supporting the survival of probiotic strains and for the development of an effective vegetable-based functional food (Valerio et al., 2006; 2010; 2011). *Lactobacillus plantarum* ITM21B (LMG P-22033) and *Lactobacillus paracasei* IMPC2.1 (also cited as LMG-P22043) survived on brined artichokes for at least 90 days and the anchorage of bacterial strains on the vegetable tissues improved their survival in simulated GI digestion. As a demonstration that fermented artichokes (served as portion of ready-to-eat lightly dressed artichoke carrying more than 1 billion of live cells) can work as an active vehicle for transporting and delivering adequate amount of bacterial populations, the *L. paracasei* IMPC2.1 strain was recovered from stools of the volunteers during a human feeding study (Valerio et al., 2011). The high cell viability shown in the study has been ascribed to the nutrients provided by artichokes that enable bacterial survival during storage and by the roughness of the vegetable structure, which may offer protection to the bacterial population in the hostile GI tract environment. Actually, a large number of bacterial cells colonizing the surface of probiotic artichokes can be observed by Scanning electron microscopic (SEM) observation (Valerio et al., 2013). The efficacy of the "synbiotic" (the combination probiotics-prebiotics) product in favoring the human gut colonization by the probiotic and in modulating microbial groups has been demonstrated during a double-blind, placebo-controlled, crossover randomized clinical trial (Riezzo et al., 2012) (Fig. 2). Actually, the presence of prebiotic substances in the food matrix, such as inulin in artichokes, can further strengthen the probiotic efficacy into the human gut (Gupta & Abu-Ghannam, 2012). Particularly, the prebiotic function exerted by inulin present in artichokes in sustaining the growth of the probiotic strain *L. paracasei* IMPC2.1 was confirmed by the ability of the strain to metabolize inulin from artichokes at 0.2% in a minimal medium where inulin was the sole carbohydrate source (S.L. Lonigro, unpublished results). The ability to metabolize inulin is possibly due to the activity of an *exo*-inulinase enzyme which has been identified in other *L. paracasei* strains (Boger, Lammerts van Bueren, & Dijkhuizen, 2018). However, other molecules in addition to inulin could contribute to the performance of the strain IMPC2.1. In fact, the addition of an artichoke phenolic extract to the culture medium determined a remarkable increase of the growth of the strain and influenced its immuno-modulatory feature (Sisto et al., 2016). From a technological point of view, probiotic strain *L. paracasei* IMPC2.1 while colonizing the artichokes also acts as a bioprotective culture able to counteract possible contamination by pathogenic microorganisms (Valerio et al., 2013). Acidified ready to-eat vegetable or fruit based products are known to vehicle human pathogens (Berger et al., 2010), since some pathogens can adapt to acidic conditions and survive during shelf life. Heat treatments while destroying microorganisms may affect the sensorial and nutritional quality of products, therefore milder preservation methods that ensure microbiological safety and high-quality products are sought. The use of LAB bioprotective cultures, and/or their antimicrobial compounds may ensure prolonged shelf-life and safety of the products (Leverentz et al., 2006). *L. paracasei* IMPC2.1 populations reduced the survival of *Listeria monocytogenes* ATCC19115, *Salmonella enterica* subsp. *enterica* ATCC13311 and *Escherichia coli* ATCC8739 on probiotic artichokes; in fact, different inactivation curves were observed at the same pH values in probiotic and not-inoculated standard products challenged with the pathogens (Valerio et al., 2013).

2.2. Table olives

2.2.1. Health-promoting properties of table olives

The incorporation of health-promoting bacteria as probiotic strains may add functional features to the intrinsic nutritional and functional properties of table olives. From a nutritional point of view, table olives are rich in monounsaturated oleic acid, fibers (approximately 3 g per 100 g of edible portion), vitamins (α-tocopherol, beta-carotene, pantothenic acid, and vitamin B1), minerals, and essential amino acids (Boskou, Camposeo, & Clodoveo, 2015). Particularly, olives represent one of the main sources of selenium (on average 22 ng/g), an essential element for the human organism since it is involved in the formation of thyroid hormones, is an essential component of seleno-amino acids and seleno-proteins and may also act as an antioxidant catalyzing the reduction of peroxides (De Bruno, Piscopo, Cordopatri, Poiana, & Mafrica, 2020; Puccinelli, Malorgio, & Pezzarossa, 2017). It should be also considered that olive fruits constitute the plant matrix from which the olive oil is extracted and whose regular intake has been associated to well-known beneficial effects for health such as antioxidant and anti-inflammatory activities and protection against the risk of cardiovascular diseases (Covas, De La Torre, & Fitó, 2015). In particular, it is relevant that the health-promoting properties of virgin olive oil resulted in direct relationship with its phenolic content (Reboredo-Rodríguez et al., 2017). In fact, a related health claim was also approved by European Food Safety Authority (EFSA) stating that olive oil phenolic compounds contribute to protect blood lipids from oxidative stress (Commission Regulation - EU, 2012). However, EFSA limited this claim only to olive oil containing at least 5 mg of hydroxytyrosol and its derivatives in 20 g olive oil, also indicating that a daily intake of 20 g of olive oil is necessary to obtain the beneficial effect. Therefore, the beneficial effects depend on the amount of ingested phenolic compounds. Actually, a recent study reports that table olives contain an amount of phenolic compounds, in terms of hydroxytyrosol and its derivatives, about 8 times higher than olive oil (D'Antuono et al., 2018a). Thus, table olives should be considered as an important additional or alternative source of those health-promoting molecules and a component of the diet potentially providing health benefits even higher than those associated to olive oil intake. In this regard, Accardi et al. (2016) demonstrated in a pilot study that table olives, as an important source of phenolic

