Fermented Milks

EDITED BY

A.Y. TAMIME

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Preface to Technical Series

For more than 60 years, the Society of Dairy Technology (SDT) has sought to provide education and training in the dairy field, disseminating knowledge and fostering personal development through symposia, conferences, residential courses, publications, and its journal, the *International Journal of Dairy Technology* (previously known as the *Journal of the Society of Dairy Technology*).

In recent years, there have been significant advances in our understanding of milk systems, probably the most complex natural food available to man. Improvements in process technology have been accompanied by massive changes in the scale of many milk/dairy processing operations, and the manufacture of a wide range of dairy and other related products.

The Society has now embarked on a project with Blackwell Publishing to produce a Technical Series of dairy-related books to provide an invaluable source of information for practising dairy scientists and technologists, covering the range from traditional to modern large-scale operation. This second volume in the series, on *Fermented Milks*, under the editorship of Dr Adnan Tamime, provides a timely and comprehensive review of this group of micro-organisms, and how they can be used to produce both beneficial and highly acceptable fermented dairy products.

Andrew Wilbey
President, SDT
January 2005

Preface

One of the main aims of the Society of Dairy Technology in the United Kingdom is 'the advancement of education in dairy science and technology, food technology and management of resources in all branches of the industry by the dissemination and application of knowledge gained from experience and experiment'. Such knowledge is disseminated to the Society's members through meetings at regional level, symposia, conferences and residential courses, publishing a scientific journal (*International Journal of Dairy Technology* in conjunction with Blackwell Publishing Ltd) and providing technical books covering selected aspects of dairy technology.

Given recent developments in dairy technology, it has become apparent that revision of some of the Society's publications is overdue, together with the desirability of adding new titles to cover other dairy products and new developments. Hence, the revised and proposed new publications will comprise a Technical Series, and will be orientated towards a more practical approach to the manufacture of a wide range of dairy products.

Fermented Milks is the second book of the Technical Series, and the range of products reviewed includes yoghurt and related products (e.g. frozen, dried and desserts), Nordic/Scandinavian fermented milk products including buttermilk, kefir and koumiss, concentrated fermentate, and milk-based fermented beverages. The increasing economic value of these products in Europe, North America and the rest of the world is a reflection of consumer acceptability, and the increased basic research in the field of starter cultures and manufacturing methods. Furthermore, these products are highly profitable and very important within the dairy industry worldwide.

Equally important is the fact that the technological developments, and the metabolic activities of the starter cultures including the different blends available for production purposes, have provided consumers in different markets with a wide range of fermented milk products. As a consequence, these products have become popular, and this is reflected in their increased per capita consumption. Although the scientific data are available in journals and books that have been published in the late 1990s, the primary aim of this text is to detail the manufacturing methods and properties of all of these products in one publication.

The authors, who are all specialists in these products, have been chosen from around the world. There is no doubt that the book will receive international recognition by dairy scientists, students, researchers and dairy operatives, and will become an important component of the Technical Series promoted by the Society of Dairy Technology.

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Technical Series Editor
December 2005

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Fermented Milks
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1 Types of Fermented Milks

R.K. Robinson and A.Y. Tamime

1.1 Background

The reasons for fermenting milk are numerous and, although the primary function is to extend its shelf-life, other advantages, such as improving the taste of milk, enhancing the digestibility of the product and the manufacture of a wide range of products (i.e. from yoghurt to concentrated yoghurt to cheese) should not be overlooked. An historical survey of the origin(s) of fermented milk products (e.g. yoghurt, kefir, koumiss, sour milk) shows that they date back to early civilisations around 10 000 BC as the way of life of humans changed from food gathering to food producing; this change also included the domestication of the cow, sheep, goat, buffalo and camel (Rašić & Kurmann, 1978; Pederson, 1979). A wide range of indigenous fermented milk products are traditionally made in rural areas worldwide, and most of these products rely primarily on spontaneous fermentation due to the presence of indigenous microflora (mainly lactic acid bacteria) in the milk. However, in the Asiatic Steppes, for example, special containers made from horse's hide that contain the micro-organisms including yeasts from the previous production batch or season are used during the manufacture of traditional koumiss, and this reliance on containers or utensils to provide an initial inoculum of starter organisms is widespread.

In addition, it is safe to assume that the evolution of any given type of fermentation is dependent on the climatic condition of the region, so that while the thermophilic lactic acid fermentations became predominant in hot and subtropical countries because of the favourable growth conditions of the lactic cultures (40–45ºC), mesophilic fermentations became more popular in colder climates, such as northern Europe (Tamime & Marshall, 1997).

Nowadays, most fermented milks are manufactured under controlled conditions with specified starter cultures, and the aim of this chapter is to provide a general background to the evolution of these products, their properties and the patterns of their consumption in some selected countries. The health aspects attributed to special probiotic micro-organisms used in fermented milks and other dairy products will not be covered in this publication, as they are included in *Probiotic Dairy Products* within this Technical Series prepared on behalf of the Society of Dairy Technology (SDT) in the UK (Tamime, 2005).

1.2 Evolution of the process

Originally, the souring of milk was by no means uniform, and fermentations brought about by mixtures of lactic and non-lactic acid bacteria gave rise to products that were insipid and stale. Furthermore, the coagula were often irregular, filled with gas holes and showed signs of whey syneresis. By contrast, pure cultures of lactic acid bacteria act on milk to produce a fermentate that is pleasant to eat or drink and, over the years, communities in different countries derived fermentation processes that brought the 'souring of milk' under control. In particular, the process of yoghurt making might have included:

- The use of the same vessel, or the addition of fresh milk to an ongoing fermentation, to ensure the build-up of a specific indigenous microflora(s) to sour the milk;
- Heating the milk over an open fire, increasing the solids content of the milk slightly, so yielding a more viscous coagulum due to the modified properties of the casein;
- Seeding of the processed milk at blood or ambient temperature with sour milk from a previous batch, so enabling the thermophilic strains of lactic acid bacteria to become more predominant;
- A gradual selection of lactic acid bacteria capable of tolerating high acidic conditions and giving the product its distinctive flavour;
- Recognition of the fact that the action of heating the milk helped to eradicate any vegetative pathogenic micro-organisms present in the raw milk (Tamime & Robinson, 1999).

