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ORIGINAL RESEARCH

Phenolic content and antioxidant activity of donkey milk kefir fortified with sulla honey and rosemary essential oil during refrigerated storage

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The aim of this work was to evaluate the phenolic content and antioxidant activity of donkey kefir fortified with sulla honey and rosemary essential oil, during refrigerated storage. The type of flavouring strongly influenced the antioxidant activity of the kefir: rosemary essential oil kefir showed the highest 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid values; sulla honey kefir showed the highest ferric-reducing antioxidant power values but, at the same time, in both fortified kefirs, the thiol content decreased, probably because of the formation of polyphenol-protein complexes that would have influence the availability of bioactive components. The antioxidant activity increased significantly during refrigerated storage, showing the highest values after 15 days. Sensory analysis highlighted the fact that the donkey kefir was well accepted by consumers. Addition of sulla honey resulted in the highest acceptability, while addition of rosemary essential oil kefir was less accepted by consumers. This knowledge provides a basis that could lead to the production of fortified donkey kefir with specific nutraceutical characteristics.

Keywords Donkey kefir, Honey, Rosemary essential oil, Phenolic content, Antioxidant activity, Consumer acceptability.

INTRODUCTION

Kefir, less well known than yoghurt in Italy, is a viscous dairy beverage, slightly fizzy and with an acidic and alcoholic taste, which originated in the Caucasus mountains and in Tibet (Jin 1999). Like yoghurt, kefir is a fermented product rich in protein, B vitamins, calcium and potassium. The main differences between kefir and yoghurt are the type and amount of microorganisms present in each of these dairy products; in particular, yoghurt cultures are all mesophilic and thermophilic bacteria, while kefir is produced with a variety of mesophilic bacterial cultures plus yeast, and it provides potentially greater amounts of probiotic cultures (Adriana and Socaciu 2008). Moreover, kefir has a more pronounced sour taste and thinner consistency than yoghurt; in fact, it is often served as a beverage. Kefir is produced by inoculating cow, goat, sheep, camel, buffalo, mare, or donkey milk with kefir grains, which contain a symbiotic mixture of yeast, lactic and acetic acid bacteria in a polysaccharide matrix (Chen *et al.* 2015). Its characteristics are: pH about 4.0; fat and protein content dependent on the type of milk used; alcohol from 0.5% to 2%; lactic acid; acetic acid; CO_2 and aromatic compounds (Irigoyen *et al.* 2005). Kefir, given its composition, is considered a source of probiotic strains. In fact, its regular consumption has been related to many health benefits (Ahmed *et al.* 2013). Moreover, Fouladgar *et al.* (2016) showed that the intake of kefir by neonatal calves improved productivity and health. The antioxidant capacity of kefir could be due to its proton-donating ability, its reducing power, and SOD-like activity (Liu *et al.* 2005). Donkey milk is a valuable product that, due to its physicochemical and microbiological properties, could be considered a good substrate for the production of fermented products (Perna *et al.* 2015). To impart desired sensory properties, fermented milks are

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frequently supplemented with fruit, flavouring and sweeteners; this type of fortification also provides a technological solution for minimising oxidative stress during product storage (Cruz *et al.* 2013; Batista *et al.* 2015; Granato *et al.* 2018). Honey is frequently used as a sweetener in fermented milks; it, in fact, can be considered to be a natural syrup, containing primarily fructose and glucose, with flavour derived from flower essences. Sulla honey is a typical product of southern and central Italy. Its smell is faint and floral and it has a sweet and slightly acidic taste. The colour varies from white to straw yellow (Gambacorta *et al.* 2014). The addition of essential oils from aromatic herbs is another means of improving the flavour and beneficial properties of dairy products (Cutrim and Cortez 2018; Khorshidiana *et al.* 2018). Essential oils are synthesised in various plant organs, constituted from a complex mixture of polyphenols, which are useful in food preservation, the fragrance industry and aromatherapy (Teixeira *et al.* 2013). One of the most important sources of natural antioxidants is rosemary (*Rosmarinus officinalis* L.) of the Labiatae family (Tavassoli and Djomeh 2011). Today, rosemary is widely used in dairy foods to improve several qualities, including antioxidant properties (Marinho *et al.* 2015; Cutrim and Cortez 2018). Rosemary essential oil (REO) is an almost colourless or pale yellow liquid that possesses the characteristic odour of the plant. Due to its major constituents, 1,8-cineole, trans-caryophyllene, camphor, and α-pinene and to the synergy among these, REO can be considered a natural compound with strong antioxidant capacity (Tavassoli and Djomeh 2011). The aim of this study was to evaluate the phenolic content and antioxidant activity of kefir made from donkey milk and fortified with sulla honey and REO during refrigerated storage.

