Kocatepe Veteriner Dergisi

Kocatepe Veterinary Journal ISSN: 1308-1594

DERLEME / REVIEW

Kefir: A fermented milk product and production methods

Hilmi YAMAN ✉

Adnan Menderes University, School of Health Sciences, Department of Nutrition and Dietetics, 0900-Aydın,TURKEY

SUMMARY

Traditional kefir can not be prepared without "kefir grains". It is agreed that the presence of kefir grains are always necessary to produce a "traditional" kefir. These must be recovered in gelatinous form from kefir after fermentation and they can not be re-constructed from individual microbial components. Any attempts to obtain new kefir grains by various combination of micro-organisms isolated from original grains have failed. Kefir differs from other fermented milk products such as buttermilk and yoghurt which are made with a suspension of growing cells evenly distributed in milk. Large scale production of kefir must therefore, require different fermentation process steps and equipment to those used for large scale production of yoghurt. Recent work has focused on the importance of the grain for consistent kefir production. However, it has been also suggested that it is possible to produce a much more uniform product using freeze-dried concentrated cultures made from kefir grains than kefir produced with grains.

KEY WORDS: Kefir, fermented milk, production, grain

••••

Kefir: Bir fermente süt ürünü ve üretim metodları

ÖZET

Geleneksel kefir, kefir taneleri olmadan yapılamaz. Geleneksel kefirin yapımında kefir tanelerinin bulunmasının gerekli olduğuna herkes hemfikirdir. Taneler fermentasyondan sonra kefirden jelatinöz formda geri kazanılmalıdır ve taneler tek tek mikrobiyal bileşenlerinden yeniden yapılamaz. Orjinal kefir tanelerinden izole edilen mikroorganizmaların çeşitli kombinasyonlarıyla yeni kefir taneleri yapma girişimleri başarısız olmuştur. Kefir, sütün içinde üremekte olan hücrelerin eşit şekilde dağıtıldığı bir süspansiyonla yapılan buttermilk ve yoğurt gibi diğer fermente süt ürünlerinden farklılık gösterir. Bu nedenle büyük miktarlardaki kefir üretimi, büyük miktarlarda yoğurt üretiminde kullanılanlardan daha farklı alet ve fermentasyon aşamalarını gerektirir. Son çalışmalar tanenin tutarlı kefir üretimi için önemi üzerine odaklanmıştır. Bununla beraber, ayrıca önerilmiştir ki kefir taneleriyle yapılmış kefirden daha birörnek kefiri, kefir tanelerinden dondurularak kurutulmuş konsantre kültürler kullanılarak üretmek mümkündür.

ANAHTAR KELİMELER: Kefir, fermente süt, üretim, tane

✉ Hilmi Yaman, Adnan Menderes University, School of Health Sciences, Department of Nutrition and Dietetics, Aydın, TURKEY
E-mail: hilmiyaman@hotmail.com

"Partial Characterisation of Lactobacilli Isolated from Commercial Kefir Grain" isimli doktora tezinden özetlenmiştir.

INTRODUCTION

Milk is an important part of many peoples' diets world-wide. Consumption of milk and fermented milk products is widespread, and even in countries where there is no tradition for milk and dairy products, consumption is increasing. This is the result of changing consumer lifestyles and attitudes to food, including awareness of the nutritive value and health benefits of dairy products. In developed countries where there is an established dairy industry, new products are being developed to sustain consumer interest. As the market develops there is a requirement from technology and research to improve the quality and manufacture of fermented milks to maintain the new markets.

Fermentation is one of the oldest methods of preserving or enhancing flavours and texture of raw food materials. Milk fermentation can be defined as any modification of the chemical or physical properties of milk or dairy products resulting from the activity of microorganisms or their enzymes. This activity can involve metabolizing cells, extracellular enzymes, or intracellular enzymes released after cell lysis that contributes to desirable flavors and textures in products or results in spoiled and degraded products. From the point view of scientific understanding of food preparation and preservation, there are two basic advantages for converting milk into fermented dairy products. The first is to preserve the high-quality nutrients present in the fluid milk in the form of relatively more stable products and the second is to provide organoleptic and sensory variety in foods. These are obtained by the microbial modification of milk constituents. To ensure development of desired fermentations, microbial cultures including lactic acid bacteria (LAB), micrococci, propionibacteria, brevibacteria, moulds and yeasts are added to milk or to dairy products substrates.

Yoghurt and cheese are the most well known of the fermented milk products, and over 400 types of fermented milk products exist worldwide.[1] However closer study will show that many products are very similar and some may have only a regional significance. A more accurate list would include few varieties especially when taking into account the type of milk used and the microbial species which dominate(s) the flora. According to Robinson and Tamime[2] fermented milks can generally be divided into three broad categories based on the metabolic products which are lactic fermentations, yeast-lactic fermentations and mould-lactic fermentations. Some closely related products are manufactured from fermented milks by: (a) de-wheying to concentrate the product which could resemble soft cheese (e.g. labneh, ymer or skyr), (b) drying of cereal/fermented milk mixture (e.g. kishk or trahana) and (c) freezing fermented milk to resemble ice cream.

In recent years there has been more interest in different fermented milk products known only to particular countries with a view to adapting them for commercial large-scale production in other parts of world. Though all the products listed in Table 1 are produced commercially, the best known of these in the Western world are yoghurt, acidophilus milk, kefir, koumiss and yakult.

Table 1. Fermented milk products that are commercially produced.*

Product	Country	Type of fermentation
Yoghurt	USA, Europe, Asia	Moderate acid
Cultured buttermilk	USA	Moderate acid
Acidophilus milk	USA	High acid
Bioghurt	Europe	High acid
Bulgarican milk	Europe	High acid
Yakult	Japan	Moderate acid
Leben	Egypt	High acid
Surati cheese	India	Mild acid
Shrikhand	India	Sour and sweet
Kefir	USA, Europe, Asia	High alcohol
Koumiss	Europe, Asia	High alcohol

*Adapted from Shanani and Friend.[3]

Few people recognize that these products are prepared by bacterial and/or yeast action and the characteristic flavours and textures of these products are results of these fermentations. Although it is evident that there is a considerable degree of similarity in respect of technological aspects, by today's knowledge of fermented milks, the manufacturing stages are still a complex process which combines the following disciplines: (1) microbiology and enzymology, (2) chemistry and biochemistry, (3) physics and engineering.[4]

Tamime[4] has considered how these products may have arisin and observes that the origions of fermented milks is difficult to establish, but it is possible to suggest that modern fermented milks production may have evolved as follows: firstly the manufacturing techniques involved the constant use of the same vessels or the addition of fresh milk to an ongoing fermentation relying mainly on the indigenous microflora to sour the milk, secondly, the heating of milk over an open fire to concentrate the milk slightly followed by seeding the cool milk to (blood or ambient temperature) with previous day's sour milk, and thirdly, the preparation of these products with the use of defined micro-organisms since the early 1900s.