Although the evolution of the process was strictly intuitive, the production of fermented milk products became an established pattern of preservation, and, since the early 1900s, defined micro-organisms have been used to prepare these products on a large industrial scale (see Chapter 2 for further details) (Marshall & Tamime, 1997; Tamime & Marshall, 1997; Tamime, 2002a). Gradually, other communities learned of this simple preservative treatment for the milk, and defined products like yoghurt, cultured/fermented buttermilk, kefir, koumiss and/or acidophilus milk gained discrete identities.

1.3 Diversity of fermented milks

A wide range of fermented milks are manufactured throughout the world and, according to Campbell-Platt (1987) and Kurmann *et al.* (1992), around 400 generic names are applied to traditional and industrialised products. Many of these products are known locally by different names but, in essence, a more accurate list may include far fewer variations, especially taking into account (a) the type of milk used (e.g. cow, goat, sheep or buffalo) and (b) the microbial species that dominate(s) the flora.

Table 1.1 Proposed scheme for the classification of all yoghurt products.

Category	Physical state	Yoghurt products
I	Liquid/viscous	Yoghurt (set, stirred and drinking)
II	Semi-solid	Concentrated/strained
III	Solid	Frozen
IV	Powder	Dried

From Tamime, A.Y. & Robinson, R.K. (1999) *Yoghurt Science and Technology*, 2nd edn, Woodhead Publishing Ltd, Cambridge, reproduced by permission of Woodhead Publishing Ltd.

For example, Tamime and Deeth (1980) proposed a scheme of classification that separated all types of yoghurt into four categories based on the physical characteristic of the product, and such an approach could be also applied to other fermented milks. The proposed system is illustrated in Table 1.1, and the yoghurt products are subdivided into groups on the basis of: (a) existing or proposed legal standards that classify the product on the basis of chemical composition or the fat content (full-fat, semi-skimmed/medium-fat or skimmed/low-fat); (b) physical nature of the product (set, stirred or fluid/drinking/beverage); (c) flavours such as plain/natural, fruit or flavoured; and (d) post-fermentation processing (e.g. heat treatment). Incidentally, some of these products are sweetened, and one dried product, kishk, may be fortified with cereals, vegetables or pulses. However, kishk will not be reviewed in this publication, and the following are recommended regarding this product (Tamime & O'Connor, 1995; Tamime *et al.*, 1997a, b, 1999a, b, c, 2000; Tamime & McNulty, 1999; Muir *et al.*, 2000).

In contrast, Kurmann (1989) sought to classify fermented milk products on the basis of the type(s) of micro-organisms used, and their initial groupings were as follows:

- Undefined microfloras;
- Defined microfloras;
- Microfloras with selected human intestinal bacteria;
- Products fortified with pre- and probiotic properties.

This basic scheme of classification was further expanded into the form of a 'tree' that included other properties such as gel/liquid, viscous/pasty, dried or frozen; the optimum growth requirements of the starter cultures (i.e. mesophilic and thermophilic microfloras) provided an additional means of subdivision.

A modification of Kurmann's scheme was used by Robinson and Tamime (1990) to classify fermented milks in a slightly different manner and, although the scheme was also based on the main micro-organisms that dominate the flora in the product, the main metabolites of the starter cultures (e.g. type of organic acid and carbonyl compounds produced, presence of carbon dioxide in the gel and/or production of ethanol) provided, as shown below, an additional principle:

- Lactic fermentations: (a) mesophilic type (cultured buttermilk, filmjölk, tätmjölk and långofil), (b) thermophilic type (yoghurt, Bulgarian buttermilk, zabadi, dahi), (c) therapeutic or probiotic type (acidophilus milk, Yakult®, ABT, Onka®, Vifit®, Actimel®, LC); products within this group constitute, by far, the largest number known worldwide;
- Yeast-lactic fermentations: kefir, koumiss, acidophilus yeast milk;
- Mould-lactic fermentations: viili.

Although not integrated into the above scheme, it could be argued that fermented milks should be subdivided into groups of products based on their physical characteristics (e.g. liquid, gel, concentrated, frozen or dried). Such an overall scheme might help marketing agents and consumers to differentiate between the different fermented milk products available in the market. For example, the name 'yoghurt' could only be applied to a product with 'gelled or viscous' physical properties, and fermented with thermophilic-type lactic cultures, and a 'bio-yoghurt' would be similar to 'yoghurt', but with added probiotic-type lactic cultures.

Whether or not the industry would adopt a unified approach remains an open question, but the casual use of the term 'yoghurt' for any gel-type fermented milk must be unacceptable.

1.4 Patterns of consumption

Consumers of these products in certain societies in Eastern Europe and the Middle East paid scant attention to their properties until the classic text by Metchnikoff (1910), which stirred the imagination of those attracted by his hypothesis. Also, in the late 1800s, Tissier and Moro had isolated *Bacillus bifidus cummunis* (currently known as *Bifidobacterium bifidum*) and *Lactobacillus acidophilus*, respectively, from the stools of breast-fed infants, and highlighted their benefits with respect to the health of humans (Rašić & Kurmann, 1983; Tamime, 2002b). In particular, Metchnikoff's proposal that the apparent longevity of the hill tribesmen of Bulgaria was a direct result of their life-long consumption of yoghurt inspired an interest in the nutritional characteristics of the product that has never abated. Even today the controversy smoulders on, and, although modern commercial yoghurt bears little resemblance to its Balkan counterpart, there are still many consumers who believe that it is more than 'just another foodstuff' (Robinson, 1989).

The validity or otherwise of Metchnikoff's views will be debated for many years to come, but one indisputable effect of his work was a marked increase in the popularity of yoghurt throughout Europe. The almost mystical properties of natural yoghurt were not, however, enough to sustain the interest of the market for very long, and it was not until the introduction of fruit-flavoured varieties in the late 1950s that yoghurt became a major dairy product. Furthermore, during the same period, the technology of manufacture of fermented milks developed to the extent that production was centred in large modern factories, and success in the marketplace was dependent on the existence of a network of retail outlets with storage facilities

at ~5ºC. Since that time, its popularity as an inexpensive and convenient dessert has increased almost annually, so that, at the present time, fruit yoghurts represent a major source of income to the dairy industry. In the UK, for example, the economic value of yoghurt and drinking yoghurt sold in 2002 was around £814 million (Anon., 2003a), and comparable figures are now commonplace in many countries. Indeed, total production is still rising, a trend confirmed by the data shown in Table 1.2. This massive growth has, of course, been accompanied by considerable changes in the process plant employed for production while, in a modern factory, control of the microbiological aspects of the fermentation have reduced the chances of unacceptable variation between batches. Nevertheless, the principles underlying the various aspects of manufacture have altered little with time, and an understanding of these basic tenets is essential for efficient control at plant level.