MATERIALS AND METHODS

Samples

Donkey milk (Martina Franca breed) provided by a farm situated in the Basilicata region (Southern Italy) was used in this study. Proximate analysis of donkey milk showed the following composition (g/100 g): 9.16 dry matter, 0.44 fat, 1.43 total protein (N × 6.38), 0.18 non-nitrogen protein (N × 6.38), 0.45 ash, and 7.02 lactose. The pH of the milk was 7.03. The sulla honey sample was collected directly from beekeepers and stored at 4 °C in the dark until analysed. The honey purity was carefully checked by pollen analysis and the predominant pollen type was *Hedysarum* spp. (frequency ≥50%). The REO (*R. officinalis* L.; Ravetllat Aromatics, Barcelona, Spain) was obtained by steam distillation of the entire plant. The REO density was 0.909 g/mL at 20 °C.

Folin–Ciocalteu (FC) reducing capacity and antioxidant activity of the honey and REO samples

The sample preparation and the FC reducing capacity determination were performed as described by Perna *et al.* (2012) for the sulla honey samples, and Proestos *et al.* (2013) for the REO samples. Each assay was carried out in triplicate and the results were expressed as mg of gallic acid equivalent (GAE) per 100 g of sample. The antioxidant activity of the sulla honey and REO samples was determined by 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and ferric-reducing antioxidant power (FRAP) assays, as reported by Perna *et al.* (2012). Each determination was made in triplicate and the results for both assays were expressed as mg of trolox equivalent (TE) per 100 g of sample.

Kefir manufacture

Three sets of kefir were produced with donkey milk: kefir fortified with sulla honey (HK), kefir fortified with REO (RK), and kefir without any addition (K). After being heat treated at 95 °C for 15 min in a temperature-controlled water bath followed by cooling to 45 °C, the milk sample (45 L) was inoculated with 1% (w/v) of kefir grains (Bionova, Piacenza, Italy). Fermentation was carried out at 25 °C for 24 h. At the end of the fermentation process, the grains were separated by filtration through a sieve, and the kefir beverage was divided into three equal aliquots. The first aliquot was maintained as a control, without any addition. To the second aliquot was added 30% (w/v) of sulla honey, the maximum concentration permitted by Italian legislation (http://www.foodinprogress.com/wp-content/uploads/2016/04/circolare_min_san_9_3_febbraio_1986.pdf), and to the third aliquot was added 0.15% (w/v) of REO. Preliminary tests had shown that higher concentrations of REO affected the viability of the microorganisms present (data not shown). Sulla honey and REO were incorporated by mechanical stirring; consequently, the product was a stirred type kefir. Finally, the samples were distributed in 500 mL plastic containers, cooled at 4 °C and stored in a refrigerator. All samples were analysed at 1, 9, and 15 days of refrigerated storage because in a preliminary study (data not shown) we observed that for up to 15 days of refrigerated storage no statistically significant differences were detected.