Kefir: A Fermented Milk Product

The names of the well-known fermented milks, such as yoghurt, ayran/airan and probably koumys/koumiss come from Turkish language,[5,6] so too is the name of kefir which is thought to originate from Turkish word "key(i)f" meaning "good feeling", for the sense of well-being experienced after drinking it.[7]

Kefir is a self-carbonated, lactic sour, fermented milk beverage and it is produced by co-incident lactic acid and alcohol fermentation. It is made from whole or skim milk and produced by adding a unique culture "kefir grain" to the fresh milk. The culture contains a group of micro-organisms dominated by a *Lactobacillus*/yeast population in the form of grains because the micro-organisms are embedded in a resilient, insoluble polysaccharide matrix called " kefiran".[8,9]

In the kefir fermentation process, alcohol and carbon dioxide are produced by lactose-fermenting and non lactose-fermenting yeast while lactic acid is formed by lactic acid bacteria. After fermentation the grains are recovered and used to ferment the next batch of milk. The fermented milk is bottled and stored. The end product, fermented milk, kefir is defined as a fizzy refreshing beverage with a smooth texture, creamy consistency, mildly acidic taste and a slight yeasty aroma. It can be consumed with or without addition of fruit, and can be used in cooking (in soups, sauces, and cakes).

Fermented dairy products represent about 20% of the total economic value of fermented foods. Although there are no exact data for the world production of kefir, it has been produced on the industrial scale in Moscow since 1930s and is one of the popular cultured drinks. Today kefir production is well known and wide-spread. In 1984, the estimated output of kefir and traditional German soured milk was 130,000 tons in Germany, with a per capita consumption of just over 2 kg. In 1988, 107,000 million litres of fermented milk was produced in Poland of which 1/3 was kefir.[5,10] It is also produced in Slovakia, Sweden, Finland, Hungry, Norway, Denmark, Switzerland and recently in USA. The market is expanding and interest is growing in Western Europe and Japan. In Turkey, kefir is made traditionally at home, but it is also available commercially on the market in Turkey by a few dairy companies. Interest in kefir is growing and there is a promotion for its large scale production due to its health benefits.[11]

History of Kefir

Traditional kefir has a long history and has been manufactured for hundreds of years in homes. The traditional fermentation was a continuous culturing process that the milk and grains were mixed in the leather sacks and fresh milk added as the finished kefir was gradually removed.

The motherland of kefir is often considered to be the northern slopes of Caucasus Mountains which are located between Black Sea and Caspian Sea on the borders of Georgia. Those people who lived in the Caucasus Mountains learnt to make kefir from cow and goat milk with the starter culture called kefir grains. It is not certain where and how these grains appeared, but only a legend tells us that kefir grains were given to the people by the Prophet Hz. Mohammed (peace be upon him) who also told them not to pass on the kefir grains because they would lose their mysteries and strength.[12] This legend might explain why kefir grains and the method for kefir preparation have been carried on at home for such a long time. This legend also gives us a time indication that Mohammed (p.b.u.h.) was told to be chosen as a prophet in 611 AD and died in 632 AD. During this time period he performed many miracles that were witnessed by people in his time. Since kefir grains has not been reconstructed artificially from isolates, it might be true that kefir grains were a gift to those nomadic people by the Prophet Hz. Mohammed (p.b.u.h.).

Kefir remained unknown until travellers took it to Europe in the eighteenth and nineteenth century. Marco Polo spoke of kefir in his travels, after which it was forgotten for several hundred years in the west. Renewed interest in kefir occurred in the West in the early nineteenth century as it was found to be useful therapeutically for the treatment of tuberculosis at sanitariums.

At the beginning of this century the All-Russian Physician's Society asked two brothers who owned cheese manufacturing factories in the Northern Caucacus town, for helping in obtaining the kefir grains. One of the brothers sent one of his employees to get access to the desired grain. In September 1908, the first bottles of kefir were brought to Moscow for sale where it was first used for medicinal purposes. In the 1930s, kefir production started on the industrial scale in Russia and from there spread to Europe and to USA.

The Importance of Kefir as a Food

In many parts of Caucasus Mountains, many of the natives who are still active beyond 100 years of age, drink kefir. As it is rich in proteins, minerals and vitamins, there is a view that it has great benefits for health and well being.

Kefir is considered to be one of the best remedies for digestive troubles by many Naturopathic doctors. Because it is such an easily digested nutritious food, it is ideal for infants, pregnant women, nursing mothers, convalescents, the elderly, people who suffer from constipation, and those who have other abnormal digestive activity.

Elie Metchnikoff,[13] an international Nobel prize winning researcher, found that kefir activates the flow of saliva, most likely due to its lactic acid content and its slight amount of carbonation. Kefir stimulates peristalsis and digestive juices in the intestinal tract. For these reasons, it has been recommended as a post-operative food since most abdominal operations cause the bowels to stop contracting and pushing food along (peristalsis).

Orla-Jenson,[14] a Danish bacteriologist who is specializing in dairy research states that kefir digests yeast cells and has a beneficial effect on the intestinal flora that kefir may aid in the prevention of a condition known as "*Candida*" and excessive growth of yeast cells.

Kefir has mild laxative properties. In Germany and many parts of Asia it is used extensively with cases of chronic constipation and is used for a wide variety of intestinal disorders. It is also recommend for restoring the intestinal flora of people recovering from a serious illness or being treated with antibiotics. It has been effective for people who can not tolerate dairy products due to lactose intolerance.[15]

Nature of Kefir

Kefir, as a fermented milk beverage has an appearance similar to low milk solids yoghurt and is pourable. It should have a creamy consistency and a discernable effervescence in consistency. Kefir is characterized by a uniform weak gelation of the milk. Although kefir has a low pH of 4.2-4.6 and is pourable, the product does not whey-off.[16] There are differences in acidity between the traditional and the modern kefirs. The latter may be prepared in high volumes by using lyophilized starters and separate fermentation steps such as a lactic acid fermentation followed by yeast/lactic fermentation,or mixing milk fermented using lactic acid bacteria with milk fermented by yeast/lactobacilli. These products may have a titratable acidity of 1% and pH 4.0 or below. Kefir is described as a "self carbonated" product with the fizzy character due to carbon dioxide (0.08-0.2%) as a result of yeast metabolism, although heterofermentative lactic acid bacteria could contribute in a small way. It has a refreshing acidity, a buttery, yeasty aroma and an alcohol content (0.08-2%).

