

## Effect of lactic acid fermentation on fruit juices: A review

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### Abstract

Consumer awareness for functional beverages that promote health has increased during the last years. There is a demand for non-dairy probiotics due to veganism and lactose intolerance. Fermented juices are an excellent source for bioactive components. The aim of this study was to review the latest reports concerning the influence of lactic acid fermentation on fermented fruit juices. The searching strategies proposed by Arksey and O'Malley were applied. Numerous studies were found on functional properties of fruit juices which showed that lactic acid fermentation can improve the product and sensory profile of fruit juices like volatile compounds, polyphenols and antioxidant activity with less sugar content. A decrease of sweet taste and occurrence of unpleasant orders were noticed.

**Keywords:** lactic acid, fruit juices

### Introduction

The World Health Organization (WHO) defines probiotics as living microorganisms, mainly bacteria but also yeasts, that when administered in adequate amounts provide health benefits to the host [1]. Probiotics are considered as functional food. Since 2007, researchers have focused on functional foods. There is an increasing trend of functional foods and probiotics represent approximately 60-70 % of functional foods [2]. Apart from probiotics, there are prebiotics and synbiotics. Prebiotics are indigested food ingredients which support growth or activity of bacteria. They can improve the gastrointestinal health and enhance calcium absorption. Prebiotics are found naturally in leeks, onions, garlic, asparagus, etc. Synbiotics are a selective fusion of both probiotics and prebiotics which improve survival of ingested microorganisms and their colonization of intestinal tract [3].

Studies have shown that probiotics may prevent various ailments like diarrhea caused by pathogenic bacteria and viruses, inflammatory diseases and bowel syndromes, cancer, cardiovascular diseases and mild depression [4, 5]. To receive health benefits from probiotics, the concentration in foods and ability to survive conditions in gastrointestinal tract is decisive. A minimum of  $10^6$ - $10^7$  CFU/ml (colony forming units per ml) of microorganisms must be reached in a food product to be considered as probiotic. The minimum bacteria level of  $10^6$ - $10^7$  CFU/ml must be kept in food products until the end of shelf life [6].

The industry produces many types of probiotics. In general, they are classified in dairy products and non-dairy probiotics. *Lactobacillus* (Lb.) and *Bifidobacterium* (B.) are the main strains used in worldwide consumed probiotic products [6], while yeasts mainly belong to *Saccharomyces cerevisiae* var. *bouardii*. The most common strains in dairy and non-dairy products are: Lb. *acidophilus*, Lb. *casei*, Lb. *crispatus*, Lb. *delbrueckii* subsp. *bulgaricus*, Lb. *fermentum*, Lb. *gasseri*, Lb. *johnsonii*, Lb. *paracasei*, Lb. *plantarum*, Lb. *reuteri*, Lb. *ramnosus*, Lb. *helveticus*, Lb. *lactis*, B. *bifidum*, B. *breve*, B. *infantis*, B. *longum*, B. *lactis*, B. *adolescentis*, B. *essensis*, B. *laterosporus* and other species

like *Escherichia coli* Nissle, *Saccharomyces cerevisiae* var. *bouardii*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Propionibacterium* spp., *Pediococcus* spp. and *Leuconostoc* spp [2].

Fruit juices offer a suitable matrix for probiotics. Numerous studies have researched suitability of fruit juices for probiotics (see table 1). Probiotic microorganisms are especially applied in orange juice, grape juice, pomegranate juice, apple juice, etc. Juices are considered as healthy and refreshing and contain essential nutrients like vitamins, minerals, antioxidants and polyphenols. They are known for their health promoting effects in reducing chronic diseases. They have good taste profiles and attract all age groups [7].

Compared to non-dairy probiotics, starter cultures are not needed in probiotic juices due to their rich source of nutrients. In probiotic juices, there is no competition for nutrients with probiotic cultures which leads to less loss in viability during storage [8]. Another advantage of probiotic juices compared to traditional dairy probiotics is that they are fortified with acidulants which could increase the shelf life for probiotic cultures by creating an anaerobic environment. Also sugars in fruit juices promote the growth of probiotics. Probiotic cultures utilize fructose as a substrate for energy to produce organic acids like lactic acid which results in a low pH. In theory, utilization of sugar by probiotics decrease sugar content and increase acidity in juices. This can be beneficial in treatment of obesity and type two diabetes. Moreover, juices pass the stomach very quickly and spend very less time in acidic environment of stomach [7].

The major challenge of developing probiotic fruit juices is retaining the viability of bacteria. Fortification of juices with probiotic cultures can also lead to sensory challenges. The survival of probiotics in fruit juices is more complex than in dairy products because of the acidic conditions of this matrix. Bacteria need to be protected from low pH, mainly during storage [9].

### Research Methods

Electronic database Scopus (Elsevier) was searched to

identify published studies investigating lactic acid fermentation of fruit juices from the last 10 years (2011 to 2021). The searching strategies proposed by Arksey and O'Malley were as follows: 1. identifying the research question; 2. identifying the relevant literature; 3. study

selection; 4. charting the data and 5. collating the results [10, 11]. The main important results of the literature review of experimental studies on probiotic fruit juices are presented in table 1.