Microbiological analysis

The K, HK, and RK samples (10 mL) were dispersed with 90 mL of a sterile solution of 0.2% (w/v) sodium thiosulfate and homogenised for 1 min in a stomacher (Seward Medical, London, UK). Further decimal dilutions were made adding in 90 mL of 0.1% (w/v) sterile peptone water 10 mL of the previous dilution (Oxoid; Unipath Ltd, Basingstoke, UK), according to the International Dairy Federation (IDF) standard method (IDF 1992). Kefir samples, after 1, 9, and 15 days of storage, were tested for three different types of potential probiotic strains: lactobacilli, lactococci, and yeasts. The entire experiment was repeated in triplicate. Lactobacilli counts were determined on de Man

Rogosa Sharpe (MRS) agar (Merck, Darmstadt, Germany) after incubation at 30 °C for 5 days under anaerobic conditions (García Fontán *et al.* 2006). Lactococci counts were enumerated on M17 Agar (Merck, Darmstadt, Germany) after incubation at 30 °C for 3 days under anaerobic conditions (Irigoyen *et al.* 2005). In both MRS and M17 plates, 200 mg/L of cycloheximide (Acros, Geel, Belgium) was added to prevent the growth of yeasts (Chen *et al.* 2008). Yeasts were enumerated on yeast extract glucose chloramphenicol agar (Sigma, St. Louis, MO, USA) after incubation at 25 °C for 5 days under aerobic conditions (Gronnevik *et al.* 2011). Afterwards, plates with 25–250 colonies were enumerated and recorded as colony forming units per mL of kefir.

Preparation of water-soluble extracts of kefir for FC reducing capacity and antioxidant activity

Water-soluble extracts of kefir samples were prepared as described by Perna *et al.* (2015) and kept at −55 °C until analysis.

Folin–Ciocalteu reducing capacity and antioxidant activity of kefir samples

The FC reducing capacity was determined for the K, HK, and RK samples as described by Sahin *et al.* (2013). Each determination was carried out in triplicate and the results were expressed as mg of GAE per 100 g kefir. The antioxidant capacity of kefir samples was determined by ABTS radical scavenging and FRAP assays, as described by Perna *et al.* (2014). Each determination and measurement was made in triplicate and the results of both assays were expressed as mg of TE per 100 g kefir. The number of free thiol groups was determined according to Ellman's method (1959), with some modifications, as reported by Perna *et al.* (2015). Each determination and measurement was made in triplicate and the results were expressed as a concentration (μM) of thiol groups (SH).

Consumer acceptability

An sensory method was used to evaluate consumer acceptability. Sensory analysis consisted of 322 untrained consumers who had been selected based on their regular consumption of fermented beverages as well as their sex (176 females and 146 males) and age (between 21 and 60 years of age), attempting to represent the distribution of the population as closely as possible. The test was conducted as described by Perna *et al.* (2015), in a sensory laboratory equipped in accordance with UNI-ISO 8589 recommendations (ISO 1988).

Statistical analysis

Data were analysed by analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software (SAS Institute 1996):

$$y_{ijk} = \mu + \alpha_i + \beta_{ij} + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the observation; μ is the overall mean; α_i is the fixed effect of the ith kefir (i = 1, 2, 3); β_j is the fixed effect of the jth storage time (j = 1, 2, 3); $(\alpha\beta)_{ij}$ is the interaction of kefir × storage time; and ε_{ijk} is the random error. A mono-factorial model was used for sensory analysis:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (2)$$

where y_{ij} is the observation; μ is the overall mean; α_i is the fixed effect of the ith kefir (i = 1, 2, 3); and ε_{ij} is the random error. Before setting the values, expressed in percentage terms, they were subjected to angular transformation. Student's test was used to compare all the variables. Results are presented as mean ± standard deviation (SD). Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSION

Folin–Ciocalteu reducing capacity and antioxidant activity of honey and REO samples

The FC reducing capacity, ABTS, and FRAP values of the added flavouring substances are reported in Table 1. Folin–Ciocalteu reducing capacity was found to a noticeable degree for REO, around three times more than that found for sulla honey (23.96 and 7.23 mg GAE/100 g, respectively). Closely related to the polyphenol content, the antioxidant activity, evaluated using both assays, was higher for REO than for sulla honey. Bozin *et al.* (2007) reported that the main constituents of REO [hydrocarbons limonene (21.7%), R-pinene (13.5%), oxygenated monoterpenes camphor (21.6%) and Z-linalool oxide (10.8%)] have a strong effect against hydroxyl radicals.

