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*Review Article*

# Microbiological, Biochemical, and Functional Aspects of Fermented Vegetable and Fruit Beverages

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In recent years, the request for the functional beverages that promote health and wellness has increased. In fact, fermented juices are an excellent delivering means for bioactive components. Their production is of crucial importance to supply probiotics, in particular, for people with particulars needs like dairy-product allergic consumers and vegetarians. This review focuses on recent findings regarding the microbial composition and the health benefits of fermented fruit and vegetable beverages by lactic acid bacteria, kefir grains, and SCOBY as well as discussing the metabolites resulting from these fermentations process. Moreover, limits that could restrain their production at the industrial level and solutions that have been proposed to overcome these constraints are also reviewed.

## 1. Introduction

Juice is defined as "the extractable fluid contents of tissues or cells." Each juice has particular chemical, nutritional, and sensorial characteristics, depending upon the type of fruit or vegetable used. Ruxton et al. [1] have indicated that consumption of fruits and vegetables or drinking their juices is correlated with the reduction of chronic diseases risk. Vegetable and fruit juices are sources of many bioactive phytochemicals such as vitamins, minerals, and phenolic compounds [2]. Many studies showed that there is a correlation between the total phenols and their antioxidant activities [2].

The consumers' requirements in the food field changed considerably. Consumers show an increased request for foods and drinks that can stimulate wellbeing, which encouraged functional food production. Well-known examples of these products are the fruit and vegetable juices with probiotics. Furthermore, these beverages are also good for people with lactose intolerance [3].

The fermentation is considered as a low-cost process, which preserves the food and improves its nutritional and sensory characteristics [4]. Many cultures were used as starter cultures for fermented juices and have been recognized as probiotics [4]. Moreover, these fermentations allow introducing new products to the market. The present paper aimed to review the information on fermented fruit and vegetable beverages, which offer good health, and investigates recent trends within this field.

## 2. Composition and Nutritive Value of Fruit and Vegetable Juice

Fruits and vegetables represent one of the essential elements for a balanced food and are known for their promoting human health. They are often regarded as "functional food" thanks to their content rich in various micronutriments such as phenolic compounds (recognized, in particular, for their antioxidant capacity), minerals, and vitamins. Many epidemiologic studies showed a relationship between the consumption of fruits and/or vegetables and prevention of various diseases like cancer, neurodegenerative diseases, obesity, and diabetes [5].

The fruits' composition is similar to that of vegetables (Table 1). They are characterized by very important water content (90% on average). However, carbohydrate content

Table 1: The main bioactive components and their properties in some fruits and vegetables.

| Fruits and vegetables | Bioactive components | Properties | References |
|---|---|---|---|
| Apple | Vitamin C<br>Polyphenolic compounds (chlorogenic acid, epicatechin, procyanidin B2, phloretin, and quercetin) | Decrease in lipid oxidation and cholesterol<br>Reduction of the risk of some cancers, cardiovascular diseases, asthma, and diabetes | [6–8] |
| Citrus fruit | Vitamin C<br>Flavonoids (hesperidin and naringin, polymethoxyflavones, and glycosylated flavones)<br>Pectin | Anticancer activity both *in vitro* and *in vivo*<br>Antiviral and anti-inflammatory activities<br>Inhibition human platelet aggregation<br>Improvement of digestive processes | [8, 9] |
| Grapes | Phenolic acids<br>Stilbenes<br>Flavonoids (flavonols, flavanols, anthocyanins, catechins, and proanthocyanidins)<br>Hydroxycinnamic and hydroxybenzoic acid derivative | High antioxidant capabilities<br>Antifungal activity<br>Inhibition platelet aggregation<br>Inhibition human LDL oxidation | [8, 10] |
| Berries | Vitamin C<br>Carotenoids<br>Flavonoids<br>Phenolic acids<br>Tocopherols. | Antioxidant activity | [8] |
| Stone fruits | Sweet cherries: anthocyanins<br>Prunes: hydroxycinnamic acids and chlorogenic and neochlorogenic acids | Scavenging hydroxyl radicals | [8] |
| Tropical fruits (mango-papaya) | Vitamins A and C<br>Carotenoids | Antioxidant activity | [8] |
| Tomatoes | Vitamins A and C<br>Carotene<br>Lycopene | Prevention of the oxidation of LDL cholesterol<br>Reduction of the risk to develop atherosclerosis and coronary heart disease<br>Protection against epithelial cancers | [8] |
| Root and tuberous vegetables | Carrots: carotene and vitamin A<br>Potatoes: vitamin C, vitamin E, and phenols<br>Beet (*Beta vulgaris esculenta*): betalains | Inhibition of LDL oxidation | [8] |
| Prickly pear | Vitamin C, carotenoids, and vitamin E. Betalain pigments | Antioxidant activity<br>Enhancement of renal function | [8] |
| Peppers | Provitamin A carotenoids | Antioxidant activity<br>Acting as prooxidant | [8] |