The flavour of kefir is a result of many components: lactic acid (0.9%), formic, succinic, acetic and propionic acids; acetaldehyde; ethanol; acetone; diacetyl. Diacetyl is responsible for the buttery aroma and may reach levels of 1 ppm.[17] The protein content is 3-3.4% and there is a slight increase in the content of vitamins B1, B2 and in folic acid during the fermentation. In the presence of propionibacterium strains a marked increase in vitamin B12 level is noted.[18] Traditional kefir can not be prepared without kefir grains. These must be recovered in gelatinous form from kefir after fermentation and they can not be re-constructed from individual microbial components. This differs from other fermented milk products such as buttermilk and yoghurt which are made with a suspension of growing cells evenly distributed in milk.[19] Large scale production of kefir must therefore, require different fermentation process steps and equipment to those used for large scale production of yoghurt. These differences are detailed in Figures 1 and 2.

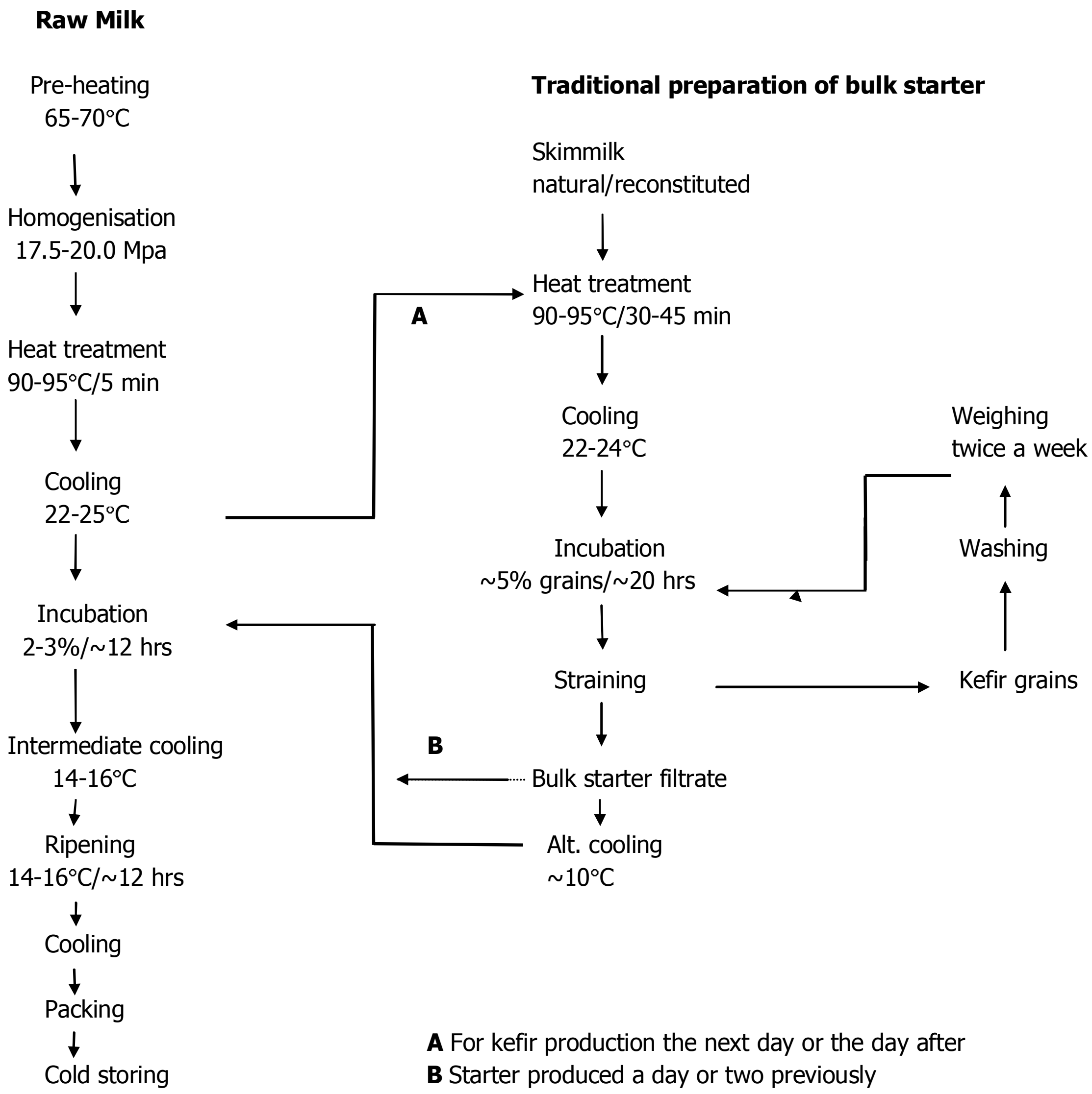

Figure 1. The manufacturing stages of commercial kefir production, courtesy of Tetra Pak (Processing Systems Division).[20, 21]

