fermentation

Review

Improving Kefir Bioactive Properties by Functional Enrichment with Plant and Agro-Food Waste Extracts

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Abstract: An increase in the number of novel fortified kefir-based beverages was observed in the last decades. Vegetables were often proposed as convenient resources of bioactive molecules able to improve nutraceutical benefits of these drinks and/or to confer them new significant features. These findings have been well accepted by the consumers, which generally reserve an important role to the quality of the assumed food and beverages. Specifically, functional fermented milk-based drinks enriched with vegetable extracts display significant biological properties, due to the presence of bioactive compounds exhibiting antimicrobial and antioxidant features. In addition, agro-industrial wastes have been also proposed as innovative resources of secondary metabolites to enrich kefir-based products. Eco-friendly extraction techniques were generally exploited to achieve the isolation of biomolecules and reducing, at the same time, economic and environmental loads. To this regard, this review deeply investigates the main findings to improve kefir bioactive properties by functional enrichment with plant and agro-food waste extracts. The nutraceutical characteristics related to the consumers' health benefits, as well as their effects on the sensorial, chemical, and microbiological properties of the products were evaluated.

Keywords: kefir fortification; agro-food wastes; plant extracts; bioactive properties

1. Introduction

Kefir is an ancient fermented milk drink originating from the Caucasus, showing a creamy consistency and an acidic taste (pH 4.6). The beverage can be prepared from a wide range of milks, including cow, sheep, camel, buffalo, or goat milk mixed with kefir grains [1] that are the raw materials for production and usually are recovered after the fermentation process [2]. In general, milk kefir grains contain different lactic acid bacteria along with yeasts and acetic acid bacteria [3]. Geographical origin, fermentation time, temperature, type and volume of the used milk, as well as the employed starter-kefir grains, largely influence the chemical composition of the final kefir beverage [4].

In the last decades, many scientific reports highlighted the nutritional properties of kefir. Health benefits were ascribed to different bioactive compounds such as proteins, lipids, vitamins, minerals, amino acids, and microelements that usually compose this beverage [5]. The fermentation process seemed to play a key role, allowing a noteworthy increase in the content of vitamins, folic acid, calcium, and amino acids [6]. Moreover, during milk fermentation different bioactive compounds like peptides and heteropolysaccharides, such as kefiran, are formed, improving and/or conferring to the beverage significant antihypertensive, antioxidant, antiallergenic, antitumor, antimicrobial, anti-inflammatory, and cholesterol-lowering activities [7].

Increasing consumers' demand for healthy foods has prompted the efforts to develop novel foods with defined health benefits. Nowadays, a great number of novel functional foods are available on the market with dairy foods and beverages representing an important segment. In order to improve nutraceutical benefits of kefir, an appropriate approach could involve the enrichment with suitable components able to confer to the drink specific and valuable properties [8,9]. To this regard, the employment of plant, soy-derived, and agro-food waste extracts, as well as juices, honey [10], and fibers represents a hedonistic way to achieve convenient functional beverages [11,12]. In this sense, improved antioxidant features are well established in samples fortified with these kinds of extracts. In addition, inhibitory effects on spoilage and pathogenic microorganisms have been underlined, while probiotic growth can be increased or preserved by selecting proper addition levels and process parameters [13].

In this context, this review aims to summarize recent studies dealing with enriched kefir, with an emphasis on the medical and nutraceutical characteristics of the final beverages. Specifically, the discussion will firstly consider plant extracts and essential oils as a source of bioactive molecules. The possibility to achieve formulation of kefir-like products manufactured using non-dairy raw materials, such as soy milk or fruit juices will be also exploited. Finally, agro-industrial wastes will be also evaluated as a precious source of secondary metabolites that, properly added to kefir beverages, allow the achieving of fortified drinks, at the same time, overcoming the environmental and economic problems usually associated with their disposal.

2. Plant Extracts for Kefir Enrichment

Medicinal plants as extracts or essential oils are suitable to incorporate into food, including dairy products due their GRAS (Generally Recognized as Safe) character [12]. These plants derived compounds are often a complex matrix of bioactive constituents. In the last decades, several studies pointed out that the plant extracts addition to kefir can improve its health properties.

An in vitro study carried out by Kim et al. [14], evidenced the effect of *Linum usitatissimum* (flaxseed) extract (rich in α-linolenic acid, lignans, and fiber) on the growth and viability of kefir-isolated lactic acid bacteria. It was observed that, after treatment with 100 μL of crude flaxseed extract, the growth of *Lactobacillus kefiranofaciens* DN1, *Lactobacillus bulgaricus* KCTC3635, *Lactobacillus brevis* KCTC3102, and *Lactobacillus plantarum* KCTC3105 was significantly higher compared to the control.

Similarly, Atalar [15] assessed that the addition of hazelnut milk (25%, 50%, and 75%) to kefir beverage improved its bioactive properties, the organic acids profile, and the viability of kefir microorganisms during storage. In particular, hazelnut milk (50–75%) showed a stimulating effect on the growth of lactobacilli and *lactococci* in kefir samples. Additionally, an increase in total phenolic compounds and antioxidant capacity were observed. Regarding the organic acids, the fortification with hazelnut milk caused a reduction in lactic and citric acid contents, at the same time increasing significantly the levels of malic and acetic acids.

The total phenolic content (TPC) and antioxidant activity of donkey kefir, fortified with honey (30% *w/v*) and *Rosmarinum officinalis* essential oil (0.15% *w/v*), were analyzed during refrigerated storage by Perna et al. [16]. A great viability in antioxidant activity was observed for samples enriched with essential oil characterized by the ABTS highest radical scavenging ability, while kefir added with honey showed the highest ferric-reducing antioxidant power. It is interesting to note that in all samples, the antioxidant activity significantly increased during refrigerated storage, with a maximum value reached after 15 days. Moreover, donkey kefir with honey was well accepted by consumers, while kefir enriched with the rosemary essential oil received lower scores.

