

Microbiological, physicochemical, and sensory characteristics of kefir during storage

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Abstract

Changes in certain microbiological, physicochemical, and sensory parameters of kefir were studied during refrigerated storage. Kefir batches were prepared using 1% and 5% added kefir grains, and samples for analysis were taken 24 h after inoculation and then after 2, 7, 14, 21, and 28 days of storage at 5 ± 1 °C. After fermentation for 24 h after inoculation, lactobacilli and lactococci were present at levels of 10^8 cfu/ml, and yeasts and acetic acid bacteria were present at levels of 10^5 and 10^6 cfu/ml, respectively. The lactic acid flora decreased by about 1.5 log units between days 7 and 14 and then stabilized at that level. Yeast and acetic acid bacterial counts, lactose, and pH all remained constant over the storage period, while the total fat content and dry matter decreased. The percentage inoculate did exert an influence, and the sample batches made using 1% added kefir grains had higher lactic acid bacterial counts, lactose, and pH, while the sample batches made using 5% added kefir grains had higher yeast and acetic acid bacterial counts and viscosity. The total fat and dry matter contents were similar in both sample batches. Sensory analysis of the kefir samples revealed maximum acceptability levels in the first 2 days of storage.

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1. Introduction

Kefir is a fermented milk product that has its origin in the Caucasian mountains, Tibet or Mongolia, many centuries ago. The Caucasian people discovered that the fresh milk carried in leather pouches would occasionally ferment into an effervescent beverage (Duitschaever, Kemp, & Emmons, 1987). In their countries the kefir until now has been produced primarily from sheep milk, whereas in Europe its production on a commercial scale is limited basically to cow milk (Wójtowski, Danków, Skrzyper, & Fahr, 2003).

The benefits of consuming kefir in the diet are numerous, as it is reported to possess the antibacterial (Zacconi, Parisi, Sarra, Dalvalle, & Botazzi, 1995), immunological (Furukawa, Matsuoka, & Yamanaka, 1990), antitumoral (Furukawa, Matsuoka, Takahashi,

& Yamanaka, 1991) and hypocholesterolemic effects (Tamai, Yoshimitsu, Watanabe, Kuwabara, & Nagai, 1996).

Kefir is the product of the fermentation of milk with kefir grains and mother cultures prepared from grains. Kefir grains are irregularly shaped, gelatinous masses varying in size from 1 to 6 mm in diameter. These grains contain lactic acid bacteria (lactobacilli, lactococci, leuconostocs), acetic acid bacteria and yeast mixture coupled together with casein and complex sugars by a matrix of polysaccharide. Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide. Kefir grains usually contain lactose-fermenting yeast (*Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Torula kefir*), as well as nonlactose-fermenting yeasts (*Saccharomyces cerevisiae*) (Angulo, López, & Lema, 1993). The principal polysaccharide is a water soluble substance known as 'kefiran'. Several homofermentative lactobacillus species including *Lb. kefiranofaciens* and *Lb. kefir* (Toba, Arihara, & Adachi, 1987) produce this polysaccharide.

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The major end products of the fermentation are lactic acid, acetaldehyde, acetoin, diacetyl, ethanol and CO₂ (Güzel-Seydim, Seydim, Grenee, & Bodine, 2000). Moreover during the fermentation, vitamin B₁, B₁₂, calcium, amino acids, folic acid and vitamin K, increase in the kefir (Ötles & Cadingi, 2003). Kefir can be made from any kind of milk (cow, goat, sheep, camel, buffalo) and has the following characteristics: pH about 4.0; alcohol from 0.5% to 2%; fat content depends on the type of milk used; the taste is acid, prickly and slightly yeasty. The sharp acid and yeasty flavour, together with the prickly sensation contributed by the carbon dioxide produced by the yeast flora can be considered as the typical kefir flavour.

The microorganisms present in the kefir grains, the chemical attributes of the milk employed, and the manufacturing technology are all factors that influence the microbiological, physicochemical and sensory characteristics of kefir during the storage (Koroleva, 1988, Chap. 2).

The object of the present study was to assess the microbiological, physicochemical, and sensory attributes of refrigerated samples of kefir manufactured using two different proportions of added kefir granules, that is, kefir grain inoculate, during storage.