**Table 1**

Product	probiotic strain(s)	conditions of fermentation	outcome of study	reference
Jamai-can cherries juice sour cherry juice cherry juice corne-lian cherry juice	lb. plantarum FNCC 0027 lb. rhamnosus GG, lb. acidophilus 150, lb. acidophilus La-5, lb. casei Shirota, lb. casei LC-01, lb. reuteri DSM 17938, lb. plantarum 2142, lb. acidophilus N2, lb. fermentum DT41 lb. plantarum, lb. casei, lb. paracasei, lb. rhamnosus industrial strains: lb. plantarum ATCC20174, lb. casei ATCC 393 and lb. rhamnosus ATCC 7469, Iranian native strains: lb. casei T4, lb. casei TD4	37 °C for 48 hours 30 °C for 24 hours and 48 hours 30 °C for lb. plantarum, 37 °C for lb. rhamnosus, lb. casei and lb. paracasei for 48 h 30 °C for 72 hours	after fermentation - 10 <sup>9</sup> CFU/ml: highest viable count after 24 hours fermentation - pH ↓ - gallic acid, dihydrokaempferol and 5,7-dihydroxyflavone are detected - phenolic content, antioxidant activity, and inhibition of diabetic-related enzymes ↑ decrease of lactobacilli strains after 24 hours fermentation (pH 3.01–3.34) good growth of lactobacilli strains after pH adjustments (pH 5.8): 10 <sup>9</sup> CFU/ml addition with yeast → better growth of strains after fermentation: - titratable acidity ↑ (significant differences between strains) - some strains (not typically mentioned): total polyphenol content ↑ cell viability remained almost unchanged after 12 days of storage at 4 °C lactic acid fermentation and storage did not affect the initial pH = 3.61 sucrose ↓, malic acid ↓ converted to lactic acid, volatile compounds ↑ (propyl acetate, an ester with fruit notes, reached highest concentration in lb. rhamnosus and lb. paracasei fermented juices) phenolics are extensively metabolized: caffeic acid is converted into dihydrocaffeic acid, p-coumaric acid into 4-ethylphenol and phenyllactic acid is produced better survival rates for Iranian native strains than industrial strains, most resistant strain: lb. casei T4 (10 <sup>8</sup> CFU/ml), other strains: 10 <sup>6</sup> CFU/ml at adjusted pH of 3.5 after 28 days of storage; no survival of lb. casei T4 under natural conditions of pH 2.6 for more than 7 days during storage at 4°C: - antioxidant activity, anthocyanin and phenolic compounds ↓ significant changes in taste, odor and overall acceptance between different strains	2021 Frediansyah, A., Romadhoni, F., Suryani, <i>et al.</i> reference nr. 12 2021 Perjessy, J., Hegyi, F., Nagy-Gasztonyi, <i>et al.</i> reference nr. 13 2019 Ricci, A., Cirlini, M., Maoloni, A., <i>et al.</i> reference nr. 14 2016 Nematollahi, A., Sohrabvandi, S., Mortazavian, A. M., <i>et al.</i> reference nr. 15
acai juice	lb. casei	28 °C for 22 hours	higher survival rate in juices with sucrose; after fermentation: 10 <sup>8</sup> CFU/ml stays above recommended minimum level during storage, lactic acid ↑, citric acid ↓, cyanidin-3-O-rutinoside, polyphenolic compounds, and antioxidant activity ↑ after 42 days storage at 4 °C - juice with sucrose: color preference ↑, flavor and overall acceptance ↓	2021 Freitas, H. V., Filho, A. L. D. S., Sueli Rodrigues, <i>et al.</i> reference nr. 16
gojy berry juicie gojy berry juice	lb. brevis CICC 6239, lb. plantarum Lp39, lb. acidophilus NCFM, lb. plantarum C8-1 b. velezensis, b. licheniformis, lb. reuteri mixed with lb. rhamnosus and lb. plantarum	23 ±1 °C for 7 days 37 °C for 20 hours	during fermentation: lb. brevis (2.2 · 10 <sup>9</sup> CFU/ml), lb. acidophilus (2.1 · 10 <sup>9</sup> CFU/ml), lb. plantarum C8-1 (1.4 · 10 <sup>9</sup> CFU/ml), lb. plantarum Lp 39 (1.1 · 10 <sup>9</sup> CFU/ml) after fermentation: 5.2 · 10 <sup>9</sup> CFU/ml, pH ↓, lactic acid ↑, glucose ↓, fructose ↑, biogenic amines ↑, amino acids ↓, darkness ↑, redness ↑, yellowness ↑, saturation ↑, polyphenol and antioxidant activity (with some fluctuation) ↑ after fermentation: 10 <sup>9</sup> CFU/ml through fermentation: lactic acid components ↑, protein contents ↑, volatile compounds (aroma) ↑, antioxidant capacity ↑, phenols ↑ antioxidant capacity correlated with phenol composition	2020 Wang, M., Ouyang, X., Liu, Y. <i>et al.</i> reference nr. 17 2019 Liu, Y., Cheng, H., Liu, H., <i>et al.</i> reference nr. 18
pome-granate juice pome-granate juice	immobilized lb. plantarum ATCC 14917 with prebiotic carrier wheat bran lb. plantarum	30 °C for 48 h 30 °C for 120 h	after fermentation: total phenolic content ↑, antioxidant activity ↑, sugars ↓, organic acids ↑, lactic acid ↑, ethanol ↑ viability of probiotic cells was well maintained after 24 h of fermentation and during 28 days of storage at 4 °C microbial strain > 10 <sup>7</sup> CFU/ml, 10 <sup>8</sup> CFU/ml at day 28, no pathogens contamination during entire storage period sensory evaluation: no significant differences in aroma, taste, and overall quality (preference) until the end of storage compared with unfermented pomegranate juice during fermentation and storage for 30 days at 4°C:	2019 Mantzourani, I., Terpou, A., Alexopoulos, A., <i>et al.</i> reference nr. 19 2017 Di Cagno, Filannino, R. P., Gobetti reference nr. 20 and Filannino, P., Azzi,