Until the early part of the 1990s, figures for *per capita* annual consumption of fermented milks and/or their annual production were compiled by the International Dairy Federation (IDF) and reported under the headings of 'buttermilk, yoghurt and others'. Some of the data should be analysed with caution; in particular, figures for consumption of buttermilk were not well classified in many countries. For example, there is traditional or natural buttermilk (a byproduct from the manufacture of ripened cream butter), cultured buttermilk, made by fermenting skimmed milk with the addition of butter flakes, and sweet buttermilk that may not be fermented. For example, the consumption/production data for India (figures not shown) included

Table 1.2 Consumption of milk drinks and fermented products including yoghurt.

	Annual consumption (kg per person)				
Country	1975	1985	1996	2000	2001
Australia	1.0	1.8	5.4	5.6	5.7
Austria	7.9	11.0	13.9	21.4	NR
Belgium	11.8	18.4	25.1	21.1	NR
Canada	5.1[a]	3.1	3.4	4.9	5.2
Czechoslovakia	3.9	9.5	11.0/4.4[b]	13.8/11.7[b]	14.3/12.6[b]
Denmark	36.1[a]	26.7	25.9	35.8	37.0
Federal Germany	16.5	11.1	23.0[c]	26.5[c]	26.2[c]
Finland	42.6	39.3	38.3	40.3	40.8
France	9.6	12.7	25.4	27.9	28.3
Iceland	1.7	20.3	NR	34.9	34.0
Israel	14.1	15.9	23.6	28.0	28.2
Netherlands	24.7	26.6	46.5	46.4	46.1
Norway	9.1	14.0	16.7	16.6	16.4
Poland	3.2	1.9	5.4	14.9	NR
Spain	3.4	5.5	12.7	15.7	NR
Sweden	24.1	27.3	28.4	32.1	33.3
Switzerland	16.4	17.4	NR	22.8	24.2
United Kingdom	1.7	3.1	NR	NR	NR

[a] Data may also include skimmed milk.
[b] Data for Czech Republic and Slovakia, respectively.
[c] Data includes German Democratic Republic.
NR Not reported.
Data compiled from IDF (1999, 2002) and Tamime & Robinson (1999).

19.5m tonnes for buttermilk in 1991, without any indication as to whether the buttermilk was sweet or traditional (see Tamime & Marshall, 1997).

Unfortunately, the approach adopted by the IDF since the mid 1990s (Table 1.2) provides only an overall figure of consumption/production of fermented milk in some member countries, as it has become too difficult to determine the exact identity of the different types of products, e.g. yoghurt, 'bio-yoghurt' or other related products, that do not contain the traditional microflora (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*).

It is of note also that the data in Table 1.2 do not include consumption of fermented milk in the Arab-speaking countries. However, taking into account that annual consumption in Jordan in 1999 was 12.2 kg per person (IDF, 2002), it is safe to assume that consumption is high in the majority of the Arab countries. Similarly, figures for annual consumption of all fermented milks in the former USSR are difficult to obtain – 7.5 kg per person in 1985 (Tamime & Robinson, 1999) – and, currently, only limited data are available from some of the Russian republics/federations such as Belarus and the Ukraine where, in 2000, consumption was 30.0 and 3.5 kg per person, respectively (IDF, 2002). Furthermore, fermented milks are just becoming popular in countries such as China and Thailand (Itsaranuwat & Robinson, 2003) where, in 2000, consumption was 0.2 and 5.8 kg per person, respectively (IDF, 2002). Nevertheless, taking into account the production data of fermented milks in some selected countries reported by the IDF (2002), and the fact that figures are missing from the USA, the Middle East, most countries of the former USSR and South America, the total figure for the world production of fermented milks must be well in excess of 20m tonnes for 2003.

1.5 Manufacture of fermented milks

There are now numerous types of fermented milk manufactured in different parts of the world, and recent publications dealing with the major types of yoghurt and related products, such as drinking-types and concentrated yoghurt (e.g. labneh), include those by IDF (1988), Tamime and Marshall (1997), Tamime and Robinson (1999), Tamime *et al.* (2001), Robinson *et al.* (2002), Anon. (2003b) and Jaros and Rohm (2003).

In general, two types of retail product dominate the market for fermented milks. One variant has a firm, gel-like like structure (set-type), whilst the other has a thick consistency and the apparent background flavour is usually modified by the addition of fruit or flavours and sugar (stirred-type); however, a variant of the stirred product, which is pourable and low in consistency, is the drinking-type. Despite the apparently contrasted nature of the end-products, the manufacturing stages for these types of fermented milk are broadly similar, and an outline of the overall process is shown in Fig. 1.1; the production of drinking yoghurt is detailed in Chapter 5.

Although many of these fermentations involve specific microfloras (Marshall, 1987; Marshall & Tamime, 1997; Tamime & Marshall, 1997), there is a considerable degree of similarity in respect of the technological aspects (Tamime & Robinson,

1988, 1999), especially when the process is a lactic fermentation. Obviously, the fine details of manufacture differ from product to product, and to some extent from plant to plant even for the same product, but certainly many of the processes have much in common.

In normal commercial practice, the stages of manufacture consist of preliminary treatment of the milk base (i.e. fat standardisation, solids-not-fat (SNF) fortification, temperature between 60 and 70ºC), followed by homogenisation at 10–25 MPa or at a pressure dependent on the type of product being made, for example (a) kefir at 10–20 MPa, (b) ymer and ylette at 18–23 MPa, or (c) yoghurt at 20–25 MPa (Tamime *et al.*, 2001).

A heat treatment of the milk base of most types of fermented milks after the homogenisation is usually applied to remove any microbial contaminants acquired from the homogeniser, but an alternative method is to homogenise the milk base aseptically after the heat treatment stage, which is the recommended method during the manufacture of buttermilk (Tamime *et al.*, 2001). One possible advantage of this system is that it may break up any protein aggregates that have formed due to the high heat treatment of the milk and, as a consequence, the coagulum of the final product has a smoother texture. However, little scientific evidence is available to justify this reversal of the normal procedure.

