4 Kefir: A Fermented Milk Product

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CONTENTS

4.1 Introduction ....................................................................................................78
4.2 Kefir Grains ....................................................................................................78
  4.2.1 The Microorganisms in Kefir Grains and Kefir ....................................78
  4.2.2 Kefiran ....................................................................................................83
  4.2.3 Electron Microscopy of Kefir Grains ....................................................84
4.3 Commercial Kefir Production ........................................................................86
  4.3.1 Size of Production ..............................................................................86
  4.3.2 Methods of Production .......................................................................86
4.4 Composition of Kefir .....................................................................................89
  4.4.1 Carbon Dioxide Content .....................................................................90
  4.4.2 Fat Content .........................................................................................91
  4.4.3 Lactose/Lactic Acid Content ................................................................91
  4.4.4 Ethanol Content ..................................................................................91
  4.4.5 Amino Acids .......................................................................................92
  4.4.6 Volatile Components ..........................................................................92
4.5 The Taste of Kefir ..........................................................................................93
4.6 Nutritional Value of Kefir ..............................................................................94
  4.6.1 Digestibility ............................................................................................94
  4.6.2 Protein Nutrition ..................................................................................94
  4.6.3 Lactose Metabolism ...........................................................................95
  4.6.4 Vitamin Content ...................................................................................95
  4.6.5 Kefir as an Infant Food .......................................................................96
  4.6.6 Other Nutritional Uses .......................................................................96
4.7 Physiological Effects of Kefir Consumption .................................................97
  4.7.1 Kefir as a Probiotic .............................................................................97
  4.7.2 Antitumor Effect in Animals ..............................................................97
  4.7.3 Antibacterial, Antifungal, and Antiviral Properties of Kefir .................99
  4.7.4 Cholesterol Metabolism .....................................................................101
  4.7.5 Other Uses ..........................................................................................102
References ..............................................................................................................103
4.1 INTRODUCTION

Kefir is a beverage produced by the action of lactic acid bacteria (LAB), yeasts, and acetic acid bacteria on milk. This complex mixture of microorganisms produces a distinctive fermented milk product with unique properties.

The first fermented foods may have been produced by accident. However, fermentation of foods such as milk became a widespread method of preservation before refrigeration was introduced or preservation procedures such as canning and pasteurization were developed and used to extend shelf life. It would appear from the oral tradition of kefir that fermentation of milk in skin bags as a way of preserving milk led to the production of the first kefir grains and started the long tradition of producing kefir. Kefir has been produced using milk from cows, ewes, goats, and buffaloes and has been sold in Europe under a variety of names including kephir, kiaphur, kefyr, képhir, kéfer, knapon, kepi, and kippe. (See Chapter 1 for more details on the history of kefir.)

Traditional home production of kefir has been joined by commercial production in many countries, and this has helped to increase the consumption of kefir and to promote its reputation as being good for health. Kefir is at the same time a functional food and a probiotic, and there is growing evidence that this unique fermented milk product may indeed be helpful in many disease/infection conditions.

Early reports on kefir quote original research that was carried out in the former Soviet Union. The composition of this unique product has not been fully described, and it is evident that more research is required on the microbiology of kefir before the product can be fully understood from a scientific point of view. Consumer acceptance may be slow because of the unique taste of kefir. The reported health properties of kefir and the desire of consumers to consume probiotic products may open new markets.

4.2 KEFIR GRAINS

Kefir is produced by adding either a starter culture called kefir grains directly or a percolate of the grains to milk. Kefir grains are a mass of several different bacteria and yeasts embedded in a complex matrix of protein and carbohydrate. The microorganisms in the kefir grains ferment the milk, and the grains can be recovered at the end of the fermentation process. The grains have been described as resembling elastic small florets similar to cauliflower in shape, yellow/white in color, and 20 to 30 mm in size. Figure 4.1 is a photograph of kefir grains. A crude analysis of the grains shows that they are a mass of bacteria, yeasts, polysaccharides, and proteins with a chemical composition of 890 to 900 g/kg water, 2 g/kg lipid, 30 g/kg protein, 60 g/kg sugars, and 7 g/kg ash. A study of the proteins in kefir grains using SDS-PAGE on acrylamide gels indicated that the major grain proteins had a higher molecular weight than milk proteins, indicating that they were not proteolysis products.

4.2.1 THE MICROORGANISMS IN KEFIR GRAINS AND KEFIR

The bacteria, yeasts, polysaccharides, and proteins in kefir grains added to milk produce kefir. Usually there is no pasteurization step after fermentation, and therefore
Kefir: A Fermented Milk Product

Live bacteria and yeasts are found in the final product. In order to understand the fermentation process better and also to evaluate the health benefits of eating kefir, the microbiological profile of kefir has been studied by many researchers. The isolation and identification of microorganisms in kefir grains and kefir depends on the choice of suitable growth media, and more recently, sophisticated methods of identification have been used. It should be noted here that the identity of several of the microorganisms found in kefir has been revised over time as more definitive methods of identification have been used. In some cases, the nomenclature assigned to various bacterial species has also changed. The names of microorganisms used in this chapter are the names used in the original scientific articles cited.

Studies on the selection of a suitable selective growth medium for *Lactobacillus* species are numerous, but the media are often not suitable for the growth of some *Lactobacillus* species found in kefir or kefir grains. Fuisawa et al. observed that *Lactobacillus kefiranofaciens* grew on KPL agar at 30°C, but not on BL and MRS agars. Kojima et al. found that Rogosa medium in 100% cheese–whey solution (Rogosa-CW) gave the best results for the isolation and cultivation of lactobacilli from kefir grains. Farnworth and Mainville compared MRS, KPL, and Rogosa-CW to the lactic acid whey medium (LAW) and found LAW to have the best recovery and growth rate for the lactobacilli present in kefir and kefir grains. This was especially true for *Lactobacillus kefirgranum* isolates.

Many studies have been carried out to identify the various bacteria and yeasts in kefir grains and in the final product. Early studies revealed that many of the bacteria isolated are closely related and therefore difficult to isolate and identify. As noted by Koroleva, studying and monitoring kefir grains is difficult because when the various microorganisms are separated as pure cultures, they do not grow in milk or have decreased biochemical activity. Because of this, kefir has been cited as an example of symbiosis; the growth and survival of individual strains are dependent on the presence of others. Toba indicated several possible metabolic products that might contribute to the symbiotic relationship in kefir grains.

The growth of *Lactobacillus kefir* was enhanced when *Candida kefir* was added, either before or simultaneously, to the milk to be fermented. Growth of the yeast, however, was not stimulated by the presence of the bacterium. When the two organisms were cultured together, the amounts of lactic acid, glycerol, and ethanol
produced were increased. The growth of several bacteria isolated from kefir grains
is improved when yeast extract is added to the growth medium, indicating that the
yeasts found in kefir grains are essential to maintain the integrity and viability of
the microflora population. Vitamins, amino acids, and other essential growth factors
for bacteria are produced by yeasts, while bacterial metabolic endproducts are used
as energy sources by yeasts. Figure 4.2 is an electron micrograph of kefir grains
showing bacteria surrounding a yeast cell; the close proximity presumably indicates
some sort of physical and chemical interaction.

The symbiosis found in the kefir grain microorganism population allows the
grains to maintain uniformity so that throughout the year the microbiological profile
of kefir grains and the kefir drink remain stable in spite of variations in milk quality
and the presence of antibiotics and other inhibiting substances. The microorganism
profile of the final product does not necessarily parallel that of the grains because
of conditions (pH and other) during the fermentation process. Also, the location of
the microorganisms in the grains may be a factor. Yeasts are generally found in the
interior of the grains, while lactococci are found on the exterior. Therefore, the
numbers of yeasts found in the final product are lower than those counted in the
grains themselves, while lactococci are numerous in the final drink. The complex
microbiological composition of kefir grains produces kefir. Therefore, unlike the
situation with yogurt, the drink kefir cannot be used as a starter to produce more kefir.

Initially, traditional selective media were used to isolate and identify individual
bacterial strains. Recent studies dealing with the microflora identification of kefir
grains and kefir drink show the importance of using the tools of molecular biology
in order to clearly characterize the bacteria and yeasts present in these types of
products. Earlier reports on the composition of kefir list many different types of
Kefir: A Fermented Milk Product

LAB, yeasts, and acetic acid bacteria. These identifications were based on phenotypic characteristics, which are often not sufficient to identify an organism to the species level.