in fruits is higher than that in vegetables. Organic acids are the second most abundant soluble solids component of the juices, and they have an impact on their sensory properties. Malic and citric acids are the main acids. Tartaric, glutamic, oxalic, and quinic acids are also present in large quantities in many fruits [11]. Moreover, juices represent an important mineral source (such as sodium, potassium, iron, zinc, magnesium, phosphorus, copper, calcium, and manganese), contributing to the acid-basic equilibrium in the blood and facilitating the neutralization of noxious uric acid reactions [12, 13].

The main juice phytochemicals are vitamins, phenolics, and pigments, which promote the chemopreventive potential of these juices [14]. The phenolic compounds have, as a common characteristic, the presence of at least one aromatic ring bearing one or more hydroxyl clusters [14]. They can be divided into groups (Table 2), according to their chemical structure, in particular, the number of carbon atoms' components and the basic structure of the carbon skeleton [23].

The main subtypes of polyphenols are flavonoids, tannins, and phenolic acids. Flavonoid can be divided into many classes such as flavones, flavonols, isoflavones, flavanones, and anthocyanidins [24]. They have powerful antioxidant activities and exhibit protective effects against many chronic diseases and infectious [25]. However, the substitution of their hydroxyl groups decreases their efficacy [26]. Quercetin is reported to be the major flavonoid that belongs to the class of flavonols. It is recognized to improve the mental and physical health. In fact, it has an antioxidant, antidiabetic, anti-inflammatory, and antiproliferative activities [27].

Tannins can be classified into two major groups: hydrolysable and condensed tannins [28]. Their antioxidant activity has been less marked than flavonoids, and it is related to their polymerization degree. Condensed and hydrolysable tannins of high molecular weight offered greater antioxidant activities than simple phenols [28].

Phenolic acids are associated with the sensorial, nutritional, and antimicrobial proprieties of fruits and vegetables

Table 2: Classification of phenolic compounds and their health benefits.

| Phenolic compounds | Groups | Health benefits | References |
|---|---|---|---|
| Phenolic acids | Hydroxybenzoic<br>Hydroxycinnamic | Antioxidant<br>Anticancer<br>Anti-inflammatory<br>Antimicrobial<br>Antidiabetic<br>Anticholesterolemic<br>Antihypertensive | [15] |
| Flavonoids | Anthocyanins | Antioxidant<br>Anticholesterolemic<br>Reduction of pancreatic swelling | [16] |
| | Chalcones | Combat diseases as well as modulate activities | [17] |
| | Flavanones | Antioxidant<br>Anti-inflammatory<br>Anticholesterolemic | [17] |
| | Flavones | Antioxidant<br>Reduction of vascular disease risk | [17] |
| | Flavonols | Antidiabetic<br>Anticancer<br>Antioxidant<br>Antimicrobial<br>Prevention of cardiovascular disorders | [18] |
| | Isoflavonoids | Phyto-oestrogens | [17] |
| Tannins | Hydrolysable tannins<br>Condensed tannins | Anti-inflammatory, antibacterial<br>Antiviral<br>Antidiabetic<br>Anticholesterolemic<br>Anticancer | [19, 20] |
| Stilbens | — | Antioxidant<br>Anti-inflammatory, antiallergenic, antiaging<br>Anticancer | [21] |
| Ligans | — | Antioxidant<br>Anticancer | [22] |

[29]. They possess one carboxylic acid functionality and contain two groups: hydroxybenzoic acids and hydroxycinnamic acids. Their antioxidant activity is related with the number and position of hydroxyl groups on the molecule. However, many studies showed that the antioxidant activity of hydroxycinnamic acids is higher than that of their corresponding hydroxybenzoic acids [30].

## 3. Juice Fermentation

Besides having an important nutrient content, fruit and vegetable juices constitute a new means of probiotic transfer. Their carbon content can facilitate culture growth and the development of appealing taste profiles. These microorganisms can also improve their physiochemical aspects and their nutritional content. Furthermore, fermentation improves their shelf life and safety.