The subsequent processing stages of stirred-types of fermented milks include heat treatment, cooling, fermentation, primary cooling of the gel, addition of fruit, packaging, cooling of the packaged product in a chill tunnel and cold storage. However, variations in plant/process design are tailored to suit the type of fermented milk being made. For example, during the manufacture of yoghurt (set- or stirred-types), kefir, ymer and ylette, the milk base is handled as shown in Fig. 1.1, whilst for the manufacture of buttermilk, the skimmed milk is heated to ~95ºC, cooled to 60ºC, homogenised at 18–20 MPa, cooled and fermented at 20ºC and, after acidification, the gel is thoroughly stirred, homogenised at 5–10 MPa pressure at 20ºC, cooled to 4ºC and packaged (Tamime *et al.*, 2001). Incidentally, post-fermentation homogenisation of the ymer and concentrated yoghurt known as labneh is highly recommended to make the product smooth and free from lumps.

1.6 Conclusion

Anyone walking around a supermarket in Europe or elsewhere cannot help but notice the prominence given to dairy products, and that cheeses and fermented milks appear to be essential components of many diets. The sheer variety and consistent quality of such products soon becomes evident as well, attributes that are, in turn, dependent on the technical and scientific expertise of the manufacturers.

How markets will develop in the future remains to be seen, for the consumer's tastes are not always predictable. The surge in demand in the UK for high-fat, Greek-style or concentrated yoghurt, for example, might not have been foreseen against the background of nutritional advice warning of the danger of consuming saturated dairy fats, and yet the product is a success. In order to meet such changes

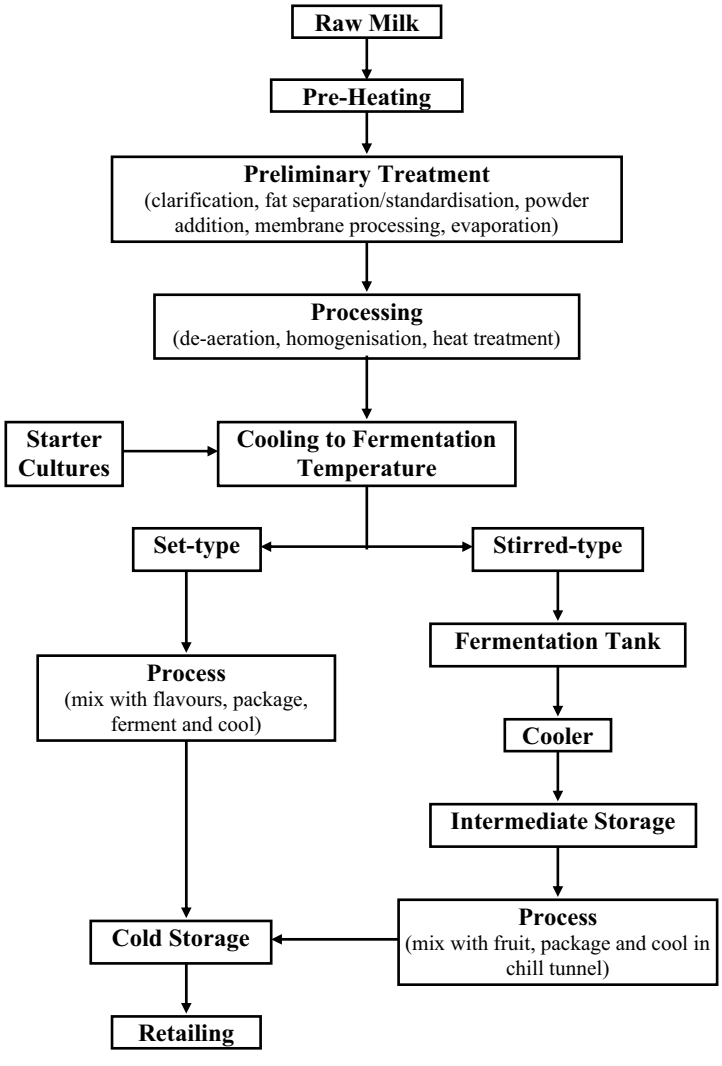

Fig. 1.1 Flow diagram showing the manufacturing stages of fermented milk products.

in demand, what is important is that fundamental knowledge about the fermentation of milk continues to expand, for only then can product diversity and quality achieve the standards that consumers deserve.

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Fermented Milks
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2 Starter Cultures

A.Y. Tamime, A. Skriver and L.-E. Nilsson

2.1 Introduction

A wide range of micro-organisms have been used in food and beverage preparations for thousands of years by humankind (see Chapter 1) and, according to Tamime (2002), the major functions of microbial starter cultures in either food or dairy products may be summarised as:

- to biopreserve the product due to the fermentation that results in an extended shelf-life and enhanced safety;
- to produce bacteriocins that may have potential uses as food preservatives;
- to enhance the perceived sensory properties of the product (e.g. due to the production of organic acids, carbonyl compounds and partial hydrolysis of the proteins and/or fats);
- to improve the rheological properties of fermented milk products (i.e. viscosity and firmness);
- to contribute dietetic/functional/nutraceutical properties to fermented milks, such as occurs with the use of probiotic micro-organisms.

Throughout the world, many names have been applied to traditional and industrialised fermented milk products. The majority of these products may have different local names, but they are practically the same. However, several micro-organisms (bacteria, yeasts, moulds or combinations of these) are employed in the manufacture of fermented milk products, and, when taking into account the microbial species that dominate the flora in the product and the type of fermentation that occurs in milk, the following can be concluded: first, the list of these products comes down to only a few varieties, and second, these products can be divided into three broad categories based on the main microflora and metabolites present in the product (i.e. lactic fermentations, yeast-lactic fermentations and mould-lactic fermentations). Hence, these micro-organisms will be referred to as starter cultures (traditional and non-traditional), and this chapter covers some of the basic microbial aspects with an emphasis on selecting, screening and blending these starter cultures, including their sensory profiling, and methods of preservation and production on-site to achieve a successful product.

2.2 Types and nomenclature of the starter organisms

As mentioned earlier, bacteria, yeasts, moulds or combinations of these are used during the manufacture of fermented milk products. The overall characteristics of these micro-organisms have been extensively reviewed, and the following are recommended for further reading (Sneath *et al.*, 1986; Cogan & Hill, 1993; Cogan, 1995; Wood & Holzapfel, 1995; Monnet *et al*., 1996; Tamime & Marshall, 1997; Salminen & von Wright, 1998; Tamime, 2002; Liu, 2003; Liu *et al*., 2004).