Bacteria such as *Lactobacillus kefir* and *Lactobacillus brevis*, which are phenotypically very similar, are a good example to demonstrate the need to use molecular tools for identification. Some researchers have reported that they were able to differentiate the two species based on the fermentation of sucrose, trehalose, and xylose, but results were not always obvious since some strains of *Lb. brevis* will not ferment one or more of these sugars. Some researchers have reported that they were able to differentiate the two species based on the fermentation of sucrose, trehalose, and xylose, but results were not always obvious since some strains of *Lb. brevis* will not ferment one or more of these sugars. 

Table 4.1 summarizes the most recent studies published on the identification of kefir microflora. Studies performed by Angulo et al., Rohm et al., Pintado et al., and Lin et al. are based on phenotypic traits of the strains isolated, while papers published by Takizawa et al., Wyder and Puhan, and Wyder et al. used restriction length polymorphism (RFLP), DNA/DNA hybridization, and other molecular tools to characterize the strains they have isolated. One would not expect the list of bacteria and yeasts composing the grains to be very extensive, nor should it vary significantly from one part of the world to another if good care, similar growth conditions, and proper sanitary conditions are maintained. One could even assume that if a contaminant species were to come in contact with the kefir population, it would probably not survive or its growth would be inhibited due to the production of compounds such as bacteriocins by the symbiotic flora of kefir. However, over time and under different growing conditions, kefir grains may change their microbial makeup and fermentation properties.

Most of the microbiological studies done on kefir and kefir grains have centered on the identification of the constituent bacteria. In many fermented milk products, yeasts are not desirable; they cause spoilage because the low pH provides a selective environment for their growth. For kefir, yeasts play a key role in the fermentation process and, even though the number of yeasts in the final drink is less than in the grains, it is important to maintain the balance of bacteria to yeasts. Yeasts contribute to the unique characteristics of kefir. Rosi was one of the first researchers to study the yeast in kefir grains using electron microscopy. She found that the yeasts tended to be located in the center and along the peripheral channels of the grains. Using morphological and physiological characteristics, DNA base composition, and electrophoresis patterns, Iwasawa et al. identified *Torulopsis holmii* in commercial kefir grains. Engel et al., in their survey of commercial and home-produced kefir, found both lactose-fermenting and nonlactose-fermenting yeasts in most products, although some commercial products called “kefir” contained no yeasts.

The stability and composition ratio of the microorganisms in kefir between the time of production and time of consumption is important to consumers who are eating kefir as a probiotic for its health-promoting properties. Figure 4.3 shows the changes in the total number of lactococci, lactobacilli, and yeasts in commercial kefir that had been stored at 4°C for 42 days. The patterns for the three categories of microorganisms studied were similar in natural (unflavored) or strawberry (15% by weight) flavored kefir. Numbers of lactococci declined by almost 2 log units by the end of 42 days. Even with this decline during storage, the number of microorganisms in the drink remained high enough for the product to be considered a probiotic.
### TABLE 4.1
Microorganisms Identified in Kefir and Kefir Grains

<table>
<thead>
<tr>
<th>Lactic Acid Bacteria</th>
<th>Yeasts</th>
<th>Others</th>
<th>Sources</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lb. kefirum</em>, <em>Lb. kefiranofaciens</em>, <em>Lb. kefir, Lb. parakefir</em></td>
<td>N.D.</td>
<td>N.D.</td>
<td>Christian Hansen (Copenhagen); kefir grains</td>
<td>30</td>
</tr>
<tr>
<td>N.D.</td>
<td><em>Kluyveromyces marxianus, Pichia fermentans, Saccharomyces cerevisiae, S. dairenensis</em></td>
<td>N.D.</td>
<td>Nineteen sources from Austria; kefir grains and kefir drinks</td>
<td>40</td>
</tr>
<tr>
<td><em>Lb. helveticus, Leuconostoc mesenteroides</em></td>
<td><em>Kluyveromyces marxianus, Pichia fermentans</em></td>
<td>N.D.</td>
<td>Taiwan; kefir grains</td>
<td>41</td>
</tr>
<tr>
<td>N.D.</td>
<td><em>Candida kefyr, Kluyveromyces marxianus, Candida colliculosa, Torulaspora delbrueckii, Saccharomyces unisporus, Brettanomyces anomalus, Saccharomyces turicensis</em></td>
<td>N.D.</td>
<td>Five sources of different origins (Toni AG, Zürich, Switzerland; Biolacta-Texrl, Poland; private sources); kefir grains and kefir drinks</td>
<td>31, 32</td>
</tr>
<tr>
<td><em>Lb. kefir, L. lactis ssp. lactis</em></td>
<td><em>Saccharomyces unisporus</em></td>
<td>N.D.</td>
<td>Portugal; kefir grains</td>
<td>38</td>
</tr>
</tbody>
</table>

*Note:* N.D., not determined.
Kefir: A Fermented Milk Product

4.2.2 KEFIRAN

Early observations of the structure of kefir grains noted that some of the bacteria were encapsulated by a polysaccharide. La Rivière et al. carried out the first studies of the polysaccharide and gave it the name kefiran. Kooiman was able to show that the water-soluble polysaccharide consisted of approximately equal proportions of glucose and galactose. The chemical structure proposed is shown in Figure 4.4.

This structure was modified by Mukai et al., who used nuclear magnetic resonance (NMR) to show that 6-O-substituted galactose may also exist in the structure of kefiran. However, to date, no complete structural analysis with NMR confirmation has been published for kefiran.

\[
\rightarrow 6)\beta-D-Gp\{1\rightarrow2(6)\}\beta-D-Galp\{1\rightarrow4\}\alpha-D-Galp\{1\rightarrow3\}\beta-D-Galp\{1\rightarrow4\}\beta-D-Gp\{1
\]

It appears that kefir grains are the only source of kefiran, but it is not known whether more than one polysaccharide or various isomers of kefiran exist in kefir grains or kefir. Koroleva quotes Russian references that claim kefir grains can contain up to 34% polysaccharide, while the final drink contains 0.2 to 0.7% polysaccharide.

The bacteria that produce kefiran have been the subject of additional research. La Rivière et al. originally suggested that *Lb. brevis* was the bacterium producing kefiran. In 1983, Kandler and Kunath identified *Lb. kefir* as the bacterium responsible. Toba et al. isolated a polysaccharide-producing bacterium, which they named *Lactobacillus kefiranofaciens*. Lactobacillus sp. KPB-167B was published by Yokoi et al., and *Lactobacillus kefirgranum* and *Lactobacillus parakefir* were reported by Takizawa et al. as the responsible organisms. Yokoi et al. were able to isolate five different polysaccharide-producing bacteria of the genus *Lactobacillus* from commercially available kefir grains. Yokoi et al. stated that this list of bacteria producing kefiran indicates the complexity of the taxonomic relationships of the bacteria isolated from kefir, and they implied that other bacteria found in kefir grains may be capable of producing kefiran-like polysaccharides, especially kefir grains originating from different geographical locations.

Toba’s group tried to produce a new fermented milk drink using the kefiran-producing *Lb. kefiranofaciens* they had isolated from kefir grains, but the product had aropy consistency and scored low on consumer acceptability criteria. In spite of this, Toba et al. stated that the health benefits of kefiran might be great enough to convince people to consume the product.

Several groups have tried to find media and growth conditions that would optimize the production of kefiran. Toba et al. were able to produce 80 mg polysaccharide/l of medium using a whey-based growth medium that had increased glucose and added trypsinase peptone compared to their standard whey-based medium. They also reported that the viscosity of the fermented drink was directly proportional to the polysaccharide content, and they used viscosity measures to gauge the efficiency of their different growth media.

The five different bacteria isolated by Yokoi et al. were capable of producing 273 to 406 mg of total (supernatant + capsule) polysaccharide/l of medium. This group also used a whey-based growth medium. Recently, Yokoi and Watanabe reported yields of 2.04 g polysaccharide/l of medium after 4 days of culturing, using a modified de Man, Roguska, Sharp Lactose (MRSL) medium that contained 10% lactose. Micheli et al. have been able to batch produce large quantities (2 g/l) of kefiran from a slime-forming rod-shaped *Lactobacillus* isolated from grains obtained from a local dairy in Italy, using the medium reported by Yokoi and Watanabe.

It is not clear how many different bacteria synthesize kefiran or kefiran-like polysaccharides. Although kefiran is found in kefir grains, few reports have been published in which the concentration of kefiran in the final drink has been reported. Kefiran may be contributing to the texture of kefir.