The kefir fortification with lyophilized wild garlic powder (1% *w/v*) was conducted by Znamirowska et al. [17] The addition of garlic slowed down the fermentation stage, changed the color and reduced the syneresis. In particular, the functional kefir displayed a significantly higher pH value and a lower lactic acid content than the control beverage, probably in relation to the antimicrobial activity exerted by the garlic powder. Moreover, significant changes of CIELab colors parameters were observed since garlic are rich in flavonoids, chlorophylls, and carotenoids. In addition, the fortification reduced the adverse effect of syneresis (−3%), probably for the garlic rehydration. Conversely, sensorial analysis showed that control kefir was preferred to its enriched counterpart.

The physico-chemical and sensory evaluations of goat milk kefir enriched with ginger (4% and 8% *v/v*) and cinnamon (6% and 12% *v/v*) extracts were investigated by Setiyoningrum et al. [18]. Results did not underline significant differences in protein content, pH value, titrated total acid, and microbial content. The highest increase in antioxidant activity (+12.4%) was observed for kefir added with cinnamon extract at a concentration of 8% *v/v*. However, the fortification with both extracts induced negative sensory results compared to the control. Similarly, Wulansari et al. [19] reported that the addition of ginger extract (2%) did not affect the composition of kefir in terms of water, ash, protein, and fat content.

Characterization of kefir beverages, enriched with yam (*Colocasia esculenta* L.), sesame seed (*Sesamum indicum* L.), and bean (*Phaseolus vulgaris* L.) extracts, were analyzed by Da Costa et al. [20]. These extracts were added at different concentrations (25%, 50%, and 75%). Results showed that fermentation of extracts containing yam, sesame, and beans by water kefir grains were suitable for the production of fermented vegetable drinks. Additionally, the formulation enriched with 50% beans was not only the best substrate for obtaining kefir beverage, but also a protein-rich drink. The development of these kefir vegetable drinks appears particularly interesting for vegans or people with allergies to dairy products.

Natural α-amylase and α-glucosidase inhibitors from food plants represent an attractive strategy to manage the postprandial hyperglycemia [21]. For this reason, kefir enriched with plants extracts was proved to inhibit carbohydrate-hydrolyzing enzymes, being useful in the prevention of inflammation and chronic diseases. In addition, plant derived compounds were able to induce an intense vasorelaxation via different mechanisms [22,23]. In this context, Kwon et al. [24] evaluated the α-amylase, α-glucosidase, and ACE inhibitory activity of kefir enriched with *Rhodiola* extract (2%). The content of salidroside and tyrosol in relation to the fermentation time was also monitored, as well as the antioxidant activity. A moderate α-glucosidase inhibitory activity was observed after 24 h of fermentation and this was probably due to an increase in the tyrosol content. The α-amylase inhibitory activity decreased to zero with fermentation time, the latter seeming the most important parameter affecting the ACE enzyme activity, irrespective of tyrosol and salidroside concentration. These results indicated that kefir culture-mediated fermentation of soymilk supplemented with *Rhodiola* extracts could be proposed as a functional product for the prevention of hyperglycemia that occurs in prediabetes and diabetes type 2. Additionally, due to the significantly reduced α-amylase inhibitory activity at the end of fermentation, this approach would have minimal gastrointestinal side effects.

Green tea and black tea supplementations at 2.0% or 4.0% (*w/v*) in kefir manufacture deal products with promising health properties. Kefir enriched with green tea showed an antioxidant activity superior to kefir enriched with black tea. This effect was stronger when the enrichment ratio increased from 2% to 4%. Therefore, fortification of kefir with green tea could be an alternative way to develop a functional dairy product having both nutritional and health benefits. Furthermore, 2.0% (*w/v*) concentration, which is approximately the level found in a common cup of tea, may improve the taste of kefir [9].

A cocoa (*Theobroma cacao*) pulp beverage inoculated with kefir grains was also to rationalize production processes and expand the range of new products on the market with possible nutritional value [25]. The microbiota of Brazilian kefir grains and kefir cocoa beverages were characterized using molecular techniques that evidenced the presence of yeast *Kazachstania unispora*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and bacteria *Lactobacillus fermentum, Lactobacillus kefiranofaciens* subsp. *kefiranofaciens, Lactobacillus kefiranofaciens* subsp. *kefirgranum*, *Lactobacillus plantarum*, as well as other microorganisms related to the genus *Acetobacter*. Kefir grains were able to reduce the sucrose concentration and produce metabolites useful for improving the product quality.

During fermentation time (72 h), the acetic, citric, lactic, malic, and propionic acid contents increased in kefir beverages, while butyric, oxalic, and tartaric acids along with glycerol were detected in similar concentrations. Moreover, samples obtained after fermentation at 10 °C for 48 and 72 h obtained the greatest acceptance (92% and 100% of the panelists, respectively). The proposed technology in this study is significant because new and diverse methods for processing fruits can be used to minimize production losses, to generate more profits and to introduce innovative nutraceutical products on the market, including the use of probiotic kefir grains as an alternative. The one key point for industrial application of the proposed technology is the promotion of fermentation by kefir of granular biomass, which provides the possibility of eliminating the use of centrifugal separators that have a high-energy demand and require high industrial investments [26].