2. Materials and methods

2.1. Production of kefir

Kefir grains were obtained from a private household in Navarre (Spain). They were washed with distilled water and inoculated in full fat UHT cow's milk (3.6% fat content). After each elaboration process, the grains were separated from the fermented milk by filtering them through a sieve, and then washed for later use. Whilst the grains were not being used, they were preserved in milk at 4 °C.

Two batches were made by adding an inoculate consisting of 1% or 5% (w/w) kefir grains. After incubation at 25 °C for 24 h, the grains were separated from the fermented milk by filtration through a plastic sieve and washed prior to the next culture incubation. Samples were taken into propylene boats and were analyzed for 24 h following inoculation and after storage at 5 ± 1 °C for 2, 7, 14, 21, and 28 days. Two replications of all batches, samples, and analyses were performed.

2.2. Microbiological analysis

Tryptone water (Difco) at a concentration of 1 g/l was used to prepare the dilutions for the microbiological analyses. Surface seeding was used in all cases. Lactobacilli counts were performed on MRS medium (pH 6.5 ± 0.2) from Difco at an incubation temperature of 30

°C under anaerobic conditions (5% CO₂) for 3 days. Lactococci counts were carried out on M17 medium (pH 7.2 ± 0.2) from Difco at an incubation temperature of 30 °C under anaerobic conditions for 2 days. Cycloheximide (200 mg/l) was added to the two above-mentioned media to inhibit yeast growth. Yeasts and moulds were grown on OGYE medium (pH 7.0 ± 0.2) from Difco with 1% added oxytetracycline at 25 °C under anaerobic conditions for 7 days. Acetic acid bacterial counts were performed on a medium prepared from 5% glucose, 1% yeast extract, and 2% agar (Difco) as described by Guillamon (2000) with added pimarin (100 mg/l) to inhibit yeast growth and penicillin (3 µg/ml) to inhibit lactic acid bacterial growth, with incubation at 25 °C under anaerobic conditions for 2 days.

2.3. Physicochemical analysis

The total fat in the kefir was measured using the method of Röse-Gottlieb according to IDF Standard 1D (IDF, 1996). Dry matter (DM) was determined according to IDF Standard No. 4 (IDF, 1986).

A continuous pH-meter with probes for fermented milks from Hanna Instruments was used to take pH readings. Readings were taken every 10 min for 24 h using the Temperanet programme from AES laboratory.

Determinations of the lactose and D-galactose in the milk and in the kefir were performed by enzymatic methods (IDF, 1991) using the kit from Boehringer Mannheim GmbH Biochemical (cat. no. 176303).

Viscosity measurements were carried out using a coaxial cylinder viscometer (Haake Viscotester VT6/7 R) with a temperature sensor using an R2 spindle at 100 rpm.

Four replications of all physicochemical analyses were carried out for each percentage inoculate of added kefir grains and batch.

2.4. Sensory analysis

All samples, until the product was deemed not acceptable, were evaluated by at least 6 assessors trained in evaluating dairy products. The attributes considered were: odour intensity, milky odour, fermented odour, vegetable odour, mouth odour, viscosity, flavour intensity, dairy taste, sour taste, bitter taste, milky taste, astringency, and acceptability. Each attribute was scored on an increasing scale of from 1 (not present) to 7 (very intense).

2.5. Statistical analysis

Analysis of variance (ANOVA) using 95% confidence intervals was run on each of the physicochemical and microbiological variables to disclose possible differences

among the samples for the two factors “percentage added kefir grains” and “storage time”. All analyses were performed using the SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA).

Correlation analysis was carried out for the different sensory attributes evaluated.

3. Results and discussion

3.1. Microbiological analyses

Fig. 1 depicts the changes in the microorganism populations during storage of the kefir. Until day 2 of storage, lactobacilli counts in the kefir were 10^8 cfu/ml, in agreement with the findings reported by other researchers (Kandler & Kunath, 1983; Kiliç, Uysal, Akbulut, Kavas, & Kesencas, 1999; Motaghi, Mazaheri, Moazami, Farkhondeh, & Goltapeh, 1997; Rea et al., 1996; Rosi & Rossi, 1978), and much higher than the levels of around 10^2 – 10^3 cfu/ml recorded by Koroleva (1982). Lactobacilli levels decreased until around day 14, the greatest decrease of roughly 1.5 log units that took place from days 7 and 14 being statistically significant ($p < 0.05$), and after that the population levelled off and held steady until day 28. This pattern of behaviour was

observed for both sample batches, i.e., made using the 1% and the 5% added kefir grain inoculates. Other studies on kefir have reported increases in lactobacilli counts until day 4, which were then followed by a decline of around 1 log unit (Kiliç et al., 1999; Wszolek, Tamime, Muir, & Barclay, 2001).