*3.1. Juice Fermentation by Lactic Acid Bacteria.* Lactic acid bacteria (LAB) are extensively used in food fermentation. Their use improves the organoleptic characteristics and nutritional values of fermented products. During the fermentation, LAB transform indigestible substances into others easier to digest and produce different antimicrobial compounds. Some of these bacteria are called “probiotic” and are known to have health-promoting attributes [31].

Different researchers studied the suitability of fruit and vegetable’s juices for the development of probiotic beverages. Reid et al. [32] showed that the fermented beverages’ consumption increases the total number of LAB in the intestinal tract, which helps in enhancing immunity against common pathogens. Among all LAB, *Lactobacillus plantarum* is the most used species for vegetable fermentation. LAB use carbon sources and free amino acids present in the medium to produce metabolites of interest [33]. Lactic acid is the major organic acid produced by LAB, and it has a role in the reduction of the proinflammatory cytokine secretion of Toll-like receptor- (TLR-) activated, bone marrow-derived macrophages and dendritic cells in a dose-dependent manner [34]. Besides organic acids’ production, many secondary metabolites are synthesized such as bioactive peptides, fatty acids, exopolysaccharides, and vitamins [35, 36]. LAB increase also the antioxidant activity of fermented products thanks to enzymes’ activity such as $\beta$-glucosidase and esterase [37, 38]. Moreover, the produced phenol derivatives are usually a source of the end products’ aroma [38].

Several juice substrates are fermented using LAB (Table 3). The tomato juice was used as a substrate for LAB by Yoon et al. [48] and Kaur et al. [49]. They found that LAB

Table 3: Effect of lactic acid fermentation on fruit and vegetable's juices.

| Fruit and vegetable juices | Strains | Effect of the fermentation on juice | References |
|---|---|---|---|
| Apple juice | *Lb. plantarum*<br>*S. thermophilus* | Reduction in polyphenolic content<br>Improvement of proteins and carbohydrates digestibility<br>Decrease in vitamin C | [39] |
| Beet-root | Coculture of *Lb. acidophilus* and *Lb. plantarum* | Increase in the concentration of polyphenols<br>Formation of kaempferol-3-rutinoside, which is known to be an effective antioxidant, anticancer, and antimutagenic compound | [40] |
| Cashew apple juice | *Lb. plantarum*<br>*Lb. casei*<br>*L. acidophilus* | Increase in the amount of isoflavone aglycone | [41] |
| Cherry juice | *Lb. plantarum* strains | Decarboxylation of protocatechuic acid to catechol<br>Conversion of caffeic and p-coumaric acids<br>Accumulation of reduced derivatives like dihydrocaffeic acid and phloretic acid | [38] |
| Cornelian cherry juice | *Free Lb. plantarum*<br>Immobilized *Lb. plantarum* | Increase in the amounts of total phenol compounds (165.02 ± 10.62 mg GAE/100 ml)<br>Increase in the total phenol compounds more important than with free *Lb. plantarum* (214.41 ± 10.56 mg GAE/100 ml) | [42] |
| Elderberry juice | *Lb. rhamnosus*<br>*Lb. plantarum* | Consumption of hydroxycinnamic and hydroxybenzoic acids<br>Production of dihydrocaffeic acid and catechol<br>Increase of anthocyanins | [33] |
| *Momordica charantia* juice | *Lb. plantarum* | Increase in the total phenolics and total flavonoids content<br>Production of dihydrocaffeic acid and decrease in the concentration of caffeic and p-coumaric acids<br>Increase of the antioxidant activity of the juice<br>Improvement of the juice sensorial characteristic by lowering aldehydes and ketones and increasing alcohols and acids concentration | [43] |
| Mulberry juice | *Lb. plantarum*<br>*Lb. acidophilus*<br>*Lb. paracasei* | Improvement of total anthocyanin and phenolic and flavonoid concentration<br>Increase in antioxidant activities. | [44] |
| *Phyllanthus emblica* juice | *Lb. paracasei* | Increase in the amount of polyphenolic compounds<br>Increase in the antioxidant properties of the beverage | [45] |
| Pomegranate juice | *Lb. acidophilus* | Increase of the antioxidant activity due to the transformation of anthocyanins to anthocyanidins<br>Increase in the content of ellagic acid due to the transformation of ellagitannins | [46]<br>[47] |

