TM 2082-1e

Technical Memorandum

Danisco kefir cultures

Kefir grains

INTRODUCTION

Danisco kefir cultures make it possible to produce traditional kefir as it has been known for centuries. Securing exactly the right characteristics to meet the needs and expectations of manufacturers and customers, these unique cultures are based on real kefir grains that have been freeze-dried and supplemented with lactic acid bacteria for an improved flavour and texture. Danisco has also developed HOWARU™ KEFIR 1 BIFIDO, an outstanding blend of the highly documented probiotic strain HOWARU™ Bifido and a kefir culture. This paper describes the technical properties of the kefir cultures and the production of kefir.

BENEFITS OF DANISCO KEFIR CULTURES

- Concentrated product for direct vat inoculation and a shorter production time
- Composition similar to the natural microflora in fresh kefir grains
- Yeast and lactic bacteria content within defined specifications, resulting in uniform final products
- Various options with regard to texture, yeast flavour intensity and fizziness

DANISCO KEFIR CULTURES

The Danisco kefir range comprises direct vat inoculation (DVI) cultures in freeze-dried format (LYO). Kefir DT, Kefir DA, Kefir DG and Kefir DC are available in pouches for inoculation of 500 liter, 1000 liter, 2000 liter and 5000 liter of milk. The shelf life of the cultures is 12 months from the production date when stored at 4°C. HOWARU™ KEFIR 1 BIFIDO is available in a pouch for inoculation of 1000 liter, with a 12-month shelf life at -18°C.

Kefir cultures vary in microbial composition. Kefir DT contains the highest amount of kefir grains and has the highest yeast content. Kefir DC and HOWARU™ KEFIR 1 BIFIDO are the mildest cultures in the range, containing 15% kefir grains (figure 1, table 1).