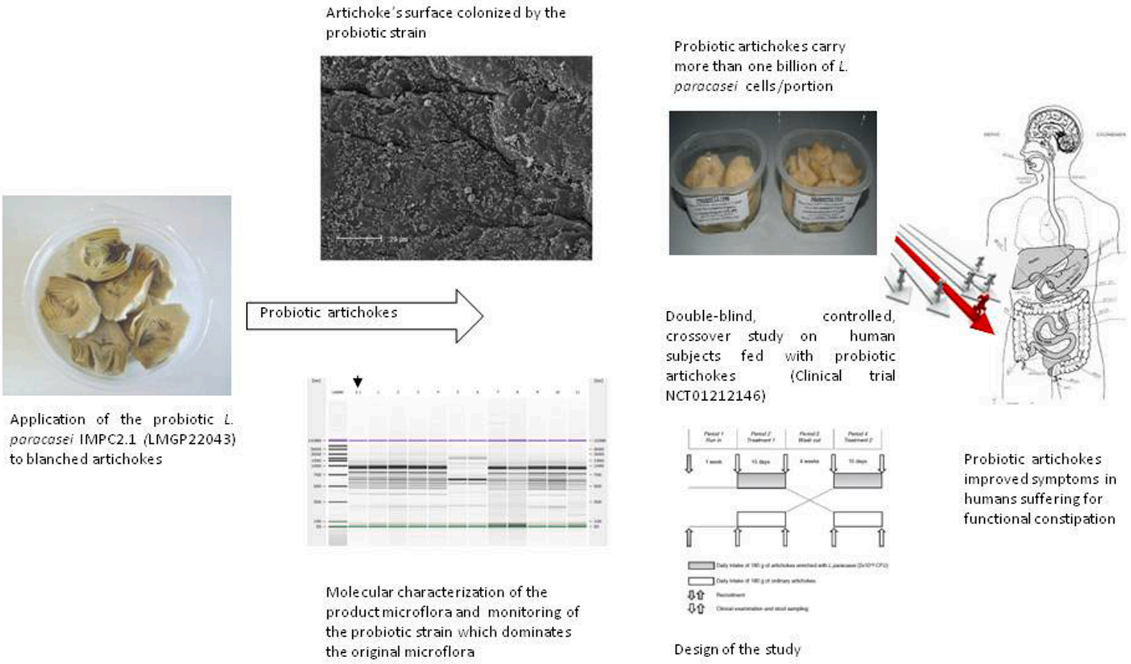

Fig. 2. Example of vegetable matrix acting as carrier for probiotics: the case of the artichoke.

molecules, potentially provide health benefits. An anti-inflammatory and anti-oxidant effect of daily consumption of green table olives Nocellara del Belice was demonstrated to be related to their polyphenol and mono-unsatured oleic acid content.

Moreover, table olives, similarly to other vegetable matrices, have also been considered a source of presumptive probiotic strains, particularly LAB, that may support piloted fermentations while providing health benefits (Argyri, Panagou, & Tassou, 2016). Many studies investigated on probiotic features of strains belonging to the olive-related species *Lactobacillus plantarum* and *Lactobacillus pentosus* and to *L. paracasei*, *Lactobacillus casei*, and *Lactobacillus paraplantarum*. Strains were able to adhere to intestinal epithelial cells (stimulating intestinal barrier integrity), to ferment prebiotic fibers, to inhibit pathogens or *in vivo* capable to exert protective effects in the model organism *Caenorhabditis elegans* (Perpetuini, Prete, Garcia-Gonzalez, Khairul Alam, & Corsetti, 2020; Botta, Langerholc, Cencič, & Cocolin, 2014; Peres, Peres, Hernández-Mendoza, & Malcata, 2012).

Furthermore, the additional advantage represented by the use of table olives as a carrier for probiotic cells, relates to the natural process underlying their production, leading to perceive the presence of beneficial microorganism not as “food additives” as for many marketed probiotic products.