could use the fruit juices for their cell synthesis. They concluded that the growth of LAB into acidic juice may help them to survive in the gastrointestinal tract. Cabbage, tomatoes, courgette, and pumpkin juices were also used to produce fermented beverages [50]. LAB growth decreased the juices' pH and increased lactic acid concentration. Moreover, about 43% to 56% of the initial content of L-ascorbic acid is found in the end products. Cabbages and courgettes seemed to be the most suitable to the development of these probiotic beverages. Kohajdová et al. [51] studied the fermentation of cucumber juice supplemented with 0.5%, 1%, and 2% onion juice by *Lb. plantarum*. They noted that onion presence in the juice enhanced organic acids' production in the initial stages of fermentation. However, a slight inhibition effect of onion was observed, especially at an elevated onion/cucumber ratio, in the further course of fermentation.

In another study, Reddy et al. [52] explored the fermentation of mango juice by four LAB (*Lb. Acidophilus, Lb. delbrueckii, Lb. plantarum*, and *Lb. Casei)*. They concluded that the four bacteria were able to resist and survive in the fermented juices during 4 weeks of storage at 4°C. The same result was observed by Nagpal et al. [53], when they used *Lb. plantarum* and *Lb. Acidophilus* for the fermentation of orange and grape juices. However, when *Lb. plantarum, Lb. delbrueckii, Lb. Paracasei*, and *Lb. Acidophilus* were used for the pomegranate juice fermentation, only *Lb. plantarum* and *Lb. delbrueckii* were able to survive after two weeks of storage at 4°C and none of them survived after four weeks. Recently, Mantzourani et al. [42] mentioned that the immobilization of *Lb. Paracasei* on wheat bran enhanced their viability during their storage at 4°C on fermented Cornelian cherry beverage.

Red beets were also used for the production of a functional beverage fermented by a coculture of *Lb. Acidophilus* and *Lb. plantarum* [40]. They observed an enhancement of some biological activities like an increase in the polyphenols concentration and an antibacterial activity against *Listeria monocytogenes*. A 64% cytotoxic activity against human liver cancer cells Hep G2 has been also reported. The increase in phenolic compounds was thought to be due to their depolymerization into more bioavailable simple compounds [54]. A release of phenolic acids was also reported in the study of Ryu et al. [55]. They showed that phytochemical contents increased as fermentation progressed. Several enzymes are involved in these hydrolysis mechanisms, such as glycosyl hydrolases, esterases, phenolic acid decarboxylases, and reductases. These inducible enzymes are considered as a specific chemical stress response to overcome the phenolic acid toxicity [56]. In the fermented cherry juice by *Lb. plantarum,* a conversion of caffeic and p-coumaric acids and an accumulation of reduced derivatives (dihydrocaffeic acid and phloretic acid) were observed [38]. In another study, an increase in the amount of catechol, protocatechuic, chlorogenic, gallic, and syringic acids was noted in the fermented juice [55]. However, lactic acid fermentation does not increase all phenolic compounds. A decrease in hydroxybenzoic and hydroxycinnamic acids was observed in the fermented elderberry juice, [38] may be due to their decarboxylation or hydrogenation by phenolic acid reductases. Strains of *Lb. rossiae*, *Lb. brevis*, and *Lb. curvatus* were found to be capable of metabolizing hydroxycinnamic acids by decarboxylation or reduction [57]. It was also supposed that hydroxycinnamic acids may be used by LAB as external acceptors of electrons to gain one extra mole of ATP [58].

In addition to phenolic acids, an enhancement of the flavonoid content has been also observed when cashew apple [41], *Momordica charantia* [43], and mulberry [44] juices were fermented by *Lb. plantarum.* Specific glycosyl hydrolases are responsible for the reduction of flavonoids glycosides into their aglycones [59]. McCue et al. [60] reported that phenolic aglycones had higher antioxidative activity than their glycosides and are absorbed faster through the intestines than their glucoside bound forms.

A significant increase in ellagic acid concentration was noticed during the fermentation of pomegranate juice by *Lb. plantarum* [46]. Ellagic acid is known as a potent antioxidant and anti-tumor compound, and its release may be due to the hydrolysis of ellagitannins [46]. It has been reported that *Lb. plantarum* can degrade tannins by a tannin acyl hydrolase named tannase [61]. This esterase can hydrolyse the ester bonds present in gallotannin, tannin polymers, and gallic acid esters, avoiding their polymerization and giving the juice high content of aromatic compounds and appropriate color [62]. In addition, tannase can modify the phenolic composition of juices and increase their antioxidant and antiproliferative activities [63].