Microbial enumeration of kefir

Kefir may contain a rich number of viable potential probiotic strains useful for enhancing health (Guzel-Seydim *et al.* 2011). Lactobacilli, lactococci, and yeast in K, HK, and RK were investigated at 1, 9, and 15 days of cold storage (Table 2). Overall, the viable counts of detected microorganisms were in line with those found by Öner *et al.* (2010) in cow, ewe, and goat milk kefir. No significant differences were detected among the three kefir types at any of these storage times. Moreover, a small nonsignificant decrease in the viable counts of all microorganisms was detected in all kefir samples during storage ($P > 0.05$). These findings were consistent with Öner *et al.* (2010) who reported that in cow kefir, the microbial counts remained stable during the first 15 days of storage. Furthermore, the microorganisms enumerated in all kefir samples were in agreement with the specifications of FAO/WHO (2003).

Table 1 Folin–Ciocalteu reducing capacity[a] and antioxidant activity[b] of sulla honey and rosemary essential oil (REO).

	Sulla honey		*REO*	
	Mean	*SD*	*Mean*	*SD*
Folin–Ciocalteu reducing capacity	7.23	0.11	23.96	1.02
ABTS	29.33	1.92	58.12	2.01
FRAP	9.87	0.26	13.51	1.33

Values are means ± SD (standard deviation). [a]Values expressed as mg of gallic acid equivalents per 100 g sample. [b]ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (values expressed as mg of trolox equivalents (TE) per 100 g sample); FRAP, ferric-reducing antioxidant power assay (values expressed as mg of TE per 100 g sample).

Table 2 Viable counts (log cfu/mL) of microrganisms in kefir during storage.

	K		*HK*		*RK*	
Storage, days	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Lactobacilli						
1	10.39	0.41	10.08	0.39	10.41	0.62
9	10.24	0.23	9.89	0.42	10.32	0.38
15	10.07	0.34	9.62	0.28	9.94	0.51
Lactococci						
1	9.72	0.68	9.56	0.57	9.75	0.66
9	9.58	0.53	9.25	0.43	9.45	0.36
15	9.13	0.39	8.98	0.51	9.07	0.53
Yeasts						
1	7.28	0.35	7.56	0.68	7.25	0.62
9	7.21	0.23	7.44	0.52	7.14	0.39
15	6.96	0.32	6.78	0.61	6.57	0.58

HK, kefir fortified with sulla honey; K, kefir; RK, kefir fortified with rosemary essential oil; SD, standard deviation.

Folin–Ciocalteu reducing capacity of kefir

The FC reducing capacity of donkey kefir without (K) and with (HK and RK) added flavourings during storage are shown in Table 3. It is known that the phenolic content of milk is associated with pasture consumption (Butler *et al.* 2008); in particular, it is strongly linked to the composition of the diet and the length of the grazing period (Fraisse *et al.* 2007). At the start of storage (day 1), donkey kefir (K) showed a FC reducing capacity of 4.96 mg GAE/100 g, while HK and RK showed a higher FC reducing capacity compared to the control ($P < 0.05$). This finding suggested that the addition of rosemary oil and sulla honey increased the FC reducing capacity of kefir, in line with findings reported by many authors (Karnopp *et al.* 2017; Ramos *et al.* 2017; Helal and Tagliazucchi 2018) who showed that the addition of natural herbal extract to fermented milk increased the phenolic content of these dairy products.

Table 3 Folin–Ciocalteu reducing capacity[1] of donkey kefir without (K) and with added sulla honey (HK) and rosemary essential oil (RK) during storage at 4 °C for up to 15 days.