			<p>antimicrobial activity ↑ after fermentation: <math>10^9</math> CFU/ml, after storage: <math>10^7</math> CFU/ml, glucose ↓, fructose ↓, malic acid ↓, antioxidant activity ↓, concentration of ellagic acid ↑, volatile free fatty acids content (acetic, propionic, isobutyric, 3-methyl-butyric, 2-methyl-butyric, and hexanoic acids) ↑, content of desirable volatile compounds like alcohols, ketones, and terpenes ↑ while content of aldehydes ↓ (may cause off-flavors) depending on strain used, concentration of free amino acids (Asp, Ser, Glu and Ala) ↑ or ↓ anti-inflammatory activity fermented juice has better browning index, health-promoting and sensory properties (flavor, sweetness, aroma and overall acceptability) compared to unstarted juice, higher intensity of floral, fruity and anise notes than the controls</p>	L., Cavoski, I. reference nr. 21
pine-apple juice pine-apple juice	b. lactis Bb12, lb. plantarum 299V, lb. acidophilus La5 lb. NRRL B442	37 °C for 24 hours 31 °C for 24 h	<p>after 24 h fermentation: lb. strains <math>&gt; 5 \times 10^9</math> CFU/ml, b. strain = <math>10^9</math> CFU/ml, lactic acid ↑, fructose ↑, total phenolic content ↑, antioxidant capacity ↑ during fermentation and ↓ during storage, microbial population did not change significantly during the first month of storage, after one month microbial population ↓ after fermentation: <math>10^8</math> CFU/ml, best condition for viability at 31 °C and pH 5.8 (optimized conditions) after 42 days of storage under refrigeration at 4 °C: - non-sweetened sample: <math>10^5</math> CFU/mL - sweetened sample: <math>10^6</math> CFU/mL - pH ↓, lactic acid ↑ - no changes in colour and no browning effect</p>	<p>2019 Nguyen, B. T., Bujna, E., Fekete, <i>et al.</i> reference nr. 22 2013 Costa, M. G. M., Fonteles, T. V., Tibério de Jesus, A., <i>et al.</i> reference nr. 23</p>
papaya juice	lb. acidophilus, lb. plantarum	37 °C for 48 hours	<p>during fermentation pH ↓, sugars ↓, large amounts of aroma-associated compounds, organic acids are produced, especially lactic acid, which increased significantly -&gt; improving product quality after fermentation lb. plantarum generated better antioxidant activities compared to lb. acidophilus</p>	<p>2018 Chen, R., Chen, W., Chen, H., <i>et al.</i> reference nr. 24</p>
star fruit juice	lb. helveticus L10, lb. paracasei L26, lb. rhamnosus HN001	30 °C for 8 days	<p>after fermentation all strains: <math>10^8</math> CFU/ml lb. rhamnosus produced highest amount of lactic acid, resulting in a significant lower pH (4.41) than compared to lb. helveticus (4.76) and lb. paracasei (4.71), aldehydes and esters ↓, production of ketones, alcohols, and fatty acids -&gt; could have influence on aroma</p>	<p>2018 Lu, Y., Tan, C.-W., Chen, D., <i>et al.</i> reference nr. 25</p>
apple cider, orange juice, grape juice	lb. rhamnosus GR-1 (prebiotic: long chain and short chain inulin)	37 °C for 72 hours	<p>during fermentation and 30 days of storage at 4 °C: <math>10^7</math> CFU/ml for all samples sensory evaluation in appearance, flavor, texture, and overall acceptability: best score except for appearance: apple cider juice with long chain inulin fiber</p>	<p>2018 White, J., Hekmat, S. reference nr. 6</p>
apricot juice	b. lactis Bb-12, b. longum Bb-46, lb. casei 01, lb. acidophilus La-5	37 °C for 24 hours	<p>after fermentation - all strains: <math>&gt; 10^8</math> CFU/mL - acetic acid ↑, antioxidant capacity ↑ (except lb. casei 01), lactic acid ↑, malic acid ↓, citric acid ↓ - glucose and fructose did not change significantly</p>	<p>2017 Bujna, E., Farkas, N. A., Tran, A. M., <i>et al.</i> reference nr. 26</p>
fig juice	lb. plantarum DSMZ 20179, lb. delbrueckii DSMZ 15996, lb. casei DSMZ 20011	30 °C for 48 hours	<p>after fermentation, all strains: <math>10^8</math> CFU/ml after four weeks of storage at 4 °C: lb. plantarum: <math>10^5</math> CFU/ml, lb. delbrueckii: <math>10^6</math> CFU/ml after two weeks of storage at 4 °C: lb. casei <math>10^3</math> CFU/ml sensory evaluation fermented samples are significantly different in taste, odor, consistency and overall acceptability compared to non-fermented juice, lb. casei reached highest score</p>	<p>2016 Khezri, S., Dehghan, P., Mahmoudi, R., <i>et al.</i> reference nr. 27</p>
mango juice	Leuconostoc mesenteroides MPL18 and MPL39	30 °C for 24 hours	<p>after fermentation: phenol ↓, antioxidant capacity ↑ during 30 days of storage at 4 °C: <math>10^9</math> CFU/ml content of total sugar and mannitol of fermented juice is lower than that of nonfermented juice fermented juice: detection of citric acid, gallic acid, lactic acid, and acetic acid, non-fermented juice: only citric acid and gallic acid were found DPPH (2,2- diphenyl-1-picrylhydrazyl) radical scavenging capacity and total phenolic compounds of fermented is higher than that of nonfermented juice sensory evaluation: acidity and sweetness has noticeable impact on overall acceptance of fermented juice</p>	<p>2016 Liao, X.-Y., Guo, L.-Q., Ye, Z.-W., <i>et al.</i> reference nr. 28</p>
apple, grape, orange juice	lb. acidophilus, lb. casei lb. rhamnosus, lb. paracasei, lb. plantarum	40 °C for 48 hours	<p>after fermentation: <math>10^8</math> - <math>10^9</math> CFU/ml (apple juice was the best medium for lactobacilli growth whilst orange juice showed lowest growth rate), during 4 weeks of storage, (4 °C) lactobacilli counts remained above <math>10^5</math> - <math>10^6</math> CFU/ml sensory evaluation apple juice has highest score for overall acceptance lb. acidophilus is the most suitable strain for apple juice fermentation</p>	<p>2015 Espirito-Santo, A. P., Carlin, F., Renard, C. M. G. C. reference nr. 29</p>