### 4.2.3 Electron Microscopy of Kefir Grains

Initially, light microscopy and later, transmission electron microscopy and scanning electron microscopy have been used extensively to study and describe the structure...
Kefir: A Fermented Milk Product

and microbiology of kefir grains. Figure 4.5 is a scanning electron micrograph of kefir grains obtained from the Moscow Dairy Institute that shows the matrix of bacteria, yeast, polysaccharide, and protein. Molska et al. used electron micrographs to estimate the composition of kefir grains. They reported that their commercial grains from Poland contained 66% bacilli, 16% streptococci, and 18% yeasts. The microbiological population appears to be different on the surface of the grains than in the interior. This may be due in part to differences in pH throughout the grain; the interior has been reported to have a very low pH that inhibits the growth of lactococci. Generally, long rod-shaped bacteria predominate on the surface, while yeasts are concentrated in the interior of the grain. This difference is important because the surface microorganisms are the ones that probably have the greatest impact on the fermentation process that produces kefir. Toba et al. observed differences in the type of bacteria on the surfaces of propagable and nonpropagable grains and concluded that long rod-shaped bacteria with filamentous appendages were necessary for kefir grains to function. However, a large variation in the bacterial population occurs between grains and even within the same grain. Lin et al. concluded that place of origin of the grains alone may explain the differences that have been reported in various electron microscopy studies.

A study of ways to preserve kefir grains showed that grains stored at −20°C for 120 days had the same microflora profile and produced kefir with the same rheological characteristics, acidity, and carbon dioxide content as kefir produced from nonstored grains. Grains stored at 4°C and then used to inoculate milk produced inferior quality kefir. Lyophilizing kefir grains has also been used as a way of producing starter cultures for industrial production.

Traditionally, kefir grains have been replicated in cows’ milk. However, Abraham and de Antoni have shown that the same grains that grow in cows’ milk can grow and replicate in soybean milk. When soybean milk is used, the resulting grains are more compact and smaller in size, and they have a yellowish color — most likely due to the protein. When the grains are added to soybean milk, the pH is lower after 30 h than it is in cows’ milk. After 20 subcultures, the soybean-grown grains had a

**FIGURE 4.5** Electron micrograph of kefir grains showing bacteria and yeasts in carbohydrate/protein matrix. Magnification × 2555, bar indicates 10 µm. (Photo courtesy of M. Kalab, Agriculture and Agri-food Canada.)
higher protein content and a lower polysaccharide content than the milk-grown grains, although the microbial profiles of the two grains were similar.

### 4.3 COMMERCIAL KEFIR PRODUCTION

#### 4.3.1 SIZE OF PRODUCTION

Kefir is a relatively new commercial product in North America, and this, combined with the fact that the majority of kefir is produced and consumed in eastern Europe, has made the gathering of statistics on kefir production and per capita consumption difficult. In addition, many data gathering organizations do not differentiate between the various types of fermented (processed) milk products, and so kefir is often included with yogurt, buttermilk, and traditional Russian dairy products such as smetana, tvarog, blinchiki, and pelmeni. A sharp decrease in milk production and a substantial fall in the spending capacity of consumers have led to a significant decrease in the consumption of milk and milk products in the Ukraine and many eastern European nations. However, the importance of kefir in the Russian diet is demonstrated by the fact that in 1992 it was included on the list of regulated commodities together with such other staples as milk, sugar, salt, and bread. The industrial production of kefir has been large enough that Germany, Austria, Brazil, France, Luxembourg, Norway, Switzerland, Czechoslovakia, and Israel have regulations defining the composition and method of production of kefir. In the United States, California has regulations concerning kefir milk. Table 4.2 is a listing of production levels of kefir from various countries.

#### 4.3.2 METHODS OF PRODUCTION

Home production of kefir is practiced in many countries, and scientific studies have often used samples gathered from homes. The traditional method of kefir making is

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**TABLE 4.2**

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Production</th>
<th>Comment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>USSR</td>
<td>1940</td>
<td>15,900 tons</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>USSR</td>
<td>1975</td>
<td>254,000 tons</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Sweden</td>
<td>1985</td>
<td>17,000 tons</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Poland</td>
<td>1982</td>
<td>22.5 million l</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Poland</td>
<td>1988</td>
<td>35.3 million l</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Poland</td>
<td>1997</td>
<td>255 million l</td>
<td>Kefir + buttermilk + flavored milk</td>
<td>71</td>
</tr>
<tr>
<td>Poland</td>
<td>1998</td>
<td>276 million l</td>
<td>Kefir + buttermilk + flavored milk</td>
<td>71</td>
</tr>
<tr>
<td>Poland</td>
<td>1999</td>
<td>327 million l</td>
<td>Kefir + buttermilk + flavored milk</td>
<td>71</td>
</tr>
<tr>
<td>Hungary</td>
<td>1996</td>
<td>116,337 tons</td>
<td>Fermented or acidified dairy products</td>
<td>72</td>
</tr>
<tr>
<td>Hungary</td>
<td>1997</td>
<td>119,255 tons</td>
<td>Fermented or acidified dairy products</td>
<td>72</td>
</tr>
<tr>
<td>Hungary</td>
<td>1998</td>
<td>138,986 tons</td>
<td>Fermented or acidified dairy products</td>
<td>72</td>
</tr>
</tbody>
</table>
performed by adding kefir grains directly, as a starter, to pasteurized, cooled milk. In home production, fermentation temperature and time are not rigidly controlled. The final product cannot be used to inoculate new milk to produce kefir because the original balance of microorganisms in the grains has been disrupted, kefir grains are essential to the process.73

The properties of kefir (chemical, physical, and organoleptic) were initially difficult to duplicate in large-scale production. Some processes were developed where no grains were used, but the quality of the product was much different from that of kefir fermented with grains.

Large-scale production of kefir has been hindered by problems involved in reproducing the kefir grains and producing a consistent product. However, production of kefir on an industrial scale is common in many European countries, and patents have been taken out in several countries describing the process.74–77 Several variations exist in the protocol used in the commercial production of kefir. Initially, the set method was used to produce kefir. In this procedure, inoculated milk was filled into bottles, fermented at a controlled temperature until a strong coagulum formed, and then cooled. However, the kefir produced was of low quality compared to that produced on a smaller scale using traditional methods. Today, kefir is produced by a stirred method where fermentation, coagulum formation, agitation, ripening, and cooling all occur in one vessel.24 The composition (chemical, organoleptic, microbiological characteristics) of the final product depends on the type of milk used, the source of grains, the preparation of the mother culture (often produced by coarsely sieving the gains and using the percolate), the length of fermentation, the inclusion of a cooling step, and the inclusion of a maturation step.

A major difference in processing involves the choice of using either kefir grains or a kefir grain-free extract as the mother culture. Traditional artisan production of kefir involves inoculating milk with a quantity of grains (2 to 10%) and allowing the fermentation to proceed for approximately 24 h, to a predetermined pH or until a desired taste/texture is obtained. Fermentation is carried out at 20 to 25°C. A maturation step, carried out at 8 to 10°C for 15 to 20 h, is often added. The grains are then sieved out and can be used for a new fermentation or preserved (1 to 7 days) in fresh milk. Leaving the grains in the final product results in excessive acid production and inferior taste.70,78,79

For the production of “Russian style” kefir, a grain-free innoculum or mother culture is prepared by carrying out a traditional kefir fermentation, sieving the finished product, and using the percolate to inoculate the milk. About 1 to 3% mother culture is added to pasteurized milk. When lyophilized starter cultures are used, the mother culture is prepared by adding 1 g of lyophilized kefir grain starter to 3 liters of milk.64 Koroleva80,81 has suggested that the fermentation step should be followed by a slow cooling step (to a temperature of 8 to 10°C) and then a ripening (maturing) step that allows microorganisms to grow and taste and aroma to develop. Korovkina et al.82 showed that as the temperature of the fermentation step increased (from 19 to 28°C), the pH of the kefir produced dropped and the viscosity increased. There was little change in the amounts of CO₂ and ethanol produced.

A third step, to produce what has been termed “industrial kefir,” involves taking some of the Russian style kefir and adding it (2 to 3%) to a new source of milk,
and again carrying out fermentation (8 to 20 h at 20–22°C) and maturation (12 h to 7 days at 8 to 16°C) steps.

Koroleva reported the effect of various variables on the production process and the composition of starters (mother culture) used to produce Russian style kefir. As the level of inoculum (as kefir grains) increased, the duration of the fermentation process became shorter. However, the changes to the microbiological profile (decreased levels of heterofermentative lactic acid streptococci and yeasts) were judged not to be favorable. The number of major groups of microorganisms increased as the quantity of kefir grains inoculated into the milk decreased. The pH of the starter was also dependent on the quantity of inoculum used: a starter pH of 3.6 to 3.8 was found using a ratio of 1:10 (starter to milk), and a pH of 4.4 to 4.6 with a ratio of 1:30 or 1:50. Garrote et al. also showed that the drop in pH during fermentation was greater when the amount of grains added was increased, and that the ratio of grains to milk affected the viscosity of the final product. The apparent viscosity of kefir produced with 10 g grains/l of milk was highest; adding less or more grains per liter of milk reduced the viscosity. The numbers of yeasts and lactobacilli did not change in the final product produced with different ratios of grains to milk, but the number of lactococci decreased as the amount of grains added increased.

