

Review

# Assessment of Pomegranate Juice as an Alternative “Substrate” for Probiotic Delivery. Recent Advances and Prospects

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**Abstract:** The probiotic products in the market are mostly milk-based products, such as yoghurts, cheese and fermented milk. However, lately, there has been an increasing demand for non-dairy probiotic products due to various reasons such as allergies, lactose intolerance, high cholesterol content and consumers turning to more natural foods. Fruit juices are considered as an appropriate new substrate for probiotic delivery. From these, pomegranate (*Punica granatum L.*) is gaining more attention in recent years. Pomegranate is a fruit known since ancient times for its therapeutic qualities, such as antioxidant, anti-inflammatory, antibacterial, antiviral and antitumor properties, among others. Pomegranate juice contains a range of bioactive compounds such as phytochemicals, like polyphenols, ellagitannins, anthocyanins and punicalagins. The fermentation of the juice with probiotic strains seems to provide beverages of high nutritional values and accepted organoleptic quality. Therefore, the aim of this comprehensive review is to present an overview of the innovative in vitro and in vivo assays that have taken place regarding fermentation of pomegranate juice by probiotic bacteria. In addition, various drawbacks are underlined and solutions are proposed and discussed regarding the feasibility of pomegranate juice as an alternative substrate for probiotic delivery.

**Keywords:** probiotic; functional foods; pomegranate juice

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## 1. Introduction

Increased consumer concern about safer and more beneficial food for humans has boosted the demand for the production of functional foods and beverages. Functional foods are food products, part of the normal diet, which may provide beneficial health effects or possible reduction of risk of disease to consumers [1].

More specifically, functional foods are mainly categorized as: i) beverages with natural bioactive compounds (e.g., dietary fibre), ii) beverages with extra added bioactive compounds (e.g., antioxidants) and iii) introduced nutrient ingredients to conventional beverages (e.g., prebiotics). Functional foods and beverages of all kinds should not be considered as medicines and should be consumed only as part of a normal daily diet [1]. Likewise, functional beverages are considered as a fast-growing category of functional foods that includes juices enriched with herbs, vitamins, aminoacids or/and vegetables. Their health benefits are numerous and namely include anti-inflammatory, anti-hypertensive [2], antitumor [3], antibacterial, antiviral and antioxidant activities [4].

The objective of this comprehensive review is to gain insights into the in vivo and in vitro trials that have taken place on the application of pomegranate juice in lactic acid fermentation through probiotic bacteria up until now, and discuss respective advantages and disadvantages.

## 2. Probiotics, Prebiotics and Synbiotics

Probiotics are defined as live single or mixed microbial cultures able to improve the composition of gut microbiota to defined strains with immunomodulatory potential [1]. Various probiotic strains have been categorized, mostly belonging to *Leuconostoc spp.*, *Lactobacillus spp.*, *Pediococcus spp.*, *Weissella spp.*, *Bifidobacterium spp.* and *Lactococcus spp.* Many of them are part of fermented dairy and non-dairy products, like yogurt, kefir and cheese [5].

Prebiotics are nondigestible or low-digestible food ingredients in that they stimulate the growth of some probiotic bacteria in the colon [6,7]. Prebiotics are considered a range of di-, oligo- and polysaccharides, some resistant starches and sugar polyols [8]. The main prebiotics included in the human diet are galacto-oligosaccharides, fructo-oligosaccharides, inulin, lactulose and malto-oligosaccharides [1]. Prebiotics have been applied to bakery products, breakfast cereals, cakes, beverages, dairy products, table spreads, butter-like products, cream cheeses, chocolate products, yogurts and fruit juices [9].

Nowadays, the combination of probiotics and prebiotics (synbiotics) is gaining space in the market, mostly because of their synergistic effects when applied to food products [9]. The main advantage of their development is to help probiotics to overcome possible survival difficulties in the host [7]. Several products of this category have been launched in markets with promising results, such as Raftiloses P95 in combination with *L. rhamnosus*, *Bifidobacterium spp.*, *L. acidophilus* and *L. casei*, and others [10,11].