2.2.2. Suitability of table olives as a carrier of probiotics

Table olives, having those substantiated health-promoting features, represent a valid biological vector for carrying viable microbial populations in the gut, including selected probiotic strains. The fermentation of table olives can take place thanks to the spontaneous microbiota, but the use of starter cultures is an advisable, widespread practice. The selected starter strains generally belong to a species component of the spontaneous microbiota and the presence of LAB on olive phylloplane was already reported by Ercolani (1978) and by Lavermicocca, Surico, Varvaro and Babelegoto (1987). At the end of fermentation, table olives still host on their surface a viable microbiota, with species able to degrade the bitter glucoside oleuropein (Lavermicocca, Gobbetti, Corsetti, & Caputo, 1998; Lavermicocca et al., 2002). The evidence that LAB can colonize and survive on the surface of olive fruits paved the way for the use of table olives as a biological carrier for beneficial strains such as probiotics (Lavermicocca et al., 2003, 2005). Particularly in the case of table olives, their surface colonization by microorganisms relates to the fruit surface microstructure, composition and release of nutrients. SEM observation revealed that the olive fruit surface is an example of microarchitecture where bacteria can survive by adhering to skin (Lavermicocca et al., 2005). In particular, SEM observation of the surface of table olive revealed a regular distribution of the inoculated *Lactobacillus rhamnosus* GG and *L. paracasei* IMPC2.1 and *L. paracasei* IMPC4.1 bacterial cells, while cells of *Bifidobacterium longum* and *Bifidobacterium bifidum* seemed to adhere to the olive surface through small aggregates of amorphous material or clusters directly on the olive surface. Actually, the surface of the olive epidermis coated by epicuticular waxes may have an important role in microorganism allocation and adhesion as observed in black olives whose surface, scanned by SEM, resulted colonized by an association between yeasts and bacteria (Nychas, Panagou, Parker, Waldron, & Tassou, 2002; Grounta & Panagou, 2014). The molecular basis of LAB adhesion to olive surface have been recently reviewed by Perpetuini, Prete, Garcia-Gonzalez, KhairulAlam and Corsetti (2020). Genes involved in the organization of a biofilm on the olive skin formed by *L. pentosus* during fermentation have been identified (Perpetuini et al., 2016). Particularly, three genes encoded for enzymes involved in the adhesion to specific components of the olive skin, including lipids of the epicuticular wax.

Moreover, technological aspects have been approached to define conditions ensuring probiotic cell survival during table olive processing and storage. In this regard, Blana, Polymeneas, Tassou and Panagou (2016) have monitored the survival of *L. plantarum* and *L. pentosus* strains selected for their *in vitro* probiotic potential, during the storage of brined green table olives in polyethylene pouches started with those strains. The procedure seems to provide a standardized method for producing probiotic table olives as revealed by the high survival rates of both strains for 6 months on olive surface. Efforts have been also made to define factors (time of inoculation, time of the processing season, pH etc.) that may affect the survival/predominance of a potential probiotic strain (*L. pentosus* TOMC-LAB2) at a large-scale level (16 cubic meter fermentation vessels) (Rodríguez-Gómez et al., 2017). Also the predominance of the potential probiotic strains during large scale processing is a real challenging task due to the current conditions prevailing in the industrial fermentation yards such as competitiveness of the environmental microbiota, the risk of contamination by alterative microorganisms, etc. Authors have depicted the main actions to be implemented to perform a suitable process: an immediate post-brining inoculation to dominate wild microflora, re-inoculation steps to replace died cells and an early processing in the season. In this regard, it is worth noting a study on the fermentation of debittered green olives cv. Bella di Cerignola piloted in an industrial plant with the probiotic strain *L. paracasei* IMPC2.1, in which the strain successfully colonized the olive surface and dominated the natural LAB populations (De Bellis, Valerio, Sisto, Lonigro, & Lavermicocca, 2010). Strain IMPC2.1 can be considered as a good example of a probiotic strain also suitable for industrial processing of table olives. It combines the health-promoting properties of a probiotic strain with the efficacy of a starter culture, that can control the fermentation process and protect table olives during storage, leading to the realization of a final palatable low-salt-probiotic product that meets commercial and functional requirements throughout its shelf-life of several months. In fact, monitoring the dynamics of microbial populations adhering on the surface of debittered green olives (4% w/v NaCl) inoculated with the probiotic strain IMPC2.1 showed that the strain, by dominating the spontaneous microbial populations, decreased the pH of brines to a value ≤ 5.0 after 30 days until the end of fermentation (90 days). No alterative processes occurred and the final probiotic product was ready to eat and suitable for packaging. Furthermore, washing steps were not required to reduce the salt content. The final product contained adequate amounts (more than one billion per portion) of the probiotic strain throughout its shelf-life of several months. Conversely, in control sets pH dropped slower and an increase of pH occurring after 30 days caused an uncontrolled fermentation with irreversible deterioration of the product (De Bellis, Valerio, Sisto, Lonigro, & Lavermicocca, 2010). Further evidence confirms the technological advantages represented by table olives as carrier for probiotics. When anchored to table olives, a good survival rate of the probiotic *L. paracasei* IMPC2.1 was observed during simulated gastric and intestinal digestion (Valerio et al., 2006). The presence of prebiotic substances and the physical structure of the vegetable may offer a protection (similarly to skim milk) to the strain from the harsh conditions characterizing the digestive process. In addition, the good survival performance on olive surface of *B. bifidum* and *B. longum* strains observed during the study of adhesion and survival on table olives of selected strains (Lavermicocca et al., 2005), suggests an alternative way to incorporate these species into the food chain, knowing their sensitivity to the environmental conditions of fermented milk-based products that limits their survival.