Lactococci levels were 10^8 cfu/ml, which agreed with the findings of Rosi and Rossi (1978) and were slightly higher than the levels found by Kiliç et al. (1999). This bacterial group followed the same general pattern as the lactobacilli. In contrast, Kiliç et al. (1999) observed a very high increase in lactococci until day 3 of storage, followed by a drop in lactococci levels.

The yeast population level was 10^5 cfu/ml, in line with the level recorded by Rosi (1978a) and Vayssier (1978) but slightly lower than the level reported by Kiliç et al. (1999). Counts remained virtually constant over the 28 days of storage with no significant differences ($p < 0.05$) in the sample batches made using the 5% added kefir grain inoculate. In the batches made using the 1% added kefir grain inoculate there was a slight decrease, with significant differences between days 14 and 21. Mould population levels were 10^3 cfu/ml on day 14 and thereafter rose progressively to 10^5 cfu/ml.

Initial levels of acetic acid bacteria were 10^6 cfu/ml, slightly higher than the levels reported by Rea et al.

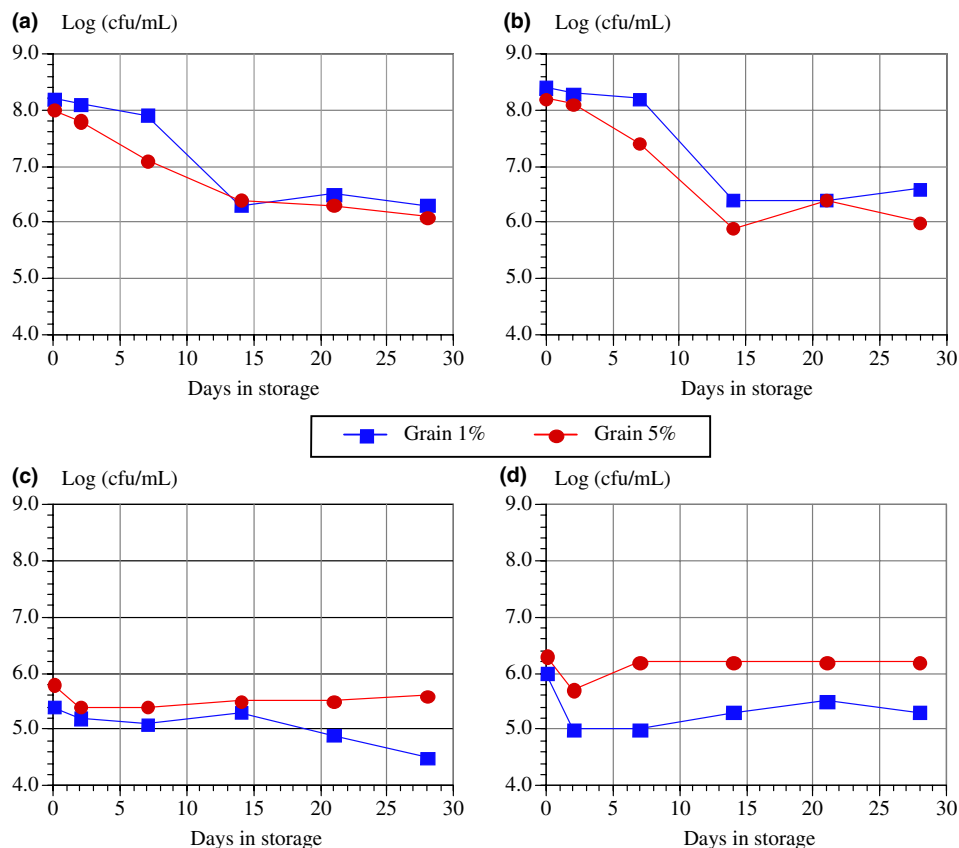


Fig. 1. Counts of A: lactobacilli, B: lactococci, C: yeasts, D: acetic acid bacteria over the storage period.