On the contrary, Thakur and Joshi [39] have noticed a decline in polyphenolic content of apple juice fermented by *Lb. plantarum* and *S. thermophilus* due to the conversion of some phenolic compounds. The phenolic compounds' concentration of papaya juice has also been reduced after fermentation [64]. This decrease could result from the precipitation of phenolic compounds and/or their adsorption with proteins or cells [65]. However, Thakur and Joshi [39] reported that phenolic compounds' decrease could improve the proteins and carbohydrates' digestibility. Therefore, the effect of fermentation on phenolic compounds appears to be highly relying on the used strain and raw material.

The antioxidant efficiency of fermented juices by LAB generally augments with the increase of total phenolic compounds' concentration, and it is related to their chemical structures; however, the dependence is not linear. Oh et al. [54] showed an increase of the antioxidant activity despite the decrease of total phenolic compounds. Furthermore, the decrease in the pH of the fermented juices might have stabilized the phenols, leading to high levels of activity compared with the nonfermented ones [44]. Nevertheless, a decrease in the DPPH radical scavenging activity of fermented carrot juices with *Lb. rhamnosus* was noted by Nazzaro et al. [66, 129].

LAB growth on juices led to the modification of flavor attributes [41]. The sensory properties of fermented juices result from molecules and metabolites produced during fermentation (exopolysaccharide, aromatic compounds, and organic acids). Aromatic compounds formed during fermentation may include alcohols, aldehydes, ketones, esters, or fatty acids, derived from the catabolism of carbohydrates, proteins, and fats in the juice. Lactic fermentation of tomato juice has led to the formation of aromatic compounds, such as alcohols, esters, ketones, alkanes, and terpenes [66, 129]. Filannino et al. [46] have found that volatile free fatty acids increased during the fermentation and the storage of pomegranate juice by *Lb. plantarum*. The same results were observed by Ricci et al. [33] during elderberry juice fermentation by *Lb. plantarum*. However, some researchers showed that probiotics could negatively affect the sensorial proprieties of some fermented juices. To improve their overall acceptance, Luckow et al. [67] proposed the supplementation of these beverages by tropical fruit juices such as pineapple at 10%.

*3.2. Juice Fermentation by Kefir Grains.* Kefir grains are composed of white or yellow irregular granules of protein, and a polysaccharide matrix named kefiran [68]. It has health-promoting effects and the ability to change the gut microbiota composition and activity [69].

The starter culture consists of a symbiotic consortium of several yeasts and bacteria. LAB are represented by the genera of *Leuconostoc, Lactobacillus, Streptococcus*, and *Lactococcus,* and yeasts include the genera of *Saccharomyces, Zygosaccharomyces* spp., *Dekkera, Candida, Kluyveromyces*, and *Pichia* [69]. Acetic acid bacteria were also isolated from kefir [70]. Among the different bacteria and yeasts found in kefir, some of them are recognized as probiotics [71]. The ratio of the viable counts of LAB to those of yeasts in the grain (2 LAB/10 yeasts) is relatively constant during the whole fermentation [72]. In this relationship, yeasts produce the essential growth factors for bacteria. However, the excessive growth of bacteria inhibits the growth of yeasts [73]. The proportion of the microorganisms in the grains differs from that in the fermented product due to many parameters such as substrate type, fermentation time, incubation temperature, agitation rate, inoculum ratio, and storage conditions [70, 74, 75]. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend that kefir should contain a minimum of $10^7$ CFU/mL microorganisms, and the final product should contain at least $10^4$ CFU/mL of yeast [76].

Several fruit and vegetables' juices were fermented by kefir grains (Table 4). The end products are slightly carbonated and characterized by the presence of organic acids, low alcoholic content, and multiple flavors [68, 78]. The desirable flavor of the beverage is due to volatile esters, which result from reaction of acids with alcohols.