## 3. Fruit Juices as Substrates for Probiotic Delivery

Dairy-fermented products are considered, nowadays, as optimum carriers of probiotic strains. However, in the last decade, several reasons, such as (i) lactose-intolerance, (ii) allergies after dairy products consumption and (iii) a general emerging food trend towards a vegan diet, have turned research to the production of innovative, alternative mediums for probiotic delivery [12,13]. Regarding this new trend, fruit and vegetables have started to gain the attention from the food industry and consumers. The contents of fruits and vegetables are rich in nutrient ingredients, vitamins and minerals. Fermented fruits and vegetables are mostly part of the Asian food tradition more than in Western food cultures. This fact can be explained by the variety of fermented products consumed in Asian countries such as tempeh, kimchi, gundruk, khalpi, sinki and sauerkraut [14].

Probiotic beverages have been produced with various fruit juices such as apples, pineapples, pears and carrots through lactic acid fermentation with probiotic bacteria as it can be observed in the literature [15–19].

Similarly, commercially probiotic juices are proposed by many manufactures lately, verifying the high interest and significance of these new novel products (Table 1). Various juices have been applied either in single or mixed from with other juices and many probiotic bacteria, mainly lactic acid bacteria (LAB) have been used in the respective lactic acid fermentation of the juices. In this aspect, pomegranate juice also poses a high place in this new innovative trend (Table 1).

**Table 1.** Commercially available probiotic juices (references [20–22]).

Product Name and Manufacture	Probiotic Strain(s)	Fruit Juice
<b>BiolaR</b> (TINE BA, Norway)	<i>L.rhamnosus</i> GG	Mixture of apple/pear and, orange/mango juice with 95% fruit and no added sugar
<b>Bio-Live Gold &amp; Dark</b> (Bio-Live/Microbz Ltd., UK)	Mixtures of 13 strains including <i>L.acidophilus</i> , <i>L.bulgaricus</i> , <i>L.casei</i> , <i>L.plantarum</i> , <i>L.fermentum</i> , <i>Lactococcus lactis</i> , <i>Bacillus subtilis</i> , <i>B.bifidum</i> , <i>B.longum</i> , <i>B.infantis</i> , <i>Streptococcus thermophilus</i> , <i>Comobcillus</i> and <i>S. cerevisiae</i>	Mixture of fruit juices such as acai berry, cherry, goji, noni, pomegranate, lemon, and various herbs
<b>Bravo Friscus</b> (Probi AB, Sweden)	<i>L.plantarum</i> HEAL9 and <i>L.paracasei</i> 8700:2	Orange, apple and tropical fruit juices

Table 1. Cont.

