

# Lactiplantibacillus (Lpb.) plantarum

Subjects: [Agriculture](#), [Dairy & Animal Science](#)

Contributor: Stavros Plessas

Lactic acid fermentation of fresh fruit juices is a low-cost and sustainable process, that aims to preserve and even enhance the organoleptic and nutritional features of the raw matrices and extend their shelf life. Selected Lactic Acid Bacteria (LAB) were evaluated in the fermentation of various fruit juices, leading in some cases to fruit beverages, with enhanced nutritional and sensorial characteristics. Among LAB, Lactiplantibacillus (Lpb.) plantarum subsp. plantarum strains are quite interesting, regarding their application in the fermentation of a broad range of plant-derived substrates, such as vegetables and fruit juices, since they have genome plasticity and high versatility and flexibility. *L. plantarum* exhibits a remarkable portfolio of enzymes that make it very important and multi-functional in fruit juice fermentations. Therefore, *L. plantarum* has the potential for the production of various bioactive compounds, which enhance the nutritional value and the shelf life of the final product. In addition, *L. plantarum* can positively modify the flavor of fruit juices, leading to higher content of desirable volatile compounds.

*L. plantarum*

probiotics

fruit juices

health benefits

enzymes

phenolics

## 1. Introduction

The focus of innovations in the food industry has turned to new functions of food, which are prevention from various lifestyle diseases, mainly through the development of dietary supplements, that can affect the intestinal microbial composition. In addition, the preference of many consumers to foods free of additives has also boosted the facilitation and promotion of various novel products by the food industry called functional foods. Functional foods are foods that can positively affect specific human body functions beyond adequate nutritional effects, leading to the delivery of various health benefits to humans <sup>[1]</sup>. Functional foods mainly include probiotics, prebiotics, and more recently symbiotics <sup>[2][3]</sup>. Dairy products such as cheese, sour milk, yogurt and others are considered as the main representatives of probiotic foods. However, other alternative food products are being developed as probiotic substrates in the last few years <sup>[4][5]</sup>. The main reason for this shift is that dairy products display some drawbacks, affecting consumers' attitudes, such as milk cholesterol content, lactose intolerance and dairy allergies <sup>[6][7]</sup>. Thus, alternative food substrates were proposed as ideal carriers or mediums for probiotics, in order to tackle these disadvantages, such as cereals, vegetables and fruits in fermented and unfermented forms <sup>[7]</sup>. Particularly, fruit juices are presumed as attractive good substrates for probiotic delivery. The advantages of fruit juices, as probiotic "vehicles" include: (i) high nutritional value, (ii) positive health effects, and (iii) wide acceptance and consumption by consumers worldwide <sup>[8][9][10]</sup>. Many fruit juices were inspected, explored and evaluated, regarding their suitability, as viable and shelf-stable probiotic carriers, through lactic acid fermentation. Nevertheless, fermentation is a complex process, which needs control and selection of appropriate conditions in order to achieve desirable food characteristics and preserve probiotic cell functionality and viability. Likewise, the

proper selection of the starter culture for the fermentation of fruit juices and possible modifications of fruit juices (before fermentation) are considered critical parameters. Lactic acid bacteria (LAB) are the most common microorganisms applied for the fermentation of fruit juice. Particularly, *Lactobacillus plantarum* or, as it has lately been denoted, *Lactiplantibacillus (Lpb.) plantarum* subsp. *plantarum* [11] is an interesting and well-studied strain in the fermentation of fruit juices.

## 2. Lactic Acid Fermentation of Fruit Juices

The preference of consumers to fruit juices, nectars and ready-to-drink juice drinks was boosted in the last decade worldwide [12]. This is mainly, due to the shift of consumers' preference to more natural foods containing less or no chemical preservatives and the better awareness of consumers, regarding the nutritional values of foods [13]. In particular, fruit juices contain appreciable amounts of dietary fibers, antioxidants, polyphenols, minerals and vitamins and meet the consumer's claims for healthy, tasty, and practical foods.