	Folin–Ciocalteu reducing capacity					
	K		*HK*		*RK*	
Storage, days	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
1	$4.96^{A,a}$	0.30	$5.91^{A,b}$	0.28	$7.28^{A,c}$	0.35
9	$5.93^{B,a}$	0.36	$7.10^{B,b}$	0.29	$8.07^{B,c}$	0.23
15	$6.81^{C,a}$	0.28	$7.98^{B,b}$	0.21	$8.95^{C,c}$	0.22

SD, standard deviation. Different uppercase superscripts (A–C) depict the statistical difference within a column ($P < 0.05$). Different lowercase superscripts (a–c) depict the statistical difference within a row ($P < 0.05$). [1]Values expressed as mg of gallic acid equivalents per 100 g kefir.

Furthermore, RK showed a higher capacity than HK (7.28 vs 5.91 mg GAE/100 g; $P < 0.05$). During refrigerated storage, the FC reducing capacity increased in all kefir samples (Table 3). This is consistent with findings reported by other authors (Shori 2013; Yilmaz-Ersan *et al.* 2016) who attributed the proteolytic activity of the microorganisms to the breakage of the protein-polyphenol complexes and thus the release of polyphenols.

Antioxidant capacity of kefir

The total antioxidant capacity of K, HK, and RK during storage at 4 °C for 1, 9, and 15 days was evaluated using three different tests (ABTS, FRAP and Thiol assays) in order to better determine the antioxidant behaviour of kefir types (Table 4). Overall, plain kefir showed antioxidant activity. The antioxidant capacity of this fermented product is due to the presence of many bioactive peptides of low molecular weight (≤5000 kDa) released from milk proteins through the proteolytic activity of lactic acid bacteria and yeasts (Edward 2005). Piovesana *et al.* (2015) observed that bioactive peptides and amino acids of donkey milk derive mainly from the enzymatic degradation of αs1- and β-casein, while the contribution of αS2-casein, β-lactoglobulin, k-casein, and of other whey proteins is less. Also, during fermentation, some antioxidant components present in the kefir grains were transferred to the milk (Liu *et al.* 2005). At the start of storage (day 1), the fortified kefir exhibited significantly higher antioxidant activity than plain K, in all the assays. The addition of flavouring resulted in an increase of polyphenol content, which is closely related to antioxidant activity (Perna *et al.* 2012). This finding is in line with those reported by several authors (Najgebauer-Lejko *et al.* 2011; Chouchouli *et al.* 2013; Frumento *et al.* 2013; Karnopp *et al.* 2017; Ramos *et al.* 2017; Helal and Tagliazucchi 2018) who highlighted a positive correlation between fermented products fortified with vegetables and antioxidant activity. Significant differences in the

Table 4 Antioxidant activity[1] of donkey kefir without (K) and with added sulla honey (HK) and rosemary essential oil (RK) during storage at 4 °C for up to 15 days.

	ABTS						*FRAP*						*THIOLS*					
	K		*HK*		*RK*		*K*		*HK*		*RK*		*K*		*HK*		*RK*	
Storage, days	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
1	$14.98^{A,a}$	1.34	$16.61^{A,a}$	0.84	$24.56^{A,b}$	0.86	$4.24^{A,a}$	0.17	$7.75^{A,b}$	0.12	$5.89^{A,c}$	0.19	$497.72^{A,a}$	3.97	$288.88^{A,b}$	6.04	$307.69^{A,c}$	6.48
9	$23.75^{B,a}$	1.05	$19.34^{B,b}$	0.47	$26.48^{B,c}$	0.57	$5.06^{B,a}$	0.13	$8.42^{B,b}$	0.11	$5.94^{A,c}$	0.11	$657.71^{B,a}$	4.25	$307.35^{B,b}$	8.17	$326.11^{B,c}$	5.57
15	$25.47^{C,a}$	0.92	$25.78^{C,a}$	0.48	$28.35^{C,b}$	0.59	$6.14^{C,a}$	0.23	$9.81^{C,b}$	0.09	$6.16^{B,a}$	0.23	$691.14^{C,a}$	5.33	$317.78^{C,b}$	5.19	$337.72^{B,c}$	8.84