Table olive paste has been also demonstrated to act as a carrier for delivering potential probiotic strains. Survival of microencapsulated *L. plantarum* cells has been monitored during storage and their tolerance to GI transit and ability to *in vitro* adhere to intestinal epithelium have been studied. Encapsulation by the extrusion method with sodium alginate and starch protected bacterial cells and prevented the loss of cells exposed to a simulated environmental stress. The microencapsulation procedure did not adversely affect the bacterial functions essential for adhesion to epithelial cells. However, free cells of a *L. plantarum* strain have been proved able to survive in olive paste during storage at refrigerated temperatures (Alves et al., 2015).

2.3. Apples

2.3.1. Health-promoting properties of apples

Non-digestible polysaccharides as well as polyphenols represent the main health-promoting compounds of the apple matrix. Apples may provide a daily intake of 20 –25% of polyphenols as well as 10 –30% of fiber and potassium, depending on individual eating habits. The soluble fiber pectin (about 50% of total fibers) has been demonstrated to have a role in the effects of apples on lipid metabolism. However, the apple phenolic fraction (mainly represented by phloridzin, chlorogenic acid, quercetin glycosides) by interacting with pectin, can lower circulating cholesterol and triglyceride concentrations, demonstrating the cooperative action that determines the biological effects in rats of the food components. Pectin and phenolic compounds when reach indigested the colon, act as substrates for colonic fermentation allowing the production of SCFA and phenolic acids (Aprikian et al., 2003). Generally, the health-promoting properties of fruit and plants are investigated using derived bioactive fractions, while the effects of whole apples on human gut microbiota have been investigated by Koutsos et al. (2017). They demonstrated, by comparing 3 apple varieties in an *in vitro* batch culture colonic model, that the bioactive apple components differently modulated gut microbiota composition and the relevant metabolic pattern. Whole apple administration resulted in a bifidogenic effect more relevant than that determined by the treatment with the prebiotic inulin. Furthermore, apples increased total SCFA as well as the microbial-derived metabolites modified in the fecal samples, thus demonstrating that whole apples can modulate fecal microbial compositions and metabolic output (Koutsos et al., 2017).

2.3.2. Suitability of apples as a carrier of probiotics

The demonstration of richness in prebiotic compounds strengthen the evidence that particularly the apple matrix can be considered a suitable functional matrix for carrying live probiotic cells into the human gut. This is also because of its large volumes within parenchyma (20–25% of total volume) filled with gas and liquid that can be replaced – using appropriate technologies - by bioactive compounds or micro-organisms (Noorbakhsh, Yaghmaee, & Durance, 2013). A process to obtain probiotic-enriched dried apples has been designed by Betoret et al. (2003) combining vacuum impregnation and air-drying techniques: dried apple samples carried about more than 7 log CFU/g of probiotic cells belonging to *L. casei* ssp. *rhamnosus* and *Saccharomyces cerevisiae*, during storage. However, even if vacuum impregnation is a recognized method that favors the inclusion of probiotic cells into a fruit matrix (Morais et al., 2018), some drawbacks related to particular pressure conditions are reported with respect to other methods (i.e. immersion) impacting on strain viability and product quality (Rêgo, Freixo, Silva, Gibbs, & Teixeira, 2013). The suitability of the apple matrix to be fortified with probiotic cells has been recently confirmed by Valerio et al. (2020). An inclusion/coating/dehydration procedure allowed obtaining a probiotic snack containing more than 9 log CFU per portion of the probiotic strain *L. paracasei* IMPC2.1 that, included in the apple matrix, survived also the simulated GI digestion. The study confirmed that the adhesion of probiotic cells to fruit tissue could be due to the structure of plant tissues, able to host bacteria in intercellular spaces that allow their survival. SEM observation showed that bacterial cells, whose size were about on average 0.5 μm in diameter and 2.0 μm in length, covered the apple surface but also penetrated inside parenchymal tissue and adhered to the cells also forming agglomerations. The application of the pectin coating did not modify the main structural aspects of the probiotic cell distribution. Interestingly, the probiotic samples showed a higher total polyphenol content with respect to the not inoculated control, suggesting a protective action of the probiotic cells covering the apple surface that slowing the passage of oxygen may limit the oxidation of polyphenols within the apple matrix. Therefore, the process including a mild procedure (soaking and stirring) represents an easy method to combine the health-promoting features of a fruit matrix with probiotic populations that survived during a 30-day storage period (Valerio et al., 2020).

In recent years, the production of probiotic fresh-cut apples has been also studied. Emser, Barbosa, Teixeira and de Morais (2017) applied the *L. plantarum* 299 v to apple cubes by osmotic dehydration. The strain maintained the viability of 7 log CFU/g during storage and also survived the simulation of the digestion. An effective carrier for probiotic lactobacilli and bifidobacteria was also fresh-cut apples coated with alginate enriched with inulin and oligofructose. The presence of probiotics and prebiotics in coatings made possible to obtain apple cubes that maintained the microbiological and nutritional quality during storage (Alvarez, Bambace, Quintana, Gomez-Zavaglia, & Moreira, 2021).