When starters were produced at 25°C, the numbers of homofermentative bacteria (particularly lactobacilli) were favored compared to preparation at 18 to 20°C. Higher temperature fermentation resulted in lower pH, which inhibited the growth of both homofermentative and heterofermentative lactic acid streptococci and yeasts. Agitation of the fermentation mix had the benefits of preventing the growth of molds on the starter surface and promoting the even distribution of microorganisms and metabolites. A tenfold increase in homofermentative lactic acid streptococci and yeasts followed agitation, but there was no effect on the numbers of heterofermentative lactic acid streptococci, thermophilic lactobacilli, or acetic acid bacteria or any changes in the quantities of volatile fatty acids in the finished starter. In some facilities, kefir grains are washed with water once a week. Data from Koroleva showed that washing reduced the numbers of the main groups of starter microorganisms. When the washed grains were then used in the production process, fermentation times were increased and the final product had poor taste and consistency.

Various schematics have been published detailing the methods for producing kefir on an industrial scale. Rossi and Gobbetti outlined the details of a continuous process for producing kefir using a multistarter made up of bacteria and yeast isolated from kefir grains. Kefir was produced over a 30-day period; it was lower in viscosity and lacked some of the volatiles normally found in kefir.

Pettersson and Pettersson et al. listed several reasons for the need for a simpler starter culture to replace traditional kefir grains in the production process, including easier handling, uniformity of starter activity, reduced risk of infection and changes in the starter, final product uniformity, uniform yeast content, and improved storage characteristics. They developed a freeze-dried starter containing 75% homofermentative streptococci, 24% citric-fermenting streptococci, 0.5% lactobacilli, and 0.1% yeasts that produced a kefir with characteristics (pH, lactic acid production, viscosity) similar to those of kefir produced with traditional grains and that had higher organoleptic scores (yeast taste, overall aroma) than the kefir produced from grains. This
freeze-dried starter was also found to produce a more stable bacterial population in prepared kefir than traditionally regenerated kefir grains did.

Other attempts have been made to produce a less complex starter culture that could replace kefir grains in the production of kefir. By using two fermentation steps — *Lb. bulgaricus, Lb. acidophilus, Streptococcus thermophilus, Streptococcus lactis,* and *Leuconostoc* — followed by *Saccharomyces cerevisiae,* products were produced, but they had different characteristics (pH, viscosity, titratable acidity, and viable yeast counts) than the control kefir product. More recently, Rossi and Gobbetti combined four lactic acid bacteria and two lactose-negative yeasts all isolated from kefir grains to produce a multistarter that allowed them to produce kefir using a continuous process.

No one to date has been successful in producing kefir grains themselves, and therefore new kefir grains can only be obtained by the propagation of existing grains. Few sources of kefir grains exist today, and although kefir drink can be found in many countries, grains are not commercially available. It has not been possible to establish whether all existing grains have a common origin. Koroleva cautioned that any attempt to replace kefir grains by pure microorganism cultures would not be effective because the unique composition of kefir grains that has evolved over time cannot be replicated or replaced. Any symbiotic relationships between bacteria and yeasts that have developed over time would be difficult to replicate.

It is evident that the characteristics of kefir eaten by the consumer — chemical, microbiological, and sensory properties — are influenced by the starting material, various process variables, and manipulations of the final product. Figure 4.6 summarizes the factors that impact on the properties of the final product.

### 4.4 COMPOSITION OF KEFIR

The reported microbiological and chemical composition of kefir varies widely because the fermentation products in the final product are greatly influenced by the
Handbook of Fermented Functional Foods

4.4.1 Carbon Dioxide Content

Yeasts and some heterofermentative lactic acid bacteria are responsible for the production of the CO₂ gas in kefir. The CO₂ content increases during fermentation as the pH drops. If the fermentation is carried on for longer than 24 hours, CO₂ production plateaus after 48 hours. The gas produced leads to “fine flake” coagulum formation and also imparts a sparkling mouthfeel to kefir. The presence of the gas bubbles in the drink has prompted some to refer to kefir as the champagne of fermented milk drinks.

TABLE 4.3
Chemical Composition of Various Kefirs

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO₂</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polish commercial kefir</td>
<td>24.74% (v/v)</td>
<td>77</td>
</tr>
<tr>
<td>Grain fermented, 24 h</td>
<td>1.33 g/l</td>
<td>43</td>
</tr>
<tr>
<td>Grain free, 24 h</td>
<td>0.65 g/l</td>
<td>43</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taiwanese kefir from grains (cows’ milk)</td>
<td>3.16–3.18%</td>
<td>62</td>
</tr>
<tr>
<td>Polish lab kefir</td>
<td>3.1%</td>
<td>78</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taiwanese kefir from grains (cows’ milk)</td>
<td>3.07–3.17%</td>
<td>62</td>
</tr>
<tr>
<td><strong>Lactose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional kefir</td>
<td>2.5%</td>
<td>92</td>
</tr>
<tr>
<td>Eastern European stir type</td>
<td>3.7–3.8%</td>
<td>92</td>
</tr>
<tr>
<td>Irish kefir from grains (diluted milk)</td>
<td>1.8%</td>
<td>61</td>
</tr>
<tr>
<td>Taiwanese kefir from grains</td>
<td>2.81–3.13%</td>
<td>62</td>
</tr>
<tr>
<td><strong>Ethanol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polish lab kefir</td>
<td>0.021–0.029%</td>
<td>78</td>
</tr>
<tr>
<td>Traditional kefir</td>
<td>0.5–1.5%</td>
<td>92</td>
</tr>
<tr>
<td>Eastern European commercial stir type</td>
<td>0.02–0.114%</td>
<td>92</td>
</tr>
<tr>
<td>Irish kefir from grains (diluted milk)</td>
<td>0.04%</td>
<td>61</td>
</tr>
<tr>
<td>Taiwanese kefir from grains</td>
<td>0.17–0.25%</td>
<td>62</td>
</tr>
<tr>
<td><strong>Lactic Acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional kefir</td>
<td>0.7–1%, 50% as l-(+) isomer</td>
<td>92</td>
</tr>
<tr>
<td>Eastern European commercial stir type</td>
<td>0.7–0.8%</td>
<td>92</td>
</tr>
<tr>
<td>Irish kefir from grains (diluted milk)</td>
<td>0.5%</td>
<td>61</td>
</tr>
</tbody>
</table>

source of kefir grains used during fabrication. In addition, different types of milk (various species, various levels of fat) and different production methods (commercial, artisan) can be used. Generally, the pH of kefir is between 4.2 and 4.6. The ingredients most commonly measured as indicators of quality are CO₂, protein, lipid (fat), lactose, ethanol, and lactic acid. Table 4.3 is a summary of the composition of kefir reported in the literature.
Early research on yeasts in kefir showed that a yeast isolated from commercial kefir grains was able to ferment glucose, galactose, mannose, and sucrose, but not lactose. Fructose was only fermented after an inductive period. The amount of CO₂ produced appeared to be dependent on the presence of constituent enzymes that Iwasawa et al., thought might be unique to this yeast. Clementi et al. studied the production of CO₂ by two yeasts as a way to better understand the factors that control the CO₂ content of kefir because they felt that even with the possibility of producing a kefir from different starters (not grains), the CO₂ content had to be maintained. They were able to show that in 2.5 h, immobilized yeast cells added to lactic acid fermented milk could produce CO₂ levels comparable to those of traditional kefir that had been fermented for 24 h.

The production of CO₂ during the fermentation step and continuing on in the finished product presents a unique packaging problem that can cause bulging of containers and leakage of contents. In some countries, kefir has been either produced or sold in glass bottles. However, polyethylene foil packs covered with aluminium foil or plastic containers with an aluminium foil cover are more flexible containers that can accommodate the gas produced after packaging.

### 4.4.2 Fat Content

The fat content of kefir can be altered based on the type of milk fermented. In the former Soviet Union, kefirs have been sold that vary from fat free up to 3.2% fat. In her studies of various fermented milk products, Alm found only minor differences in the fat content and composition (mono-, di-, and triglycerides, free fatty acids, and steroids) of kefir compared to the starting milk. The fact that free fatty acids were found in all the fermented milks analyzed (including kefir) indicated a disruption of the fat molecules in milk during fermentation, and Alm speculated that this could result in an increase in the digestibility of milk fat in fermented products compared to other sources of fat.