2.2.1 *Traditional lactic acid bacteria*

Lactic acid bacteria are the main group of micro-organisms that has been used successfully for decades for the production of fermented milks, and these organisms belong to the genera of *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Lactobacillus* (Table 2.1). Based on their morphology these micro-organisms can be classified into cocci and rods, and according to their optimum growth requirements they are divided into mesophilic and thermophilic starter cultures that can grow at 20–30ºC or 37–45ºC, respectively.

Although the traditional approach to blending organisms in a starter culture is based on their optimum growth temperature, the current approach is different. Thus, the availability of concentrated freeze-dried or frozen direct-to-vat set (DVS; alternatively known as direct-to-vat inoculation – DVI) in the market has provided the manufacturer of fermented milks with the opportunity to mix different bacterial strains into the product. Some examples include: (a) the addition of probiotic micro-organisms into yoghurt and other dairy products (Tamime, 2005); (b) the incorporation of mesophilic microflora into yoghurt to modify the characteristic of the product; (c) *Streptococcus thermophilus* is sometimes added with sour cream starter cultures to increase the viscosity of the product; the same organism is blended with *Lactococcus* species during the manufacture of Cheddar cheese (Tamime, 2002).

The micro-organisms which are employed in fermented milks (including probiotic products, alcoholic/lactic beverages, cultured cream, and products containing moulds) and the cheese industry are used singly, or in pairs or multiples, or in a mixture, thus giving the industry the opportunity to manufacture different products. The historical evolution, development and terminology of starter cultures systems have been recently reviewed by Tamime (2002).

2.2.2 *Non-traditional microflora*

Many bacterial species have been incorporated into dairy starter cultures for specific applications, and some examples belong to the genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus* (Table 2.1). These micro-organisms are used in the manufacture of fermented milks and other dairy products because of the alleged health benefits for the consumers. However, these products will not be reviewed in this chapter (for further details, see Tamime 2005). Nevertheless, certain non-traditional species of lactobacilli and yeasts are more commonly used in fermented milks to contribute

Table 2.1 Some selected characteristics of micro-organisms and their principal metabolic products used in fermented milks.

Starter organism	Metabolic product	Lactose fermentation	Examples of fermented milk products
I. Lactic acid bacteria			
Traditional			
Lactococcus spp.[a]	L(+) lactate	Homofermentative	Buttermilk, sour cream, ymer, Nordic milks
Leuconostoc spp.[b]	D(–) lactate, diacetyl	Heterofermentative	Buttermilk, sour cream, ymer, Nordic milks
Pediococcus acidilactici	DL lactate	Homofermentative	Fermented milk, kefir[g]
Streptococcus thermophilus	L(+) lactate, acetaldehyde, diacetyl	Homofermentative	Yoghurt, skyr, labneh, sour cream
Lactobacillus delbrueckii spp.	D(–) lactate, acetaldehyde, diacetyl	Homofermentative	Yoghurt, skyr, labneh
Non-traditional			
Lactobacillus spp.[c]	DL lactate	Homofermentative	Yoghurt, kefir, buttermilk, sour cream
Lactobacillus spp.[d]	DL lactate	Heterofermentative	Yoghurt, kefir
Bifidobacterium spp.[e]	L(+) lactate, acetate	Heterofermentative	Yoghurt, buttermilk, sour cream
Enterococcus spp.[f]	L(+) lactate	Homofermentative	Fermented milk
Acetobacter aceti and *rasens*	Acetate, CO_2		Kefir
II. Yeasts			
Candida spp., *Saccharomyces* spp., *Kluyveromyces* spp. and *Debaromyces* spp.	Ethanol, CO_2, acetone, amyl-alcohol, propanal		Skyr, kefir
III. Moulds			
Geotrichum candidum	Mould		Viili, kefir[g]

[a] *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* produces diacetyl and CO_2.
[b] *Leuconostoc mesenteroides* subsp. *cremoris* produces also ethanol and CO_2.
[c] *Lactobacillus acidophilus*, *gasserie*, *helveticus*, *johnsonii*, i.e. probiotic, and *kefiranofaciens*.
[d] *Lactobacillus casei*, *reuteri*, *plantarum* and *rhamnosus* [produces L(+)], i.e. probiotic, and *fermentum* and *kefir*.
[e] *Bifidobacterium adolescentis*, *animalis*, *bifidum*, *breve*, *infantis*, *lactis* and *longum*, i.e. probiotic.
[f] *Enterococcus faecium* and *faecalis*, i.e. probiotic.
[g] These organisms could be present in the product.
Data compiled from Tamime & Marshall (1997), Walstra *et al.* (1999) and Tamime (2003).

to new flavour profiles in these products, and a typical example is kefir (Table 2.1). Furthermore, propionibacteria (*Propionobacterium freudenreichii* subsp. *shermanii*) has also been recognised as probiotic (Mantere-Alhonene, 1995); some strains are used in the manufacture of Swiss cheese, and there are very limited applications in fermented milks.

2.2.3 *Yeasts and moulds*

Some of the non-traditional microflora of yeasts that have been used during the manufacture of fermented milk products are shown in Table 2.1. The total microbial spectrum of strains and species (i.e. lactic acid bacteria and yeasts) that have been identified in kefir grains by many researchers are numerous (Tamime & Marshall,

1997; Witthuhn *et al*., 2004), and only some commercially available strains are listed in Table 2.1.

A single mould is used during the manufacture of fermented milk products in Finland (e.g. viili), and is known as *Geotrichum candidum*. The physiological characteristics of lactic acid bacteria (i.e. traditional and non-traditional), yeasts and moulds have been recently reported by Tamime (2002).

2.3 Partial characterisation of the starter microflora

2.3.1 *Carbohydrate metabolism*

The metabolic pathways (i.e. homolactic and heterolactic fermentation including the bifidus pathway) of the lactic acid bacteria are well established (see Cogan & Hill, (1993); Poolman, 1993; Roussis, 1994; Monnet *et al*., 1996; Tamime & Marshall, 1997; Tamime & Robinson, 1999; Hemme & Foucaud-Scheunemann, 2004). The brief description of microbial metabolism of carbohydrates that follows, and Figs 2.1–2.4, are modified from Jensen (1999); similarly, for citrate metabolism, this section has been modified from Curic (1998).