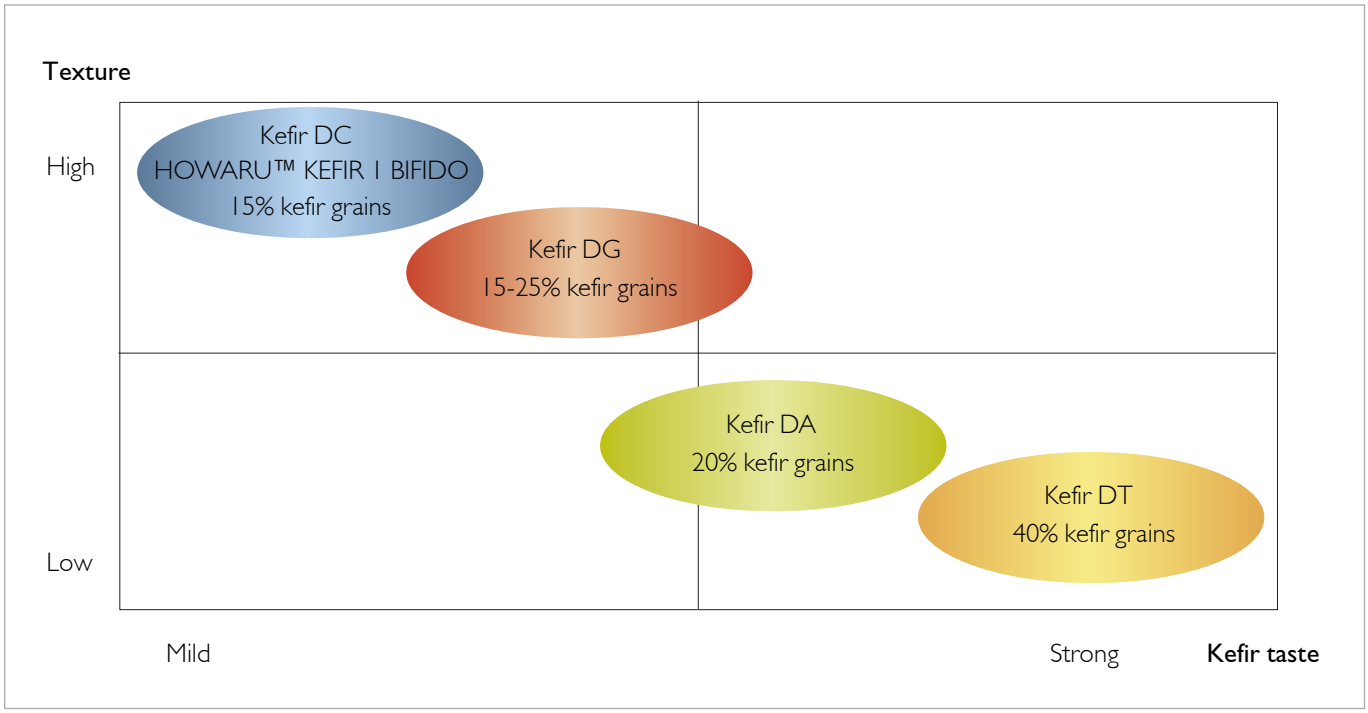

Figure 1. Overview of the kefir culture range.

PRODUCT NAME	COMPOSITION	APPLICATION
Kefir DT	Blend of kefir grain microflora, kefir yeast (10^6-10^7cfu/g) and lactic acid bacteria (10^9-10^{10}cfu/g) mainly isolated from kefir grains	For kefir drinks with a strong taste, low viscosity and fizzy sensation
Kefir DA	Blend of kefir grain microflora, kefir yeast (10^5-10^6cfu/g) and lactic acid bacteria (10^9-10^{10}cfu/g) mainly isolated from kefir grains	For kefir drinks with a mild taste, low viscosity and slightly fizzy sensation
Kefir DG	Blend of kefir grain microflora, kefir yeast (10^5-10^6cfu/g) and lactic acid bacteria (10^9-10^{10}cfu/g) mainly isolated from kefir grains	For kefir drinks with a mild taste, medium viscosity and slightly fizzy sensation
Kefir DC	Blend of kefir grain microflora, yeast (10^5-10^6cfu/g) and lactic acid bacteria (10^9-10^{10}cfu/g) mainly isolated from kefir grains and viscous *Streptococcus thermophilus* strain	For kefir drinks with very mild taste, high viscosity and slightly fizzy sensation
HOWARU™ KEFIR I BIFIDO	Kefir DC culture blended with *Bifidobacterium lactis* HNO19	For probiotic kefir drinks with very mild taste, high viscosity and slightly fizzy sensation *Bifidobacterium lactis* HNO19 benefits the immune system

Table 1. Composition and application of Danisco kefir cultures.

PROCESSING

Kefir is traditionally produced from cow milk but can also be made from sheep, goat or buffalo milk. The product can be manufactured from full-fat, low-fat or skimmed milk using the stirred or set method. The stirred type is more common.

Fermentation is usually at 23-26°C and takes 15 to 18 hours before pH4.6 is reached. A lower fermentation temperature favours the development of yeast and a specific yeasty flavour. At higher temperatures, lactic acid fermentation will dominate. Adjusting the fermentation temperature results in sour milk with a low alcohol content (~0.1% ethanol) or a fizzy, light alcoholic drink (~0.5% ethanol). The product does not obtain its characteristic features until after maturing for at least 24 hours, during which the lactic acid bacteria and yeast produce sufficient concentrations of metabolites to give the final product its typical flavour, taste and texture. The majority of consumers prefer a creamy, smooth texture, but the intensity of the yeasty flavour may vary. Mature kefir contains 0.8-1% lactic acid, 0.04-0.15% acetic acid, up to 0.4% CO_2 and several milligrams/l of diacetyl and acetaldehyde. Other minor volatile compounds include propionaldehyde, 2-butanone, iso-amyl alcohol, acetone and short chain fatty acids. The levels of vitamin B_1, B_2 and folic acid in kefir are higher than in milk. Kefir shelf life is limited due to the complex microflora and, particularly, the yeast content. Changes in flavour during prolonged storage are noticeable as a bitter off-flavour and cheese notes develop. The typical shelf life of kefir is 10-14 days, depending on the selected culture and storage conditions.