### 4.4.3 Lactose/Lactic Acid Content

The lactose found in milk is degraded to lactic acid during fermentation; this causes the pH to drop and the milk to thicken. As much as 30% of the milk lactose is broken down during fermentation. β-galactosidase is the enzyme that hydrolyzes lactose into glucose and galactose. β-galactosidase is found in yogurt and kefir grains, but β-galactosidase activity is very low in kefir. The bacteria in kefir are able to break down glucose to lactic acid. Both D-(−)-lactic and L-(+)-lactic acid are produced from the milk lactose, and in kefir the L-(+) form tends to predominate. However, the ratio of L to D depends on the source and microbial composition of the grains.

### 4.4.4 Ethanol Content

The production of ethanol in kefir is complex; both yeasts and heterofermentative bacteria produce ethanol. The quantity of ethanol produced is dependent on the fermentation process and the type of container used (tightly capped or not). Kefir produced in small dairies in the former Soviet Union in the early twentieth century
contained alcohol levels between 1 and 2%. Present-day methods of production result in much lower levels of alcohol. This may be due in part to the fact that fermentations are stopped at higher pH levels than previously. The final alcohol concentration is determined for the most part by the number of yeasts present in the grains added to the milk and the duration of fermentation. Ethanol appears to be produced toward the end of the fermentation process, and its formation can continue even when the pH has decreased to the point where the lactic acid bacteria in the product are no longer active. The ethanol concentration can be increased in the final product by increasing the temperature during fermentation. Kefir produced in the laboratory from grains had a higher ethanol content (0.040 to 0.30%) than kefir produced commercially in Germany (0.002 to 0.005%).

Kwak et al. studied ways to control the production of ethanol in kefir made from a defined starter culture and found that a two-stage fermentation was best. They used a nonlactose fermenting yeast — *Saccharomyces cerevisiae* — initially to ferment glucose added to milk and then to carry out a lactic acid bacterial fermentation using a mixture of lactobacilli, lactococci, leuconostocs, and propionibacteria obtained commercially. During the yeast fermentation stage, the pH remained stable; it only dropped when the lactic fermentation started. Adding 0.4 or 0.5% glucose to the starter milk resulted in ethanol production only during the yeast fermentation stage, yielding a final product with 0.07 or 0.08% ethanol. Storage experiments showed that kefir produced with the addition of 0.4% glucose was the most stable. When 1.0% glucose was added, the production of ethanol continued into the lactic fermentation and resulted in an ethanol concentration of 0.24% in the final product.

### 4.4.5 Amino Acids

Kefir has the same pattern of amino acids as milk. Kefir proteins are easily digested due to the action of acid coagulation and proteolysis of milk proteins. Free amino acids found in milk are consumed during the first hours of fermentation by selective bacteria. As the fermentation slows and the kefir enters the ripening stage, the proteolytic activity of other microorganisms such as acetic acid bacteria and yeasts causes more peptides and free amino acids to be formed in a manner seen in other fermented milk products. (See Chapters 5 and 7 for more details on the hydrolysis of milk proteins.)

### 4.4.6 Volatile Components

Minor volatile constituents have been studied because of their possible contribution to kefir’s unique taste. Compositional data are inconsistent, however, because of the variety of sources of the kefir analyzed. Acetaldehyde and diacetyl are two important contributors to flavor, but propionaldehyde, 2-butanone, *n*-propanol, iso-amyl alcohol, acetic acid, and ethanol found in kefir may also influence the aroma. Görner et al. noted that the levels of volatiles found in kefir, particularly ethanol, changed during the course of the fermentation. Data from laboratory-produced kefir showed that maturation following fermentation lowered pH and acetaldehyde concentrations, but increased lactic acid and diacetyl levels. Commercial kefir contains half as
much orotic acid, twice as much pyruvic acid, nine times as much acetic acid, and about an equal amount of uric acid as does commercial yogurt. Some kefir also contains propionic acid. Douset and Caillet followed the concentrations of seven organic acids during the fermentation process and found that propionic acid was produced only in the last stages of fermentation (pH 4.33 and below) and that when the temperature during fermentation was increased from 20 to 30°C, the concentrations of citric acid, lactic acid, acetic acid, propionic acid, and isobutyric acid increased in the final product, while pyruvic acid levels declined.

Guzel-Seydim et al. followed the production of organic acids and volatiles during the fermentation of kefir produced in the laboratory from grains obtained from Turkey. Lactate production started slowly but the concentration of lactate rapidly climbed to 6000 µg/g by the end of the 22 h fermentation. Citrate, the next most abundant organic acid, declined during fermentation from 1760 to 1440 µg/g at the end of the fermentation. Pyruvate levels increased during fermentation to a final level of 18 µg/g. Levels of orotate and urate declined over the 22 h period; hippurate was totally consumed after 15 h. Acetic acid, propionic acid, and butyric acid were not found in any samples. Diacetyl was also absent. Acetaldehyde and acetoin levels increased as the fermentation progressed. However, ethanol production did not begin until after 5 h into the fermentation. Guzel-Seydim et al. also studied the changes in organic acids and volatile flavor compounds in kefir stored at 4°C for up to 21 days. Lactate was the organic acid in highest concentration (>6000 µg/g), followed by citrate (1500 µg/g). Lactate increased slightly during storage. Pyruvate was found at day zero, but by day seven of storage it had disappeared. The conversion of pyruvate to ethanol and CO₂ may account for this observation. The stored kefir contained no hippuric acid, acetic acid, propionic acid, or butyric acid. Acetaldehyde levels doubled (to 11 µg/g) during storage, while acetoin levels decreased from 25 to 16 µg/g during the storage period. This group found no diacetyl in their kefir.

Linossier and Douset showed that the yeast *Candida kefir* was capable of producing malic acid, citric acid, and pyruvic acid in milk with *Lb. kefir*, but levels of lactic acid, fumaric acid, and butyric acid were low. Citric acid was not found after 70 h of fermentation. There appeared to be a symbiotic relationship between the *C. kefir* and the *Lb. kefir*, as the growth of the bacterium was enhanced by the presence of the yeast in the medium. The addition of *C. kefir* at levels as low as 0.5% of the total microbial population stimulated the growth of *Lb. kefir*.

### 4.5 THE TASTE OF KEFIR

The taste of unflavored kefir has been described as “yeasty,” and the terms “prickling” and “sparkling” have been used to describe the mouthfeel of kefir caused by the liberation of trapped CO₂. Complaints from long-time consumers of kefir about the taste of traditional kefir occur only when the yeast taste is either very pronounced or absent. However, the taste has not scored high in sensory evaluations by North American consumers. Kefir that scores higher than unflavored kefir in consumer acceptability tests can be produced by adding flavor — either as as flavor itself or as fruit preserve. Starter cultures that contain bacteria that produce flavors can also be added to improve product acceptability. Although flavored kefir may appeal
to the North American consumer, the addition of fruit or other sources of sugars may cause unwanted fermentation by yeasts after packaging.4

In a sensory evaluation study carried out in Scotland, Muir et al.105 showed that both the chemical composition and the scores given by sensory panelists differed between commercial traditional kefir made from kefir grains and modified kefir made from a defined blend of bacteria and yeasts. Traditional kefir had higher levels of lactic, acetic, and propionic acids. The sensory panelists judged the modified kefir as less acidic, with a creamier taste and less serum separation compared to the traditional kefir. This led the researchers to conclude that modified kefir might be more acceptable to Western European consumers than traditional kefir.

Kefir produced using buffalo milk and traditional grains was reported to have chemical properties similar to kefir produced from cows’ milk, and it had acceptable organoleptic properties and was deemed suitable for Egyptian tastes.1

4.6 NUTRITIONAL VALUE OF KEFIR

The protein, fat, and mineral content of kefir is similar to that of the milk from which it is made, and therefore kefir has an inherently high nutritional value as a source of protein and calcium. Kefir also has a reputation as being palatable with a high digestibility, allowing for consumption of large quantities without intestinal disturbance.106

4.6.1 DIGESTIBILITY

The fermentation process brings about denaturation of milk proteins and hydrolysis of some of the proteins, resulting in smaller structures that are more susceptible to digestion by gastric and intestinal juices. In vitro experiments carried out by Alm107 showed that kefir developed a very small curd size when exposed to simulated gastric juice even after a three-hour exposure to acidic conditions. Kefir was widely used in hospitals and sanitaria in the former Soviet Union as part of the diet for patients with gastrointestinal and metabolic diseases, hypertension, ischemic heart disease, or allergies. Evenshtein108 reported that kefir (250 ml/day) was used successfully to stimulate gastric secretions and acid formation in patients with pulmonary tuberculosis. Kefir is particularly recommended for babies and patients with malabsorption syndromes, presumably because of the small curd size that forms in the stomach and the observation that fermented dairy products in general are digested without the secretion of large amounts of gastric juices.93 Mann2 quotes two Russian publications that highlight the nutritional value of kefir especially for infants and indicate that products based on kefir were being produced for this market.

