

Kefir and Microbiology of Kefir Grains



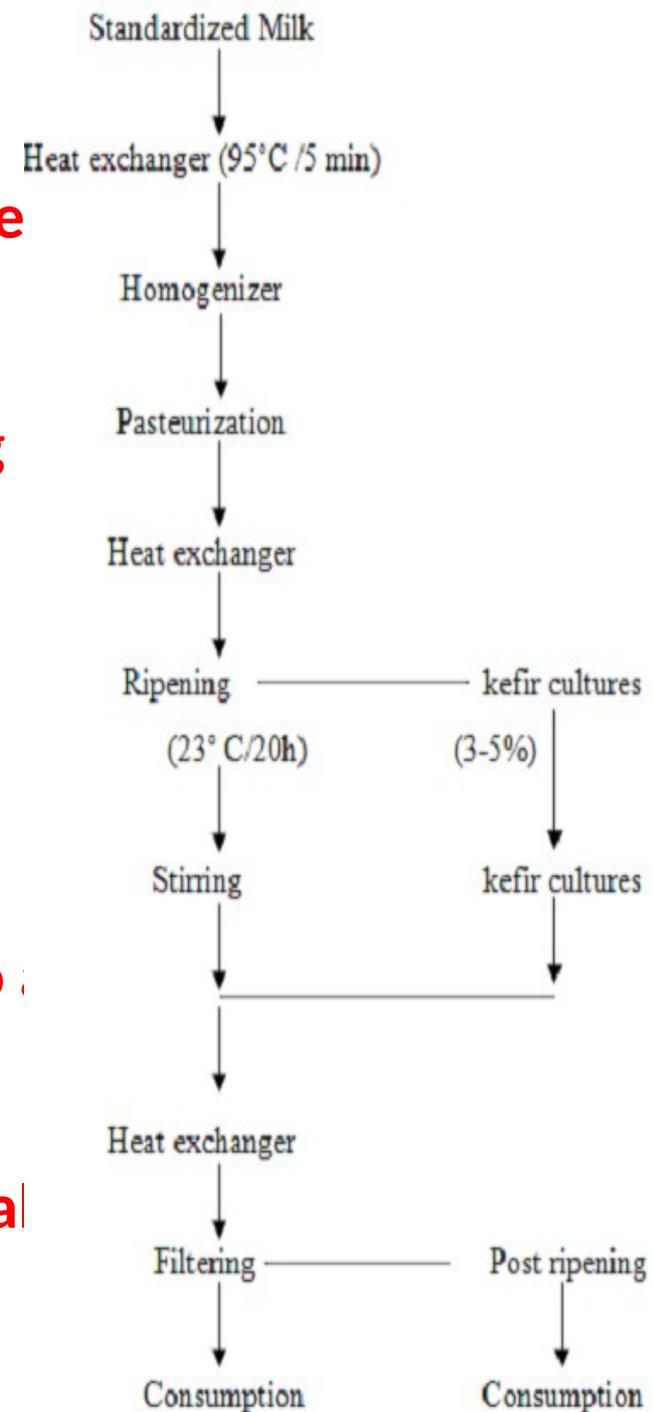
- 1. Kefir is a viscous, slightly carbonated dairy beverage that contains small quantities of alcohol and originated in the Caucasian mountains. It is originally a Russian liquid fermented milk product.**
- 2. It is manufactured under variety of names including kephir, kiaphur, kefer, knapon, kepi and kippi with artisanal production of kefir occurring in countries (Argentina, Taiwan, Portugal, Turkey and France).**
- 3. It is a viscous, acidic, and mildly alcoholic milk beverage produced by fermentation of milk with a kefir grain as the starter culture (FAO/WHO 2003). The Codex Alimentarius describe it as starter culture obtained from kefir grains (*Lactobacillus brevis*, *Leuconostoc*, *Lactococcus*) growing in a strong specific relationship.**
- 4. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces delbrueckii* and *Torulopsis holimii*).**

Codex Standard for Kefir

Milk protein (% w/w)	min. 2.8
Milk fat (% m/m)	<10
Titratable acidity, expressed as % of lactic acid	min. 0.6
Ethanol (% vol. /w)	not stated
Sum of specific microorganisms constituting the starter culture (cfu/g, in total)	10^7 (minimum)
Yeasts (cfu /g)	10^4 (minimum)

Making of Kefir

1. Commercial kefir is traditionally manufactured from cow's milk. Also made from the milk of ewes, goats buffaloes as well as soymilk.
2. Traditionally, kefir is produced by adding kefir grains (a mass of proteins, polysaccharides, mesophilic, homofermentative and heterofermentative lactic acid streptococci, thermophilic and mesophilic lactobacilli and yeast) to quantity of milk.
3. The size of the initial kefir grain inoculum affects the pH, viscosity and microbiological profile of the final product.