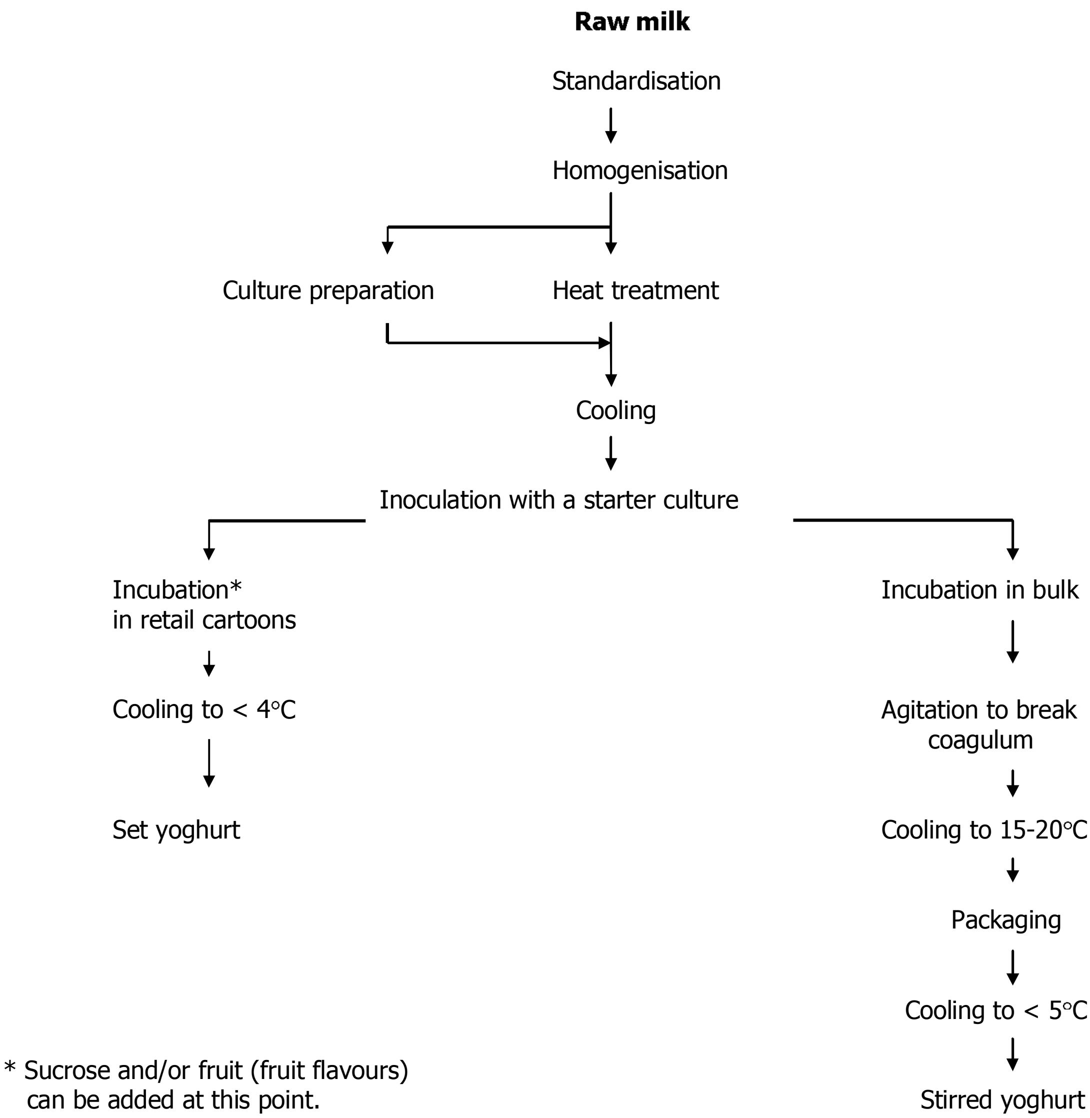

Figure 2. Outline of the principal steps necessary for the production of yoghurt (Robinson and Tamime, 1993).[19]

Macroscopic Structure of Kefir grains

Kefir beverage and kefir grains have a long history, consumed and produced across a wide geographical region. The products and grains have therefore been studied in different regions. It is not certain that there is comparison between different samples. In some studies, the grains that have been studied come from an identified source, in some the data has been obtained from products of "home production" where asepsis will not be observed and other studies have been on commercially available products. The published data, therefore on morphology and microbial content of kefir and kefir grains using different samples from different sources can be expected to vary. It has been found very complex, variable and not constant. Under naked eye observations, kefir grains on recovery from milk are large, shiny and globular, with milk solids adhering to their surfaces. They are quite large, up to 6 cm, but on washing with water they are of various smaller sizes (0.5-3.5 cm diameter) and are characterised by an irregular form. Mature grains resemble miniature cauliflower florets in shape. They are white or yellowish in colour and have an elasticity when pulled apart. The grains, recovered after a fermentation process and washed with water may also show thin sheet-like forms and scrolls.[22,23] The sheet-like forms show an asymmetry: one side is smooth while the other side is rough and convoluted. The mechanism of grain formation is not clear and recently a distinct kefir grain formation of a globular, sagy structure resembling small pouches has been reported.[24]

Making Kefir Beverage From Kefir Grains

Making kefir was a simple process for those people who lived on the Caucasian mountains. Kefir was traditionally made in goat or sheep skin bags by continuous fermentation in a natural and uncontrolled way, where fresh milk replaced the aliquots drawn off for consumption. Natural ambient temperatures were sufficient for fermentation to proceed. The finished product was characterised by high acidity, by low or high CO_2 and an alcohol content dependant on the holding time.

Although kefir has been in production for a many decades, there is not a single identified common standard for the production of kefir as there is with yoghurt and some other fermented products, probably because of its complex microflora. Many dairies produce kefir with a culture from which the grains have been removed or from bacterial cocktails prepared in the laboratory. This is very different from the traditional kefir, because the typical yeast flavour and effervescence is absent and the product has more of the characteristics of buttermilk rather than kefir.[25] There is a general standard of identity for fermented milks published by International Dairy Federation (IDF) determining starter to be used for kefir.[26] Standard makes reference to kefir grains which can be used to make kefir. Standard however, dose not describe how the kefir grains used. It specifies only that different species of lactobacilli and yeast have been identified. Qualitative and quantitative analysis of kefir from different countries show that the microflora of kefir products differs greatly.

Vayssier[27] catalogued the various bacteria found in kefir beverages from different countries and called for a defined starter. Twenty years later studies are still required to identify the micro-organisms of the kefir grains and to produce a product that has similar characteristics to that of traditional kefir.

In general, it is difficult to define a single process for the manufacture of kefir. There have been many attempts to produce kefir at industrial scale similar to traditional kefir made with kefir grains. The starters and methods used by different researchers in different countries are variable and it is possible that commercial companies may have their own starters and procedures.

The traditional way of making kefir had three steps, (1) inoculation (adding fresh milk to kefir grains), (2) fermentation under sunlight and (3) ripening. During each at these stages the kefir was shaken. But on an industrial scale, the method of kefir production differs from the traditional process and from country to country. However, there are stages which are common unit operations.

Maintaining of Kefir Grains

The main source of kefir grains is from a previous batch. During the fermentation, kefir grains float on top of fermentation vessel. Following fermentation the grains are sieved and used to make kefir at home or to provide a mother culture in order to produce bulk culture for large scale industrial production. Although kefir made from kefir grains may be used as an inoculum, kefir made from mother culture cannot be successfully used for subsequent inoculations to obtain an acceptable product, because the original balance of micro-organisms is disrupted.