CHOOSING THE RIGHT CULTURE

The most relevant characteristics of the kefir culture range relate to acidification speed, post-acidification and the viscosity and sensory properties of the final product. In Danisco's trials, the same fermentation substrate was used throughout while the optimal fermentation temperature was chosen for each culture. Fermentation was stopped at pH4.6 and the kefir stored at 6°C for 28 days. For comparative yeast enumeration, the samples were stored at 4°C and 8°C (table 2). The microbial enumerations were carried out in accordance with the relevant IDF standard. The technical characteristics of HOWARU™ KEFIR I BIFIDO are identical to Kefir DC and are, therefore, not given separately. Confirmation of the survival rate of *Bifidobacterium lactis* in HOWARU™ KEFIR I BIFIDO was achieved by enumeration using the TOS Propionate Agar Method after inoculation on day 1, day 7 and day 14.

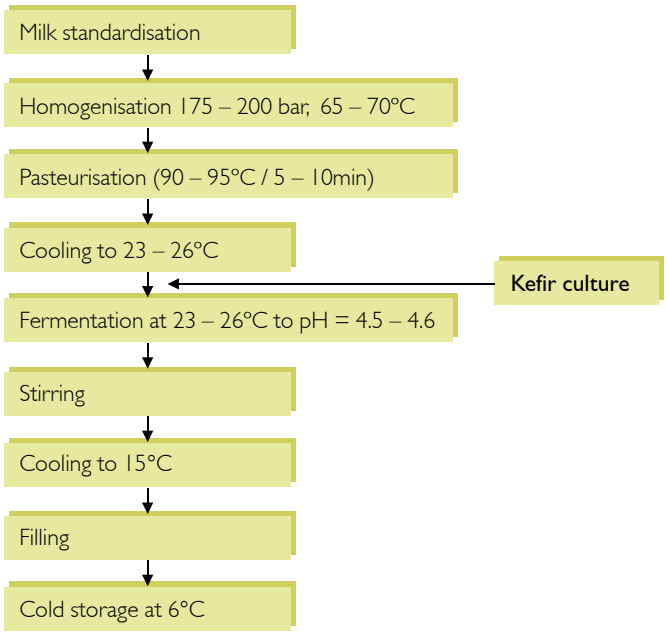

Figure 2. Flow diagram for the production of stirred kefir.

CULTURE	INCUBATION TEMPERATURE, °C	SUBSTRATE	FINAL pH	STORAGE TEMPERATURE, °C
Kefir DT	23	2% fat UHT milk	4.6	4, 6, 8
Kefir DA	23	2% fat UHT milk	4.6	4, 6, 8
Kefir DG	25	2% fat UHT milk	4.6	4, 6, 8
Kefir DC	26	2% fat UHT milk	4.6	4, 6, 8

Table 2. Description of kefir production.

Acidification characteristics

Acidification behaviour is described at the optimum fermentation temperature of the kefir cultures. The fermentation time at optimum temperature is 16-18 hours, depending on the culture and formulation (figure 3 and 4).

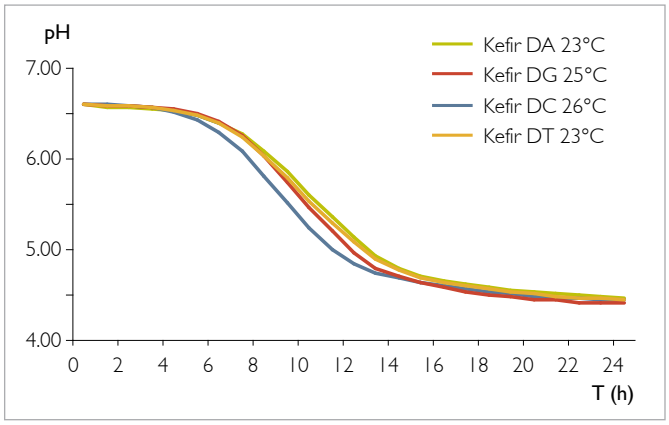

Figure 3. Acidification curves of kefir cultures at optimum temperature.