Product Name and Manufacture	Probiotic Strain(s)	Fruit Juice
<b>Gefilus Fruit drinks</b> (Valio Ltd., Finland)	<i>L.rhamnosus</i> GG and <i>Propionibacterium freudenreichii</i> <i>ssp. shermanii</i> JS	Berries
<b>Golden Circle Healthy Life Probiotic Juice</b> (Golden Circle, Australia)	<i>L.paracasei</i> 8700:2 and <i>L.plantarum</i> HEAL9	Mixture of apple juice with mango puree or orange, apple, pineapple, passionfruit with banana puree
<b>Goodbelly® Carrot Ginger Flavor</b> (Goodbelly, USA)	<i>L.plantarum</i> 299v	Carrot juice, ginger extract, and cane sugar contains 2% or less gluten-free oat flour
<b>KEVITA</b> (KEVITA, USA)	<i>L.rhamnosus</i> , <i>L.plantarum</i> , <i>L.paracasei</i> , <i>B. coagulans</i> GBI-30 6086	Various fruit-based mixtures such as strawberry and coconut, lime, mint and coconut, pineapple and coconut
<b>Malee Probiotics</b> (Malee Enterprise Company Ltd., Thailand)	<i>L.paracasei</i>	Prune, grape and orange juice
<b>PERKii Probiotic Water</b> (PERKii, Australia)	<i>L.paracasei</i> Lc431	Fruit juice mixtures such as raspberry and pomegranate, lime and coconut, mango and passionfruit and strawberry and watermelon
<b>Probiotic Naked Juice</b> (Naked® Juice, USA)	<i>Bifidobacterium</i>	Mixture of apple, orange, pineapple juices and mango and banana puree with fructooligosaccharides
<b>ProViva</b> (EMEA Probi AB, Sweden)	<i>L.plantarum</i> 299v	Orange, strawberry or blackcurrant juice and fortified with 5% oat flour
<b>Rela Fruit Juice</b> (Biogaia Global, Sweden)	<i>L.reuteri</i> MM53	Fruit juice
<b>Tropicana probiotics</b> (Tropicana, USA)	<i>B.lactis</i>	Fruit juice mixtures such as strawberry and banana, pineapple and mango, peach and passionfruit
<b>Vita Biosa</b> (Biosa, Denmark)	<i>B.lactis</i> , <i>B.longum</i> , <i>L.acidophilus</i> , <i>L.casei</i> , <i>L.rhamnosus</i> , <i>L.salivarius</i> , <i>L.lactis</i> , <i>Streptococcus thermophilus</i>	Ginger, blackcurrant, blueberry juice with ginger extract enriched with 19 different aromatic herbs

#### 4. The Case of Pomegranate Juice as Substrate for Probiotic Bacteria Fermentation

Pomegranate (*Punica granatum* L.) and its juice exhibit high antioxidant capacity because of their rich polyphenol content. In comparison with other widely consumed fruit juices like grape, cranberry, grapefruit and orange juice, pomegranate juice demonstrated 3 times higher antioxidant capacity [23]. Ellagitannins and anthocyanins are the responsible substances for the observed antioxidant activity [24]. Punicalagins are the major ellagitannins in pomegranate and after the consumption of the fruit they can be hydrolysed to ellagic acid in vivo [25]. The amount of punicalagins contained in the juice depends on the fruit cultivar, processing and storage conditions [26,27]. The total phenolics calculated either with HPLC or with the Folin–Ciocalteu method is approximately 2500 mg/L [27].

Other chemical constituents in pomegranate juice include sugars (glucose, fructose, sucrose), organic acids (citric acid, malic acid, tartaric acid, fumaric acid, succinic acid, ascorbic acid, etc.), hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and their glycosides (catechin, epicatechin, quercetin, rutin), aminoacids (proline, valine, methionine, glutamic acid, aspartic acid), indoleamines (tryptamine, serotonin, melatonin) and tocopherols [28,29]. Minerals in traces are also included, such as Fe, Ca, Cl, Cu, K, Mg, Mn, Na, Sn and Zn.

The antioxidant capacity of pomegranate juice is of major significance for the agro-food industry because of the fact that it can be used as a natural biopreservative instead of synthetic antioxidants. The latter are frequently accused as having toxic side effects, meanwhile isomers of punicalagin, tannin derivatives and anthocyanins scavenge free radicals and inhibit lipid oxidation in vitro [25].

Commercial pomegranate juices showed an antioxidant activity (18–20 TEAC) three times higher than those of red wine and green tea [27].

Likewise, many scientific data have been published lately regarding lactic acid fermentation of pomegranate juice with probiotic bacteria. Mousavi et al. [12] used *L. plantarum*, *L. delbrueckii*, *L. paracasei* and *L. acidophilus* for the fermentation of pomegranate juice. The results demonstrated that *L. plantarum* and *L. delbrueckii* had higher viability levels during fermentation and storage time compared to the other LAB ( $2.8 \times 10^5$  cfu/mL and  $1.5 \times 10^5$  cfu/mL after the second week of storage, respectively). The maximum viability level was retained for two weeks storage and after the fourth week fell dramatically for both of the strains mentioned above. Authors concluded that pomegranate juice is a suitable medium for the production of a fermented probiotic drink.