Kefir grains can be either preserved dry or wet and can be re-used several times if proper sanitation is observed in recovering, drying and storing the grains from batch to batch. One simple method which can be followed at home is washing the excess grains with potable water and drying at room temperature. This crude method of preservation can lead to contamination of the dried grains, and it is possible that the symbiotic microflora may be altered. It is advisable to use boiled water where water sanitation is not good enough to prevent contamination.

Alternatively, the washed grains are suspended in a sterile 0.9% saline buffer solution which can be stored for a few months at ordinary refrigeration temperature (4°C) without any appreciable loss in activity. The wet preservation of kefir grains in 0.9% sterile saline (buffered sodium chloride) solution can be easily practised in a small laboratory environment.

However, use of freeze-dried cultures is a better method and it is more practical for both home and commercial production of kefir. The preserved culture is in the form of a powder or small crystals. After two or three sub-cultures, the grains start to form in the milk.[28]

The starter cultures in sterile 0.9% sodium chloride and freeze-dried grains which have been standardized by addition of yeast isolated from kefir grain are available commercially and can be obtained from specialist starter companies. Addition of yeast is necessary because more than 80% of the yeasts can be lost on freezing and freeze drying of kefir grains.

Recently, a comparative study of storage of kefir grains under different conditions suggested that an alternative method can be used to maintain the grains for household kefir production.[29] These authors found that grains stored in milk at -20°C and -80°C for 120 days maintained their microflora and increased their weights at a rate comparable to non-stored grains. Kefir made with the grains stored in milk at -20°C and -80°C showed the same microflora, rheological behaviour and carbon dioxide content as kefir obtained with non-stored grains.

The Processing of Kefir

I. Use of Dry Grains

Dried grains require activating, and this can be achieved by soaking dry kefir grains in previously boiled tepid water for 2-3 hours, followed by soaking in a 1% solution of sodium bicarbonate for a few hours, preferably in covered steel or glass vessels.[30] Alternatively dry kefir grains are rehydrated in warm water that has been previously boiled and cooled to 25-30 oC for 24 hours with 2-3 changes of water. Following these treatments, grains that are swollen, elastic and clear in appearance are added to boiled or pasteurised milk available from the market-place and incubated at room temperature (25-30°C). After 24 hour or as soon as the milk curdled, the grains are transferred to a new supply of milk and continued making such transfers every 24 hours until grains tend to rise to the surface during fermentation. This is an indication that the grains have regained normal activity.

II. Use of Fresh Kefir Starters

Kefir grains are added to pasteurised skim milk or sterilised skim milk at ratio of 1:10, 1:30 or 1:50 (w/v).[12] After incubation at 20-22°C for 24 hours the grains are filtered through a sieve and the recovered grains are used as a mother culture for the preparation of the bulk starter. Pasteurised and cooled milk is inoculated with 3% (w/v) starter culture and incubated at 20-22°C for 20 hours. Then the grains are recovered and directly introduced into fresh milk. Recovered grains may be washed with water or with skimmed milk. Koroleva[12] reports that weekly washing caused a sharp decrease of the main groups of the starter microflora. After washing, the composition of the normal flora is restored after further cultivation of the grains for 3-5 days. Yeasts were the only group of micro-organisms which did not decrease after washing. As similar results were reported by Pintado et al.,[31] yeasts are apparently the least affected by environmental conditions.

III. Use of DVI Starter Cultures

DVI commercial cultures can be obtained in the concentrated freeze-dried form, or as concentrated cultures deep frozen at -196°C. The latter can be stored between -40°C and -80°C. The former type of culture can be stored at < 5°C or at -20°C, and the latter temperature extends the self life of the culture.[32] Petterson et al.,[28] suggested that it is possible to produce a much more uniform kefir using defined DVI cultures made from kefir grain.

There are a number of publications on kefir starter and production of kefir.[25,28,33,34,35,36,37] These studies suggest different and possible methods for production of kefir which could be similar to those of traditional kefir.

Kefir production varies from country to country due to absence of an international standard method for the production of kefir.

Preparation of Milk

Milk is firstly skimmed to remove fat from milk or standardized to a particular fat content (e.g. 1,2 or 3%). After homogenization, milk is pasteurised at time/temperatures which may vary according to the particular country and the particular dairy. Minimum permitted legal treatments in the UK and the US are 62.8°C for 30 min (low temperature-long time; LTLT) or 71.7°C for 15 s (high temperature-short time: HTST). Milk is either heated at 63°C and held at this temperature for 30 min or passed through a pasteuriser where it is heated quickly to a temperature of 72°C for 15 seconds. In recent years concern over the possible survival of some pathogens (e.g. *Listeria monocytogenes*) has led to some processors increasing the pasteurizing temperature to above the legal minimum. It may be 20-30 min at 85-87°C, 5-10 min at 92-95°C.[17] For preparation of mother culture from grains the higher heat treatment is normally used. Consistency of final kefir may be improved by a higher treatment because of the greater heat denaturation of whey proteins which occurs. This leads to a greater contribution from the whey protein to formation of a good coagulum. Denaturation of whey protein may also be achieved by a double pasteurization, i.e. by heating to 87°C, cooling to 77°C and holding for 30 min then raising the temperature to 87°C again.[17] Some dairies prefer to use UHT (ultra high temperature) milk where milk is subjected to superheated steam. UHT milk varies from country to country. In UK for example milk is heated no less than 135°C for at least 1 s and elsewhere processes vary from 130-150°C for 1-4 s.[38,39]

Preparation of Starter Culture

Fresh grains are added to cooled milk at a ratio of 1:10 by weight. Grain cultivation is carried out at 20°C for 24 h after which grains sieved or filtered through a cheese cloth and washed with sterile water before adding to further batches of milk. Steel or glass vessels are advised for grain cultivation. Some dairies may use different ratios of kefir grain (4-5%). Nowadays lyophilized cultures are used, a 1 g kefir starter is added to 1 quart of milk or 1 g sachet is added to 3 liters of heat -treated milk and incubated at 20°C for 18-20 hours till milk is clotted.

Kefir Production on a Commercial Scale

Basically the production methods of kefir can be divided in two broad sections. (1) Those which have been developed from traditional methods, and (2) those resulted from new starter development.