2.4. Cabbage

2.4.1. Health-promoting properties of cabbage

The health benefits of cruciferous vegetables are particularly attributed to a group of secondary plant metabolites, the glucosinolates, which are considered associated to the reduction of the risk of the colon and rectal cancer (Verkerk et al., 2009). Mainly the *Brassicaceae* family is rich in polyphenols and carotenoids but the glucosinolate fraction represents its most important dietary group of bioactive molecules. The basic chemical structure of glucosinolates is a β-thioglucoside-*N*-hydroxysulfate with a sulfur linked β-d-glucopyranose moiety with an aromatic, indolic, or aliphatic side chain. In the case of white cabbage (*Brassica oleracea* var. *capitata*), glucosinolates are represented by glucoiberin, progoitrin, sinigrin, glucobrassicin and 4-methoxyglucobrassicin, and their derivatives, particularly isothiocyanates, that are the main responsible for the health benefits of *Brassicaceae* (Verkerk et al., 2009). White cabbage is also source of significant amounts of phenolic acids, particularly hydroxycinnamic and hydroxybenzoic acid derivatives (Abu-Ghannam & Jaiswal, 2015). The preservation of these antioxidant and health-promoting compounds is highly dependent on the processing they underwent; in fact, besides the enzymatic hydrolysis occurring in industrial or domestic processing, glucosinolate and polyphenol levels in *Brassica* vegetables can also be affected by the heat treatments due to their thermal degradation and leaching into the processing solutions (Sarvan et al., 2013; Abu-Ghannam & Jaiswal, 2015). Particularly, an appropriate heat treatment inactivating the myrosinase and a piloted fermentation can help saving glucosinolates in fermented cabbage which are completely lost during conventional fermentation of sauerkraut.

2.4.2. Suitability of cabbage as a carrier of probiotics

The probiotic strain *L. paracasei* LMG-P22043 has been successfully used to pilot the fermentation of blanched white cabbage leading to a final product containing about 8 log CFU/g of product. During fermentation, the probiotic strain colonized the vegetable surface and dominated the spontaneous lactic fermentation thus fast decreasing the pH of brines to a safe pH value. Furthermore, the strain grew without nutrient supplementation and efficiently acidified the product even when a low inoculum load (about 4 log CFU/g), 1000 times lower than that generally used in industrial vegetable fermentation, was applied. Therefore, also from a technological point of view, the fermentation process settled up by Sarvan et al. (2013), using the probiotic *L. paracasei* LMG-P22043, represents a convenient procedure to limit the enzymatic breakdown of glucosinolates determined by the traditional processing in which cell tissues are destroyed and the endogenous enzyme myrosinase comes in contact with glucosinolates. A mild cooking processing (blanching) can inactivate myrosinase allowing the ingested glucosinolates to reach human gut flora, where the deriving bioactive breakdown molecules can be absorbed by the colon (Sarvan et al., 2013). In fact, the glucosinolate content in blanched cabbage was reduced only by 35% after the probiotic fermentation, while glucosinolates are usually completely destroyed in traditional sauerkraut. This is a further example of a vegetable matrix that can be combined with a strain playing the dual role of starter and probiotic culture thus obtaining the control of the fermentation processes and the realization of final products with functional appeal for the presence of glucosinolates and probiotic populations (Sarvan et al., 2013). Furthermore, it is interesting to note that the fermentation process piloted by the probiotic strain conferred a good firmness and crunchiness to the product as well as an overall more natural lighter color than the non-inoculated cabbage. The fast decreasing of pH of started brines and the colonization of the vegetable surface by the probiotic strain ensured a microbiological stabilization of the product. On the contrary, the pH of the non-started control remained quite unvaried during storage (no LAB were found during the entire experiment) and, as a consequence, a complete alteration finally occurred probably due to the growth of spore-forming bacteria which survived the blanching step (Sarvan et al., 2013).

Another interesting result was obtained by Beganović et al. (2011). They applied the probiotic strain *L. plantarum* L4 and strain *Leuconostoc mesenteroides* LMG 7954 for a fast controlled fermentation of cabbage heads, thus obtaining a final product with more than 10^6 CFU of probiotics/g and a reduced NaCl concentration (2.5% w/v). Therefore, their process combined economic advantages with improved health-promoting properties of the final product.

2.5. *Other plant matrices*

Concerning other solid plant matrices tested for developing probiotic foods, it can be supposed that also in the case of the probiotic foods developed with dried yacon root (*Smallanthus sonchifolius*), an important role in sustaining the probiotic *L. casei* LC-1 can be played by inulin and the fructooligosaccharides, and by the matrix texture. Cells adhered to natural cavities of the surface characterized by the irregularities normally occurring in dried vegetables. Both matrix structure and composition played a role in the cell survival during the simulated GI digestion, indicating that probiotic cells carried by dried yacon would be able to survive the human digestive process and colonize the intestine, providing benefits to the host (De Souza Leone et al., 2017). Other local crops may offer matrices eligible for developing vegetable functional products with a favorable impact in regional economies. Purees from Churqueña potato, a variety of Andean tuber, have been fermented with a probiotic *Lactobacillus brevis* to obtain a “Potato Cheese” with a firm texture. More than 8 log CFU/g of the strain survived 4 weeks of storage. A good survival rate of the strain was also registered after GI digestion of this probiotic food (Mosso, Lobo, & Samman, 2016).