apple juice, red fruit juice	lb. plantarum c19, b. animalis subsp. lactis DSMZ 10140, b. animalis subsp. animalis DSMZ 20104, b. bifidum DSMZ 20456, b. longum subsp. infantis DSMZ 20088, yeast: z. bailii DSMZ 70492	37 °C for 96 hours	storage at 4 °C and 37 °C b. animalis subsp. lactis: highest reduction time (23.21 d) and death time (96.59 d) in red-fruit juice at 4 °C, lb. plantarum: best growth rate in apple juice at 37 °C no significant effect of biocitro and lemon extract	2013 Bevilacqua, A., Campaniello, D., Corbo, M. R., <i>et al.</i> reference nr. 30
cashew apple juice cashew apple juice fuji and gala apple juice	lb. plantarum, lb. casei, lb. acidophilus lb. casei NRRL B-442 lb. casei, lb. acidophilus	30 °C for 48 hours 30 °C for 16 hours 37 °C for 0-20 hours	within 24 hours fermentation growth declined significantly for all strains after fermentation: 10 <sup>6</sup> CFU/ml for all strains during fermentation: - vitamin C ↑, - after 48 hours fermentation vitamin C ↓ - phenolic metabolites: condensed tannin ↑, hydrolysable tannins ↓ - antioxidant activity ↓ - fruity odor ↓, whiskey and acid odor ↑ fermented juices: 10 <sup>8</sup> CFU/ml throughout storage for 42 days at 4 °C fortification with sucrose (8% w/v) increased cell count pH ↓, ascorbic acid ↓, antioxidant activity and total polyphenolic compounds ↑ during fermentation and ↓ during storage, polyphenol oxidase ↓ after fermentation: 10 <sup>8</sup> CFU/ml, lb. casei presented higher cell counts (0.7 log cycle CFU/mL) as compared to product fermented with lb. acidophilus optimal conditions for fermentation: 37 °C for 10 hours sensory evaluation reference: caramel color, apple aroma, acidic apple taste fresh fermented probiotic apple beverage: thick texture and sweet taste sensory evaluation for probiotic juice after storage for 28 days at 7 °C: thick texture and acidic taste consumer acceptance of 96%	2017 Kapasoba, R., Kerdchoechuena, O., Laohakunjita, N. reference nr. 31 2013 Pereira, A. L. F., Almeida, F. D. L., Tibério de Jesus, <i>et al.</i> reference nr. 9 2012 Ellendersen, L., Granato, D., Bigetti, K., <i>et al.</i> reference nr. 32

### Fermentation and Microbial Growth

Fermentation by lactic acid bacteria contributes to improvement of nutritional value and digestibility of various foods. Fermentation also decreases lactose intolerance and controls potential infections. It can also enhance functional bioactives like vitamins and antioxidants and flavor attributes in fruit juices [26, 31]. The main reason for probiotic growth are nutrients of fruit juices especially high content of amino acids and polysaccharides with probiotic effect [17]. A positive effect of fermentation is the metabolic activity that convert high-calorie sugars into low-calorie sugars and that can degrade lipids and cholesterol [26].