1) Methods Derived From Traditionally-made Kefir

The traditional or original manufacturing procedures consist of inoculating the milk with kefir grains and then recovering these grains from the finished product for the next production of kefir or future use. This is a cumbersome practice for large scale production of kefir. The composition of the microflora is in a constant flux and may vary from one production to the next. It is difficult to maintain consistently the same high quality. Several methods of producing kefir exist. Pijanowski,[33] and Puhan & Vogt[34] described two methods of kefir production. In the first method, a 0.5% to 10% inoculum of kefir grains is added to heat-treated milk which is kept at 20-23°C for 12 to 24 hours. During this stage the predominant process is lactic fermentation, after which the kefir grains are strained, the product is bottled and held at 10-15°C for 1 to 3 days. During this time the carbon dioxide content from yeast activity increases. The kefir is then refrigerated and distributed to retail outlets.

The second method is widely practised in Europe (Figure 3). The kefir grains are used to produce a bulk starter culture. The grains are then removed and the "grain free" bulk culture is used for high volume production of kefir. The composition of kefir produced by this method is different from that produced with fresh kefir grains. The ethanol and diacetyl contents are much lower, but the lactose content is higher than in kefir 3.7%-3.8% compared to 2.5% for kefir made with grains.[34]

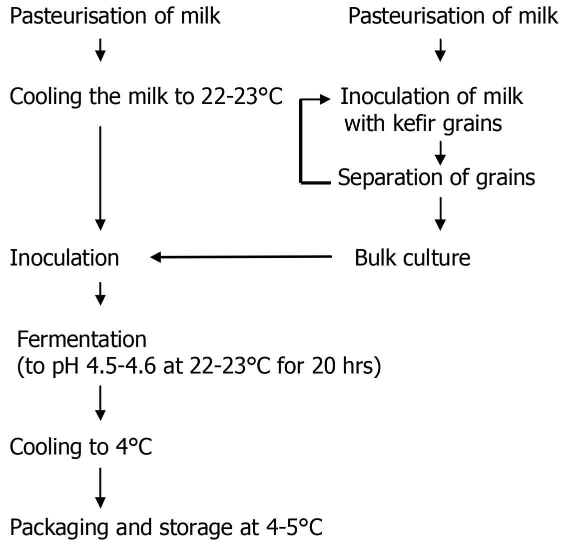

Figure 3. Production of kefir.[34]

In the method of Koroleva,[12] a Russian researcher suggested two types of kefir starters (Figure 4) could be used. Starter (I) is prepared by fermenting milk with kefir grains and starter (II) is prepared by fermenting milk with starter (I). In this case, the starter is prepared in a vessel with a stirrer. Milk is pasteurized and then cooled to 18°C in summer and to 22°C in winter. The milk is inoculated with 2-3% of the starter (I) and carefully agitated. After 18 hrs fermentation, the starter ripens in the course of slow cooling to 8°C over 12-24 hours. Kefir is made by adding 1-3% of starter (I) or 3-5% of starter (II) to new supply of milk. Fermentation lasts 8-12 hours until the coagulum acidity reaches 90-100°N. The fermented milk is than agitated and slowly cooled during 10-12 hours to 8-10°C. Ripening provides the specific taste and aroma. According to Koroleva,[12] kefir made from starter (I) has the typical taste and aroma, therefore it is advised to manufacture kefir even on an industrial scale by using starter (I) whereas, other researchers report that kefir grains are necessary for the production of traditional kefir. Figure 4 illustrates the preparation of starter (I) and starter (II) and production of kefir.

Marshall[17] reported that in Poland, traditional large-scale manufacture of kefir is practised as follows. In winter , heat-treated (pasteurization) milk is cooled to 21-23°C, in summer to 19-20°C, and bulk starter added at 2-3% in summer and 2-7% in winter. This is mixed gently to distribute the starter and the milk dispensed into returnable glass bottles with crown cap closures. Incubation is carried out at 19-23°C for 12-14 hours, followed by a ripening period of 12 hours at 8-10°C. Fermentation may also be carried out in bulk tanks with similar inocula incubated for 6-8 hours. When titratable acidity reaches 0.85-0.9%, the soured milk is mixed and cooled to 14°C, dispensed into polyethylene containers with aluminium foil lids. The kefir is then ripened at 8-10°C for 12-14 hours before distribution to retailer outlets.

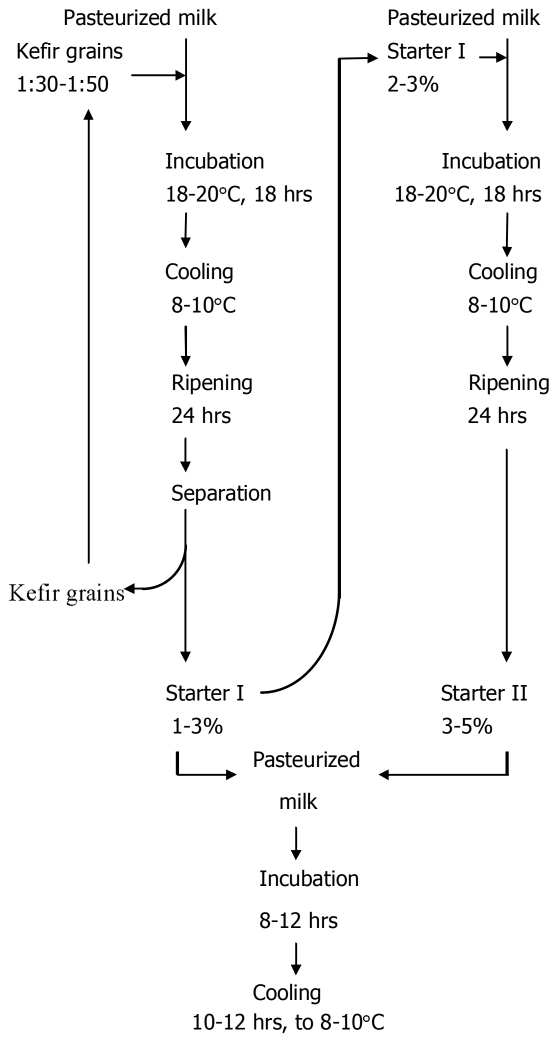

Figure 4. Preparation of Starter I and starter II.[12]

2) Methods Derived From Defined Starter Culture

Recent work has focused on the importance of the grain for consistent kefir production. It is agreed that the presence of kefir grains are always necessary to produce a "traditional" kefir. Any attempts to obtain new kefir grains by various combination of micro-organisms isolated from original grains have failed. However, Petterson et al.[28] suggested that it is possible to produce a much more uniform product using freeze-dried concentrated cultures made from kefir grains than kefir produced with grains. The composition of the freeze-dried culture was chosen in order to obtain a balance of micro-organisms such as lactic acid producing lactococci, citric acid fermenting lactococci, lactobacilli and yeasts (Table 2).