Moreover, fresh-cut fruits and vegetables have been considered and studied as interesting alternative vehicles for probiotics (Dávila-Aviña, Ríos-López, Aguayo-Acosta, & Solís-Soto, 2020) (Table 1). Also these minimally processed products can exert both a protective/nutritional activity on probiotics and a beneficial effect on consumers due to their health-promoting components (vitamins, minerals, antioxidant compounds and fibers). An example of these products is the fresh-cut cantaloupe inoculated with *L. plantarum* B2 and *Lactobacillus fermentum* PBCC11.5 (Russo et al., 2015). Both the probiotic strains showed high viability during storage at the same time increasing the nutritional value of the product by *in situ* fortification of the riboflavin content. Moreover, the LAB strains were suitable to enhance the safety of the minimally processed melon (Russo et al., 2015). Carrot has also been demonstrated as a suitable vegetable matrix for carrying probiotics. An edible coating based on sodium alginate with a probiotic was applied to minimally processed carrot slices (Shigematsu et al., 2018). After 19 days of storage at 8 °C, the viability of *Lactobacillus acidophilus* La-14 was 7 log CFU/g. Moreover, the probiotic coating improved the quality of the minimally processed carrots by reducing their metabolism, conserving their moisture and minimizing color changes during storage. Barbu et al. (2020) obtained beetroot products enriched with *L. plantarum* BL3. A ready-to-eat single dosage (100 g of fresh or dried chips) ensured a daily intake of 10^8–10^9 probiotic cells; moreover, the functional products showed a high antioxidant activity and an increased content of betalains and polyphenols.

3. Health-promoting effects of probiotics associated to plant-based matrices.

Health-promoting effects of probiotics are the results of complex mechanisms, sometimes overlapping, which are generally strictly strain-specific even if some of them can be commonly shared among different strains belonging to the same taxonomic group such as the genus *Lactobacillus* (Sanders, Benson, Lebeer, Merenstein, & Klaenhammer, 2018a). The potential beneficial role of this genus in the intestine as well as of the treatment with probiotics has been also evaluated in a recent review by Heeney, Gareau and Marco (2018). It should be considered that probiotics are not chemical compounds but biological organisms whose beneficial effects may be due to diverse activities also of interaction with the human host and its gut microbiota. Therefore, the benefit for health can be an indirect result of more related biological activities and the mechanism responsible for an observed clinical result may be difficult to establish exactly. A number of potential mechanisms of action of probiotics have been identified even if they have been demonstrated only *in vitro* or using animal models (Sanders, Merenstein, Merrifield, & Hutkins, 2018b). These mechanisms include: i) enhancement of epithelial barrier function, ii) modulation of gut microbiota, iii) competitive exclusion and inhibition of pathogens, iv) production of SCFA (considered a typical common mechanism shared among strains), v) production of other small compounds with also a systemic activity and (vi) modulation of the immune system with strain-specific diverse activities (Borchers, Selmi, Meyers, Keen, & Gershwin, 2009; Lebeer, Vanderleyden, & De Keersmaecker, 2008). The reinforcement of the gut barrier can be considered a frequent mechanism shared at the species level among strains (Sanders, Merenstein, Merrifield, & Hutkins, 2018b). In fact, while gut epithelial cells, interconnected by tight junction proteins, constitute the main gastro-intestinal barrier protecting the host from harmful microorganisms and toxicants, impairment of the gut barrier increases intestinal permeability causing diseases of the gastrointestinal tract and dysfunction of other organs including the central nervous system (Parker, Fonseca, & Carding, 2020). In particular, certain microbial species composing the gut microflora may be mainly responsible for impairing gut permeability causing organ dysfunctions. For example, the overgrowth of *Clostridium* species or other potential pathogens may be associated to microbial dysbiosis, which determines in turn, an increased intestinal permeability (“leaky gut syndrome”), leading to inflammatory bowel diseases, immuno-related intestinal disorders and dysfunction of other organ systems including the brain (microbiota-gut-brain communication) (Parker, Fonseca, & Carding, 2020; Tsai et al., 2019). Moreover, recent scientific advances clearly indicate that several gut microbes (mainly belonging to the dominant phyla Gram-negative *Bacteroidetes*) are source of neurotoxic and pro-inflammatory biomolecules such as lipopolysaccharide (LPS), enterotoxins, microbial-derived amyloids and small non-coding RNA (Parker, Fonseca, & Carding, 2020; Lukiw, 2020). In this context, probiotics may play a proved role in gut microbiota modulation favoring implantation of beneficial microbial populations and preventing alteration of permeability of the GI barrier. In fact, modulation of the gut microbiota represents a potential tool for health promotion and restoration and it can be obtained by tailoring nutrient availability towards specific beneficial bacteria, by using prebiotics (Cantu-Jungles & Hamaker, 2020), or by supplementing the resident population with selected probiotic strains, such as *Lactobacillus* and *Bifidobacterium* strains, which are known to contribute to the healthy status of the gut bestowing health benefits to the host. In both strategies, which can be also successfully combined, an undisputed role is played by the produced SCFA, the main products of bacterial fermentation that may regulate the enteric neuron functions influencing gastrointestinal motility, and by metabolites crossing through the epithelial barrier that may be implied with several functions (Tsai et al., 2019; Parker, Fonseca, & Carding, 2020). Dietary and bacterial metabolites are therefore crucial for immune tolerance maintenance and in counteracting inflammatory reaction in the gut. Filosa, Di Meo and Crispi (2018) reported that the onset and progression of age-related disorders is prevented by modulating the gut microbiota which regulates the enteric neuroimmune system. Thus, beneficial intervention on the microbiota could contribute to prevent neurodegeneration through the production of bacterial metabolites. As examples, SCFA can exert a neuroactive action, GABA can modulate brain chemistry, while microbial transformation of tryptophan in the neurotransmitter serotonin is essential in maintaining mood and cognitive behavior.