The same authors [30] applied two probiotic strains of *L. plantarum* and *L. acidophilus* as starter cultures in pomegranate juice fermentation. Their viability in the juice reached to  $3.07 \times 10^8$  cfu/mL and  $3.9 \times 10^8$  cfu/mL respectively, after 72 h of fermentation. As far as the consumption of sugars is concerned, glucose degradation rate was higher than fructose. Lactic acid was the most abundant acidic metabolite. It was found that probiotic pomegranate juice had increased antioxidant activity. The free radical scavenging effect of the juice was increased through fermentation; however, the enhanced effect of fermentation varied with the employed bacteria since *L. acidophilus* improved the antioxidant capacity of the juice more extensively than *L. plantarum*.

In another assay, a yogurt drink was supplemented with different concentrations of inulin, as a prebiotic (0%, 0.5%, 1%, 2%) and with pomegranate juice (0%, 3%, 8%, 12%) [31]. The probiotic strain that was added for fermentation was *L. casei* 431. High levels of pomegranate juice addition resulted in high levels of antioxidant activity. Additionally, high levels of inulin resulted in low acidity and lactic acid amounts. Overall, supplementation with inulin and pomegranate juice had positive impact on *L. casei* viability. Especially for yogurt drink samples containing 12% juice, the mean value of viability was 7.93 log cfu/mL compared to controls where the mean value was 7.11 log cfu/mL.

Sabkobar et al. [32] supplemented a mixture of pomegranate juice and whey with kefir grains. Two different fermentation temperatures (19 °C and 25 °C) and level of kefir grains inoculum (5% and 8% w/v) were examined through 32 h of fermentation. The best combination regarding the achievement of high viabilities was kefir grains inoculum 8% and 25 °C fermentation temperature. The sensorial characteristics of this novel probiotic dairy and fruit juice beverage were also acceptable.

Encapsulation of *L. rhamnosus* GG in whey protein microbeads has also been examined, as far as the probiotic protection is concerned, by Doherty et al. [33]. After encapsulation, storage studies were performed in cranberry and pomegranate juice in combination with ex vivo porcine gastric (pH 1.6) and intestinal digestion (pH 6.6). During storage, results revealed that coated microbeads enhanced probiotic viability (approximately 8.6 log cfu/mL) and high gastric survival (approximately 9.5 log cfu/mL) with 30 min delayed intestinal release in comparison to non-coated microbeads, justifying their role for targeted intestinal probiotic delivery.

Immobilization of probiotic bacteria was also applied in lactic acid fermentation of pomegranate juice. Specifically, *L. paracasei* K5 was immobilized on delignified wheat bran (DWB) and was used for pomegranate juice fermentation at three different pH values (3.3, 3.6, 3.9) for 24 h. Storage of the fermented pomegranate juice for 4 weeks at 4 °C followed. As proven, immobilization enhanced the viability of *L. paracasei* K5, especially at pH 3.9 where the range varied between 9.8 and 11.7 log cfu/mL. The phenolic content of the produced beverage increased as well [34].

In addition, the co-culture of two different LAB (*L. plantarum* VITES07 and *L. acidophilus* NCIM2903) for the fermentation of pomegranate juice has been examined as well by Murthy et al. [35]. The initial viability value of the co-culture was  $2.75 \times 10^5$  cfu/mL and reached the value of  $6.75 \times 10^5$  cfu/mL after 72 h of fermentation at room temperature. Various organic acids were detected, including formic acid (antibacterial activity), oxalic acid (antitumor activity), tartaric acid (antioxidant activity), gallic acid (antifungal and antiviral activity), citric acid (preservative and flavor enhancer), vanillic acid (flavor enhancer), malic acid (flavor enhancer). These results indicate high antioxidant (initial value

for raw pomegranate juice was 63% and for fermented pomegranate juice, 79.84%), antibacterial, and antitumor activities as well as polyphenol content (concentration for raw pomegranate juice was 75 µg/mL and concentration for fermented pomegranate juice was 160 µg/mL) for the pomegranate juice produced with fermentation with co-culture of the two probiotic strains.