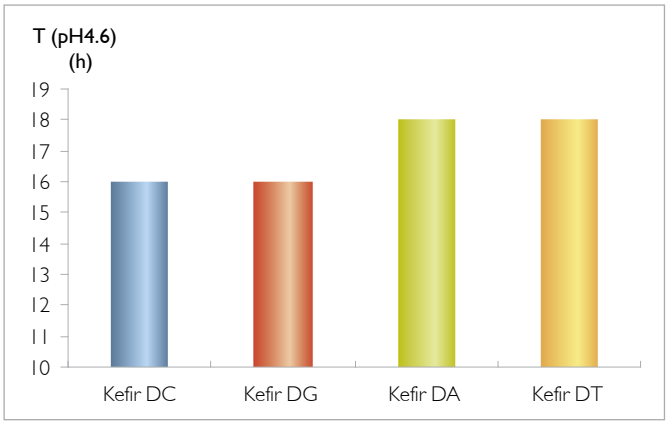

Figure 4. Time taken to reach pH4.6 at optimum fermentation temperature.

Post-acidification

The pH and lactic acid content were measured on day 3, 14, 21 and 28 to evaluate the post-acidification of the kefir range. The kefir samples were stored at 6°C for 28 days.

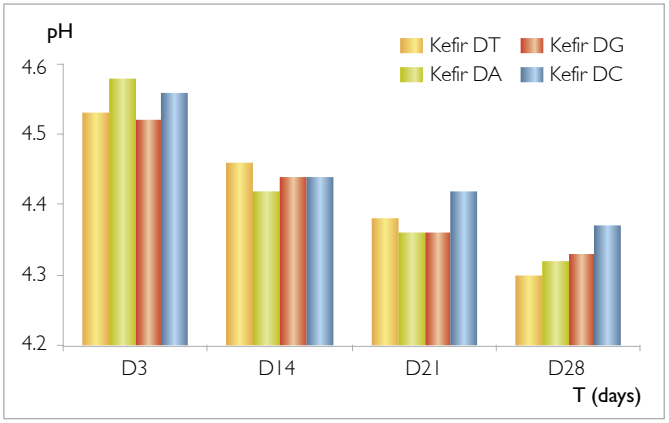

Figure 5. pH changes in kefir stored at 6°C for 28 days.

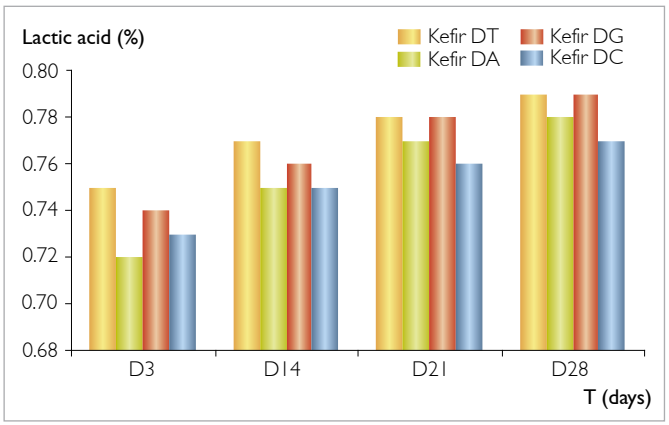

Figure 6. Changes in the lactic acid content of kefir stored at 6°C for 28 days.

Kefir microflora composition

The kefir microflora was analysed on day 14 and 28 after production to illustrate the growth dynamics of microflora in storage. All cultures had a lactic acid bacteria (LAB) count above 10^9cfu/g and a yeast content of 10^4cfu/g after 14 days. The total count of aroma-forming LAB illustrates the content of *Lactococcus lactis* sups. *lactis* var. *diacetylactis*, *Leuconostoc* spp. and *Lactobacillus* spp. in kefir at day 14 and 28 (figure 9). A high content of *Leuconostoc* spp. and *Lactobacillus* spp. is typical of the kefir cultures (figure 10). Yeast content increases and lactic acid microflora decreases during kefir storage.