Oxygen, fermentation temperature and storage affects bacteria growth [22]. As a response to oxidative stress, Lactobacilli bacteria and bifidobacteria can produce antioxidant substances like NADH, NADPH, glutathione and superoxide dismutase (SOD) enzyme. These substances have the ability to decrease the risk of accumulation of reactive oxygen species (ROS). The result is an increase of bacteria cell viability and an increase of antioxidants [29]. The genus Lactobacillus can grow in temperatures from 2 to 53 °C with optimum temperature range of 30 to 40 °C. During fermentation Lactobacillus Casei could produce enzymes like amylase, lactate dehydrogenases, peptidases and proteinase which can transform primary food matrix into functional moieties [34].

By viewing the studies, an increase of lactic acid bacteria and bifidobacteria (10<sup>7</sup> CFU/ml to 10<sup>9</sup> CFU/ml) in cherry juice, acai juice, goji berry juice, pomegranate juice, pineapple juice, papaya juice, star fruit juice, orange juice, grape juice, apricot juice, fig juice, mango juice and apple juice after 10 hours to 8 days fermentation time was observed with two exceptions [4, 7, 9, 12-32].

It was observed that sour cherry juice and cornelian cherry

juice with pH of 3.01–3.34 and 2.6 in its natural form does not provide an adequate environment for the growth of Lactobacillus, already after 24 hours, the fermentation showed a decrease of the viable cell number of Lactobacillus in sour cherry and cornelian cherry juices. pH of fruit juices is typically low (2.5–3.7) and the optimum pH range of Lactobacillus is between 5.5–6.2. Due to pH adjustments to 5.8 and 3.5 microbial growth improved to 10<sup>6</sup> CFU/ml - 10<sup>8</sup> CFU/ml and 10<sup>9</sup> CFU/ml [13].

A decrease of the microbial rate also happened during storage of fermented fig juice at refrigerated temperatures of 4 °C. The microbial rate dropped from 10<sup>8</sup> CFU/ml to 10<sup>5</sup> CFU/ml for lb. plantarum and to 10<sup>6</sup> CFU/ml for lb. delbrueckii after four weeks of storage at 4 °C. The content of lb. casei in fig juice was only 10<sup>3</sup> CFU/ml after two weeks of storage at 4 °C. The decline in the viable cell counts might be due to a decrease of pH value and shortage of nutrients which induces stress of the microorganisms and thereby decreases microbial growth of bacteria strains [27].

In pineapple juice fermented with b. lactis, lb. plantarum, and lb. acidophilus, no significant decrease was observed after one month storage. First, after one month storage microbial population decreased [22].

In cashew apple juice, lb. casei strain remained at a level of 10<sup>8</sup> CFU/ml throughout storage for 42 days at 4 °C [7].

Microbial growth can be enhanced by adding sugar to fruit juices. Acai juice fortified with sucrose showed higher survival rate of bacteria during fermentation than samples without sucrose. After 10 hours of fermentation Lactobacillus Casei stopped growing in acai juice probably due to lack of carbon source in applied matrix [16].

Studies show that microbial survival during fermentation and storage depends on the fruit matrix especially on pH value of fruit juices, probiotic strain and fermentation



temperature and time.

### PH and organic acids

Lactic acid bacteria catabolize sugars through fermentation and produce organic acids like lactic acid and acetic acid causing decrease in pH, with the end product of ethanol [24]. During fermentation saccharides are being utilized as carbon sources by bacterial strain to generate lactic acid and other chemicals by chemical reactions [18]. It is well-known that strong acidification effect is correlated with high density of bacteria strains. Low pH value might be beneficial to extension of shelf life. Organic acids and low pH are responsible for sour taste in fruit juices [17]. In cherry juice lactic acid fermentation and storage (12 days at 4 °C) did not affect the initial pH [14].

It is also known that lactic acid bacteria metabolize citric acid and malic acid, producing lactic acid, diacetyl, acetoin and acetic acid. Higher content of acetic acid can also be due to metabolism of some amino acids such as serine and alanine [24]. Lactic acid in fruit juices is responsible for sourness whereas acetic acid causes the odor taste that makes products unacceptable. The increase in acidity during fermentation is probably also due to the bacterial metabolism in media containing malic acid. Malic acid is transformed into D- and L-lactate and CO<sub>2</sub> [7].