The modulation of the immune system can also be considered as one of the most important mechanisms responsible for the beneficial effects of probiotic bacteria on human health, even if distinct immunomodulatory properties can be ascribed to strains belonging to a species. In particular, this is the case of the probiotic strain *L. paracasei* IMPC2.1 (suitable in combination with plant-based matrices), when compared to other strains of the same species (D'Arienzo et al., 2011). Although all those strains stimulated phenotypic maturation of mice dendritic cells, they acted differently on cytokine secretion, and the evaluation of the different types and ⁄or levels of cytokines allowed to evaluate the pro- or anti-inflammatory properties of each strain (D'Arienzo et al., 2011). Moreover, the culture filtrates obtained from different *L. paracasei* strains also showed diverse immuno-modulatory abilities, which were also modified by the addition of artichoke phenolic compounds to the growth medium (Sisto et al., 2016). In particular, a remarkable anti-inflammatory activity was exerted by the culture filtrate of the probiotic strain IMPC2.1 grown in the presence of artichoke phenolic compounds, indicating that the use of a plant-based food as a carrier for a probiotic strain or its association with plant health-promoting components can be considered as a potential tool to join the anti-inflammatory activity of a probiotic strain with the antioxidant and the other health-promoting properties of plant bioactive compounds (Sisto et al., 2016).

To claim the probiotic feature of a product, a demonstration of *in vivo* efficacy needs to be also given, providing evidence on the ability of the strain, carried by the investigated matrix, to transiently colonize the GI tract of human subjects and determine beneficial effects. Moreover, the influence and the relevance of the matrix on the efficacy of a probiotic food (also plant-based) should be considered, even if providing their *in vivo* demonstration is undoubtedly a real challenge. In this regard, the available data are mainly referred to milk or milk-based products but they are quite difficult to be compared due to the different size and design of the studies (Saxelin et al., 2010). As outlined by Sanders et al. (2014), few studies have been conducted to compare the same probiotic strain delivered in different matrices to demonstrate the influence of the matrix on the health-promoting features of the strain. On the contrary, evidence has been reported on the contributions of the components of food matrices to the potential (strain-related) health benefits of a probiotic product (Flach, van der Waal, van den Nieuwboer, Claassen, & Larsen, 2018).

Concerning the studies of probiotics combined to plant-based matrices, there is scientific evidence that the modulation of the gut microbiota is a mechanism by which the probiotic/plant matrix combinations may provide health benefits. In fact, Valerio et al. (2011) observed that the consumption of artichokes enriched with the probiotic strain *L. paracasei* IMPC2.1 determined a general increase in presumptive lactobacilli and bifidobacteria, a general reduction in *Enterobacteriaceae* and a significant decrease in counts of *Clostridium* spp. and *E. coli*. Furthermore, Riezzo et al. (2012) evaluated the effects of the probiotic-enriched artichokes (daily dose of 2×10^{10} CFU) integrated in the usual diet on treatment preference, symptom profile, and SCFA production in constipated subjects when compared with control ready-to eat artichokes during a randomized double blind crossover human study (Fig. 2). The administration of probiotic-enriched artichokes determined a significant increase of propionic acid fecal concentration and was sufficient to permit gut colonization by the probiotic strain *L. paracasei* IMPC2.1 and a satisfactory relief of symptoms was observed. Moreover, the relief of symptoms resulted significantly higher during the period in which the probiotic-enriched artichokes were administered in comparison to the administration of the artichoke control.

Likewise ready-to-eat artichokes, table olives were validated as a probiotic carrier for transporting *L. paracasei* IMPC2.1 cells into the human GI tract during a 10-days human trial in which volunteers fed about 10–15 olives per day carrying more than one billion viable cells (Lavermicocca et al., 2005). The LAB population of the human gut was enriched when probiotic table olives were introduced in the daily diet demonstrating that the temporary gut colonization by the probiotic alters the composition of the gut microflora that switched towards beneficial populations (Lavermicocca, Rossi, Russo, & Srirajaskanthan, 2010).