4.6.2 PROTEIN NUTRITION

Rat studies carried out by Vass et al.109 confirmed that kefir had a superior biological value (protein efficiency ratio — PER) than milk, which could be explained as being due to its better protein digestibility. Also, as a result of bacterial metabolism, both the total nitrogen (TN) and the nonprotein nitrogen (NPN) in kefir are higher than those in the milk it is produced from. Schmidt et al.110 reasoned that kefir, like yogurt, had
a higher protein digestibility that contributed to its higher nutritional value and capacity to regenerate liver tissue in rats that had undergone partial (70%) hepatotectomy.

During fermentation and storage, the amounts of free amino acids in kefir increase, particularly lysine, proline, cystine, isoleucine, phenylalanine, and arginine.111–113 The bacteria and yeasts used to produce kefir also result in the isomerization of the L-amino acids liberated from milk proteins to D-amino acids, particularly D-alanine, D-leucine, D-aspartic acid, and D-allo-isoleucine. D-amino acids are less common in yogurt.114 The addition of sodium caseinate to milk results in a product that is even higher in protein content (up to 3.5%), and this has been proposed as a possible dietetic drink.115

4.6.3 LACTOSE METABOLISM

Kefir contains a variety of microorganisms that have the potential to aid lactose digestion. People with lactose intolerance have an insufficient activity in their intestines of the enzyme β-galactosidase (EC 3.2.1.23) or lactase — the enzyme responsible for the hydrolysis of lactose into glucose and galactose. Fermented milk products offer hope to such people because some of the microorganisms used in the fermentation of these products possess lactase activity. Yogurt is often suggested as a dairy product that can be consumed by people with lactose intolerance because the lactase activity in yogurt microorganisms breaks down some of the milk lactose during yogurt production and storage and because lactase activity increases after consumption of unpasteurized yogurt due presumably to the presence of yogurt bacteria in the intestines that retain lactase activity.116 Alm117 studied the decrease in lactose content in several fermented milk products including kefir and found that compared to yogurt, acidophilus milk, and bifidus milk, the decrease in kefir lactose was less following fermentation and storage for up to 14 days. In their study with pigs, de Vrese et al.118 showed that while kefir grains did have some β-galactosidase activity, kefir drink did not. Farnworth and Mainville117 have found that the galactosidase activity in kefir is very low compared to that of yogurt containing live bacteria.

Lactic acid is found in all fermented dairy products as a result of the action of homo- and heterofermentative microorganisms. Lactic acid can exist in either the L-(+) or D-(−) isomeric forms and as a 50/50 DL racemic mixture. L-(+)-lactic acid is completely metabolized by the body, but D-(−)-lactic acid is used more slowly by the body, and excess D-(−)-lactic acid can cause metabolic disturbances. Alm119 quotes World Health Organization documents that suggest that infant nutrition products containing D-(−) or the DL mixture should be avoided, although it is admitted that more research needs to be done to verify this.120 Kefir contains almost exclusively L-(+)-lactic acid; in yogurt the ratio of L(+)D(−) is 58:42.119

4.6.4 VITAMIN CONTENT

Cows’ milk is generally considered a good source of most water-soluble vitamins, except for ascorbic acid and vitamin B12. Several investigators have measured the quantity of vitamins in kefir to determine whether fermentation changed levels compared to milk, but the results have not been consistent. An early study of the
vitamin B\textsubscript{12} content of kefir indicated that both during the fermentation and maturation stages, the vitamin B\textsubscript{12} content went down.\textsuperscript{121} Alm\textsuperscript{122} used commercially available grains to produce kefir that had increased folic acid content compared to the starting milk but decreased concentrations of vitamin B\textsubscript{6}, vitamin B\textsubscript{12}, and biotin. Of the various fermented products studied by Alm, kefir had the lowest reduction (17\%) in orotic acid (also referred to as vitamin B\textsubscript{13}) as compared to the starting milk. Yogurt had a 47.8\% reduction in orotic acid. Bossi et al.\textsuperscript{112} reported declines in vitamin A, thiamin, riboflavin, nicotinamide, and vitamin C in laboratory-prepared kefir compared to the starting milk.

Kneifel and Mayer,\textsuperscript{123} using ten different sources of grains collected from Austrian households, reported increases in pyridoxine (9/10), vitamin B\textsubscript{12} (5/10), and folic acid (7/10) of laboratory-prepared kefir compared to the starting cows’ milk. Most kefir samples had folic acid contents over 20\% higher than those of the starting milk. Thiamin (8/10), riboflavin (10/10), niacin (8/10), pantothenic acid (7/10), and orotic acid or vitamin B\textsubscript{13} (9/10) levels in the finished kefir were lower than in the starting milk. When different sources of milk were compared (cow, ewe, goat, mare), ewes’ milk had the largest percent increases of thiamin, pyridoxine, and folic acid, while goats’ milk had the largest percent losses of thiamin, riboflavin, vitamin B\textsubscript{12}, niacin, pantothenic acid, and orotic acid. The source of the milk may influence the growth of particular microorganisms which, in turn, determine the final vitamin content of kefir.

Mann quotes Russian, Czech, and Polish references that describe attempts to increase the nutritional quality of kefir.\textsuperscript{65} The total protein content of kefir can be increased by adding sodium caseinate to the starter milk. Specific bacteria can also be added to the initial mother culture to increase the levels of folic acid and vitamin B\textsubscript{12}.

### 4.6.5 Kefir as an Infant Food

A Russian team studying premature infants found that a mixture of kefir and “Similac” type formula was well tolerated and produced adequate weight gain when fed to healthy premature infants. The blood fatty acid pattern in the infants was similar to that found in the kefir–Similac mixture that was fed.\textsuperscript{124} Kefir is used widely in Russian hospitals, particularly in neonatal wards, both for mothers and newborns. It is often recommended as the first food for babies after breastfeeding because of its high digestibility. Goncharova et al.\textsuperscript{125} reported that kefir fed to premature infants did not restore their intestinal bifidobacteria nor reduce the number of disturbances of biocenosis. It appears that kefir was also used in the treatment of biliary tract diseases associated with diseases of the pancreas in children.\textsuperscript{126} Ormisson and Soo\textsuperscript{127} attempted to reduce the decline in buffer bases in children up to 2 years old suffering from acute pneumonia or acute bronchitis. Kefir and sour milk both shifted the acid–base balance to acidosis, which is not desirable in sick children. It appears that some kefir products were produced specifically for children.\textsuperscript{128}

### 4.6.6 Other Nutritional Uses

Kefir has been used as part of a weight reduction program in the former Soviet Union, where obese patients are given only kefir to consume every second day of
Kefir: A Fermented Milk Product

treatment. Kefir has also been used in the former Soviet Union as a vehicle for increasing the essential fatty acid (EFA) intake of patients with metabolic diseases and intestinal tract disorders. This EFA-enriched kefir has been prescribed to patients with atherosclerosis, ischemic heart disease, obesity, peptic ulcers, and liver and gallbladder pathologies.

A Japanese patent has been granted for the formulation of a health food that contains kefir together with enzyme inhibitors such as lipase or α-amylase. This product is purported to prevent and control obesity.

4.7 PHYSIOLOGICAL EFFECTS OF KEFIR CONSUMPTION

Kefir has a long tradition of being regarded as good for health in the countries where it is a staple in the diet. However, published human feeding trials to substantiate this view are not numerous. Kefir, kefir grains, kefiran, and the bacteria found in kefir have been the subject of scientific studies to demonstrate beneficial effects on humans.

4.7.1 Kefir as a Probiotic

A probiotic is defined as a microbial preparation which contains live and/or dead cells including their metabolites, which is intended to improve the microbial or enzymatic balance at mucosal surfaces or to stimulate immune mechanisms. Kefir contains many different bacteria and yeasts. To date, however, no scientific studies have been published showing health benefits of kefir microorganisms. The numbers of microorganisms in kefir are large enough (>10⁷ CFU/g) that kefir can be considered a probiotic. During the fermentation process, microbial metabolites and/or degraded milk constituents may be produced that are also beneficial to human health.