Kefir DC contains more than 10^8cfu/ml *St. thermophilus*, compared to 10^5cfu/ml in other kefir cultures at day 14 (figure 11). Kefir DT, DA and DC cultures contain more than 10^5cfu/ml *Lb. acidophilus* at day 14, a bacteria generally recognised as being probiotic (figure 12).

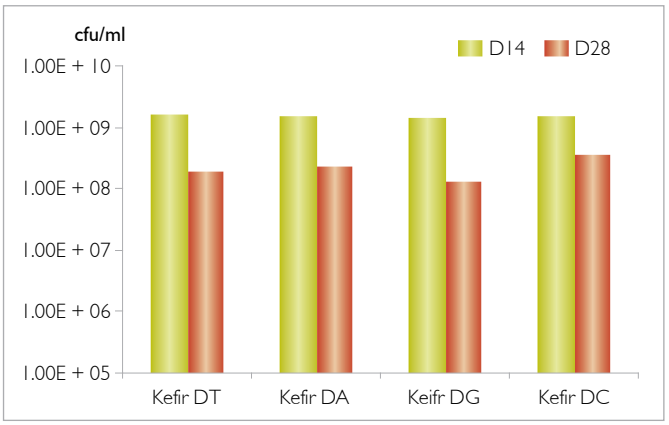

Figure 7. Total microbial count on day 14 and 28 stored at 6°C.

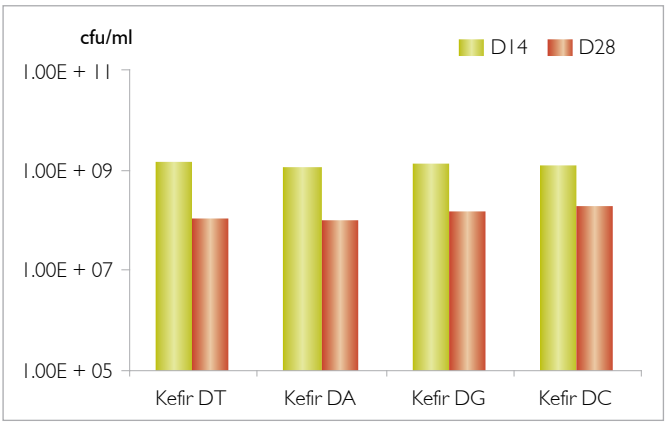

Figure 8. Total LAB count on day 14 and 28 stored at 6°C.

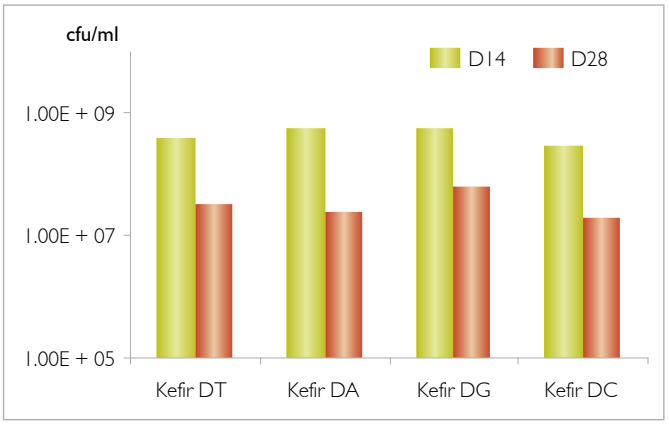

Figure 9. Total count of aroma-forming LAB on day 14 and 28 stored at 6°C.