(1996) and Rosi (1978b). On the other hand, other workers have not recorded these bacteria and have suggested that their presence stems from poor hygiene during manufacture (Angulo et al., 1993; Takizawa et al., 1998). However, the presence of acetic acid bacteria could not be ascribed to poor hygiene in this study based on the manufacturing process used to make the kefir samples employed. In another study Babina and Rozhokova (1973) found that acetic acid bacteria and lactobacilli of kefir grains increased viscosity and thus enhanced the consistency of kefir. Levels of acetic acid bacteria remained nearly constant over the course of storage.

Significant differences ($p < 0.05$) in the counts of the different microorganisms were found in this experiment depending on the percentage of added kefir grains inoculated. Lactococci and lactobacilli levels were higher in the batches made using the 1% inoculate, while yeast and acetic acid bacterial counts were higher in the batches made using the 5% inoculate. According to Koroleva (1988), the number of lactic acid bacteria tended to increase the fewer the kefir grains inoculated into the source milk. Garrote, Abraham, and De Antoni (1998) observed a rapid increase in acidity with a sharp drop in lactococci for an inoculate level of 100 g/l, much higher than the level used in this study. This was probably due to the sensitivity of this bacterial strains to low pH levels. The present findings indicated that levels of yeasts and acetic acid bacteria were directly proportional to the quantity of grains inoculated, whereas levels of lactococci and lactobacilli were inversely proportional and thus higher in the samples made with the smaller percentage inoculate.

3.2. Physicochemical analyses

Table 1 presents the values of the main physicochemical parameters in the kefir samples made using the 1% and 5% kefir grains.

3.3. Fat

After fermentation for 24 h, the kefir samples made using the 1% and 5% inoculates had fat contents of 3.51 and 3.60 g/100 ml, respectively. The fat content of the kefir did not differ significantly ($p > 0.05$) from the fat content of the milk it was made from. This finding was consistent with reports by other researchers (Alm, 1982; Gambelli, Manzi, Panfili, Vivanti, & Pizzoferrato, 1999; Huerta-González & Wilbey, 2001; Walstra & Jenness, 1987), who observed that in macronutritional terms, the nutritional composition of fermented milks, including the fat content, was the same as that of the source milk. In contrast, Ching-Yun and Ching-Wen (1999) observed the fat content of recently cultured kefir to be lower than that of the milk, a difference possibly ascribable to the

Table 1
Changes in physicochemical values in the kefir samples made using different amounts of kefir grain during storage

	1%						5%						<i>p</i>	
	24 h	2 d	7 d	14 d	21 d	28 d	24 h	2 d	7 d	14 d	21 d	28 d		
pH	4.5	4.7	4.6	4.7	4.7	4.7	4.4	4.5	4.6	4.6	4.5	4.5	4.5	ns
Viscosity (mPa s)	425	293	361	288	179	188	501	372	396	348	296	357	357	***
Lactose (%) (w/v)	3.51	3.75	3.70	3.20	n.a.	n.a.	3.41	3.59	3.34	2.94	n.a.	n.a.	n.a.	ns
Fat (%) (w/w)	3.51	3.52	3.39	3.59	3.48	3.23	3.60	3.47	3.41	3.54	3.55	3.48	3.48	*
DM (%) (w/w)	11.7	11.6	11.5	11.5	11.4	11.3	11.7	11.4	11.4	11.4	11.3	11.5	11.5	***

ns: non-significant $p > 0.05$; n.a.: not analysed.

* $p < 0.05$.

*** $p < 0.001$.

lipases produced by the kefir grains during fermentation (Vujicic, Vulic, & Konyves, 1992).

The fat content was observed to undergo a decrease of 7.9% and 3.3%, respectively, by the end of the storage period in the kefir batches made using the 1% and 5% inoculates. This decrease, which is sharper after 14 days of preservation, could be related to the growth of moulds, since those ones are the principal lipolytic agents in fermented milks (Tamime & Deeth, 1980). Formisano (1974) reported that the fat content of yoghurt decreased by 3.4% between culture and day 21 of storage.

The percentage of kefir grains inoculated did not significantly influence the fat content of the samples.

3.4. Dry matter

Dry matter in the kefir after 24 h of fermentation was 11.7 and 11.7 g/100 ml, respectively, in the samples made using the 1% and 5% kefir grain inoculates. These values were similar to those recorded for other fermented milks (Gambelli et al., 1999) and to those reported by Ching-Yun and Ching-Wen (1999) for kefir made using a 5% inoculate. These values were not significantly different from the dry matter content of the source milk. Accordingly, as was the case for the fat content, fermentation did not affect the dry matter content of the source milk used. Ottogalli, Galli, Resmini, and Volonterio (1973) found that the dry matter in recently manufactured kefir differed according to the geographic origin of the granules, with variations in dry matter of between 9.4% and 11.1%.