Studies have been published where kefir grains, kefir itself, or kefiran have been given to animals and humans to ameliorate a variety of conditions and diseases. The Russian literature contains articles describing the effects of kefir consumption, but these studies are not always readily available.

4.7.2 Antitumor Effect in Animals

The antitumor effect of kefiran was first reported by Shiomi et al. They gave mice a polysaccharide isolated from kefir grains dissolved in drinking water for 7 days prior to injection with Ehrlich carcinoma (EC) cells and continuing for 24 days, or starting the same day as the injection of tumor cells and continuing for 23 or 24 days. Forty to 59% inhibition of tumor growth was found in the mice receiving 0.02 to 0.1% polysaccharide in their drinking water. The positive effect was observed for mice receiving the polysaccharide either starting before the administration of the tumor cells or at the same time as the tumor cell dose. In a second experiment, similar results (30 to 81% tumor growth inhibition) were found when mice were dosed with Sarcoma 180 (S180). Groups of mice were also given intraperitoneal injections of polysaccharide (0.05 to 2.0 mg/mouse). The polysaccharide was administered starting 7 days before or 1 day after tumor injection, and again the growth
of both EC and S180 tumors was significantly reduced. Shiomi et al. carried out cytotoxicity tests in which they incubated EC and S180 cells with a solution of kefir polysaccharide (1 mg/ml) and showed no effect, and this led them to conclude that the antitumor effect was host mediated.

This same group carried out another study in order to define the mechanism of the antitumor effect of kefir polysaccharide.\textsuperscript{134} They showed that when mice were given polysaccharide isolated from kefir grains either by gastric intubation or dissolved in the drinking water (on days 1 and 11 of the experiment), their cell-mediated immune response was increased as measured by the delayed-type hypersensitivity (DTH) test. As little as a single dose of 5 mg/kg body weight was sufficient to produce a significant effect. This effect was shown to be dependent on the total dose administered and not the duration or frequency of the dose. When these same mice were then inoculated with EC cells, mice receiving as low as 10 mg/kg body weight had significantly reduced tumor weights after 14 days. This relation between increased immune response and reduced tumor growth was shown to be active in tumor-bearing mice but not in intact mice. It was suggested that kefir polysaccharide could be useful against tumor growth when administered either before or after the initiation of the tumor. However, larger doses of polysaccharide might be necessary if administered after tumor inoculation.

In a third paper, Murofushi et al.\textsuperscript{135} investigated the antibody response of mice given kefir polysaccharide. The polysaccharide was shown to significantly enhance the antiserum red cell (SRBC) response, but only at a low antigen dose ($5 \times 10^6$ SRBC/mouse). A treatment of 100 mg polysaccharide/kg body weight was shown to be most effective, and the data indicated that the polysaccharide was exerting its effect in early events of the antibody response. A lack of response in nu/nu, nu/+ and conventional mice to 2,4-dinitrophenyl-alanylglycylglycyl-Ficoll (DNP-Ficoll) as a TI-2 antigen or trinitrophenyl (TNP)$_2$-lipopolysaccharide (TNP–LPS) prepared as a TI-1 antigen indicated that the kefir polysaccharide was perhaps enhancing only primary T-dependent B cells and not B cells responsive to TI-1 or TI-2 antigens. Using $^3$H-labeled polysaccharide, they were able to show that the water soluble polysaccharide was absorbed into the body within 3 hours of oral administration. Radioactivity was found in all major organs, and it was concluded that intact polysaccharide reached the spleen or thymus to activate the immune system.

The Japanese literature contains a series of articles in which the antitumor properties of kefir itself are described. Furukawa et al.\textsuperscript{136} found that mice given a subcutaneous inoculation of Lewis lung carcinoma had significantly smaller tumor weights and a 62% tumor inhibition rate 2 weeks after tumor inoculation. The mice received a gastric intubation of pasteurized kefir from day 1 to day 9 after tumor cell inoculation. Spleen weights and the number of leukocytes in tumor-bearing mice increased compared to control mice, but not in the mice receiving the kefir. This same group showed that feeding mice kefir (2 g/kg body weight) for 1 to 6 days after tumor inoculation resulted in an increase in delayed-type hypersensitivity response. However, no difference was found in the survival period of control mice vs. those receiving the kefir.\textsuperscript{137} This group’s data showed that kefir consumption significantly increased the number of leukocytes in normal mice and significantly decreased delayed-type hypersensitivity as measured by foot pad swelling in mice.
Kefir: A Fermented Milk Product

bearing Meth-A fibrinoma. Using similar protocols and tests, Kubo et al.\textsuperscript{138} showed that oral administration of kefir (100 or 500 mg/kg body weight for 10 day starting 1 day after tumor inoculation) significantly reduced Ehrlich carcinoma tumor weight and inhibited tumor growth by up to 54%. When mice were given kefir together with mitomycin C, the average tumor weight was even further reduced.

Furukawa et al.\textsuperscript{139} have also looked at antimetastatic effects of two polysaccharide fractions of kefir grains. Both young (5 wk) and old (30 wk) female mice receiving the water-soluble fraction had significantly reduced pulmonary metastases of Lewis lung carcinoma. This fraction inhibited tumor growth when it was given to the young mice both before and after the tumor cell challenge. Mice given the insoluble fraction for 9 days before a challenge with B16 melanoma had an inhibition rate of 39% compared to the control mice. However, the water-soluble fraction was not protective against this highly metastatic cell line.\textsuperscript{139}

Recent studies have looked at the antimutagenic properties of bacteria isolated from kefir in an attempt to understand their mechanism of action.\textsuperscript{140} Starting with kefir manufactured in Mongolia, researchers isolated strains of \textit{Streptococcus lactis} (5), \textit{Str. cremoris} (3), \textit{Str. faecalis} (1), \textit{Lb. plantarum} (1), \textit{Lb. brevis} (1), and \textit{Leuconostoc dextranicum} (6). Using a binding assay in which the bacteria were incubated with mutagenic amino acid pyrolyzates, they showed that all the bacteria isolated from kefir had remarkable binding ability (>98.5%) to the mutagens 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole and 3-amino-1-methyl-5H-pyrido [4,3-b] indole but a lesser binding ability to 2-amino-6-methylpyrido [1,2-a:3',2',-d] imidazole. Hosono et al.\textsuperscript{140} concluded that these findings, along with similar results obtained with bacteria isolated from yogurt, supported the idea that the consumption of fermented dairy products had a negative correlation with the risk for the development of colon cancer.

Miyamoto et al.\textsuperscript{141} were able to isolate 31 strains of bacteria from kefir grains and kefir produced in western Europe, of which three strains of \textit{Lactococcus lactis} ssp. \textit{cremoris} had the strongest desmutagenic properties. These bacteria were also found to be slime-forming bacteria, and their antimutagenic properties appeared to be due to the binding affinity of AF-2 to bacterial cells. Cevikbas et al.\textsuperscript{142} showed that intraperitoneal injection of 0.5 ml kefir/day for 20 days after the establishment of fusiform cell sarcomas significantly reduced the size of the tumors in mice and even resulted in the disappearance of tumoral necrosis in some animals.

Yoon et al.\textsuperscript{143} used the Ames test to measure the antimutagenic properties of \textit{Lactobacillus} spp. isolated from kefir and yogurt and were able to show that 36 out of 40 strains found in European kefir and yogurt protected \textit{Salmonella typhimurium} TA 98 from the mutagen 2-nitrofluorene.

To date, no human studies have been carried out to verify the antitumor effect of kefir and kefiran in humans.

4.7.3 \textbf{Antibacterial, Antifungal, and Antiviral Properties of Kefir}

Recent studies looking at the production of bacteriocins by kefir microflora found different types of bacteriocins produced. A study on 33 sources of kefir grains from
Ireland showed the presence of at least three different types of bacteriocins produced by lactococcal isolates. The first bacteriocin had a narrow spectrum, inhibiting only lactococci. A second bacteriocin inhibited strains of *Lb. casei*, *Lb. helveticus*, and *Pedicococcus pentosaceus* of the strains tested. The third bacteriocin had a broad spectrum of effectiveness and inhibited all ten strains tested along with a number of other strains of *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus thermophilus*, and *Staphylococcus aureus*. This bacteriocin was named lacticin 3147 and is produced by *Lactococcus lactis* strain DPC3147. Lacticin differs from nisin in that it is plasmid encoded. Lacticin 3147 and nisin have similar inhibitory properties, and like nisin, lacticin is heat stable. Lacticin could therefore be a key factor in maintaining the integrity of the microflora of kefir grains by inhibiting the growth of foreign organisms. A recent study by Morgan et al. investigated the antimicrobial potential of 38 Irish kefir grains against the pathogens *Listeria innocua* DPC1770 and *Escherichia coli* 0157:H45. It was found that 18 grains were able to fully inhibit the growth of *L. innocua*, 13 could slightly inhibit growth, and seven grains had no inhibitory effect. Against *E. coli*, 34 of the grains completely inhibited growth, three slightly inhibited growth and one source of grains had no inhibitory activity. The inhibitory effect of the kefir starters against *L. innocua* was attributed to the production of bacteriocins by the microflora. However, the inhibitory effect on *E. coli* was attributed not to a bacteriocin, but to the combined effect of acid and hydrogen peroxide.