Fermentation pathways

One may generalise that the main activity of lactic acid bacteria is to degrade the carbohydrates present in milk to lactic acid for the generation of energy required for biomass synthesis (Jensen, 1999; see also Fig. 2.1). The carbon in the microbial biomass stems from the energy-delivering carbohydrate and from building blocks, such as amino acids or oligopeptides, present in the growth medium besides the carbohydrate. Figure 2.1 indicates the sugar uptake, the fate of carbohydrate carbon,

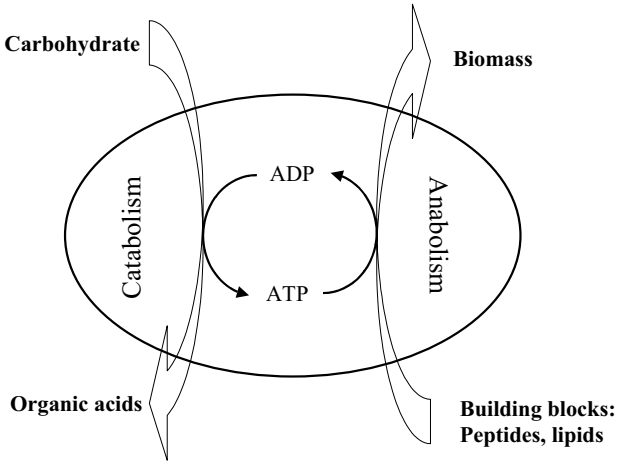


Fig. 2.1 An illustration showing how the energy-rich carbohydrate is degraded in order to fuel the reactions of anabolism. As a rule of thumb, more than 90% of the carbon from carbohydrate ends up in the product. The remainder is used to synthesise certain precursors, for example, nucleic acids. Reproduced from Jensen (1999) with permission.

and the requirement for redox balancing, which will lead directly to one of several roles for the electron acceptor, oxygen.

Lactic acid bacteria are not equipped with the enzymes necessary for respiration, and they are, therefore, unable to perform oxidative phosphorylation. The energy demand is, consequently, satisfied solely through substrate-level production of adenosine triphosphate (ATP) or the equivalent of ATP. The yield YsATP of ATP from glucose is, therefore, much smaller than would have been the case if a complete combustion/catabolism of glucose had been possible (an upper theoretical limit being 36 units ATP *per* unit glucose). Nevertheless, the bacteria are able to sustain a high growth rate ($> 0.70\ h^{-1}$), but this requires a high throughput of carbohydrate in order to satisfy the energy requirement of anabolism. An efficient system of sugar uptake is, therefore, called for.

The uptake of carbohydrates is ensured by one of several possible mechanisms, depending on the growth conditions and the sugar in question. For the sake of simplicity the only carbohydrates that will be discussed are glucose and lactose.

Sugar transportation and hydrolysis

Two known transport systems mediate the uptake of glucose (Fig. 2.2). The first system is a proton-gradient-driven permease that translocates glucose across the bacterial membrane. Glucose does not undergo any change in this process. However, the glucose molecule undergoes phosphorylation by a glucokinase after having entered the cell, yielding glucose-6-phosphate (G-6-P), which is then catabolised, for example, via the Embden-Meyerhoff-Parnas (EMP) pathway. Glucose will, in total, yield two ATP in its conversion to pyruvate. However, the process requires ATP to keep up the level of the proton motive force (PMF), for which reason the net yield in ATP per molecule of extracellular glucose is difficult to assess (Benthin, 1992; see also Titgemeyer & Hillen, 2002). The permease is not energy conserving since both translocation and the subsequent phosphorylation of glucose are ATP-consuming processes.

The second system is the mannose-phosphotransferase system (mannose-PTS). With this system, glucose is transported into the cell while at the same time being phosphorylated to G-6-P by the PTS. The high-energy phosphate bond stems from phosphoenolpyruvate (PEP), which is converted to pyruvate. This mechanism is energy conserving since translocation and phosphorylation occur at the total cost of one PEP. The net energy yield from one extracellular glucose molecule is two ATP. This system is also used for transport of mannose (but with a much lower affinity than for glucose), and similar systems exist for the transport of other sugars that can be metabolised (Benthin, 1992). PEP participates in the phosphorylation of glucose transported by the PTS system and, moreover, it is the substrate for the pyruvate kinase enzyme. Therefore, PEP plays an important role in the regulation of the metabolic pathway (Fig. 2.2).

Similarly, the transport of lactose molecules across the microbial cell membrane may be taken up by two different systems: (a) a lactose-PTS system distinct from the mannose-PTS, and (b) a permease system as described earlier (Garrigues *et al*., 1997). However, before catabolism of lactose via the EMP pathway, the moieties of the carbohydrate need to undergo several additional steps, as shown in Fig. 2.2.