Table 2. Microbial content of freeze-dried kefir starter.*

Bacteria	Number/Percentage
Total amount of micro-organisms/g	$1\text{-}4 \times 10^{11}$
Lactic lactococci	90%
Citric acid fermenting lac.	9%
Lactobacilli	<0.5%
Yeasts	<0.3%

*Modified from Petersson et al.[28]

The production method of kefir using freeze-dried starter is essentially the same compared with the conventional method using grains. After heat-treatment of the bulk milk to 90-95°C for 30 minutes and cooling to the cultivation temperature of 22°C, the freeze-dried starter is added in an approximate amount of 10 g per 100 l of milk. The bulk starter is cultivated for 20-22 hours to a pH of 4.4-4.6. For production of kefir, milk (3% fat) is homogenized and heated to 93-95°C for 3-5 minutes. After cooling to 22-24°C, 2% (v/v) bulk starter is added and incubated for 18-20 hours. The product is then cooled, stirred and packed (Figure 5).

Duitschaever et al.[25] proposed that the microflora of grains is extremely variable but that this does not seem to be critical as long as lactic acid fermentation takes place followed by a yeast fermentation. Therefore, Duitschaever et al.[25] formulated a pure culture and procedure that would produce a milk beverage with characteristics similar to those of traditional kefir. Their culture formulation consisted of a yoghurt culture (*Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus*), *Lb. acidophilus, Lactococcus lactis* ssp. *lactis* and *Leuconostoc*. The yeast was *Saccharomyces cerevisiae* as shown in Figure 6. The coagulum is pumped to a structurizer and cooled to 18-20°C.

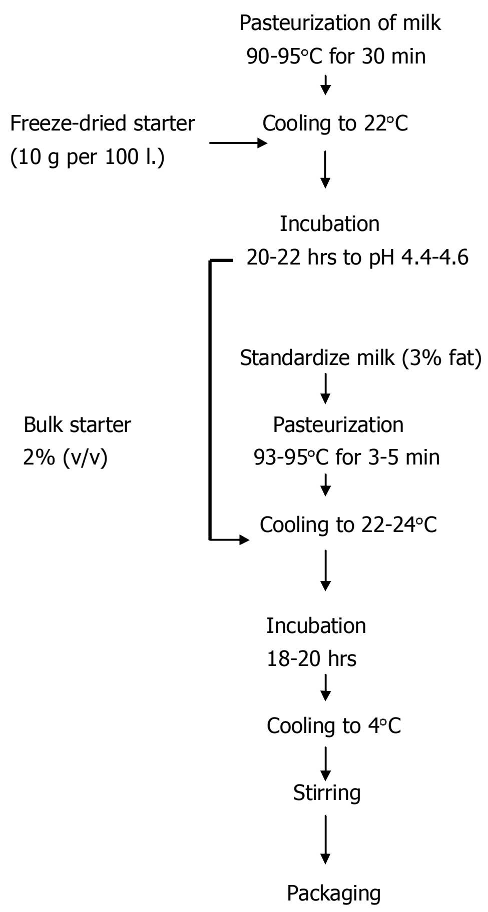

Figure 5. Kefir production, without grain.[28]

Sucrose (5.5g/l) is added and inoculated with *S. cerevisiae* R-107 (1%). After thorough mixing, the kefir is filled into bottles and incubated at 20°C for 24 hours before storage at 4°C.

The analysis of kefir and sensory evaluation indicated that kefir made of this method was acceptable and could be produced successfully on pilot scale (500 l). Storage at 5°C for 42 days did not show any sign of deterioration in quality.[25]

The variety of methods suggested for kefir production show clearly that it is difficult to determine from existing literature, the actual fermentation processes used. However, Duitschaever's[25] process is the only one which seems to provide an understanding of how kefir can be produced from defined cultures. But it is not clear how it relates to large-scale production in kefir producing countries such as Poland, Slovakia and Finland.

Standardise milk (25% fat)
↓
Homogenise
↓
Pasteurise at 85°C for 20 min
↓
Cool to 28-32°C
↓
Inoculate
Yoghurt culture 3%
Lb. acidophilus 3%
Lc. lactis ssp.*lactic-Leuconostoc* ssp.1%
↓
Incubate at 28-32°C (till pH 4.7)
↓
Flash cool to 5°C
↓
Add sucrose (5.5g/l) and inoculate with yeast culture (1%)
↓
Fill into bottles
↓
Incubate at 20°C for 18-24h
↓
Store at 2-4°C

Preparation of yeast inoculum:

Stage 1 : To 200 ml clarified, sterile apple juice, add 2-3 loopful of yeast growth from agar slant, plus 2 ml of a 20% dibasic ammonium phosphate solution. Incubate on reciprocating water shaker bath at 20-22°C for 48 h.

Stage 2 : Add 5% of this aerated culture to the required amount of apple juice to which dibasic ammonium phosphate was added (1 ml of 20% solution per 100 ml of juice) and incubate under static conditions at 20-22°C for 24-48 h. This constitutes the yeast inoculum for the kefir base.

Figure 6. Recommended procedure for the manufacture of kefir on industrial scale.[25]