Kazakos et al. [36] applied kefir grains for the fermentation of pomegranate juice alone or in combination with orange juice. Several parameters, such as sugar consumption, ethanol production, lactic acid formation and LAB viability were recorded during storage at 4 °C for 4 weeks. Results revealed that orange juice improved the fermentative activity of kefir grains and the viability of LABs during storage period. More specifically, 75% of the cells survived (6.48 log cfu/mL) after 4 weeks of storage of the fermented mixed substrate, while only 24% of the cells survived when only pomegranate juice was fermented. Lactic acid formation was observed in respectable amounts, especially in the mixed substrates, indicating metabolic activity even during cold storage.

An assay of similar experimental design was conducted by Shubhada et al. [37], where pomegranate juice alone and blended with different proportions of kokum juice was fermented with *L. plantarum*, *L. delbrueckii* and *L. acidophilus* at 37 °C for 72h. Acidity, antioxidant capacity, pH, total phenol content and viability at cold storage (4 °C) conditions were examined. *L. plantarum* reached the highest population after 30 days at storage ( $2 \times 10^6$  cfu/mL) as far as the viability is concerned. Fermented pomegranate juice exhibited enhanced antioxidant activity, acidity and total phenol content. As far as the sensorial evaluation of the produced beverage is concerned, the best scores were achieved by fermented beverage with 15% blend of kokum juice. The best results of antioxidant activity were indicated when 85% of pomegranate juice and 15% of kokum juice and *L. plantarum* was used (77.07%) compared to the unfermented pomegranate juice with the respective value of 59.05%.

From our team, three more research papers have been published lately. In the first two papers, a potential probiotic strain *L. paracasei* K5, previously isolated from feta-type cheese, was applied for pomegranate juice fermentation [38,39]. Fermentation for 24 h followed, and afterwards storage of pomegranate juice at 4 °C for 4 weeks occurred. Volatile composition appeared enhanced, probably because of the lactic acid fermentation, even after the fourth week of storage. Increased antioxidant activity was observed for all the storage times in comparison with the initial pomegranate beverage. The viability of the strain was retained in levels higher than 7 log cfu/mL of juice in all time periods studied. Finally, the fermented beverage achieved a higher score by consumers compared to the unfermented juice.

In the third assay, the main differentiation with the previously mentioned research is that the probiotic strain applied for fermentation of the pomegranate juice was *L. plantarum* ATCC 14917 [40]. Fermented pomegranate juice exhibited higher percentages of viability, even at the fourth week of storage (8.83 log cfu/mL), better volatile compound composition, higher antioxidant activity and total phenolic content compared to the unfermented juice. Finally, the fermented beverage achieved higher scores by consumers compared to the unfermented juice, in terms of aroma, taste and overall quality, especially during the 4th week of storage.

Similar findings have been recorded by Di Cagno et al. [41], who studied the lactic acid fermentation of pomegranate juice with *Lactobacillus plantarum* C2, POM1 and LP09. The fermented pomegranate juice exhibited higher concentrations of alcohols, ketones, terpenes, benzene derivatives and lower concentrations of non-desirable aldehydes. This chemical profile was associated in a second phase with the sensory features of fermented and unfermented pomegranate juices. A consensus modified flavor profile based on 13 attributes was followed and fermented samples were characterized by higher intensity of floral, fruity and anise notes compared to controls.