Germinated (GL) and non-germinated wrinkled brown lentils (NGL) were used as functional ingredients during kefir drinks production [27]. Total dietary fiber in GL (17.4 ± 0.4 g/100 g) was significantly lower than NGL (28.0 ± 2.6 g/100 g). Both GL and NGL contained more insoluble dietary fibers than soluble dietary fibers. Total titratable acidity (TTA) values of kefir samples enriched with lentils, as well as control kefirs were determined on days 1, 7, and 14 of fermentation. On day 1, the pH of the kefir treatments varied from 4.73 to 5.00. Kefir enriched with GL showed higher TTA compared to all other kefir treatments. After 14 days, all lentil supplemented kefirs showed lower pH values and higher values of TTA. These observations confirmed a more pronounced bacterial activity in both GL and NGL kefirs compared to the control. Moreover, the authors evidenced that bacteria in the kefir grew more rapidly with whole lentils than with the dietary fibers of lentils as substrate, probably due to its content in more easily fermentable polysaccharides. In particular, starch can be converted to simple sugars during the germination of seeds and can be hydrolyzed by *Lactobacillus* generating a more evident pH reduction [28]. Regarding polyphenolic content, after day 1 of fermentation, the TPC of kefir enriched with GL was significantly higher than that of kefir added with NGL (64.3 vs. 56.8 Gallic Acid Equivalent (GAE)/100 g). The high bioactive content of GL enriched kefir also persisted after 14 days of fermentation (65.2 vs. 40.7 GAE/100 g). This might be due to the increased release of bounded phenols during fermentation as previously reported [29]. The high TPC content can explain the reason why the evaluation of the antioxidant activity by both Oxygen Radical Absorbance Capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays revealed that kefir enriched with GL and had a higher activity than kefir enriched with NGL.

In another study, Skorkina et al. [30] created a new kefir formulation based on skim milk enriched with hawthorn puree and stevia syrup. Hawthorn puree chemical constituents are recognized for their ability to expand the blood vessels of the heart, to improve the absorption of oxygen by the heart muscle, to reduce the blood pressure, and to show a calming effect. It contains vitamins C and PP, carotene, some acids, and plenty of sugars (fructose) and pectin, and it is able to remove heavy metal salts and other harmful substances from the body [31]. Stevia is rich in glycosides, helping to improve the carbohydrate metabolism and stimulating the insulin secretion. It also contains β-carotene, vitamin C, and minerals characterized by antioxidant activity [31]. The addition of natural additives to kefir increased the acidity throughout its shelf-life, and this parameter remained within the normal range [30].

In Table 1 the main findings are reported regarding the kefir enrichments with plant extracts. In particular, it can be noted that phytochemicals can exert different actions, including antioxidant and hypoglycemic effects, also selecting probiotic bacteria in the gut by promoting their growth. Moreover, their addition can change the sensory attributes of the matrix often in a positive way.

Table 1. Kefir enrichment with plant extracts.

Extract	Enrichment	Microorganism	Effect	Reference
Kefir + *Linum usitatissimum* (flaxseed)	1% (*w/v*)	*L. kefiranofaciens* *L. bulgaricus* *L. brevis* *L. plantarum*	↑ Growth and viability	[14]
Kefir + hazelnut milk	25–50–75% (*w/v*)	Lactobacilli and lactococci	↑ Growth and viability	[15]
Kefir + honey	30% (*w/v*)	N.R.	↑ Antioxidant activity	[16]
Kefir + Rosmarinum officinalis L. EO	0.15% (*w/v*)	N.R.	↑ Antioxidant activity	[16]
Kefir + leaves garlic powder	1% (*w/v*)	N.R.	↓ Sensorial parameters ↓ Adverse effect of syneresis	[17]
Kefir + cinnamon	8% (*v/v*)	N.R.	↑ Antioxidant activity ↓ Sensorial parameters	[18]
Kefir + yam (*Colocasia esculenta* L.), sesame seed (*Sesamum indicum* L.), and bean (*Phaseolus vulgaris* L.) extracts	25–50–75% (*w/v*)	N.R.	↑ Fermentation process	[20]
Kefir + *Rhodiola*	2% (*w/v*)	N.R.	↑ Hypoglycemic activity ↑ Anti-inflammatory activity	[24]
Kefir + black and green tea	2–4% (*w/v*)	N.R.	↑ Antioxidant activities ↑ sensory characteristics	[9]
Kefir + cocoa-pulp	25.5 (*w/v*)	*Lactobacillus kefiranofaciens subsp. kefirgranum* *Lactobacillus plantarum* *Lactobacillus fermentum* *L. kefiranofaciens* subsp. *Kefiranofaciens*	↑ Sensorial parameters	[25]
Kefir + brown lentils	2% (*w/v*)		↓ pH values ↑ TTA ↑ Antioxidant activity ↑ TPC ↑ Bacterial activity	[27]
Skim milk enriched with hawthorn puree and stevia syrup	0.5% (*w/v*)	N.R.	↑ Antioxidant activity	[30]

N.R. not reported. ↑: increase ↓: decrease.

Soy-Derived Extracts Addition

Soybean derivatives gained much attention during recent years and are considered an important alternative in replacing food of animal origin for people with lactose metabolism deficiency. Soybean is an oilseed with excellent nutritional value and stands out for its high protein content. For these reasons, the market demand for the development of soy-based beverages increased a lot in recent years and several studies investigated the effects of soy-derived products on kefir properties (Table 2).

Table 2. Kefir fortification with soy-derived extracts.

Extract	Enrichment	Microorganism	Effect	Reference
Kefir + soymilk	(5 UC/L)	*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* spp. *lactis* biovar *diacetylactis*, *Lactobacillus brevis*, *Leuconostoc*, and *Saccharomyces cerevisiae*	↑ TPC ↑ Aglycone isoflavones	[32]
Soybean beverage fermented with kefir + inulin	3.5% (*w/v*)	N.R.	↓ TSS	[33]
Water kefir + soy whey	5% (*w/v*)	N.R.	↑ Phytochemicals content ↑ Antioxidant activity ↑ Sensorial properties ↑ Gut microbiota growth	[34]

N.R.: not reported. ↑: increase ↓: decrease.