SD, standard deviation. For each parameter, different uppercase superscripts (A–C) depict the statistical difference within a column ($P < 0.05$). For each parameter, different lowercase superscripts (a–c) depict the statistical difference within a row ($P < 0.05$). [1]ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (values expressed as mg of trolox equivalents (TE) per 100 g kefir); FRAP, ferric-reducing antioxidant power assay (values expressed as milligrams of TE per 100 g kefir); Thiols, thiol assay [values expressed as μM of thiol groups (SH)].

antioxidant activity were found among the kefir samples as a function of the type of added flavouring ($P < 0.05$; Table 4). Considering the percentage change of antioxidant activity of fortified kefirs compared to the control sample (K; Figure 1), the ABTS value increased by about 64% and 11% in RK and HK, respectively. For the FRAP assay, the situation was reversed: HK showed an increase of more than 80%, whereas in RK the FRAP value increased by about 39%. The observed difference in results between the two assay types is related to the fact that the ABTS assay highlights the activity of both hydrophilic and lipophilic antioxidants, whereas the FRAP assay uses reductants in a redox-linked colorimetric method using an easily reduced oxidant in stoichiometric excess (Perna *et al.* 2012). Moreover, it is important to specify that the binding affinity of polyphenols for milk proteins and peptides depends on amino acid composition and phenol types (Prigent *et al.* 2003). Consequently, the different polyphenols in honey and REO may react differently with the milk proteins or peptides, and this could influence the bioactivity and bioavailability of these compounds.

The antioxidant activity increased significantly during refrigerated storage ($P < 0.05$), showing the highest values at 15 days, for all assays (Table 4). Park *et al.* (2016) showed that the *in vitro* antioxidant activity of Greek-style yoghurt fortified with 1% (v/w) of ethanolic stevia leaf extracts increased during refrigerated storage. In this study, the ABTS radical scavenging activity at 15 days of refrigerated storage increased by about 70%, 55%, and 15% from the initial value, in K, HK, and RK, respectively. Comparing the kefir types, RK showed the highest ABTS values in all considered storage times ($P < 0.05$), whereas HK showed similar values to the control. In fact, no significant differences were detected between HK and K at 1 and 15 days, while at 9 days the ABTS value was lower in HK ($P < 0.05$). With regard to the FRAP assay, at the end of storage, the increase was about 45%, 27%, and 5% of the initial value, in K, HK, and RK, respectively. Comparing the kefir samples, HK showed the highest values at all storage times tested ($P < 0.05$). The differences among the studied kefirs could be partly due to the different release of polyphenols from the protein-polyphenol complex during storage. Also, during refrigerated storage, the increase of ABTS and FRAP values may be attributed to the metabolism of the microorganisms of the kefir grains that, even at low temperatures, in fortified kefirs, may have modified some phenolic compounds and hence their antioxidant activities (Blum 1998). However, our results are in disagreement with Karaaslan *et al.* (2011) who, in fortified yoghurt with grape and callus extracts, observed a decrease in antioxidant activity, measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, during storage. During refrigerated storage the thiol content increased by about 39% in K, and 10% in HK and RK from the initial value (Table 4). Interestingly, it was

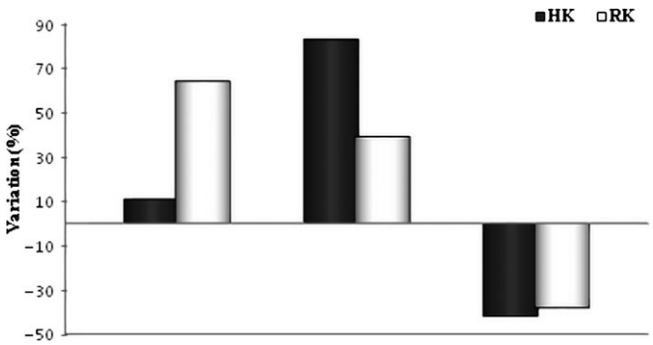

Figure 1 Percentage change of antioxidant activity[1] at 1 day of refrigerated storage of donkey kefir fortified with sulla honey (HK) and rosemary essential oil (RK) from the control sample. [1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (values expressed as milligrams of trolox equivalents per 100 g kefir); FRAP = ferric-reducing antioxidant power assay (values expressed as milligrams of trolox equivalents per 100 g kefir); Thiols = thiol assay [values expressed as μM of thiol groups (SH)].