The dry matter content decreased by 4.2% and 2.0% over the storage period in the samples made using the 1% and 5% inoculates, respectively. Accordingly, as was the case for the fat content the dry matter decreased. This diminution is sharper after 14 days of conservation, which is related with the growth of moulds.

The percentage of kefir grains inoculated did not significantly influence the dry matter in the samples.

3.5. Lactose

Lactose was consumed during the 24 h fermentation period, and lactose levels decreased by 20–25% with respect to the initial lactose levels present in the milk. Levels then held practically constant over the storage period. These results were consistent with those reported by Alm (1982) in a 4% kefir culture, in which lactose levels remained constant over the 16-day storage period in that study. Alm (1982) did not detect galactose in the kefir samples, a finding repeated here in our study. This is because the galactose formed by hydrolysis of the lactose is employed by the kefir microflora to form the polymer kefiran characteristic of kefir, used to make the new granules formed during the fermentation process.

Assadi, Pourahmad, and Moazami (2000) manufactured kefir in the same conditions employed here using a 5% inoculate and reported lactose levels of around 1.4% after 24 h of fermentation, much lower than the levels recorded in the present experiment. Larger decreases in the lactose content brought about by the bacteria in the culture have been observed during the storage of other fermented milk products like yoghurt (Alm, 1982; Katsiari, Voutsinas, & Kondyli, 2002). This one corresponds with a value of pH (around 4) smaller to that observed in our work (around 4.5). The percentage kefir grain inoculate used did influence the lactose content, with a higher lactose level being recorded in the kefir made using the 1% inoculate on all the sampling dates.

3.6. Viscosity

The viscosity (measurement in mPa s) decreased appreciably over the course of storage in both kefir sample batches. This finding differed from the findings for yoghurt made by various researchers, who have reported increased viscosity in yoghurt samples during storage (Abrahamsen & Holmen, 1980; Katsiari et al., 2002; Parnell-Clunies, Kakuda, Mullen, Arnott, & de Man, 1986). The observed viscosity was higher for the kefir batches made using the higher percentage added kefir grain inoculate. Other researchers, e.g., Thompson, Johnston, Murphy, and Collins (1990) and Garrote et al. (1998), have reported similar findings, although the latter reported a decrease in viscosity at very high grain concentrations.

3.7. pH

The pH did not vary significantly during storage. There was a sharp decrease of around 2 pH units during fermentation itself. As already related above, the lactic acid bacterial population declined with time, which is why the kefir did not become more acidic. In addition, the lactose level held practically constant throughout storage. The pH decreases with storage time in other fermented milks like yoghurt (Abrahamsen & Holmen, 1981; Katsiari et al., 2002) because of lactose breakdown by the bacteria in the culture. The pH of the kefir did not vary during the storage, which is possible because at the presence of yeasts. Collar (1996) found that lactic acid bacteria multiply and produce lactic and acetic acids more slowly in mixture with yeasts than in pure culture (Collar, 1996). The percentage kefir grain inoculate added did significantly ($p < 0.05$) affect the pH values, the kefir made using the 1% inoculate having higher pH values. This agrees with the finding reported by Irigoyen, Ortigosa, Torre, and Ibáñez (2003), who recorded significant differences during kefir manufacture according to the percentage kefir grain inoculate added.

3.8. Sensory analysis

Table 2 gives the sensory analysis results for the samples. The study was intended to last until day 28 of storage, but the sensory analysis was terminated on day 14, because the samples developed surface mould, reducing their acceptability to very low levels. Both sample batches had peak acceptability levels in the initial days of storage. Studying two kefir samples over a 5-day storage period, Kiliç et al. (1999) found that the scores of all the sensory attributes decreased significantly with time, and they concluded that kefir kept under refrigeration should be eaten within 3 days of manufacture. In this study the samples made using the 1% added kefir grain inoculate had higher odour intensity and viscosity than the samples made using the 5% inoculate on day 2 of storage. This difference was still to be observed for

odour intensity on day 14, but this was not the case for viscosity. Flavour intensity increased during storage inversely to the acceptability of the product to the panellists. Sample astringency increased with storage time. In contrast, Katsiari et al. (2002) found that storage did not significantly affect the sensory attributes of yoghurt samples. This difference is ascribable to the different floras present in kefir and yoghurt.