The fermentation of soymilk with kefir culture promoted an increase in lactic acid bacteria (*Lactococcus lactis* and *Leuconostoc* spp.), as the count remained higher than 7.8 log CFU/g [32]. Although most microorganisms remained viable during storage at 4 °C for 1, 2, and 4 days, the kefir culture did not provide sufficient resistance against gastric secretions. On the contrary, the count of *Saccharomyces cerevisiae* was less than 6 log CFU/g at the moment of inoculation and did not change during the entire storage of the product. After in vitro digestive system simulation, the count of all microorganisms was less than 6 log CFU/g. Thus, to achieve the opportune probiotic concentration, an ingestion of 100 g of ready-to-eat/day is necessary. Regarding isoflavone and TPC in kefir-fermented soymilk, it is interesting to note that their content increased 2-fold and 9-fold, respectively, after 4 days of fermentation. The rising of phenols could be explained by the action of microorganism with β-glucosidase, catalyzing the hydrolysis of the phenolic compounds, thus leading to their formation/accumulation [35]. This effect in fermented soymilk products was also observed with different microorganisms, such as *Streptococcus thermophilus* 14,085, *Bifidobacterium infantis* 14,603, and *Lactobacillus paracasei* KUMBB005 [35,36]. Therefore, kefir fermented soymilk can be considered a good source of isoflavones and other phenolic compounds, since the content of these phytochemicals was significantly increased after the in vitro digestion of the product.

The water-soluble soy extract added with macaúba palm pulp (*Acromia aculeata* (*Jacq.*) *Lodd.*) and inulin, was used to develop a fermented kefir beverage. Biomass from macaúba palm fruit was obtained after cooling, freezing, and dehydration and characterized in terms of physicochemical and technological properties. Dehydrated macaúba palm pulp with high content of lipids, carotenoids, and phenolic compounds, was used. The beverage was produced from water-soluble soy extract (9° Brix) after fermentation (12 h at 25 °C) with kefir (4.0 g/100 mL). The drink features were evaluated during 16 days storage (0, 6, 11, and 16 days) at 7 °C; five treatments were developed labelled CONT (without macaúba palm pulp and inulin addition), IN (3.5% inulin), BO (3.5% dehydrated macaúba palm pulp), BO (7% dehydrated macaúba palm pulp), and BO + IN (3.5% dehydrated macaúba palm pulp and 3.5% inulin). The microbial population development in the formulations pointed out that the latter substrate was better for probiotic growth, showing values always above 10^7 CFU/mL [9].

Costa dos Santos et al. [33] proposed a soy-based beverage obtained after fermentation with kefir grains (4 g of kefir grains/100 mL of soymilk (9° Brix and a fermentation time of 12 h) and enriched with 3.5% (*w/v*) of inulin. This formulation was chemically, microbiologically, and sensory monitored for 28 days at 7 °C. The aroma, flavor, and texture of fermented soymilk products depend on the pH. It was found that the optimum pH value for soybean beverage was 4.5, since, at this pH value a gel formation was observed while maintaining the flavor of the product. A decrease in Total Soluble Solids (TSS) was observed as result of the fermentation process (TSS ranged from 1.88 to 1.45° Brix and from 5.26 to 3.49° Brix in the control and inulin treatments, respectively). This reduction is indicative of the post acidification microbiological process. Moreover, the addition of inulin determined a significant decrease in sedimentation percentage after day 14. During the period of observation, in both enriched and unenriched soymilk beverages, the *Lactobacillus* and *Lactococcus ssp.* cell count was higher than the cell count at the beginning of storage (i.e., 10^7 CFU/mL). In particular, a significant increase in *Lactobacillus* cell survival in both control and inulin added products was observed after 14 days. Moreover, previous studies evidenced the protective role on probiotic bacteria of inulin-type fructan when these microorganisms are subjected to gastrointestinal secretions [37,38].

Norberto et al. [39] investigated the effect of partial and total replacement of milk (M) by water-soluble soybean extract (S) on fermentation and growth parameters of kefir microorganisms. The following combination were prepared: 100% S, 50% S + 50% M and 100% M. In all formulations, a decrease of TSS was observed (from 6.40 to 5.67° Brix) and an increase of lactic acid concentration (from 0.600 to 0.738 g of lactic acid/100 mL) deriving from carbohydrates consumption by kefir microorganisms. Both formulations 100% S and 100% M presented *Lactobacillus* counts of 8 log CFU/mL. *Lactobacillus* lag time increased in formulation 50% S + 50% M compared to 100% S and 100% M samples with values of 2.20, 1.03, and 1.06 h, respectively. Although *Lactobacillus* initial counts in both formulations (100% S or 100% M) were similar, these microorganisms grew faster in soybean formulation. Soybean extracts and milk are both rich in oligosaccharides (raffinose and stachyose for soybean extract and lactose for milk), amino acids, and peptides that stimulate *Lactobacillus* growth. At the same time, yeasts showed the highest growth rate in formulation 50% S + 50% M.