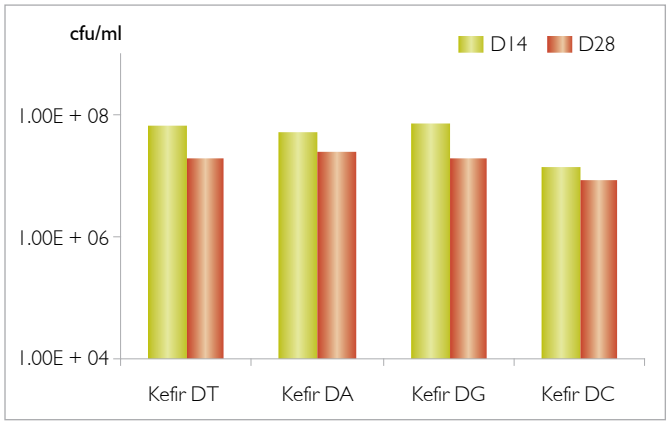

Figure 10. *Leuconostoc* spp and *Lactobacillus* spp count on day 14 and 28 stored at 6°C.

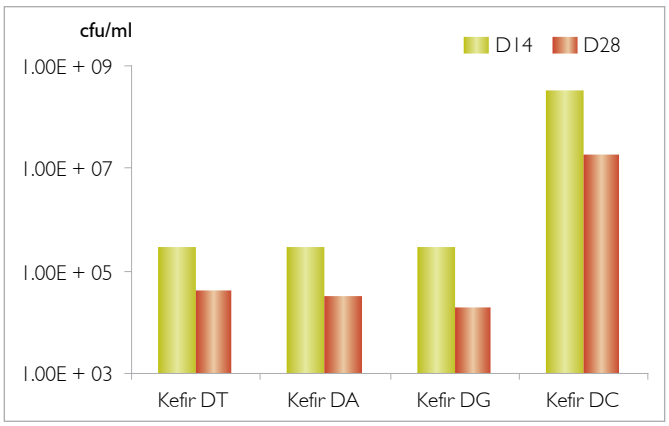

Figure 11. *St. thermophilus* count on day 14 and 28 stored at 6°C.

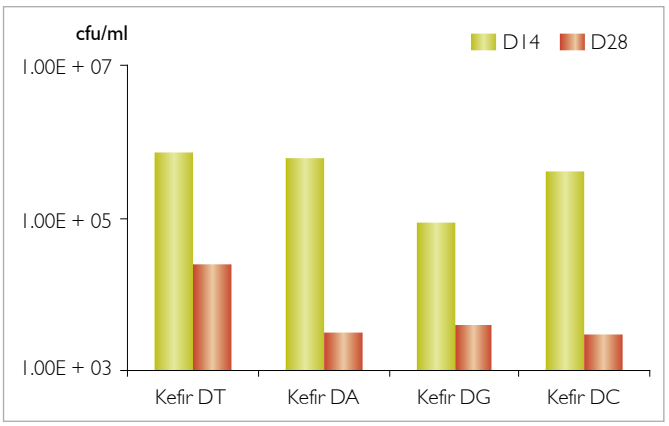

Figure 12. *Lb. acidophilus* count on day 14 and 28 stored at 6°C.

The yeast content of the kefir depends on the storage temperature and initial inoculation rate. Figure 13 and 14 illustrate the effect of varying storage temperatures on yeast growth.

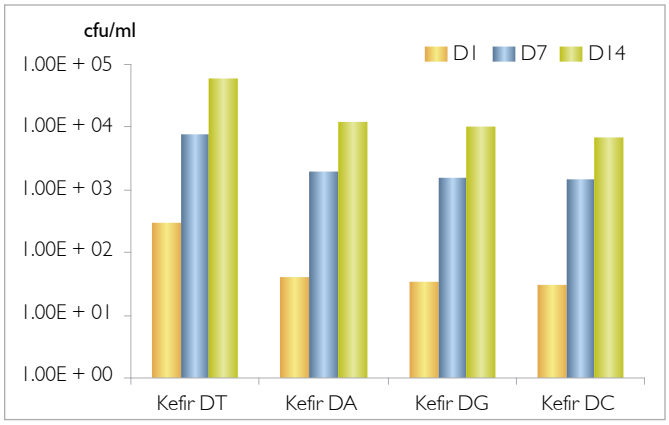

Figure 13. Yeast count on day 1, 7 and 14 stored at 4°C.