During fermentation of apricot juice a decrease of malic acid occurred. This could be attributed to malolactic reaction by decarboxylation of malic acid to lactate [25]. Most lactobacilli could decarboxylate malic acid directly into lactic acid by a single malolactic enzyme. For homofermentative lactobacillus bacteria major pathway for lactic acid production could be the transformation of a hexose into two pyruvic acids through the Embden-Meyerhof pathway, followed by the reduction in pyruvic acid into lactic acid by NAD<sup>+</sup>-dependent dehydrogenases [24].

Wang *et al.* found seven organic acids in goji berry juice: citric acid, lactic acid, tartaric acid, oxalic acid, acetic acid, malic acid and shikimic acid whereby citric acid represents highest acid content and shikimic acid lowest acid content [17]. Dependence on the organic acid, fermentation caused increases and decreases of the organic acids or they remained at the same level [17].

In fermented mango juice citric acid, gallic acid, lactic acid, and acetic acid were detected while in nonfermented mango juice only citric acid and gallic acid were found [27].

In acai juice [16] and apricot juice [26] a decrease of citric acid was observed. This could be ascribed to the citrate fermentation pathway via citrate lyase [25].

During storage ascorbic acid reduced in cashew apple juice. Reduction was less for fermented cashew apple juice (with and without sucrose) compared to nonfermented sample. [7]. Ellagic acid increased during *Lb. plantarum* fermentation in pomegranate juice [21].

### Volatile compounds

In general, esters, e.g. ethyl acetate and ethyl butyrate, ketones, phenols, alcohol and terpenes cause the desirable and satisfactory flavour of fermented products [17, 32]. These volatile compounds manifest in form of sweet and fruity notes [33]. Some *Lactobacillus* strains have the ability to produce ethanol by alcohol dehydrogenase enzymes which can metabolize acetaldehyde into ethanol [24].

High content of alcohols such like 1-octanol, linalool, (Z)-3-

nonen-1-ol, 1-nonanol, levomenthol, geraniol, and 2,4-decadien-1-ol were found in fermented goji berry juice. 1-octanol is responsible for mushroom aroma while linalool has a floral odor. New aroma compounds were detected in goji berry juice during fermentation. These new aromas are acetoin and (E)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-on. Acetoin can be converted from 2,3-butanedione by diacetyl reductase. It can be produced by citrate metabolism where it causes a creamy and vanilla aroma. Content of nonanal and hexanal decreased during goji berry fermentation. Nonanal could not be detected after fermentation anymore. It was only present in nonfermented goji berry juice. These compounds smell fatty and grassy and cause rancid odor [18].

In probiotic cashew apple juice alcohols, esters and acids are the major group of flavor volatile compounds produced throughout fermentation. 12-13 flavor volatile compounds were identified in fermented cashew apple juice fermented with three *Lactobacillus* spp. at 0 h incubation. Main compounds are 2,6-dimethyl-4-heptanol (fruity and sweet aroma), ethyl-3-methyl-butanoate (fruity aroma), and 3-methyl-1-butanol (whiskey, malt, burnt aroma). A decrease in fruity odor and an increase in acid odor could be observed. It has been shown that off-flavors in probiotic fruit juices can be overcome by adding 10 % (v/v) of tropical fruit juices. 3-hydroxy-2-butanone compound (acetoin) was found in cashew apple juice as most important ketone [31].

In papaya juice alcohols were the most abundant volatiles. Mainly, alcohols in papaya juice consisted of ethanol, 3-methyl-1-butanol, and linalool. The aroma of 3-Methyl-1-butanol is pleasant and is a major volatile of cheese. 3-methyl-butanol is catabolized by amino acids. Also 25 esters were identified in papaya juice before and after fermentation. Acetate esters are built by alcohol acetyltransferases from the reaction between acetyl-CoA and alcohols. Especially ethyl acetate can enhance fruit flavor. *Lb. acidophilus* and *Lb. plantarum* in papaya juice produced ethyl acetate and butanoic acid ethyl ester (pineapple fragrance). Ketones and lactones were identified in papaya juice before and after fermentation. Acetone, 2-butanone, acetoin, and 6-methyl-5-hepten-2 were dominant volatile compounds in papaya juice. Acetone and 2-butanone decreased during fermentation. Acetone and 2-butanone are responsible for pungent odors. The concentration of acetoin (3-hydroxy-2-butanone) increased during fermentation. Acetoin has a milk aroma and is produced from the metabolism of citrate. 2-Heptanone, 1-hydroxy-2-propanone, 2-hydroxy-3-pentanone, 2-nonanone, 2-dodecanone, and 2-tetradecanone were produced during fermentation. They are derived from  $\beta$ -oxidation of saturated free fatty acids and the further decarboxylation of  $\beta$ -ketoacids. Content of aldehydes increased throughout fermentation. High levels of acetaldehyde and benzaldehyde were detected in fermented papaya juice. Acetaldehyde and benzaldehyde can cause pleasant flavors like almond flavor, cherries flavor and sweetness. Also volatile phenols like 2,4-di-tert-butylphenol were found in papaya juice before and after fermentation [24].