Apple, quince, grape, kiwifruit, prickly pear, and pomegranate juices were used by Randazzo et al. [68] to produce kefir beverages. Results showed that both LAB and yeasts can grow in these fermented juices, but the highest levels of microorganisms were reached with prickly pear fruit juice. The ethanol content of the fermented beverages is higher than 1.2% (v/v), except that obtained from kiwifruit. *S. cerevisiae* is the primary responsible species for the alcohol production. Some researchers showed that species within the genus of *Lactobacillu*s could produce ethanol, due to the alcohol dehydrogenase enzyme, which could transform acetaldehyde to ethanol [79]. Kiwifruit and pomegranate fermented juices have been shown to possess the highest antioxidant activity. However, the products mostly appreciated by the tasters were apple and grape kefir beverages. The fermentation of pomegranate juice as a single or mixed substrate with orange juice using kefir grains was proposed by Kazakos et al. [80]. They showed that orange juice addition improved the kefir grains' ability to ferment pomegranate juice. Moreover, all kefir juice beverages have been shown to have a high nutritional value deriving from both the substrate with important antioxidant potential and the culture having probiotic properties. It has been reported that fermentation by kefir could enhance the level of total phenolic content [81, 82]. This increase is related to the metabolic activities of microorganisms in kefir grains. During the fermentation enzymes such as $\beta$-glycosidase derived from the fermentative microorganisms are responsible for hydrolyzing complex phenolic compounds and increasing the amount of total phenols. Furthermore, the fermentation with kefir grains had a positive influence on DPPH radical scavenging. Synergistic effect of phenolic compounds with produced compounds during fermentation seems to improve the antioxidant activity of fermented juices. Lin and Yen [83] indicated that many strains of LAB have antioxidative ability. In fact, they can detoxify reactive oxygen species, thanks to antioxidant enzymes (such as superoxide dismutase and peroxidase), and are capable of chelating metal ions and scavenging reactive oxygen species [84]. A relationship between redox regulation mechanisms and production of exopolysaccharides (EPS) was also shown by Zhang and Li [85]. The development of antioxidant activity is a strain-specific characteristic [86].

Corona et al. [77] developed kefir beverages using carrot, fennel, melon, onion, tomatoes, and strawberry juices. The fermentation process induced a decrease of the soluble solid content and an increase of the number of volatile compounds. High esters' amounts were obtained in strawberry, onion, and melon beverages; and an increase of terpenes was registered in the fermented carrot and fennel juices. The beverages obtained from strawberry, onion, and tomato juices retained an important antioxidant activity. However, carrot kefir beverage was the product mostly appreciated by the judges.

The therapeutic aspects of these beverages were evaluated by many researchers, and many bioactive compounds have been detected, such as bioactive peptides and exopolysaccharides [70, 87–89]. These bioactive compounds have many effects such as anti-inflammatory, antiulcerogenic, antioxidant, and cicatrizing effects.

Kefir beverages have also antibacterial properties due to the combination of several factors, like organic acids and bacteriocins produced during the fermentation process. This activity has been effective at various species of pathogenic bacteria [89].

*3.3. Juice Fermentation by SCOBY.* Kombucha beverage is generally prepared from black or green teas. The tea leaves are rich in polyphenols and supply the amount of nitrogen needed for microorganisms' growth. *Echinacea purpurea* L. tea and Lemon's balm tea have also been used for the Kombucha fermentation, and the obtained beverages have outstanding antioxidant properties [90, 91].

The kombucha beverage is prepared by a symbiotic consortium of acetic acid bacteria and yeasts, known as SCOBY [92]. LAB were also isolated from some kombucha beverages [93]. The main acetic acid bacteria isolated from kombucha are *Komagataeibacter xylinus*, which is known to produce cellulose in the culture, *Gluconacetobacter europaeus, Gluconobacter oxydans, Gluconacetobacter saccharivorans, Gluconacetobacter entanii, Gluconacetobacter kombuchae, Acetobacter peroxydans, Komagataeibacter intermedius,* and *Komagataeibacter rhaeticus* [94–96]. In another study, Marsh et al. [97] indicated that the dominant bacteria found in five kombucha samples belong to *Gluconacetobacter* and *Lactobacillus* species and that *Acetobacter* concentration was lower than 2%.

The main yeasts identified corresponded to *Saccharomyces, Brettanomyces, Dekkera, Hanseniaspora*, and *Zygosaccharomyces* [94, 96]. Many types of these yeasts are mentioned as potential probiotics such as *S. cerevisiae* [98]. In fact, Javmen et al. [99] showed that the glucan polysaccharides from the walls of *S. cerevisiae* play a role in the stimulation of the immune system and the adsorption of toxic substances. Yeasts produce ethanol and carbon dioxide from fructose and glucose [100]; and acetic acid bacteria transform glucose into gluconic acid and fructose into acetic acid. The acetic acid produced stimulates the ethanol production by yeasts [100]. Both ethanol and acetic acid are known to have good antibacterial activity against pathogens [101].