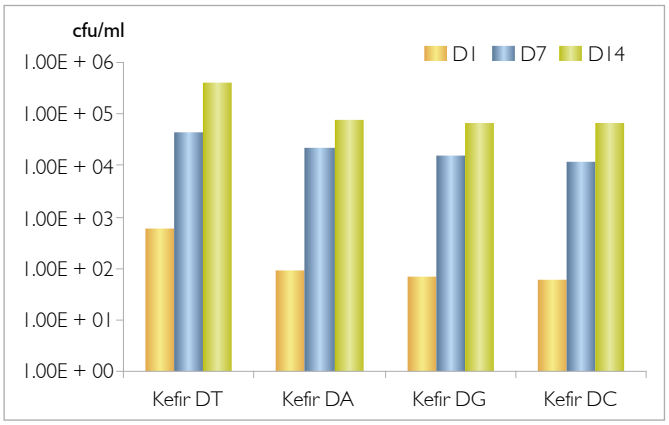

Figure 14. Yeast count on day 1, 7 and 14 stored at 8°C.

HOWARU™ KEFIR 1 BIFIDO increases the value of kefir by providing health benefits. The *Bifidobacterium lactis* HN019 in this culture blend is a highly documented probiotic strain, described in more than 15 clinical studies and recognised for its immune modulating properties. In HOWARU™ KEFIR 1 BIFIDO, the *Bifidobacterium lactis* was enumerated after inoculation on day 1, 7 and 14 to confirm the survival rate. The results are presented in figure 15.

Viscosity

After 14 days of shelf life, the viscosity of the kefir was measured using a Haake Viscotester VTL 5L. The high level of exopolysaccharides produced by the *St. thermophilus* strain of the Kefir DC culture gives kefir a higher viscosity (figure 16).

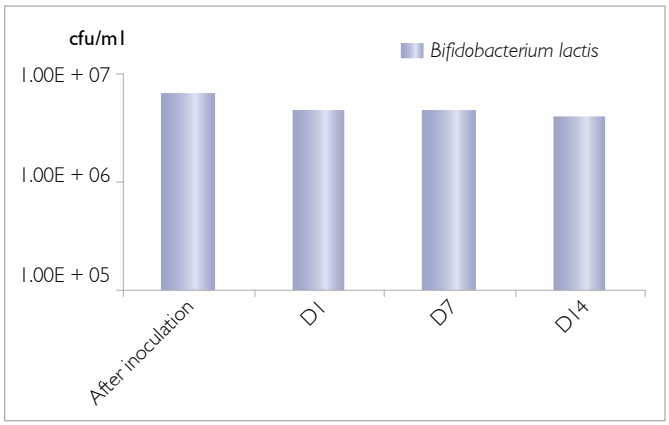

Figure 15. *Bifidobacterium lactis* count stored at 6°C.

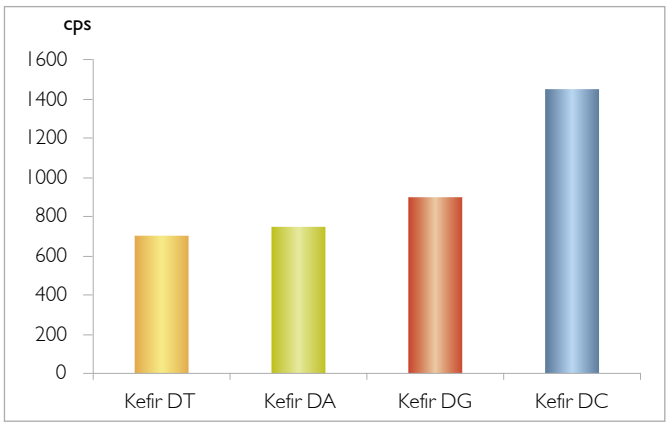

Figure 16. Haake viscosity on day 14.