On the other hand, fresh fruit juices are susceptible to spoilage by different microorganisms [14]. The shelf life of fresh fruit juices is very short and varies between 5 to 7 days at 4 °C [15]. Therefore, the preservation of fruit juices should be controlled by the addition of various synthetic preservatives, such as potassium sorbate and sodium benzoate [16]. Nevertheless, fresh juices extracted from fruits are commercialized as refrigerated products, without preservatives and with a very short shelf-life [17]. However, currently, consumers prefer fruit juices free of chemical additives and safe for consumption. Lactic acid fermentation of fruit juices seems a good alternative and could satisfy consumers' demands and preferences. Several studies were reported in the literature, verifying a clear positive impact in the extension of self-life of fruit juices, through fermentation by LAB. This impact depends on the kind of fruit juice and its chemical composition, the strain applied and the conditions of fermentation and storage (time, temperature, etc.). Lactic acid fermentation of fruit juices can keep or ameliorate: (i) the self-life [18], (ii) the nutritional, and (iii) the sensorial properties of the final product [19][20]. It is also considered as a mild processing method for preservation, which meets the standards regarding the consumption of fresh-like minimal processed beverages [21]. The most common microbiocidal group applied for lactic acid fermentation of fruit juices is LAB. Several studies were conducted and published in the literature and some examples are presented in **Table 1**, with the respective advantages.

**Table 1.** Examples of fermented fruit juices with various LAB strains (including *L. plantarum*) in single or mixed cultures with the respective effects.

Fruit Juices	Strains	Main Positive Effects	References
Mulberry juice ( <i>Morus nigra</i> )	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. paracasei</i>	Increase in total anthocyanin, phenolic, antioxidant activity.	[22]
Pomegranate juice ( <i>Punica granatum</i> L.)	<i>L. plantarum</i>	Increase in antimicrobial activity. Volatile free fatty acids content increased. Better	[23]

Fruit Juices	Strains	Main Positive Effects	References
		organoleptic properties and composition of volatile compounds.	
Pomegranate juice ( <i>Punica granatum</i> L.)	<i>L. plantarum</i>	Improved sensorial characteristics. Improved TPC and antioxidant activity.	[24]
Pomegranate juice ( <i>Punica granatum</i> L.)	<i>L. paracasei</i>	Improved sensorial characteristics. Improved TPC and antioxidant activity.	[13][25]
Cornelian cherry ( <i>Cornus mas</i> L.)	<i>L. paracasei</i>	Improved TPC and antioxidant activity.	[26]
Cashew apple juice ( <i>Anacardium occidentale</i> L.)	<i>B. bifidum</i> , <i>B. longum</i> subsp. <i>infantis</i> , <i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. mesenteroides</i> , <i>L. johnsonii</i>	Improved antioxidant activity. Modification of the type and content of phenolic. Possible prebiotic action of phenolics to lactic acid bacteria. Contained prebiotic oligosaccharides ( <i>mesenteroides</i> ) enhanced the growth of <i>L. johnsonii</i> .	[27]
Cantaloupe melon ( <i>Cucumis melo</i> L.) and cashew apple juice ( <i>Anacardium occidentale</i> L.)	<i>L. casei</i>	Emergence of new volatile compounds.	[28]
Apple juice	<i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>L. casei</i> , <i>L. plantarum</i>	Generation of new aromatic compounds.	[29]
Apple juice	<i>L. plantarum</i>	Enhanced antioxidant capacity and bioavailability of polyphenols.	[30]
Elderberry juice ( <i>Sambucus nigra</i> L.)	<i>L. plantarum</i>	Improved composition of volatile compounds.	[20]
Jujube juice ( <i>Ziziphus jujuba</i> Mill.)	<i>L. plantarum</i>	Enhanced sensorial features.	[31]
Noni juice ( <i>Morinda citrifolia</i> )	<i>L. casei</i> , <i>L. plantarum</i> , <i>B. longum</i> , <i>L. acidophilus</i>	Slight increase in total antioxidant activity. ACE inhibitory potential decreased during fermentation. The content of ascorbic acid and antioxidant activity remained stable.	[32]
Cranberry juice ( <i>Vaccinium macrocarpon</i> )	<i>L. paracasei</i>	Synergistic and additive antibacterial effects of the combination of fermented cranberry juice and antibiotics.	[33]

Fruit Juices	Strains	Main Positive Effects	References
<i>Phyllanthus emblica</i> fruit juice	<i>L. paracasei</i>	Increased Total polyphenol content and antioxidant activity.	[34]
Jujube juice ( <i>Ziziphus jujuba</i> Mill.)	<i>L. rhamnosus</i> , <i>L. plantarum</i> , <i>L. paracasei</i> , <i>L. mesenteroides</i> , <i>L. plantarum</i>	Increased flavonoid content.	[35]
Cactus pear juice ( <i>Opuntia fcus-indica</i> )	<i>L. plantarum</i>	Ameliorated insulin resistance.	[36]

fermented juices, can be affected by exposure to certain types of stress, such as acid and cold. Specifically, some fruit juices are very acidic, such as cranberry (pH 2.7), pomegranate (pH 3.0-3.5), lemon and lime juices (pH 2.8) [39], exhibiting severe impact on the viability of LAB, [40] during the production process and storage [41]. Especially, in the case of probiotic LAB strains, survival in harsh conditions is an essential prerequisite for probiotic delivery [42]. These obstacles could be tackled, with mainly 5 ways: (i) pre-adaptation or adaptive evolution, (ii) encapsulation, (iii) physical treatments (iv) mixing with a second juice and (v) proper selection of a probiotic LAB.