Several factors can affect the microbial and biochemical composition of kombucha beverage; one of them is temperature. According to Fu et al. [102], storing kombucha at 4°C during 14 days decreases slightly the acetic acid bacteria content and significantly the LAB content. Neffe-Skocińska

Table 4: Chemical characteristics of some fruit and vegetable juices fermented by Kefir grains.

| Sample | pH | Ethanol (% v/v) | CO2 | TTA (g/l acetic acid) | Lactic acid (g/l) | Acetic acid (g/l) | TP (mg GAE/l) | DPPH (%) | References |
|---|---|---|---|---|---|---|---|---|---|
| Apple | 4.04 | 2.67 | 1.51 | 1.88 | 0.02 | 0.06 | 176.4 | 37.56 | [68] |
| Carrot | 4.1 | 3 | 1.51 | 10.23 | 4.81 | 1.9 | 206.4 | 14.53 | [77] |
| Fennel | 4.4 | 0.63 | 0.87 | 4.47 | 3.55 | 0.18 | 101.83 | 20.12 | [77] |
| Grape | 3.81 | 4.44 | 1.83 | 2.66 | 0.02 | 0.16 | 61.96 | 15.13 | [68] |
| Kiwifruit | 3.48 | 1.03 | 0.9 | 12.81 | 0.13 | 0.11 | 843.42 | 89.51 | [68] |
| Melon | 4.4 | 2.56 | 3.39 | 5.33 | 4.80 | 0.59 | 160.03 | 20.24 | [77] |
| Onion | 5.0 | 0.09 | 0.14 | 1.5 | 1.24 | 0.03 | 515.94 | 78.67 | [77] |
| Pomegranate | 3.83 | 4.96 | 3.21 | 4.29 | 0.05 | 0.07 | 898.7 | 88.04 | [68] |
| Prickly pear | 4.11 | 2.31 | 1.88 | 1.92 | 1.00 | 0.16 | 374.13 | 59.65 | [68] |
| Straw-berry | 3.6 | 2.35 | 1.71 | 8.82 | 0.58 | 0.10 | 619.86 | 95.38 | [77] |
| Tomato | 4.2 | 1.48 | 1.29 | 6.7 | 2.41 | 1.25 | 268.31 | 78.31 | [77] |
| Quince | 3.61 | 4.51 | 2.42 | 7.43 | 0.18 | 0.11 | 322.71 | 60.53 | [68] |

TTA : total titratable acidity; TP: total phenols content.

et al. [103] revealed that optimal conditions for the Kombucha fermentation are a temperature of 25°C and a period of 10 days. However, Ayed et al. [104] showed that six days of fermentation are enough to ameliorate the nutritional and sensory characteristics of red grape juice fermented with SCOBY. The investigation for a fermentation media rich in antioxidants led to new trends in the research. Indeed, using fruit juice as a substrate for SCOBY improves the nutritional effect of the obtained beverage. The beneficial effects of Kombucha are due, among others, to its acidic composition like gluconic, glucuronic, acetic, and lactic acids [105, 106]. The acids' concentration depends on both fermentation time and substrate type used to prepare the beverage.

Jerusalem artichoke tuber extract was used as substrate for SCOBY [107, 108]. The obtained beverage had a high L-lactic and L-ascorbic acid contents and was considered as a dietetic product due to the presence of inulooligosaccharides. However, the disadvantage of this beverage is the absence of glucuronic acid and its bitter taste [107].

Sour cherry, grape, and pomegranate juices which are a source of biologically active ingredients were also used to produce functional beverages with a high glucuronic acid content [109–111]. Glucuronic acid is one of the most valuable healthy Kombucha components, which has a detoxifying effect [112]. Glucuronic acid is also a precursor of vitamin C [113], and it takes part in the prevention of chronic degenerative cardiovascular and neurodegenerative diseases and cancer [92, 100]. The maximum yield of glucuronic acid was obtained on sour cherry and pomegranate juices supplemented with 0.8% of sucrose [109–111] and on grape juice supplemented with 0.7% of sucrose [110]. The yield of glucuronic acid obtained on the different juices was higher than that obtained in the black tea. This increase was explained by the high carbohydrates' content in these juices. Yavari et al. [110] showed a correlation between the temperature increase from 27°C to 37°C and the increase of glucuronic acid content. According to the researchers, the obtained beverages might have a high pharmaceutical value.