In conclusion, lactic acid fermentation can enhance the flavor profile of fruit juices.

### Sugars

Most studies show that during lactic acid fermentation

carbohydrates are being metabolized due to microbial growth [7, 14, 17, 18, 19, 21, 28]. In fruit juices like cashew apple juice, pomegranate juice, mango juice, goji berry juice and cherry juice fermentation caused a decrease of glucose and fructose content. In apple juice a higher decrease of sugars was observed for fuji apple juice than gala apple juice [7]. A decrease of disaccharides could also be observed in apricot juice while content of glucose and fructose stayed at the same level during fermentation [26]. In cashew apple juice, content of glucose, fructose and sucrose decreased during storage in the sample with sucrose addition while in the sample without added sucrose glucose and fructose stayed at the same level. In the sample with sucrose addition a higher microbial survival rate was observed compared to sample without sucrose addition. The higher microbial rate could be responsible for the decrease of glucose and fructose in samples with sucrose addition [7].

Not all studies show a decrease of sugars during fermentation. In star fruit juice, an increase of fructose could be observed during fermentation. This increase can be explained by hydrolysis of fructooligosaccharides (FOS) by probiotic strains during fermentation [25]. Also in pineapple juice an increase of glucose and fructose was observed whereby content of sucrose decreased during fermentation. An explanation for this could be that sucrose hydrolysis was faster than sugar consumption which results in an increase of sugars [22].

### Amino acids

Amino acids are the elementary nitrogen resource in fruit juice for the growth of lactic acid bacteria during fermentation. Lactic acid bacteria consume amino acids by complex reactions like Maillard reaction and enzymatic conversion [17]. Studies show that decreases as well as increases of amino acids can be observed which is correlated to strains, fermentation conditions or plant matrices [32].

Wang *et al.* detected 21 amino acids in goji berry juice. Threonine, valine, isoleucine, tryptophan, leucine, phenylalanine and lysine showed a decrease behavior during fermentation. Aspartic acid, glutamic acid, asparagine and glutamine are the acidic amino acids in goji berry juice. The content of glutamine increased during goji berry fermentation while aspartic acid, glutamic acid and asparagine decreased during goji berry fermentation. Moreover, histidine, arginine, ornithine, glycine,  $\beta$ -alanine, L-alanine,  $\gamma$ -aminobutyric and proline were found in fermented goji berry juice [17].

In pomegranate juice the major amino acids are aspartic acid, serine, glutamic acid and alanine. Isoleucine, leucine, valine, tyrosine and phenylalanine decreased during fermentation while aspartic acid, alanine and arginine increased. During storage all free amino acids significantly decreased in fermented pomegranate juice [20].

### Biogenic amines

Biogenic amines are produced during fermentation due to decarboxylase which is released by microorganisms. Consuming products with high level of biogenic amines can cause health issues like headaches, heart palpitation and respiratory stress. In goji berry juice spermidine and tyramine were detected after fermentation caused by *lb. brevis* and *lb. plantarum* [17].

### Polyphenols and antioxidant activity

It has been reported that phenolic compounds have health benefits for humans, exhibit antimicrobial activity and impact flavor, taste and color of plant products [14]. They also have antioxidative, antimutagenic and anticarcinogenic effects and act as protective roles against cardiovascular diseases and cataract [26]. Beneficial activities of phenolic compounds are correlated with microbial metabolism which can occur during fermentation process. Their metabolism may contribute to bacterial stress response when microorganisms are in hostile conditions [14].

Lactic acid fermentation caused an increase of total polyphenol [7, 12, 17 - 19, 22, 25], flavonoids [18, 24] and antioxidant activity [12, 13, 15, 17-19] in fruit juices with some fluctuations, followed by a decrease during storage [20, 22]. Enzymes and carboxylic acids produced by fermentation may destroy structure of cells to release phenolic substances. Antioxidant activities are affected by the molecular structure of phenols and phenolic compounds have different sites that are active with diverse free radicals [17, 18]. This can be due to an increment of small molecules with high antioxidant activity in fermented juices [17]. Various enzymes are produced during lactic acid bacteria fermentation. For example  $\beta$ -glycosidase is produced which leads to degradation of complex phenolic compounds usually initially conjugated to simpler types and increasing the total phenolic content [19].

In apple juices antioxidant activity, total polyphenolic compounds were higher in fermented samples than in nonfermented samples. Phenolics of cashew apple juice were gallic acid, caffeic acid, and flavonoids which might combine or bind with sugar or amino acids. Therefore they are very stable. In cherry juice, phenolics were extensively metabolized: caffeic acid was converted into dihydrocaffeic acid, *p*-coumaric acid into 4-ethylphenol and phenyllactic acid was produced [14].