Tofu production processes generate a large amount of soy whey every year. To avoid environmental pollution, a reuse model for this by-product was recently proposed by the formulation of a water kefir beverage added with soy whey (WKFS) [34]. After 2-days of fermentation, both total phenols and flavonoids were significantly higher and this observation may justify the increase in antioxidant activity, with values ranging from 40.55%, 38.65% and 46.50 µg/L to 72.34%, 87.06% and 56.22 µg/L for DPPH, 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Ability Power (FRAP), respectively. Metagenomic sequencing was applied for monitoring the relationship between microbiota composition and WKFS quality. WKFS was dominated by *Lactobacillus*, *Acetobacter*, and *Saccharomyces cerevisiae*. *Bifidobacteria*, were also identified. This genus supports the growth and multiplication of other functional gut microbiota, reducing at the same time, the concentration of putrefactive and carcinogenic compounds, as well as pathogenic microorganisms in the digestive tract. Sensorial analysis revealed that the 2-day WKFS had better sensory quality, due to new aromatic volatile compounds produced during fermentation, as well as a bright and clear color.

All considered, similar effects already observed for plant extract enrichment, can be ascribed to soy derived products addition to kefir.

3. Kefir Fortification with Juices and Honey

Juice is defined as “the extractable fluid content of tissues or cells.” Each juice shows particular chemical, nutritional, and organoleptic features, depending on the fruit or vegetable type used. The consumption of fruits and vegetables or drinking their juices is linked to the risk reduction of chronic disease outbreak. On the other hand, these beverages are also good for people with lactose intolerance. Prompted by the consumers request for foods and drinks that can stimulate wellbeing, the functional food industry formulated new products including fruit and vegetable juices added with probiotics. Many microorganisms were used as starter cultures for fermented juices and proved to be useful as probiotics [26]. In this context, several Mediterranean fruit juices were used as fermentable substrates to develop new non-dairy fermented beverages. Results indicated that both lactic acid bacteria and yeasts were able to grow in the considered fruit juices. Among the tested juices, the highest levels were registered with prickly pear fruit juice. As expected, the fermentation increased the concentration of alcohols. Kiwi fruit and pomegranate juices possessed a high antioxidant activity. Esters compounds were present at higher level after fermentation, especially in grape, pomegranate, and quince. The overall quality evaluation indicated that, among the Mediterranean fruit juices evaluated, apple and grape beverages were the products most appreciated by the panelists. These findings support the possibility to develop fruit-based kefir-like beverages (KLB) with high benefits and functional properties [40]. Similar results were obtained for other fermented beverages based on carrot, fennel, melon, onion, tomato, and strawberry juices that underwent back slopping fermentations carried out by water kefir microorganisms. Melon juice recorded the highest number of microorganisms. Almost all juices underwent a lactic fermentation. The concentration of alcohols increased, while aldehydes showed an opposite trend. The overall quality assessment indicated that carrot KLB was the product mostly appreciated by the judges. Positive outcomes were observed using papaya juice as the main substrate for fermentation carried out by two lactic acid bacteria; both strains produced a large amount of volatiles with generally similar changes. The pH decrease was 0.05 units. *L. acidophilus* was proved to be less effective than *L. plantarum* as the action of the latter is very sensitive to the organic acids content, especially lactic acid. Through comparisons of the pH, the organic acids, the volatile compounds, the antioxidant components and especially the antioxidant activity, it was found that *L. plantarum* is more suitable for the production of fermented papaya beverages with probiotic features [41].

The sensorial effects of fir tree honey and pomegranate juice added to kefir was also investigated by Dimitreli et al. [42] The enrichment with pomegranate juice (15% *w/w*) determined a reduction of the pH and brightness (L*) values, a gain of the a* value, with higher acidity (lactic acid concentration) and flowing behavior index (*n*). Sensory analysis showed that the honey addition to kefir reduced the red color intensity and the acidity, while increasing the viscosity and the sweetness. Similar effects were observed for kefir enriched with pomegranate juice.

Du and Myracle [43] suggested the potential application of aronia and elderberry juice to enriched kefir products. These edible berries are rarely consumed as such, due to safety concerns and their unpalatable taste. In this study, kefir products were only formulated with juice (13 and 10% *w/w*, respectively, for aronia and elderberry) and sweetening agents (sucrose, stevia extract, or monk fruit extracts). All samples were evaluated for their sensory attributes, quality parameters, and phytochemical properties. Sensory evaluations on both aronia and elderberry kefirs pointed out that the product sweetened with sucrose received the best overall acceptability. Phytochemical analyses showed that aronia kefir samples contained high amounts of TPC and anthocyanins. Interestingly, a valuable antioxidant capacity was observed for all products, indicating that the enrichment can be advantageous in order to decrease the oxidative stress.

The same authors conducted further experiments by fermenting cow milk with aronia juice and changes in bioaccessible polyphenols of aronia kefir during digestion were measured. The outcomes revealed that the bioaccessible polyphenols in aronia kefir raised during digestion and, as a foreseeable consequence, the antioxidant capacity. These results indicated that during fermentation could be produced metabolites with higher antioxidant capacity and better α-glucosidase inhibitory activity. Utilizing aronia kefir in the diet seems a good strategy to help the blood glucose level control, without abdominal side effects [43].

Recently, another study proposed the enrichment of kefir with different juices to evaluate the antioxidant activity, shelf-life, and palatability. Kefirs were fortified with black carrot, black mulberry, pomegranate, and strawberry juices at different concentrations (10%, 25%, and 50%, *w/w*) and stored at 4 °C for 12 weeks to monitor different parameters. Addition of black mulberry, pomegranate, and strawberry juices enhanced the antioxidant performances of the samples, although under the sensorial point of view, only pomegranate- and strawberry-enriched kefirs resulted improved. Furthermore, black mulberry-enriched kefir was associated to the highest anthocyanin stability [44].

In conclusion, the addition of juices and honey to kefir mainly seemed to affect the organoleptic parameters and the antioxidant activity of the fortified beverage. The main results underlined in the above-mentioned studies are collected in Table 3.

Table 3. Kefir fortification with juices and honey.