4. A grain to milk ratios of 1:10 is found optimum.
5. In manufacturing procedures, a percolate of the grains from a coarse sieve is used as the mother culture to inoculate fresh milk. Fermentation of the milk by the inoculum proceeds for over 12-16 h, during which time homofermentative lactic acid streptococci grow rapidly, initially causing a drop in pH. This low pH favours the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the mixture, together with fermentation temperature (21-23°C), encourages the growth of aroma producing heterofermentative streptococci. As fermentation proceeds, growth of LAB is favoured over growth of yeasts and acetic acid bacteria.

6. Kefir grains are key to kefir production, and finished product has a different microbiological profile from the grains and therefore cannot be used to inoculate a new batch of milk.
7. Some researchers produced a starter consisting of two bacteria (*Lactobacillus helveticus* and *Lactococcus lactis subsp. lactis*) and one yeast (*S. cerevisiae*) isolated from kefir grain and combined with two yoghurt strains (*Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus thermophilus*). Yeast was added to the starter with sucrose either at the beginning, or after lactic acid fermentation.

Some Brands of Kefir



Characteristics of Kefir

- The flavour, viscosity and microbial/chemical composition of the final kefir product can be affected by the size of the inoculum added to the milk, the occurrence of any agitation during fermentation, and the rate, temperature and duration of the cooling and ripening stages following fermentation.
- A good quality kefir has pourable consistency, sour taste, marginally yeasty flavor and prickly sensation of CO₂.
- Modern manufacturing procedures for kefir result in ethanol levels in the finished product of 0.01–0.1% although kefir with ethanol concentrations as high as 0.25% have been produced from grains in the laboratory.

- The amounts of ethanol and CO₂ produced during fermentation of kefir depend on the production conditions used. CO₂ content of kefir has been said to be comparatively low' in relation to other fermented drinks.
- The distinctive taste of kefir results from the presence of several flavour compounds produced during fermentation. Kefir produced from pure cultures did not receive high sensory evaluation scores.
- Acetaldehyde and acetoin are of particular attention during kefir manufacture because of their contribution in the taste and increase in concentration during kefir fermentation. During storage, acetaldehyde increases in concentration and acetoin decreases.

Kefir Grains

- Kefir grains resemble small cauliflower florets: they measure 1/4-3/4 inch in diameter, lobed, irregularly shaped, white to yellow-white, slimy, firm texture.
- Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 h; which will have increased mass by 25%. Grains must be replicated to retain viability, since old and dried kefir grains have little or no ability to replicate. Washing grains in water also reduce viability.
- In commercial operation, grains should be kept viable through daily transfers and should only be replaced if their ability to ferment milk becomes impaired.



Preservation of Kefir Grains

- **Kefir grains** are mostly **preserved by lyophilisation**, dry or wet.
- **Dried grains** keep up their activity **for 12-18 month** while **wet grains 8-10 days**.
- Kefir grains are **generally preserved in milk at refrigerated condition for 3-5 day** and subsequently it can be **further reactivated with replacement of milk**



Microbiology of Kefir Grains

The microbial population found in kefir grains have been used as an example of a symbiotic community.

<u>Lactobacilli</u> <i>Lactobacillus kefir</i>	<i>Lactobacillus delbrueckii</i>
<i>Lactobacillus kefiranofaciens</i>	<i>Lactobacillus rhamnosus</i>
<i>Lactobacillus kefirgranum</i>	<i>Lactobacillus casei</i>
<i>Lactobacillus parakefir</i>	<i>Lactobacillus paracasei</i>
<i>Lactobacillus brevis</i>	<i>Lactobacillus fructivorans</i>
<i>Lactobacillus plantarum</i>	<i>Lactobacillus hilgardii</i>
<i>Lactobacillus helveticus</i>	<i>Lactobacillus fermentum</i>
<i>Lactobacillus acidophilus</i>	<i>Lactobacillus viridescens</i>
<u>Lactococci</u> <i>Lactococcus lactis</i> subsp <i>lactis</i> <i>Lactococcus lactis</i> subsp <i>cremoris</i>	<u>Enterococci</u> <i>Enterococcus durans</i>
<u>Streptococci</u> <i>Streptococcus thermophilus</i>	<u>Leuconostocs</u> <i>Leuconostoc mesenteroides</i>

Bacteria found in kefir grains and kefir

The yeasts in kefir grains provide an environment for the growth of kefir bacteria, producing metabolites that contribute to the flavour and mouth feel of kefir. To prevent excessive CO₂ production after fermentation, a two stage fermentation process starting with a non-lactose fermenting yeast such as *S. cerevisiae* can be done. Some of the yeast found in kefir grains are capable of fermenting lactose, while some are not. Some type of yeasts are located at the surface of the grain, while others inhabit interior. Yeasts located at different locations in the kefir grains play different roles in the fermentation.