The most commonly way for stress adaptation is the modification of the growth medium and/or incubation conditions. Pre-adaptation or adaptive evolution [43] involves the treatment of a microorganism to a sublethal stress (pH, cold, osmotic press etc,) for a limited time; this treatment would act on strain resistance, when exposed to a higher level of stress or to another stress. This method has been applied in lactic acid fermentation of fruit juices by probiotics, with very promising results [44][45].

Microencapsulation is considered as a promising method for the improvement of probiotics' viability in functional beverages. Microencapsulation of probiotics leads to high preservation of the probiotic load and strengthen cells, versus various physicochemical changes, such as pH, temperature, bile salts, etc. [46][47][48]. Another target of probiotic microencapsulation, is the improvement of the resistance of the probiotic cells in the gastro-intestinal tract, besides enhancing the viability of the bacterial strains in the food products. Various methods have been proposed for probiotic cell microencapsulation, such as emulsification, spay freeze drying and extrusion, with numerous encapsulating agents [49]. The most commonly employed encapsulating agents are natural biopolymer, such as alginate and κ-carrageenan, as well as prebiotics, such as resistant starch, inulin, fructooligosaccharide and fiber [50]. Application of prebiotics as encapsulating agents seems more attractive, because it is a cost-effective technology for the industrial scale and offers encouraging results [47][49]. The functionality of LAB could be significantly ameliorated employing physical treatments. The most common applied technology is ultrasound (US). Probiotic *L. casei* NRRL B442 manage to survive for at least 21 days at 4 °C in a sonicated pineapple juice [51]. In another report, *L. reuteri*, *L. plantarum*, *L. casei*, bifidobacteria, and propionibacteria were treated with US, before the inoculation in an organic rice beverage and maintained the pH and sensory scores for at least 7 days [52][53]. Addition of a second juice (fresh or fermented) to the main one is a quite interesting perspective. The main reason for this treatment, is the slight increase of the low pH value of the main juice, in order the survival of the probiotic

strain to be ameliorated. Likewise, carrot juice has been proposed for this purpose, since it has approximately a pH value of 6 [24]. Furthermore, addition of 5% acerola juice to orange juice, prevented the production of carbon dioxide gas for three weeks and has no impact on the probiotic content during four weeks of storage at 8 °C [54].

### 3. Main Advantages of *L. plantarum* Application in Food Fermentations

*L. plantarum* is a safe microorganism (Generally Regard as Safe — GRAS) and has been widely used in food-fermentation technologies [55][56]. It has also been employed in probiotic food production, such as *L. plantarum* 299v strain, which is widely marketed [57]. It is a facultative heterofermentative LAB, that can tolerate the combination of high acidity and ethanol concentration and survive under conditions, that are usually fatal to LAB [58]. The adaptability of *L. plantarum* to a fermentation process and its metabolic flexibility and versatility are some of the critical attributes, that makes it unique among the other LAB [59]. *L. plantarum* has been isolated through numerous food sources, such as cereals, meats, dairy products, vegetables, fruits and drinks [60][61][62][63][64], as well as human and mammals niches [65]. *L. plantarum* can adapt to various niches, probably due to its genome size (average 3.3 Mb), which is one of the largest detected within *Lactobacillus* genus [66].

In addition, *L. plantarum* can be involved in several biochemical reactions, usually ended in desirable metabolites, due to its specific enzymatic composition. *L. plantarum* contains a variety of extracellular enzymes, that contribute to the secretion and modification of proteins and to the modification and degradation of extracellular compounds, allowing for the use of such molecules as a source of nutrients [67][68]. Specifically, *L. plantarum* possesses enzymes, such as tannase,  $\beta$ -glucosidase,  $\alpha$ -glucosidase and  $\beta$ -galactosidase *p*-coumaric acid decarboxylase and general decarboxylase, that catalyze the production of high added-value compounds, such as phenolic compounds leading to the production of compounds, that influence positively food aroma and increase the antioxidant activity [69]. The production of aryl  $\beta$ -glucosidases, by *L. plantarum* initiates an increase in the functionally (antioxidant activity and bioavailability) of glycosylated phenolic compounds. Besides, employment of *L. plantarum* to various plant-based products, such as fruit juices, with high content of tannins, attenuated the phenolic astringency, which is responsible of the unpalatability of many fruit juices [70].