Extract	Enrichment	Effect	Reference
Kefir + vegetable juices	4% (*w/v*)	↑ Alcohols ↑ Antioxidant activities ↑ Volatile compounds	[40]
Papaya juice + lactic acid bacteria	45% (*w/v*)	↑ Antioxidant activities ↑ Aroma associated compounds	[41]
Kefir + pomegranate juice	15% (*w/w*)	↓ pH values ↑ Acidity ↓ Colorimetric parameters	[42]
Kefir + honey	3% (*w/w*)	↓ Acidity ↓ Colorimetric parameters ↑ Viscosity ↑ Sweetness	[8]
Kefir + aronia juice	13% (*w/v*)	↑TPC ↑ Anthocyanins content ↑ Antioxidant activity	[45]
Kefir + elderberry juice	10% (*w/v*)	↑ Phytochemicals content ↑ Antioxidant activity	[43]
Kefir + black carrot juice	25% (*w/w*)	↑ Antioxidant activity	[44]
Kefir + black mulberry juice	25% (*w/w*)	↑ Antioxidant activity ↑ Palatability	[44]
Kefir + pomegranate juice	25% (*w/w*)	↑ Antioxidant activity ↑ Palatability	[44]
Kefir + strawberry juice	25% (*w/w*)	↑ Antioxidant activity ↑ Palatability	[44]

↑: increase ↓: decrease.

4. Kefir Fortification with Agro-Food Waste Extracts

Agricultural and agro-industrial wastes are considered huge environmental and economic problems, but nowadays they are becoming a precious resource of secondary metabolites. In particular, the secondary metabolites have been widely exploited as new ingredients to fortify many foods or to obtain innovative functional foods. Moreover, fermentation may be an effective method to increase the bioavailability of dietary polyphenols in food. Many studies about the effects of fermentation on polyphenol-rich food are still needed to optimize the potential health-promoting properties of these products. In this context, a fortified kefir can be prepared both by the direct addition of the extract to the beverage or by a co-fermentation process using kefir grains. Several studies were found dealing with this topic (Table 4).

Table 4. Kefir fortification with agro-food waste extracts.

Extract	Enrichment	Microorganism	Effect	Reference
Kefir grains + whey and deproteinized whey from cow, goat and sheep milk	3% (*w/v*)	*Lactococcus lactis* *Leuconostoc mesenteroides* *Kluyveromyces marxianus*	↑ Kefir grains growing	[46]
Kefir grains + Hydrolyzate Annona seeds	0.07% (*w/v*)	Ferments kefir, Lacto Labo, France	↑ Kefir grains growing	[47]
Kefir + whole faba bean (*Vicia faba* L.) and its de-hulled fractions hulls and cotyledon; whole chickpea (*Cicer arietinum* L.) and its crude mucilage	4% (*w/v*)	N.R.	↑ Antioxidant activity	[48]
Kefir + *Vicia faba* L. bean	3% (*w/v*)	*L. plantarum* *L. rhamnosus*, *L. lactis* *L. cremosis*	↑ Microbial count	[49]
Kefir + *Vicia faba* L. bean	4% (*w/v*)	*L. acidophilus* and *B. lactis*	↑ Growth and viability	[50]
Kefir + mango peel	5% (*w/v*)	N.R.	↑ Antioxidant activities	[51]
Kefir + wine pomace	0.1% (*w/v*)	N.R.	↑ Antioxidant activities	[52]
Kefir + wine pomace	0.1% (*w/v*)	N.R.	↑ Antioxidant activities ↑ Inhibitory activity α-amylase, α-glucosidase, pancreatic lipase	[53]
Kefir + eggshell	0.90% (in $CaCO_3$) (*w/v*)	N.R.	↑ Ca^{2+}	[54]
Kefir + pine bud syrup	2–10% (*w/v*)	*Lactobacillus kefiranofaciens* subsp. *kefirgranum*, *Lactobacillus kefiri*, *Lactobacillus parakefiri*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Acetobacter* sp., *Kazachstania exigua*	↑ Sensorial parameters	[55]
Kefir + proteins of tomato seed	100 mL tomato seed extract + water kefir microbial mixture (0.5, 0.75, 1, 1.25, or 1.5% *v/v*)	*Lactococcus lactis* sp. *lactis*, *L. lactis* sp. *lactis biovar diacetylactis*, *L. lactis* sp. *cremoris*, *Leuconostoc mesenteroides* sp. *cremoris*, *Lactobacillus kefyr*) and *Candida kefyr* and *Saccharomyces Unisporus*	↑ Antioxidant activities	[56]
Kefir + apple and lemon fiber	0.25–1% (*w/v*)		↓ pH values ↑ Viscosity	[57]

N.R. not reported. ↑: increase ↓: decrease.

Whey, a by-product of the cheese industry, is still disposed despite its nutritional value. Recently, whey proteins have been considered as a source of healthy compounds. Many studies have shown that miscellaneous liquid and solid wastes can be exploited as a cheap source of functional food-grade microorganisms (microalgae, lactic acid bacteria, and yeasts). The possibility of using kefir grains to ferment whey in order to obtain a natural acidic drink with microbial inhibitory properties has been frequently carried out at 37 °C for 24 h with 10% and 16% (*w/v*) reconstituted whey powder. In these conditions, most of the lactobacilli released amino acids and small peptides during the first 6 h of incubation [58]. To this purpose, whey and deproteinized whey from cow, goat, and sheep milk were compared to the pasteurized milk of the same animal species during kefir preparation. Each substrate was inoculated with 3% (*w/v*) of kefir grains, cultivated in ultra-high temperature cow milk and evaluated for pH reduction, total TTA increase, and development of lactic acid bacteria (LAB) and yeasts released in the matrices after 24 h of incubation at three different temperatures (20, 25, and 30 °C). The highest kefir grains increase (almost 50%) was obtained using deproteinized sheep whey.