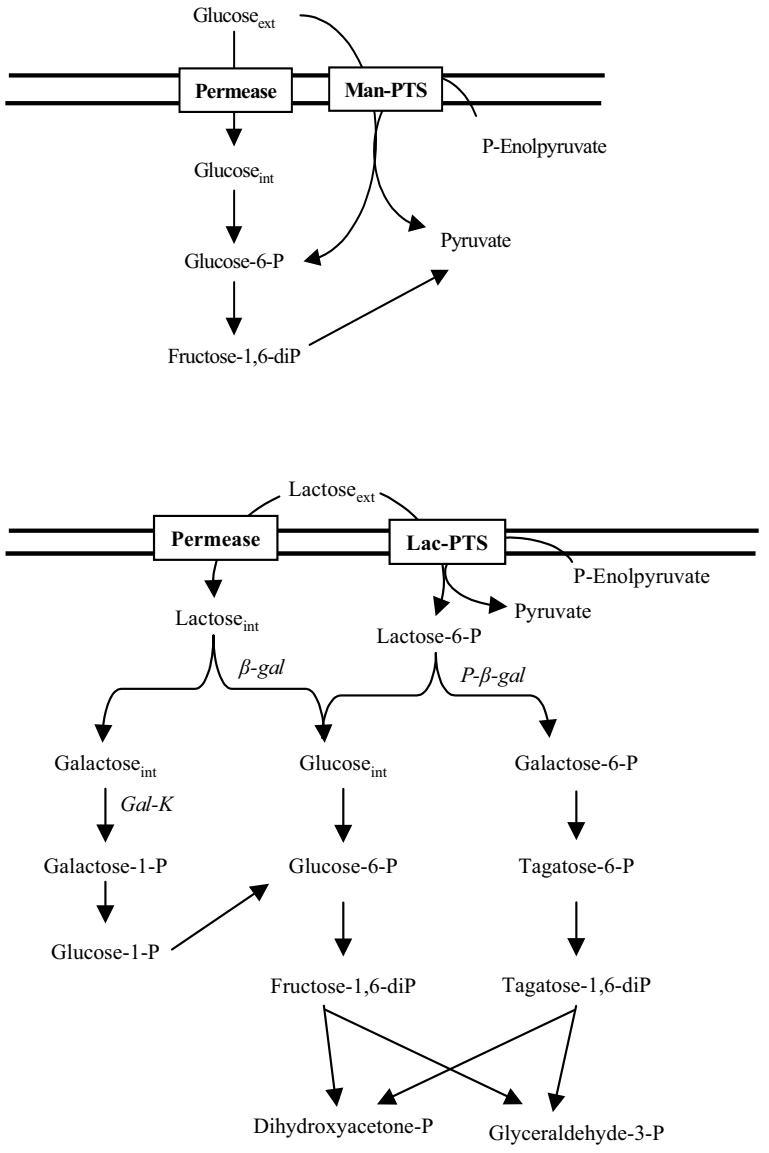


Fig. 2.2 The permease and phosphotransferase systems for glucose and lactose. β-gal, β-galactosidase; P-β-gal, phospho-β-galactosidase; Gal-K, galactokinase. *Lactococcus lactis* subsp. *cremoris* MG1363 does not have the plasmid-borne gene encoding for the lactose-PTS, leaving only the less efficient permease as uptake system. Adapted from Garrigues et al. (1997); reproduced from Jensen (1999) with permission.

Generation of energy

The usually rapid conversion of carbohydrate to metabolic products by the starter cultures is concomitant with the generation of energy required for growth. Fig. 2.3 represents the central substrate level of energy-generating pathways of lactic acid

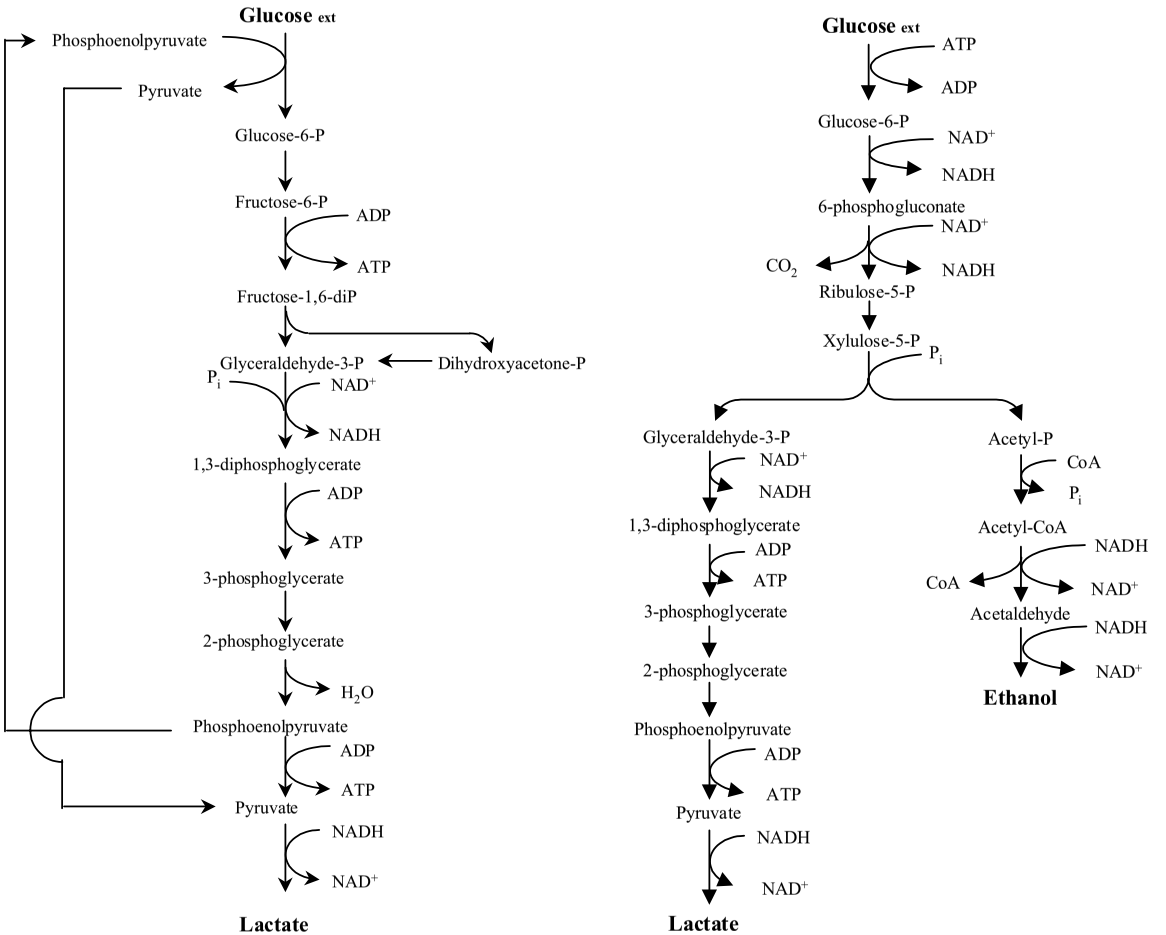


Fig. 2.3 Homolactic and heterolactic fermentation of lactose by lactic starter cultures. Reproduced from Jensen (1999) with permission.

bacteria; the different pathways provide an opportunity to distinguish between two types of fermentation sequences, homolactic and heterolactic, based on the identity of the main products formed from glucose.