The correlations of the different sensory attributes with acceptability indicated that the panel was positively influenced by milky taste, milky odour, and viscosity and negatively influenced by astringency, bitter taste, sour taste, and fermented odour (Fig. 2). Miscellaneous tastes and odours also adversely affected product acceptability. The results for certain attributes agreed with the results reported by Muir, Tamime, and Wszolek (1999), who studied the sensory profiles of various fer-

Table 2

Mean odour, viscosity, taste, and acceptability attribute values in the kefir samples made using different percentage (1% and 5%) added kefir grain inoculates during storage

	1%					5%				
	24 h	2 d	7 d	14 d	<i>p</i>	24 h	2 d	7 d	14 d	<i>p</i>
Odour intensity	3.9	4.6	3.3	4.5	***	3.6	3.3	3.6	3.5	***
Milky odour	3.1	3.8	3.4	3.3	***	3.4	3.1	3.5	3.1	***
Fermented odour	3.7	3.8	3.4	4.0	***	3.3	2.8	3.8	3.4	***
Vegetable odour	1.7	1.9	1.7	2.0	ns	1.8	1.5	1.7	1.9	**
Mouth odour	2.2	2.1	1.9	2.4	**	1.1	1.5	1.7	1.9	**
Misc. odours	1.5	1.7	1.8	2.1	***	1.2	1.6	1.5	2.0	***
Viscosity	4.1	4.3	3.5	3.8	***	3.9	3.6	3.1	4.1	***
Flavour intensity	3.9	4.5	4.0	4.6	***	4.1	4.1	4.6	4.3	***
Bitter taste	2.7	2.1	2.1	3.0	***	2.1	2.2	2.8	2.7	***
Sour taste	3.5	4.0	3.0	4.8	***	3.7	3.5	4.3	4.3	***
Milky taste	3.5	3.8	3.8	3.0	***	4.5	3.7	4.0	2.9	***
Misc. tastes	1.5	1.7	1.5	2.4	***	1.6	1.6	1.6	2.0	**
Astringency	3.1	2.6	2.6	3.2	***	2.8	2.2	3.3	3.4	***
Acceptability	4.0	4.6	3.7	2.9	***	5.0	4.6	3.0	2.5	***

ns: non-significant $p > 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

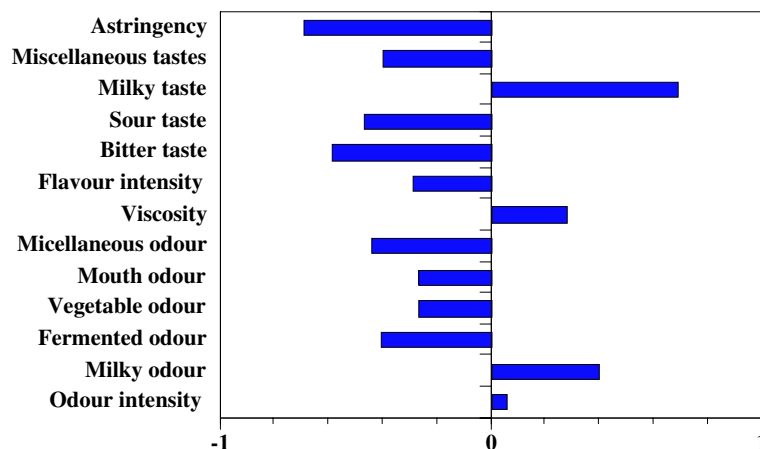


Fig. 2. Correlations of sensory attributes with acceptability.

mented milk products, including kefir. It therefore appears that the panellists preferred kefir with a pronounced milky taste and odour and a certain viscosity level.

4. Conclusions

During refrigerated storage, yeast and acetic acid bacterial counts remained constant, while lactic acid bacteria decreased between 7 and 14 days of storage. Concerning physicochemical analysis, the total fat, lactose, dry matter and pH, remained constant until 14 days of storage.

The sensory analysis in both kefir samples batches revealed the best acceptability level in the first days of storage. Nevertheless, the samples were acceptable until the first week of storage.

The percentage of kefir grains inoculated significantly influenced on viscosity, lactose, pH and microbiological counts, whereas it did not affect total fat and dry matter.

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