<i>Kluyveromyces marxianus</i>	<i>Candida friedrichii</i>
<i>Saccharomyces sp.</i>	<i>Candida pseudotropicalis</i>
<i>Saccharomyces cerevisiae</i>	<i>Canida tenuis</i>
<i>Saccharomyces unisporu</i>	<i>Candida inconspicua</i>
<i>Candida maris</i>	<i>Candida tannotelerans</i>
<i>Saccharomyces turicensis</i>	<i>Candida lambica</i>
<i>Saccharomyces delbrueckii</i>	<i>Candida holmii</i>
<i>Torulaspota delbrueckii</i>	<i>Candida kefir</i>

Nutritional Significance of Kefir

- The composition of kefir depends greatly on the type of milk that was fermented.
- L(+) lactic acid is the organic acid in highest concentrations after fermentation and is derived from approximately 25% of the original lactose in the starter milk.
- The amino acids valine, leucine, lysine and serine are formed during fermentation, while quantities of alanine and aspartic acid increase as compared to raw milk.
- Appreciable amounts of pyridoxine, vitamin B₁₂, folic acid and biotin were synthesized during kefir production, depending on the source of kefir grains used, while thiamine and riboflavin levels were reduced.

Bioactive Ingredients in Kefir: There are several compounds in kefir that may have bioactive properties.

- **Exopolysaccharides** of differing structures and compositions are produced by *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Leuconostoc*.
- These cell-surface carbohydrates confer protective and adaptive properties on their bacterial producers; since they are often loosely bound to the cell membrane and easily lost to their environment.
- In food products, exopolysaccharides often contribute to organoleptic and stability characteristics.
- A unique polysaccharide called **kefiran** is found in kefir grains. Grains may also contain other exopolysaccharides. Kefiran contains D glucose and D-galactose only in ratio of 1:1. Kefiran dissolves slowly in cold water and quickly in hot water, and forms a viscous solution at 2% concentration.

- **Bioactive peptides:** Many organisms possess enzymes (e.g. proteinases and peptidases) that are able to hydrolyze the protein in a medium, thereby supporting growth of the organism by liberating peptides and amino acids.
- Several isolates have high proteinase activities which increases the possibility that bioactive peptides may be present in kefir.

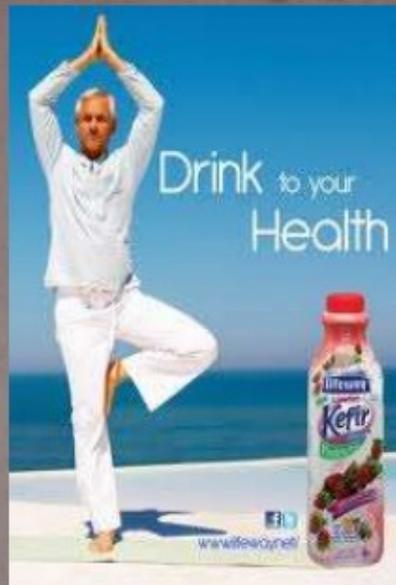
Therapeutic significance of Kefir:

- **Exopolysaccharides found in kefir grains and Bioactive peptides formed during the fermentation process stimulate immune system in animal models. Kefiran have antibacterial, antitumoral, antidiabetic properties as well as technological roles as a thickener, gelling agent and emulsifier.**
- **Anti-tumour effects of a water-soluble polysaccharide isolated from kefir grains is reported.**
- **A water soluble polysaccharide fraction from kefir grains inhibits pulmonary metastasis of Lewis lung carcinoma, whether the kefir-derived polysaccharide was given orally before or after tumour transplantation.**
- **Some kefir grains possess β -galactosidase activity which remains active when consumed and thus can be beneficial for lactose intolerant people.**

- Many lactobacilli are capable of producing antimicrobial compounds, including organic acids (lactic and acetic acids), CO_2 , H_2O_2 , ethanol, diacetyl and bacteriocins that may be beneficial not only in the reduction of food-borne pathogens and spoilage bacteria during food production and storage, but also in the treatment of gastrointestinal disorders and vaginal infections.
- Fresh kefir grains inhibit the growth of pathogens *S. aureus*, *K. pneumoniae* and *E. coli*. *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, isolated from kefir grains, produce heat-stable antimicrobial compounds that inhibit Gram-positive and Gram-negative bacteria.
- Lactobacilli isolated from kefir grains had antimicrobial activities against *E. coli*, *L. monocytogenes*, *Salmonella Typhimurium*, *S. Enteritidis*, *S. flexneri* and *Yersinia enterocolitica*.

antibiotic and antifungal

treatment of a variety of conditions



metabolic disorders, atherosclerosis, and allergies, tuberculosis, cancer,

poor digestion, candidiasis, osteoporosis, hypertension, HIV and heart disease.

HEALTH BENEFITS

contains many vitamins, minerals

calcium, phosphorus, magnesium, B2 and B12, vitamin K, vitamin A and vitamin D.



lactose intolerant people

healthy nervous system