A comparative assay has taken place by Srisukchayakula et al. [42] regarding acidic fruit juices, such as cranberry (pH 2.7), pomegranate (pH 3.5) and lemon and lime (pH 2.8), that were evaluated for the survival of *L. plantarum* NCIMB 8826. The findings indicate that cells died more quickly in pomegranate juice compared to lemon and lime juice (4 weeks versus 5 weeks) despite the fact that the pH in the former was higher, probably because of the presence of phenolic compounds. Nevertheless,



the pomegranate and the lemon and lime juice may be used as probiotic carriers, since they provide a friendlier environment for cells compared to cranberry juice.

In addition, studies with the application of pomegranate juice fermented with probiotic strains and in vivo experiments have also taken place. Esmailinezhad et al. [43] examined the impact of a pomegranate synbiotic (with *L.rhamnosus GG bacillus koagolans indicous*) juice on glycemic indices, sex hormone profile and anthropometric measurements in polycystic ovarian syndrome (PCOS) patients. In summary, this randomized, triple-blind, controlled trial showed that the synbiotic pomegranate beverage and its consumption improved some metabolic syndrome features including dysglycemia and anthropometric indices, and also reduced testosterone levels in women with PCOS. It should be underlined that a 12-week trial of synbiotics with inulin and a blend of *Lactobacillus spp.* and *Bifidobacterium spp.* could improve insulin resistance, body mass index (BMI), waist circumference and symptoms of hyperandrogenism.

In the same manner, fermented milk containing whey protein, *B.animalis subsp. lactis BB12* and pomegranate juice was found to restore intestinal microbiota and protect rats from the undesirable effects of intense acute exercise. [44]. Specifically, the researchers observed that exercised rats exhibited a decrease in the proportion of *Lactobacillus* species and an increase in *Clostridium* species. Supplementation maintained the natural microbiota proportions of the exercised rats. Finally, the final product could be prospected to humans in acute exercise or in situations of hypermetabolic states, e.g., malnutrition.

## 5. Feasibility of Pomegranate Juice Selection as Potential Substrate for Probiotic Delivery. Advantages and Drawbacks

From all the above, it becomes clear that fermentation of pomegranate juice with bacteria is feasible and can lead to a novel functional beverage, mostly because of its saccharides content, which are necessary for the growth of probiotic bacteria, and due to high levels of bioactive compounds that display antioxidant, antimicrobial and antimutagenic properties [45–48]. More specifically, fermented pomegranate juice exhibits antimicrobial properties due to punicalins, ellagic acids and punicalagins [49] and due to the contribution of short-chain fatty acids with free hydroxyl groups [50]. In addition, the presence of probiotic bacteria in high levels in pomegranate juice contributes even more to the antimicrobial activities of the product and adds nutritional value. In particular, *Lactobacillus spp.* produce antifungal substances, like benzoic acid, methylhyolantoin, mevalonolactone and short-chain fatty-acids [51,52]. It is known that probiotics bind to toll-like receptors (TLR) under epithelial cells and release defensins from the cells in order to stabilize the function of gut barrier [53].

On the other hand, the main problems regarding lactic acid fermentation of pomegranate juice with or without probiotic bacteria are: (i) the preservation of viability in high levels, approximately 6–7 log cfu/mL at the time of consumption [54] and (ii) consumers' acceptance [21]. Cell viability is usually influenced by many parameters such as pH value, phenolic content and time and temperature of storage [13]. Temperature of storage or fermentation and agitation are also involved in the viability and stability of probiotics [54]. However, an acidic environment is considered the main problem for some fruit juices such as pomegranate. Pomegranate juice is considered as an acidic fruit and therefore this may lead to significant loss of probiotic viability [49]. This drawback can be tackled mainly in two ways: (i) the proper selection of probiotic wild-strain bacteria, able to survive under acidic conditions, and (ii) the application of microencapsulation methods such as immobilization. Regarding the proper selection of a probiotic strain, there are examples in the literature proving their impact. For instance, Shubhada et al. [37] studied the fermentation of pomegranate juice with various LAB (*L. acidophilus*, *L. plantarum*, and *L. delbrueckii*). Finally it was concluded that *L. plantarum* preserved its viability for 30 days. In addition, the application of *L. plantarum* c19 led also to high preservation of viabilities [13]. The same outcome was observed in the case of the application of a novel potential probiotic *L. paracasei* K5 by Plessas et al. [38].