Several residues are still without any current valorization, as an example cherimoya (*Annona cherimola* Mill.) seeds, which are lipid-rich (ca. 30%) and have a significant lignocellulosic fraction [46]. By a series of extractions to obtain oil, the remaining lignocellulosic fraction underwent two different fractionation processes. Auto-hydrolysis extracted principally oligosaccharides (10 g/L) with promising properties for food/feed/pharmacological applications. The residual solid was enzymatically saccharified, with a saccharification yield of 83%. The hydrolysate obtained by diluted acid hydrolysis contained mostly monosaccharides, mainly xylose (26 g/L), glucose (10 g/L), and arabinose (3 g/L), and had a low content of microbial growth inhibitors. This hydrolysate is suitable to be recycled as a culture media for exopolisaccharide production, using bacteria or microbial consortia. The maximum conversion of monosaccharides into xanthan gum was 0.87 g/g and maximum productivity of kefir was 0.07 g/L [47].

Kefir is an excellent vehicle to deliver several bioactive ingredients. Saadi et al. [48] investigated the modification of kefir during storage at 4 °C for 28 days. Kefir was enriched with whole fava bean (*Vicia faba* L.) and its de-hulled fractions hulls and cotyledon; whole chickpea (*Cicer arietinum* L.) and its crude mucilage. Results evidenced that the supplementation with 4% faba bean flour, stimulated the bifidogenic microbial growth, increased the TTA linearly from day 1 to 21 days, and reduced the pH during 28 days of storage. Inulin and other supplementations improved lactate production and increased the proteolytic activity as the fermentation time increased. Moreover, the antioxidant activity of kefir depended solely on the TPC of the supplements, irrespective of the storage time. This hypothesis was confirmed by the observation that the high antioxidant capacity of the faba bean hull supplemented kefir (13.03 mmol trolox eq/g kefir) was related to its high TPC (57.53 mg GAE/g). Moreover, supplementation with whole faba beans exerted a better activity in maintaining kefir stability during refrigerated storage, with respect to commercial inulin. An increase in microbial count (*L. plantarum*, *L. rhamnosus*, *Lactoccocus lactis,* and *Leuconostoc cremosis*) during kefir storage (day 1–8) was observed and also, the supplementation with faba bean cotyledon, chickpea flour, and chickpea mucilage contributed to short-chain fatty acids (SCFA) production and proteolytic activity. Successive studies assessed the viability of kefir enrichment with mucilage extracted (3%) from faba bean and chickpea [49]. The number of bacteria significantly increased during the storage period in all the evaluated formulations. Kefir supplemented with mucilage showed slightly lower, but not significantly different, sensory acceptability scores in comparison to the control. The results are in accordance with those previously obtained by Boudjou et al. [50] that investigated the effects of faba bean (*Vicia faba*) flour on viability of probiotic bacteria during kefir storage for 28 days. Supplementation with 4% of faba bean flour stimulated microbial growth (*Lactobacillus acidophilus* and *Bifidobacterium lactis*), increased TTA linearly from day 1 to 21, and reduced pH during kefir storage.

Biotransformation processes by kefir fermentation have increased the interest in obtaining products with good biological properties also from fruit wastes. In this context, mango peel, an agro-industrial waste with excellent nutritional value and a considerable content of bioactive compounds, could be recycled as a source of antioxidants during kefir production. In particular, the effect of milk supplementation with mango peels on the growth rate of kefir microorganisms during fermentation and the antioxidant properties of the obtained fermented products have been investigated. After a comparison between fermented samples in the presence of mango peels (KMP) and in their absence (KC), significant increases ($p < 0.05$) in antioxidant activities for KMP were observed equally to those of KC fermented samples. According to microbiological analyses, the microbial growth was 3-fold higher in KMP samples in comparison to KC samples, reaching values of 10^8 CFU/mL for lactic acid bacteria. Results also showed that the products obtained from the fermentation process using milk combined with mango peels as substrate, presented improved antioxidant properties and potential probiotic properties [51].

The effect of the addition of fruit peels derived fibers were also investigated. The influence on the rheological, microbiological, and sensorial properties of apple and lemon fibers addition (at different concentrations ranging from 0.25% to 1%) to kefir stored at 4 °C for 20 days was analyzed [57]. These fibers include oligosaccharides, lignin, resistant starch, and tannins. Addition of apple and lemon fibers resulted in a decrease of pH values. In particular, kefir enriched with apple fibers at concentration of 0.5% had the lowest pH. That is related to a more prominent effect on lactic acid bacteria. This concentration allowed the correct value of water activity that is necessary for bacteria growth. The viscosity of the enriched kefir with both fibers was higher than that of the control kefir, probably in relation to the contribution of both soluble and insoluble fibers. Kefir enriched with apple fibers was characterized by the highest viscosity value, since apples had higher insoluble fibers and pectin content. Storage significantly reduced the pH, due to the lactose conversion into lactic acid. *Lactococcus* spp. counts ranged from 10.73 to 9.79 log CFU/mL, after 1 and 20 days of storage, respectively, in kefir (control). Samples enriched with apple fibers had slightly higher *Lactococcus* spp. counts in comparison to all other samples. This effect was observed up to a 0.5% fibers fortification; then an opposite trend was recorded. During storage, *Lactococcus* spp. counts reduced about a 0.8 log cycle due to the high acidity of kefir. Addition of fibers did not change the *Leuconostoc* spp. and the yeast counts in kefir samples. A similar situation was previously observed by Ertekin and Guzel-Seydim [59] and Montanuci et al. [60] for inulin enriched kefirs. Moreover, according to Montanuci et al. [60] *Leuconostoc* spp. and yeast counts increased about 1.5 log cycle during the storage period, due to the metabolism of lactose by *Leuconostoc*.