Moreover, the fortification of "functional matrices" (i.e. vegetable ones) with probiotics, poses new research challenges to understand the possible modifications that bioactive components undergo during processing due to the probiotic metabolic activities. The influence of the fermentation process started with the probiotic *L. paracasei* IMPC2.1 on the fate of health-promoting polyphenols in artichoke was compared with not started fermented artichokes (Garbetta et al., 2018). In this case, even if an isomerization process for some compounds was observed (probably due to the different pH values of the two processes), the polyphenol compositions resulted unvaried but total polyphenol bioaccessibility of started artichoke was lower than that of not started artichoke after an *in vitro* simulated digestion in small intestine. Actually, both isomerization and bioaccessibility influenced the antioxidant activity which was significantly lower for started artichoke, indicating a possible bioprotective action exerted by the probiotic strain on the food matrix that limited the release of polyphenols in the digestion liquids of small intestine, making them available for further metabolism and absorption in the colon (Garbetta et al., 2018). It should be also considered that, if on one hand food bioactive compounds modulate the gut microbiota composition and consequently its effects on gut functionality, on the other hand gut microbiota by transforming the bioactive compounds, particular polyphenol types, strongly influences their bioavailability and biological activities (Laparra & Sanz, 2010).

4. Conclusions

Even if additional human clinical trials are required to demonstrate, in each single case, the efficacy *in vivo* of the combination between a probiotic strain and a plant-based matrix, the data thus far available indicate that the intrinsic health-promoting properties of diverse plant-based matrices can be successfully exploited and improved developing effective association with probiotics. In this case, the beneficial activity and the efficacy of the probiotic could be in turn also improved and modulated by components of the plant-based carrier obtaining a final functional product in which the health-promoting properties of the matrix are added, even synergistically, to the beneficial effect of the probiotic strain. Actually, a number of specific aspects should be considered in selecting the appropriate matrix suitable for a successful combination with a probiotic strain to provide health benefits. It fact, the efficacy a probiotic/plant-carrier combination depends on many factors among which the composition of the food matrix must be primarily taken into account for sustaining and protecting probiotic cells during product processing, digestive process and gut implantation, also taking into consideration that survival and stability of a probiotic in a food carrier are highly strain-dependent. In this regard, preference should be for those matrices containing molecules with already recognized health-promoting properties (such as phenolic compounds) and prebiotic activity (such as inulin) also able to positively modulate the gut microbiota. Moreover, the matrices traditionally used for the production of naturally fermented food have a higher possibility to be accepted by the consumers as well as to support the growth of microbial populations, which could include probiotic strains, or the growth of probiotic strains of human origin, provided that they are able to work as starter cultures dominating the natural microbiota. In general, a probiotic strain isolated from the fermenting natural microbiota could be more adapted to the processing conditions, while a strain of human origin will show a higher resistance to the GI environment. Moreover, a probiotic strain with fermentation ability similar to those of microorganisms composing the natural fermenting microbiota probably will not markedly modify the taste of the original product. In fact, in studying a new combination of a probiotic/plant-based matrix, a further relevant aspect is the sensory appeal of the final product, that is strictly related to the consumer acceptance, also taking into account that the metabolic activity of the probiotic strain could improve but also worsen the sensory features of the product. It is also noteworthy that vegetable processed foods (in particular the traditionally fermented ones), in general, show an original acidic taste that will be probably only slightly modified by the probiotic strain. Consequently, the innovative probiotic product will be more likely appreciated by the market as its taste will be similar to that of the original product. In this regard, a paradigmatic example of a successful combination probiotic/vegetable is represented by the above-mentioned *L. paracasei*-enriched table olives in which the probiotic strain IMPC2.1 is also suitable as a starter culture for the industrial processing of the product. An effective combination probiotic/vegetable is also represented by the *L. paracasei*-enriched artichokes, administered to help patients with functional constipation (Riezzo et al., 2012) (Fig. 2; Table 2). In fact, the dietary fibers composing the vegetable matrix, particularly inulin functioning as a prebiotic, can be metabolized by the carried probiotic populations facilitating their implantation, also modulating in this way composition and activity of the gut microbiota. As a matter of fact, a modification of SCFA pattern, with an increase in propionic acid was observed. Moreover, the other characteristics of the vegetable matrix also potentially contributed to alleviate symptom of constipation. As a demonstration of the efficacy of probiotic artichokes in relieving symptoms, the randomized controlled trials by Riezzo et al. (2012) has been evaluated as unique example of plant-based probiotic food in the systematic review and *meta*-analysis aimed at investigating the effect of probiotics on functional constipation in adults (Dimidi, Christodoulides, Fragkos, Scott, & Whelan, 2014). No adverse events occurred in either the probiotic or the placebo group and > 95% compliance was reported with the probiotic- containing artichokes, confirming that the probiotic addition did not affect the sensorial quality of the product. Therefore, even if the demonstration of *in vivo* efficacy of a probiotic/food combination still represent a real challenge, current research could provide scientific evidence on the advantages in combining the complementary health-promoting features of probiotics with those of plant-based matrices to develop innovative functional foods. Fruit and vegetable matrices can be transformed in probiotic foods providing the everyday diet with valid alternatives to milk-based products. In fact, the possibility to utilize those matrices as carriers for probiotic strains represents a relevant opportunity to provide the consumers with diverse probiotic foods that can be part of a varied diet ensuring satisfying sensory characteristics as well as a regular intake of probiotic bacteria in appropriate amount to provide health benefits.

Ethics Statement

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

CNR project NUTR-AGE (FOE-2019, DSB.AD004.271).

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