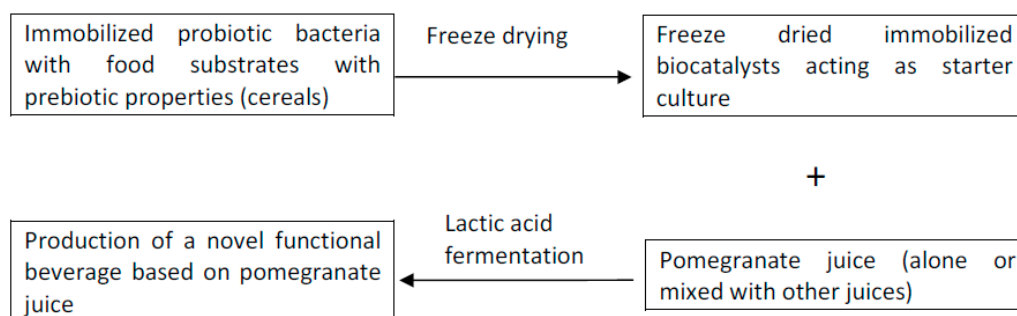
Another way to overcome viability losses is the selection and mixing of pomegranate juice with another juice with higher pH values (above 4) compared to pomegranate juice. For instance, carrot juice has relatively high pH value (pH 6).

On the other hand, the application of probiotics immobilization on various food-grade carriers exhibiting prebiotic properties such as cereals seems to overcome viability losses [55]. It is well known that cereals exhibit prebiotic properties; likewise, they can enhance probiotic viability. Indeed, recently, immobilization of the probiotic *L. plantarum* ATCC 14917 and potential probiotic *L. paracasei* K5 on delignified wheat bran enhanced cell viability above levels required for probiotics during fermentation and cold storage of pomegranate juice [34,40].

The food industry and market cares not only for technological feasibility and health-promoting properties of a new probiotic formulate but also for its sensorial properties which defines consumer acceptance. Likewise, the sensory traits of the juices fermented with LAB should be faced due to the possible identification of probiotic off-flavors by consumers [56]. However, lactic acid fermentation of pomegranate juice has not revealed problems regarding the sensorial characteristics of the final product until now. A general explanation is that pomegranate juice contains high levels of aroma precursors, such as phenolic compounds, sugars and fatty acids, which are considered as good substrates for aroma compound production during lactic acid fermentation [57]. A more persisting explanation for this point was revealed by Di Gagno, et al. [41], who observed that lactic fermentation of pomegranate juice seems to increase the abundance of desired volatile compounds (mainly ketones) and limit the numbers of non-desired volatile compounds (mainly aldehydes). The same outcome was confirmed by our team [34,40].

## 6. Conclusions and Future Perspectives

Pomegranate juice seems to offer a very good alternative option as a substrate for the delivery of probiotic bacteria to consumers who do not prefer dairy products. Lactic acid fermentation of pomegranate juice seems to (i) enhance its nutritional value and (ii) increase its preservation time compared to unfermented juice during cold storage for approximately 4 weeks. [58]. However, future work is needed in order to further optimize the lactic acid fermentation of pomegranate juice in terms of preservation of cell viability during cold storage and acceptable sensorial properties. In our opinion, a proposed strategy regarding the exploitation of pomegranate juice as a substrate for probiotic delivery should include the following steps (Figure 1): (i) probiotic bacteria should be immobilized in cereals exhibiting prebiotic properties and afterwards freeze dried for the achievement of higher preservation time, and (ii) pomegranate juice should be mixed with other fruit juices of higher pH values, in order to surpass its acidic conditions and simultaneously lead to better organoleptic properties.



**Figure 1.** Proposed diagram for functional pomegranate juice production through fermentation with probiotic bacteria.

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