Flavours – volatile compounds

Kefir contains a high variety of flavour compounds that give the product its distinctive taste. The volatile compounds produced by the culture range have been measured using headspace gas chromatography on kefir that is 14 and 28 days old. Acetaldehyde, ethanol, dimethyl sulfide, propan-1-ol, diacetyl, 2-butanone, ethyl aceteate, isovaldehyde, 2,3-pentanediol, acetoine, 2,3-butanediol were detected. The production of ethanol is specifically related to the metabolism of lactose (+) yeasts. The quantities measured ranged from 30 to 460ppm ethanol, 0.39-0.56ppm acetaldehyde, 1.80-3.47ppm acetone and 0.03-2.10ppm diacetyl. The kefir produced using different cultures varied mainly in ethanol content (figure 17).

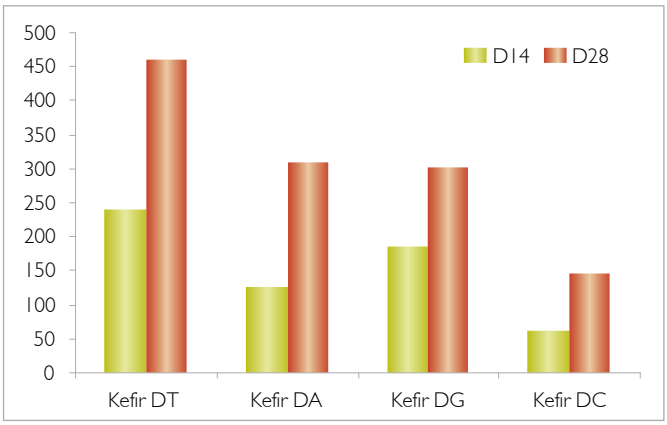

Figure 17. Ethanol content on day 14 and 28 stored at 6°C.

BACTERIOPHAGE BACKUP CULTURES

The bacteriophage backup cultures for Kefir DC and Kefir DG are Kefir DC1 and Kefir DG1 respectively. For HOWARU™ KEFIR 1 BIFIDO, the backup culture is HOWARU™ KEFIR 2 BIFIDO. Bacteriophage backup cultures are available for other kefir cultures on request.

MAIN CHARACTERISTICS

CULTURE	PROCESSING	PRODUCT CHARACTERISTICS			
	Acidification speed	Flavour	Viscosity	Gas	Recommended shelf life of kefir in days
Kefir DT	■	■■■■	■■	■■	10
Kefir DA	■	■■■	■■	■■	14
Kefir DG	■■	■■	■■■	■	14
Kefir DC	■■	■	■■■■	■	14
HOWARU KEFIR 1 BIFIDO	■■	■	■■■■	■	14
■ Low ■■■■ High					

TROUBLESHOOTING

Common defects	Cause	Corrective action
Non-specific taste	• Insufficient growth of yeast and aroma-forming LAB • High fermentation temperature • Bacteriophage attack on aroma-forming LAB	• Reduce fermentation temperature • Use bacteriophage backup culture • Cleaning and disinfection
Increased gas production or foaming	• Intense growth of yeast and aroma-forming LAB • Low fermentation temperature • Contamination of milk with high gas-forming bacteria in combination with bacteriophage attack on starter culture	• Increase fermentation temperature • Use bacteriophage backup culture • Cleaning and disinfection
Low viscosity and syneresis	• Milk-containing inhibitors • Low MSNF level • Insufficient homogenisation pressure • Insufficient heat treatment of the milk • Excessive mechanical treatment • Wrong starter culture • Bacteriophage attack on starter culture	• Examine raw milk for inhibitors • Increase MSNF level • Increase homogenisation pressure (>200 bar) of the raw milk • Increase pasteurisation temperature and prolong holding time (90-95°C/5-10 minutes) • Avoid agitating for too long or too vigorously when breaking pH, cooling and filling • Change the culture • Use bacteriophage backup culture • Cleaning and disinfection
Grainy texture	• Uneven mixing of coagulum • Bacteriophage attack on starter culture	• Agitate the coagulum more evenly • Cleaning and disinfection • Use bacteriophage backup culture

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DANISCO

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