Phenolics in cashew apple juice are present in form of complex structures and bound such as flavonoids and tannins (hydrolysable tannins and condensed tannins) where degradation to simple soluble mono-phenolics could be a possible detoxification mechanism for bacteria and yeast. Total soluble phenolics present in the fermented fruit substrate might also be mobilized and degraded to small molecules during fermentation, resulting in lower phenolic contents. Lactic acid bacteria has a range of enzymes which might help in degrading certain phenolic compounds [31].

Not all studies showed an increase of polyphenol and antioxidant activity throughout fermentation. In mango juice, total phenols decreased significantly after fermentation for 24 hours at 30 °C. Although polyphenols decreased, DPPH radical increased significantly probably due to other phenolic compounds like gallic acid, mangiferin, anthocyanin, and/or vitamin C. Anthocyanins are mostly responsible for the increase in ABTS scavenging activity, while phenolic acids are responsible for DPPH scavenging activity [15]. The content of mannitol which has several health benefits as an antioxidant was higher in fermented mango juice than in non-fermented mango juice [28].

During storage a reduction of antioxidant activity was observed in all samples. This reduction was higher in the nonfermented juice compared to fermented juices [9]. The decrease or increase in antioxidant activity of phenolic compounds is affected by their chemical structure which depends upon the group attached to a basic aglycone [31].

In a study on pomegranate juice fermented with *Lb. plantarum* a significant decrease of polyphenolic compounds and antioxidant activity during fermentation and storage for 30 days at 4 °C was observed. However, the concentration of ellagic acid significantly increased during fermentation. After 30 days of storage, the concentration of ellagic acid significantly decreased <sup>[21]</sup>.

In cornelian cherry juice antioxidant activity (phenolic and anthocyanin content) dropped during storage of 28 days at 4 °C. This could be due to the slight activity of probiotic bacteria in refrigerated temperatures as well as the presence of dissolved oxygen in samples, which results in oxidation of phenolic compounds <sup>[12]</sup>.

Antioxidant activity did not increase in apricot juice during *Lactobacillus Casei* fermentation <sup>[26]</sup>.

It should be considered that different lactic acid bacteria strains and matrices could have different effects on phenolic content and antioxidant activity.

### Sensory evaluation

Studies show that lactic acid fermentation can enhance sensory properties of fruit juices but negative sensory effects can occur as well. The metabolism of the probiotic culture can lead to the production of components that may contribute negatively to the aroma and taste of the product (probiotic off-flavor) <sup>[7]</sup>.

In apple juice, fermented products had a less intense caramel color. Also intensity of apple aroma, flavor and taste decreased in the fermented sample. During storage of 28 days the fermented apple beverage showed a more acidic flavor than the fresh beverage. This increase in acidity is due to bacterial metabolism. There was no difference in sweetness between the pure apple juice in comparison to the fresh fermented apple juice. However, panelists noticed a less sweet taste after 28 days of storage of the fermented product. The decrease of sweet taste is due to sugar consumption that occurs for the maintenance of cells during storage. Panelists also noticed a thick texture for the fermented apple juice which can be explained by the added bacterial biomass <sup>[13]</sup>.

Fermented mango juice showed significant differences in aroma, color, acidity, sweetness, and overall acceptability compared to non fermented mango juice. Fermented mango juice was preferred regarding acidity and sweetness. These attributes had a noticeable impact on the overall acceptance of fermented mango juice. Fermentation also caused a change in color from yellow to light brown. Browning is mainly attributed to non-enzymatic reactions. This may involve degradation of antioxidant compounds (e.g. ascorbic acid) and Maillard reaction <sup>[20]</sup>. Significant reduction in sweetness of fermented mango juice could also be observed with the result that panelists did not like it. The odor was agreeable of the fermented mango juice <sup>[28]</sup>.

Pomegranate juice fermented with *Lb. plantarum* did not show any differences in aroma, taste, and overall quality (preference) from unfermented pomegranate juice during 28 days storage at 4 °C. Desirable chemical groups such as alcohols, ketones and esters are increased while fewer undesirable aldehydes are produced during fermentation. Lactic acid fermentation improved sensory profile of pomegranate juice and ensured better control of flavor changes during juice processing <sup>[19]</sup>.

Overall, lactic acid fermentation can improve sensory profile of fruit juices. However, decrease of sweet taste and

occurrence of unpleasant odors were noticed especially during storage.

### Conclusions

Review of the studies show that fruit juices offer a suitable matrix for lactic acid fermentation. In almost all reviewed studies the microbial rate stayed above the minimum required level of 106-107 CFU/ml. During storage microbial growth decreased which was determined by microbial strain and type of fruit juices. Especially pH had a strong influence on microbial rate. Lactic acid fermentation led to an increase of volatile compounds, polyphenols and antioxidant activity. Sugars decreased during lactic acid fermentation which could be beneficial to treatment of diabetes and obesity. In general, organic acids increased during fermentation which was noticed by a drop of pH. Regarding sensory evaluation lactic acid fermentation can improve the sensory profile of fruit juices but a drop of sweet taste and occurrence of unpleasant odors were noticed. The optimization of the sensory profile of fermented fruit juices could be a goal for future research. To sum up, lactic acid fermentation can enrich the product profile of fruit juices.

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