REFERENCES

1. **Kurman JA, Rasic JL, Kroger M** (1992) *Encyclopedia of Fermented Fresh Milk Products*, Van Nostrand Reinhold, New York.
2. **Robinson RK, Tamime AY** (1990) Microbiology of fermented milks, In: Robinson RK, editor. *Dairy Microbiology-The Microbiology of Milk Products*, Vol: 2, 2nd edition, Elvesier Science Publishers, London, pp. 291-343.
3. **Shanani KM, Friend BA, Bailey, PJ** (1983) Antitumor activity of fermented clostrum and milk. *J Food Prot*, 46, 385-386.
4. **Tamime AY** (1990) In: Robinson RK, editor. *Dairy Microbiology*, 2nd edition, Vol: 2, Elvesier Applied Science Publishers, London, pp. 131-201.
5. **Kurmann JA** (1984) Consumption statistics and aspects of the production of fermented milks. *IDF Bull*, 179, 8-26.
6. **Gerhard JH** (1976) Alcoholic beverages and fermented foods. In Miller BM and Litsky W, editors, *Industrial Microbiology*, McGraw-Hill Inc., New York, US, pp. 165-191.
7. **Novil S** (1998) Kefir. Lifeway Foods, Inc, 7625 N. Austin Ave. Skokie, IL 60077, USA, Personal communication.
8. **La Riviere JW, Kooiman P, Schmidt K** (1967) Kefiran: a novel polysaccharide produced in the kefir grain by *Lactobacillus brevis*. *Archiv Microbiol*, 59, 269-278.
9. **Kooiman P** (1968) The chemical structure of kefiran, The water-soluble polysaccharide of kefir grain. *Carbohyd Res*, 7, 200-211.
10. **Libudzisz Z, Piatkiewicz A** (1990) Kefir production in Poland. *Dairy Indust. Int*, 55 (7): 31-32.
11. **Karagozoglu C** (1990) Kefir. Master thesis (in Turkish), The Department of Dairy Technology, Agriculture Faculty, Ege University, Bornova, Izmir, Turkey.
12. **Koroleva NS** (1988) Technology of kefir and kumys, *IDF Bull*, 227, 96-100.
13. **Metchnikoff E** (1908) The prolongation of life, New York: GP Puthnam's Sons, cited by Shanani KM & Friend AB (1983) Properties of and prospects for cultured dairy foods. In: Roberts TA & Skinner FA, editors. *Food Microbiology Advances and Prospects*, Academic Press, pp. 257-269.
14. **Orla-Jensen S** (1919) The lactic acid bacteria, Copenhagen, Andr. Fred. Host & Son, cited by Petterson HE, Christiansson A, Ekelund K (1985) Making kefir without grains, *Scandinavian J Dairy Technol* and Know-How/Nm 2-85 (Nordisk Mejeriindustri 1985 Vol:12 PT:8 PP(58-60).
15. **Novil S** (1997) Kefir. Lifeway Foods, Inc, 7625 N. Austin Ave. Skokie, IL 60077, USA, Personal communication.
16. **Kneifel N, Mayer HK** (1991) Vitamin profiles of kefirs made from milks of different species. *Int J Food Sci Tech*, 26, 423-428.
17. **Marshall VM** (1993) Kefir. In: Macree R, Robinson R, and Sadler, MS, editors. *Encyclopaedia of Food Science Food Technology and Nutrition*, Vol: 3, Academic Press.
18. **Pijanowski E** (1971) Zarys Chemii I Technologii Mleczarstwa, PWR i L, Warszawa, Vol. I.
19. **Robinson RK, Tamime AY** (1993) Manufacture of yoghurt and other fermented milks, In: Robinson RK, editor. *Modern Dairy Technology-Advances in Milk Products*, Vol: 2, 2nd edition, Elvesier Science Publishers, Essex,
20. **Tamime AY, Muir, DD, Wszolek, M** (1999) Kefir, koumiss, *Dairy Indust Int*, May, pp.32-33
21. **Bylund G** (1995) In: *Dairy Processing Handbook*, Tetra Pak Systems A/B, Lund, Sweden.
22. **Marshall VM, Cole WM, Farrow JAE** (1984) A note on the heterofermentative Lactobacillus isolated from kefir grains. *J Appl Bact*, 56, 503-505.
23. **Marshall V** (1984a) Observations on the structure of kefir grains and the distribution of the microflora. *J Appl Bact*, 57, 491-497.
24. **Yaman H, Elmalı M, Karadağoğlu G, Çetinkaya A** (2006) Observations of kefir grains and their structure from different geographical regions: Turkey and Germany. *Atatürk Üni Vet Bil Der*, 1(1-2): 11-15.
25. **Duitschaever CL, Kemp N, Emmons, D** (1987) Pure culture formulation and procedure for the production of kefir. *Milchwissenschaft*, 42(2): 80-82.
26. **IDF** (1992) General standard of identity for fermented milks, Brussels, Belgium, 163:1992.
27. **Vayssier V** (1978) Kefir qualitative and quantitative analyses, *Revue Lait Franc* No. 361, 73-75, cited by Angula L, Lopez, E, and Lema C (1993) Microflora present in kefir grains of the Galician Region (North-west of Spain). *J Dairy Res*, 60, 263-267.
28. **Petterson HE, Christiansson A, Ekelund K** (1985) Making kefir without grains, *Scandinavian J Dairy Technol* and Know-How/Nm 2-85 (Nordisk Mejeriindustri 1985 Vol:12 PT:8 PP(58-60).

29. **Garrote GL, Abraham, GA, De Antoni, GL** (1997) Preservation of kefir grains, a comparative study. *Lebensm-Wisseu-Technol*, 30, 77-84.
30. **Burkey LA** (1948) Preparation of kefir fermented milks, *Milk Plant Monthly*, January.
31. Pintado ME, Lopes Da Silva JA, Fernandes, BP, Malcata, FX, Hogg AT (1996) Microbiological and rheological studies on Portuguese kefir grains. *Int J Food Sci and Technol*, 31, 15-26.
32. **Tamime AY, Marshall VME, Robinson RK** (1995) Microbiological and technological aspects of milks fermented by bifidobacteria. *J Dairy Res*, 62, 151–187.
33. **Pijanowski E** (1980) Outline of Dairy Chemistry and Technology. (Zarys chemii technologii mleczarstura), Vol.1. PWRL, Warszawa.
34. **Punah Z, Vogt O** (1984) Milchwirsch, Ber. Bundesanst. Wolfpassing & Rotholz, No. 78, p. 7, cited by Mann JE (1985) Kefir and Koumiss, *Dairy Indust Int*, 50 (12).
35. **Marshall V, Cole WM** (1985) Methods of making kefir and fermented milks based on kefir. *J Dairy Sci*, 52, 451-456.
36. **Duitschaever CL, Kemp N, Smith AK** (1988) Microscopic studies of the microflora of the kefir grains and of kefir made by different methods. *Milchwissenschaft*, 43(8): 479-481.
37. **Gobetti, Rosi J** (1994) Batchwise fermentation with Ca-alginate immobilized multistarter cells for kefir production. *Int Dairy J*, 4, 237-249.
38. **Varnam AH, Sutherland JP** (1994) Liquid milk and milk products, In: Varnam AH and Sutherland JP, editors. *Milk and Milk Products-Technology,ChemistryandMicrobiology*,Chapman & Hall, London, UK, p 46.
39. **Varnam AH, Sutherland JP** (1994) Fermented milks. In: Varnam AH and Sutherland JP, editors. *Milk and Milk Products-Technology, Chemistry and Microbiology*, Chapman & Hall, London, UK, p 383.