Many authors noted an increase of the total phenolic content and antioxidant activities in all media used for kombucha fermentation. The rise in the amount of total phenolics could be attributed to the degradation of polyphenol complexes, as a consequence of the increased acidity during fermentation, and to the enzymes produced by the kombucha consortium [114]. The health benefits of kombucha beverage are also associated with its antioxidant activity. Antioxidants are known to prevent many disorders and metabolic diseases due to oxidative stress [115, 116]. Ayed and Hamdi [117] showed that the antioxidant potential of the cactus pear juice fermented by SCOBY increased significantly as the fermentation result. These results are comparable with those reported by Ayed et al. [104] and by Khosravi et al. [118] who used respectively grape juice and date syrup as substrates. The increase of antioxidant activity of kefir beverage is attributed to the sum of the antioxidant activities of many compounds present in the fruit, to the synergistic effects between certain metabolic products formed during the fermentation, and to some peptides released by yeasts during autolysis. Jaehrig et al. [119] showed that the antioxidant activity of yeast is believed to be mainly due to the high content of $\beta$-glucans found in their cell wall, as well as wall proteins; however, the antioxidative activity of proteins exceeds that of $\beta$-glucan greatly.

Antimicrobial activity of SCOBY was proven against several pathogenics [101, 104, 117, 120]. It is attributable to phenolic compounds and to organic acids' presence, responsible for the cytoplasmic pH decrease. Bacteria cytoplasm acidification may avoid the growth by glycolysis inhibition and by prevention of active transport [120].

## 4. Opportunities and Challenges for Functional Juices Production

The challenge for industrial functional beverage production is to select a starter culture that grants reproducing the specific characteristics of the beverage produced by spontaneous fermentation. Unfortunately, spontaneous microflora can generate the appearance of organoleptic incidents and cannot give safety assurance. The use of selected strains upon their technological and functional properties is an attractive approach to reproduce the traditional beverages for mass production and to standardize their organoleptic characteristics. However, their uses can be accompanied by the loss of their typicality.

The functionality of these beverages is due to the presence of probiotic microorganisms and due to the formed metabolites. Nevertheless, there are some limits that could restrain their production at industrial level, namely, maintaining the viability and the activity of these starters in the end products to the end of their shelf life [4]. However, Ranadheera et al. [121] found that the LAB incorporation into acidic fruit juices can improve their resistance to subsequent stressful acidic conditions.

Some solutions have been proposed by some researchers to protect the cells against exposure to stress. Rakin et al. [13] proposed yeast autolysate addition to juice to improve both the bacteria number and lactic acid production during fermentation. In other studies, it has been shown that fortification of fruit juices with prebiotics improved probiotic culture's viability. According to Saarela et al. [122], adding oat flour to juice could increase its resistance during the storage at low pH juice. The same results were obtained by White and Hekma [123], when they studied the viability of probiotic bacteria in the presence of galactooligosaccharides. Microencapsulation and nanoencapsulation techniques can also be used to protect bacteria against acidic media. The importance of encapsulation systems in fruit juices' fortification and preservation has been largely demonstrated in the review of Ephrem et al. [124].

The new trend in juices' manufacturing is adding several bioactive components. Ahmad and Ahmed [125] discussed in their review the potential of the most important vegetable processing by-products as a source of valuable compounds for beverage fortification. For example, mixing an appropriate amount of by-product with fruit or vegetable juices can be considered as a good approach for juices' fortification and waste management problem. Cheese whey is among these by-products that are used successfully due to its important characteristics. It is a source of proteins, which have the highest biologic value among all proteins present in food [126]. The uses of fermented whey and fruit beverages as a carrier for probiotic bacteria are the subject of a large number of publications in recent years [127, 128]. Furthermore, adding whey to juice allowed enhancing the beneficial bacteria growth and increased the probiotic properties of the end products.

## 5. Conclusion

Studies showed that fruits and vegetables' juices are getting more attention due to their nutritional value and for their health-promoting attributes, in particular fermented ones, because they can serve as suitable carriers for prebiotics and probiotics. In parallel to their high antioxidant activity, these fermented beverages had a reduced sugar content, which is a real addition in terms of nutritional quality, especially for people with diabetes.

Mixed fermentations with high variability confer greater complexity to the end product, but they are difficult to control. That is why they are generally replaced by pure cultivation to achieve large-scale production. Others challenges remain the maintenance of probiotics viability in the end products during their manufacture and throughout their shelf life. The combination of fermentation with new methods such as juice fortification with some ingredients or encapsulation can be considered successful strategy to overcome this problem.

## Conflicts of Interest

The authors declare no conflicts of interest.

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