Recently, probiotic strains of *L. plantarum* has been successfully applied in medical fields, with encouraging outcome. Specifically, the efficacy of *L. plantarum* strains in the cure or treatment of gastrointestinal disorders, cholesterol lowering and reduction in the irritable bowel syndrome (IBS) symptoms has been highlighted in human trials [71].

Several strains of *L. plantarum* have been exhibited antimicrobial and antagonistic activity, against some adverse microorganisms, antifungal activities and antiviral effects [72]. In addition, it should be highlighted the wide range of bacteriocins and exopolysaccharides (EPS), that *L. plantarum* is able to produce [73]. Bacteriocins show a broad antimicrobial activity spectrum against Gram-positive and Gram-negative bacteria, while EPS provide potential health-promoting properties in the advances of functional foods [74].

## Application of *L. plantarum* Strains in Various Fruit Juices Fermentations

A great variety of fruit juices has been successfully fermented by *L. plantarum* strains leading to final products with potentially functional properties. Most of these reported positive effects are presented in **Table 1** and are recapitulated to: (i) enhanced antioxidant activity, (ii) increased total phenolic and total anthocyanin content, (iii) extension of the shelf-life of fruit juices and (iv) better sensorial features. It has been reported, that *L. plantarum* ATCC14917 modified the phenolic composition of apple juice after fermentation and enhanced its overall antioxidant capacity, as well as the bioavailability of polyphenols of apples [30]. The absorption of food phenolics in humans, is necessary for the exhibition of their beneficial effects. It is mainly evaluated by their chemical structure, which depends on factors such as the degree of glycosylation and conjugation with other phenolics [75].

On the other hand, food industry is seeking ways to produce novel products with increased nutritional value. Moreover, there are many reports, that application of a probiotic bacteria in the fermentation of fruit juices may lead to a final product, with functional properties and specific health benefits [76][77][78].

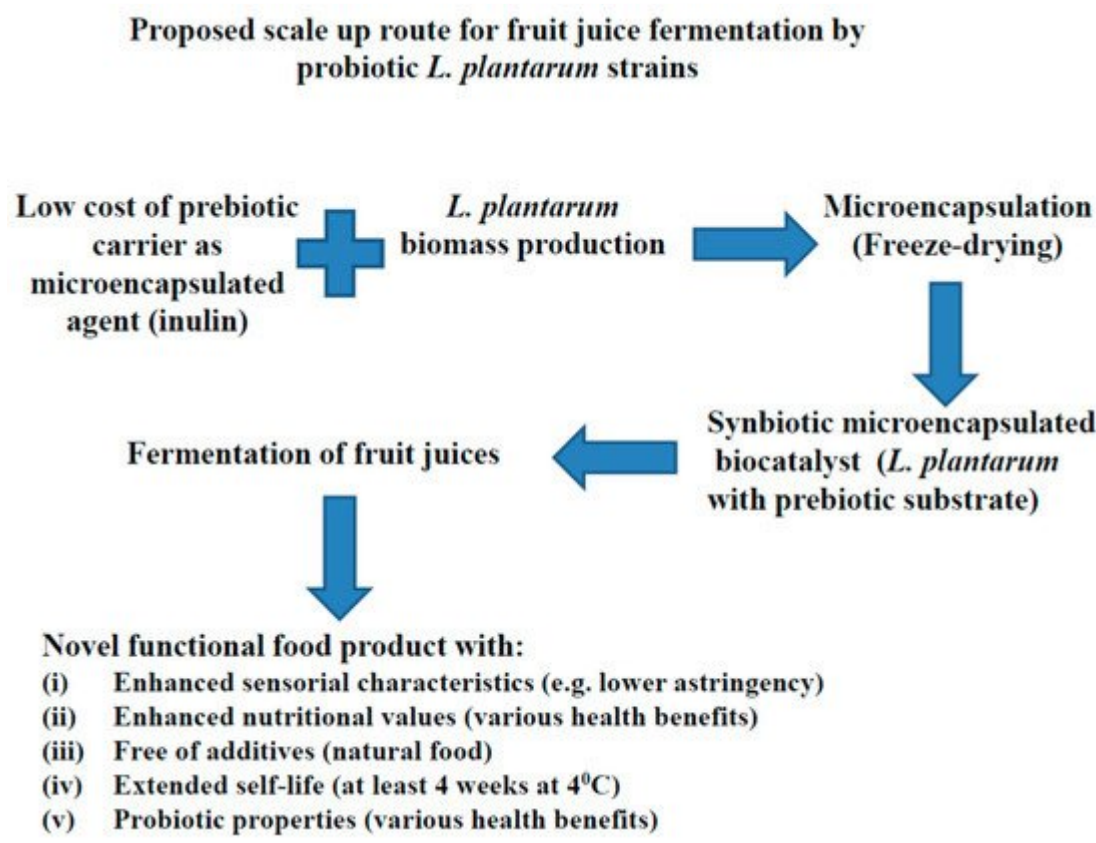
Even though, *L. plantarum* can grow generally at temperatures between 15–30 °C and at pH values near to 4 [79], there are specific probiotic strains of *L. plantarum* with respectable tolerance at low pH values (approximately 3.2) and at low temperatures in the matrix of fruit juices (4–8 °C) [80]. For instance, viability of probiotic *L. plantarum* NCIMB 8826 decreased during cold storage (4 °C) of cranberry, pomegranate and lemon & lime juices with initial pH values approximately 3. However, only in the case of lemon & lime juice cells were viable until the 35<sup>th</sup> day [39]. This could be explained by: (i) the high levels of phenolic compounds in cranberry juice [39] and pomegranate juice [81], which are known to have strong antimicrobial properties [82] and (ii) the fact that cells were pre-adapted to citric acid, which is the main antimicrobial compound in the lemon & lime juice, leading to higher viabilities to this juice compared to the others [39]. In another recent study, cell viability of probiotic *L. plantarum* ATCC14917 was remained at high levels throughout the 21 days of cold storage (4 °C) of fermented pomegranate juice (initially 11.43 log cfu/mL) and decreased to 8.83 log cfu/mL at the 4<sup>th</sup> week of storage, above the limit for the exhibition of probiotic properties. The same observations were made, during cold storage (4 °C) of fermented Cornelian cherry [83] and pomegranate juice [24], with *L. plantarum* ATCC 14917 and fermented sweet lemon juice (*Citrus limetta*) with *L. plantarum* LS5 [84].