found that the addition of flavourings to the donkey kefir resulted in a relevant reduction of antioxidant capacity assessed by thiol assay, contrary to what was observed for the other assays. In fact, K showed the highest values in all considered storage times compared with the fortified kefirs ($P < 0.05$). In particular, the addition of sulla honey to kefir caused a higher reduction of SH content compared with that detected in kefir with REO ($P < 0.05$). This different behaviour could be due to the presence of polyphenol compounds in sulla honey, such as gallic acid (Gambacorta *et al.* 2014), which showed a greater affinity for sulphur peptides forming covalent bonds through a mechanism mediated by quinine including the thiol group (Hassan *et al.* 2013), thus resulting in a masking effect of the total antioxidant capacity. In support of this, Gallo *et al.* (2013) observed that, for whey proteins, the formation of the protein–polyphenol complexes occurs through a covalent binding of SH-free group of the free cysteine residue of the protein, while noncovalent interactions take place between casein and polyphenols.

Consumer acceptability

Sensory analysis based on consumer perception is generally used in the initial stage of development of complex food matrices. In this study, the hedonic test showed that the fortification of kefir with sulla honey and REO had a significant effect on all sensory properties ($P < 0.05$; Table 5) except for appearance, where no difference between kefir samples was detected. In particular, both HK and RK showed a lower colour score than traditional kefir. The addition of flavourings to donkey kefir led to a colour change from white to a slightly straw yellow. Consequently, the lower colour score for HK and RK can be explained by the fact that consumers are used to eating kefir that is a bright white colour. The honey-fortified kefir showed significantly higher scores for taste and overall acceptability than K and RK ($P < 0.05$); this could be due to the ability of the honey to decrease the sourness and improve the flavour profile of the product (National Honey Board 2011). Meanwhile, RK showed the lowest scores for odour, taste and overall acceptability ($P < 0.05$), indicating that the addition of REO has a negative effect on consumer acceptability. This is consistent with the results obtained by Olmedo *et al.* (2013), who reported that in cheese prepared with a cream cheese base using REO, essential oil supplements increased the bitterness and sourness. From an industrial perspective, further studies should be performed using panels of people trained to optimise the sensory profile and consumers to increase the probability of acceptance of the kefir studied.

Table 5 Sensory acceptability[1] of donkey kefir without (K) and with added sulla honey (HK) and rosemary essential oil (RK).

	K		*HK*		*RK*	
Descriptor	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Overall acceptability	7.03[b]	0.76	7.91[a]	0.84	5.29[c]	0.89
Appearance	7.16[a]	0.91	7.03[a]	0.93	6.89[a]	0.86
Colour	7.43[a]	0.71	6.54[b]	0.63	6.47[b]	0.77
Odour	6.93[a]	1.03	7.02[a]	0.93	4.79[b]	0.86
Taste	6.25[b]	0.81	7.57[a]	1.12	5.07[c]	0.91

SD, standard deviation. Different lowercase superscripts (a–c) depict the statistical difference within a row ($P < 0.05$) between means for different kefir batches. [1]Each attribute was evaluated on a hedonic scale from 1 (dislike extremely) to 9 (like extremely).

CONCLUSIONS

Donkey kefir has a high antioxidant potential that interacts with a wide range of species directly responsible for oxidative damage. This study highlighted the fact that the antioxidant activity of donkey kefir was strongly influenced by both the type of added polyphenols and the formation of polyphenol–protein complexes, which influence the availability of bioactive components. Sensory analysis demonstrated that the donkey kefir was well accepted by consumers; also, that kefir fortified with sulla honey showed the highest acceptability, whereas the one fortified with REO was less acceptable to consumers. This knowledge forms a basis that could lead to the production of fortified donkey kefir with specific nutraceutical characteristics.

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