Bossi et al. tested lab-produced kefir and various combinations of bacteria found in kefir for their inhibitory effect against several intestinal pathogenic microorganisms. The kefir inhibited the growth of eight pathogens but was not effective against two strains of *E. coli* and one strain of *Staphylococcus aureus*.

Kefir grains have antibacterial activity greater than kefir itself especially against Gram-positive cocci, including staphylococci, and Gram-positive bacilli. In addition, kefir was shown to have antifungal activity against a variety of fungi, yeasts, and molds. Cevikbas et al. concluded that the antibacterial and antifungal activity of kefir helped to explain the wide use of kefir against infectious and neoplastic diseases. They quoted the work of Ormisson and Soo as a human study that supports their conclusions. However, a translation of this latter article indicates that the use of kefir in children with pneumonia and acute bronchitis as a means of improving acid–base balance was not successful.

Serot et al. were able to isolate and partially characterize two antimicrobial agents from bacteria found in kefir grains. These two substances were found to be effective against Gram-positive and Gram-negative bacteria, had molecular weights of approximately 1000 daltons, and were inhibited by some proteolytic enzymes. Zacconi et al. showed that chicks challenged with *Salmonella kedougou* were protected by live kefir but not irradiated kefir, yogurt, or acidified milk. The treatment was most effective if the kefir was given at the same time as the *Salmonella* challenge, but it also offered protection if given 1 day or 6 days after infection.

The antimicrobial activity of fresh kefir differs from that of reconstituted lyophilized kefir. Fresh kefir had an intrinsic inhibitory power against *Staphylococcus aureus*, *Kluyveromyces lactis*, and *E. coli*, but not against *Saccharomyces cerevisiae* or *Candida albicans*, but kefir that had been lyophilized and then reconstituted in
distilled water or reconstituted powdered milk had lost this intrinsic inhibitory factor. The addition of ribitol as a cryoprotective agent appeared to be the best way of preserving the inhibitory properties of kefir after lyophilization.\(^\text{149}\)

The antibacterial properties of kefir may be due to a combination of factors including competition for available nutrients or the production of inhibitory metabolites, such as organic acids, during fermentation. Garrote et al.\(^\text{150}\) observed that although the bacteria in kefir have been shown to produce a bacteriocin, lactic acid and acetic acid may also contribute to the antibacterial properties of kefir. Supernatant collected from kefir produced using grains collected from Argentinian households had inhibitory effects against Gram-positive and Gram-negative bacteria, although the effect was greater against Gram-positive bacteria. Milk had no effect. Analyses of the kefir supernatants showed that they contained both lactic acid and acetic acid. When milk supplemented with lactic acid and acetic acid was incubated with \(E. \ coli\) \(^3\), the inhibitory effect was again observed, while nonsupplemented milk had no effect. Milk with its pH adjusted to that of fermented kefir or milk with added citric acid alone had no inhibitory effect against \(E. \ coli\) \(^3\). Since the pH of the mixture determines the percentage of the acid in protonated or dissociated state, the pH of kefir supernatant (3.6 to 3.7) resulted in up to 1.5% of the lactic acid and 0.11% of the acetic acid being in the more powerfully inhibitory undissociated form. Garrote et al.\(^\text{150}\) noted that previous research had shown that even these low levels were capable of inhibiting the growth of nonpathogenic \(E. \ coli\).

Russian researchers have used a product called acipole that contains both \(Lb. \ acidophilus\) and kefir grains to manage antibiotic dysbacteriosis that is an adverse reaction to antibacterial therapy.\(^\text{151}\) It was observed that patients suffering from pneumonia and chronic bronchitis treated with antibacterials often were susceptible to undesirable bacterial infections. Treating patients with an antibiotic and acipole lowered the frequency and severity of dysbacteriosis events.

Interferons initiate intracellular production of several kinds of antivirus proteins to indirectly induce the cell’s virus-resistant state. Kefir has been shown to contain a sphingomyelin that is capable of enhancing the production of interferon-\(\beta\) from the human osteosarcoma cell line MG-63 treated with a chemical inducer poly I:poly C. Activity was found in the range of 2 to 100 \(\mu g/ml\) with a maximum secretion enhancement (14 times) occurring at 25 \(\mu g/ml\).\(^\text{152}\) However, insufficient experimental protocol details were included to allow a full evaluation of the credibility of these findings.

Recently, Besednova et al.\(^\text{153}\) added to kefir a peptide isolated from nervous tissue of squid and found that this mixture, when fed to laboratory animals, stimulated their cellular and humoral immune systems. In mice that had originally been immune deficient, the mixture restored their immune function to normal.

### 4.7.4 Cholesterol Metabolism

The consumption of fermented milk products has long been proposed as a way of reducing serum cholesterol levels. Several human studies have been published in which yogurt was consumed. Taylor and Williams\(^\text{154}\) summarized the results of 13 yogurt/cholesterol feeding trials and found that yogurt consumption lowered blood
cholesterol in eight trials, had no effect in four trials, and increased blood cholesterol in one trial compared to control. Several biochemical mechanisms can be proposed that would predict that consumption of fermented milk will lower blood cholesterol levels. It was argued that probiotic bacteria could cause an increase in the production of short-chain fatty acids, which would in turn decrease circulatory cholesterol levels either by inhibiting hepatic cholesterol synthesis or by redistributing cholesterol from the plasma to the liver. In addition, bile acid deconjugation could be increased in the large intestine. Deconjugated bile acids then would not be absorbed, but excreted from the body. This would stimulate an increase in bile acid synthesis, taking more cholesterol out of circulation.

Vujicic et al. studied the ability of kefir grains to take up cholesterol from milk during a 24-h incubation at 20°C or after incubation and a 48 h storage at 10°C. Grains were obtained from Yugoslavia, Hungary, and the Caucasus. At the end of the fermentation, between 22 and 63% of the cholesterol in the starting milk had been assimilated; by the end of the 48 h storage period, between 41 and 84% had disappeared.

Recently, a randomized crossover feeding trial was carried out with 13 moderately hypercholesterolemic men (serum cholesterol levels 6.0 to 10 mmol/l). Subjects were fed 500 ml/day of kefir for 1 month or the same volume of milk and then the opposite after a 4-week washout period. By monitoring bacteria in fecal samples it was shown that 73% of the subjects were colonized by Leuconostoc sp. bacteria found in kefir (see Figure 4.7). Analyses of fecal samples after the washout period indicated that once the feeding of kefir was stopped, the microbial composition of feces returned to normal. Total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and blood triglyceride levels were not changed during the period of kefir consumption.

4.7.5 Other Uses

Kefir, like other probiotic products, may be most effective at influencing conditions in the gastrointestinal tract. Sukhov et al. fed kefir (500 ml, 5 times/day) to 38 patients suffering from enteritis and found that there were no significant changes to the intestinal microflora population. In 1971, Russian researchers used kefir to treat patients with peptic ulcers in the stomach and duodenum.

The abstract to a patent filed in 2000 claims that a dietary supplement consisting of milk fermented with two different sources of kefir grains (the first grains rich in bacteria and the second grains rich in yeasts) that had been filtered and then dried could be used to prevent osteoporosis. It was stated that the supplement could be used in the prevention and treatment of osteoporosis and other diseases that result from calcium, magnesium, and potassium deficiencies. No data were included in the abstract to support this claim.

Kefir may also stimulate the mucosal immune system. It has been recently reported that young rats (6 months old) fed kefir ad libitum and then immunized intraduodenally with cholera toxin (CT) had significantly higher (86%) serum anti-CT antibodies compared to controls. This response was attributed to an enhanced in vitro antibody secretion by cultured lymphocytes isolated from the Peyer’s patches.
and intestinal propria. The effect of kefir was not observed in older rats (26 months old) in the same experiment. Total serum IgA titers were also not changed due to kefir consumption, but in both young and old rats anti-CT IgG titers were lower in the kefir-fed animals. It was speculated that the immunomodulation effect of kefir may be due to bacterial wall components.

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Kefir: A Fermented Milk Product

Kefir: A Fermented Milk Product


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