Furthermore, *L. plantarum* has a positive effect on the flavor of fruit juices, leading to higher content of desirable volatile compounds during fermentation. Lactic acid fermentation of jujube juice by *L. plantarum* significantly enhanced the composition and production of desirable volatile compounds, leading to aroma complexity and better sensorial characteristics [31]. *L. plantarum* 285 exhibited interesting features, in terms of total aromatic potential, as well as in the type of volatiles compounds produced through lactic acid fermentation of elderberry juice [20]. Fermentation of blueberry juices with *L. plantarum* enhanced the acceptability of the final product [85].

Besides, Food Industry has selected probiotic *L. plantarum* strains in single or mixed culture, in order to produce a variety of probiotic fruit beverages, through fermentation (**Table 2**). The commercialization of the whole fermentation system, which includes *L. plantarum* verifies the eligibility of this important LAB. A proposed route for



the scale up of the whole procedure could be feasible and it is presented in **Figure 1**. However, research is continuing and it is possible, that through in vivo tests and human trials, more interesting outcomes may be recorded. The main reason for this assumption is the biological activities, that this microorganism has exhibited so far.



**Figure 1.** Proposed scale-up route for fruit juice fermentation by probiotic *L. plantarum* strains.

**Table 2.** Global commercial probiotic products based on fruit matrices fermented by probiotic *L. plantarum*. Source: [\[86\]](#).

Food Matrix	Commercial Name	Origin	Active Probiotic Culture
Fruit drink	Probi-Bravo Friscus	Sweden	<i>L. plantarum</i> , <i>L. paracasei</i>
Fruit drink	Danone-ProViva	Sweden, Finland	<i>L. plantarum</i>
Raw organic fruits and vegetable blend	Garden of Life RAW Organic Kids Probiotic	Florida, USA	<i>L. gasseri</i> , <i>L. plantarum</i> , <i>L. casei</i> , <i>L. acidophilus</i>
Fruit juice drink	GoodBelly	Colorado, USA	<i>Lb. plantarum</i> 299v
Fruit- and vegetable-based	KeVita active probiotic drink	Oxnard, USA	<i>B. coagulans</i> , <i>L. rhamnosus</i> , <i>L. plantarum</i>

Food Matrix	Commercial Name	Origin	Active Probiotic Culture
Fruit juice	Healthy Life Probiotic juice	Australia	<i>L. plantarum</i> , <i>L. casei</i>

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