Among the agrochemical wastes, the winemaking process generates large amounts of by-products that are still a potential source of bioactive compounds to be used as functional food ingredients. Pomace extracts (skin and seeds) of Sangiovese red grape, were proved to be rich in (+)-catechin and (−)-epicatechin and were used to design a fortified cow milk kefir by Carullo et al. [52]. The addition of the skin extract to kefir was very effective in enhancing the TPC (+43.2%), thus increasing the total antioxidant capacity (+47.7%) and lowering ABTS (−36.0%) and DPPH (−31.45%) values. An increased inhibitory activity towards α-amylase, α-glucosidase, and pancreatic lipase was also achieved [52]. Furthermore, Sangiovese a pomace seeds extract-fortified kefir demonstrated interesting properties in a validated in vitro model of inflammation-impaired intestinal barrier [53]. Among the wine pomaces, white grape pomace (WGP) contained a high amount of glucose and fructose useful as a carbon source in the fermentative process. In fact, this waste was valorized by lactic acid (LA) production by *Lactobacillus casei*. Adding WGP directly, or its water extract, into the culture medium at a solid loading of 10% yielded 33.3 g/L LA. In all cases, fructose was consumed at a slower rate as compared to glucose, concluding that WGP can be considered as a sustainable plant-based feedstock for LA production by *L. casei*. Usual insufficient calcium intake in adulthood leads to osteoporosis and augmented fracture risk. The valorization of a poorly investigated waste, the eggshell, was suggested to ripen a calcium-enriched reduced lactose milk, using eggshell and kefir. This high calcium beverage can be easily prepared at home [54].

A value-added kefir by the addition of pine bud syrup (2–10% *w/w*) was also proposed. The pine bud syrup is rich in polyphenols and terpenes and has a high antioxidant activity. The addition of pine bud syrup resulted in an increase in total solids and a decrease in fat content, proteins, and pH. Furthermore, the kefir sample with 10% pine bud syrup was the most appreciated by panelists [55].

The use of protein isolate from tomato seeds as a medium for the growth of water kefir mixture was also explored. The evaluation of the radical scavenging activity of the isolate after 24 h of fermentation showed an improvement of about 74%. Chemical analysis showed a significant decrease in the concentration of total amino acids exceeding 155%, especially for glutamic acid and aspartic acid. After 24 h of fermentation, the protein isolate contained about 41.27 and 20.29 mg/100 g of glutamic and aspartic acid, respectively. Fourier-transform infrared spectroscopy (FT-IR) results showed that the fermentation increased the production of new amides and aromatic compounds [56].

Generally, it is possible to conclude that, depending on the different starting agro-food waste, several effects have been underlined, mainly related to gut microbial growth and antioxidant and hypoglycemic activity increment.

5. Conclusions and Future Perspectives

Increasing consumers' demand for healthy food products has triggered the efforts to develop novel foods with defined health effects. Today, a great number of novel functional foods are available, and dairy foods and beverages have a distinct place among them. This review analyzed the different strategies proposed in order to obtain functional kefir beverages able to produce significant health benefits to the consumers.

For this purpose, plants represent a suitable source of bioactive compounds that conveniently added to the kefir, improve the health benefits of the beverages also conferring essential nutraceutical and biological properties. In particular, functional milk-based drinks enriched with phytochemicals are a promising segment of the dairy industry. They improve the immune system and can be used as part of supportive therapies. They are also suitable for daily use to replenish the balance of essential nutrients. The addition of bioactive components present in essential oils and plant extracts confers to kefir significant biological properties, due to the presence of many antimicrobial and antioxidant compounds. Moreover, the combination of these components with lactic acid bacteria improve the viability of probiotic organisms also extending the shelf-life of the functional beverages. On the other hand, sensory changes should be taken into account as well, as the acceptance by the consumer is an essential factor to be considered. To this regard, it has been found that sometimes these functional ingredients improve the sensorial characteristics of the novel beverage as in the case of cocoa pulp or pine bud syrup.

Additionally, the possibility to develop functional fruit or cereal-based kefir-like enriched beverages represents a suitable and convenient alternative to the simple drink, in order to achieve the preservation in the final beverages of vegetable and kefir synergistic properties that exert benefits to the human health [40,61].

Lately, innovative sources of secondary metabolites, proposed to enrich the kefir beverage, were the agro-industrial wastes. Eco-friendly extraction techniques were exploited with the aim to achieve the isolation of biomolecules and reduce, at the same time, the economic and environmental impacts of these matrices. Excellent nutritional value and considerable content of bioactive compounds was found in mango peel, winemaking process wastes, and tomato seeds as these matrices were all suggested for functional kefir production. Another largely used matrix to enrich kefir is soy and its derivatives. These substrates increased the antioxidant activity of the drink, supported the gut microbiota growth, and improved the sensory aspect of the enriched product.

In conclusion, the fortification of the kefir with a suitable source of bioactive molecules allowed to achieve beverages with specific nutraceutical characteristics, able to retain sensorial, chemical, and microbiological peculiarities over time, significantly increasing the consumers' health benefits. Thus, functional milk-based drinks enriched with plant components are a promising direction in the novel foods market. They appear appropriate for daily use to refill the balance of essential nutrients in the human body; they also improve the immune system and can be useful as part of healthy diet. For a more effective improvement of kefir fortified beverages, future attempts should be done in order to ensure the preservation of the added bioactive compounds over time, for example by using suitable macromolecular systems able to protect them from the external agents.

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