In the homolactic fermentation sequence, the lactic acid produced may account for more than 90% of glucose carbon. However, as the fate of glucose catabolism in a homolactic fermentation sequence is different and under anaerobic conditions, equimolar amounts of lactate, ethanol and carbon dioxide result from the degradation of glucose based on the following reaction:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{lactic acid}}{C_3H_6O_3} + \underset{\text{ethanol}}{C_2H_6O} + \underset{\text{carbon dioxide}}{CO_2}$$

Furthermore, the difference between the two metabolic pathways, i.e. the EMP and the phosphoketolase, is illustrated by the difference in energy yield – 2 moles compared with 1 mole of ATP per mole of glucose, barring the loss due to maintenance of the PMF.

Bacterial strains (i.e. homolactic fermenters) are able to convert the fermented carbohydrate into products other than lactate. The biochemistry, as it is presently known, is shown in Fig. 2.4, and the many end-products are represented with the enzymes catalysing the reactions.

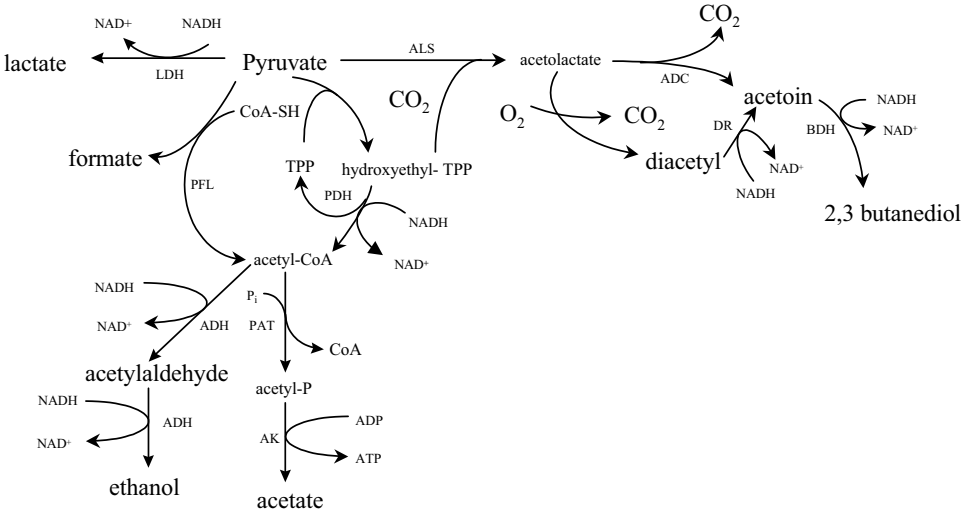


Fig. 2.4 Alternative end-products of pyruvate catabolism – products found extracellularly are given in large letters. LDH, lactate dehydrogenase; PFL, pyruvate formate lyase; ADH, alcohol dehydrogenase; PAT, phosphotransacetyl transferase; AK, acetate kinase; ALS, acetolactate synthase; ADC, acetolactate decarboxylase; DR, diacetyl reductase; BDH, butanediol dehydrogenase; PDH, pyruvate dehydrogenase. Reproduced from Jensen (1999) with permission.

2.3.2 *Citrate metabolism*

Even though the level of citrate metabolism in lactic acid bacteria is very low, it is an important property of some mesophilic strains. The citrate is metabolised by lactococci and *Leuconostoc* species, but not by thermophilic cultures. The ability of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* as well as *Leuconostoc* spp. to metabolise citrate is due to the presence of citrate permease (Monnet *et al*., 1996). Genes encoding for this enzyme are located on a 7.9 kb plasmid (Kempler & McKay, 1979; Gasson & Davies, 1984 ; David, 1992; Bourel *et al*., 2001). Citrate utilisation is regulated by the activity of citrate permease and has a strong pH dependency (David *et al*., 1990). This enzyme has a narrow pH optimum with activity between pH 5.0 and 6.0 (David, 1992; Starrenburg & Hugenholtz, 1991; Smith *et al*., 1992). Current knowledge suggests that citrate is consumed from the very beginning of the growth at pH 6.9; however, citrate uptake at pH 4.5 is reported in resting cells, but it is ascribed rather to an increased cell permeability at low pH than to the active transport system (Hugenholtz, 1993). Citrate uptake and its degradation also depend on temperature (Bassit *et al.*, 1995; Cashon *et al*., 1995).

Once the citrate molecule is transported into the cell, it is degraded to oxaloacetate and acetate by citrate lyase (CL) (Fig. 2.5). In *Lac*. *lactis* subsp. *lactis* biovar *diacetylactis*, citrate lyase is constitutively expressed (Harvie & Collins, 1961; Cogan, 1981; Hugenholtz & Starrenburg, 1992) whereas it is inducible in *Leuconostoc* spp. (Mellerick & Cogan, 1981; Hugenholtz & Starrenburg, 1992). Oxaloacetate, which is produced during the catabolism of citrate, is subsequently converted by oxaloacetate decarboxylase to pyruvate and CO_2 (Fig. 2.5).

Certain carbonyl/flavouring compounds, such as acetate, diacetyl, acetoin and 2,3 butanediol, and CO_2 (Figs 2.4 and 2.5) are produced in milk through the metabolism of citrate. While acetoin and butanediol are tasteless and not involved in flavour, diacetyl is an important flavour component in buttermilk and sour cream, which are made with mesophilic starter cultures; a quality defect of these products is attributed to the absence of diacetyl.

Lactic acid bacteria metabolise not only citrate but also sugars, and citrate is metabolised rapidly in the presence of fermentable sugars. Citrate is first hydrolysed by citrate lyase to acetate and oxaloacetate, which is decarboxylated to pyruvate (Figs 2.4 and 2.5). Acetolactate is formed from pyruvate, and decarboxylation of acetolactate produces acetoin, which can be further reduced to butanediol by butanediol dehydrogenase. Furthermore, diacetyl can also be easily produced from acetolactate because the latter is not a stable molecule. It is believed that diacetyl is produced non-enzymatically (i.e. chemically), as the enzyme diacetyl synthase has never been shown to exist in lactic acid bacteria. Diacetyl, acetoin and butanediol represent three oxidation stages of a four-carbon skeleton. In addition, diacetyl can be reduced to acetoin and butanediol by acetoin dehydrogenase and by butanediol dehydrogenase, respectively. The activity of the latter reaction is much more significant as the K_m value is higher.

Mixed strains of mesophilic starter cultures (i.e. D, DL and L types including the citrate producing lactococci) produce much more acetoin than diacetyl from citrate. In comparison, pure L strains do not produce any diacetyl and acetoin at all.

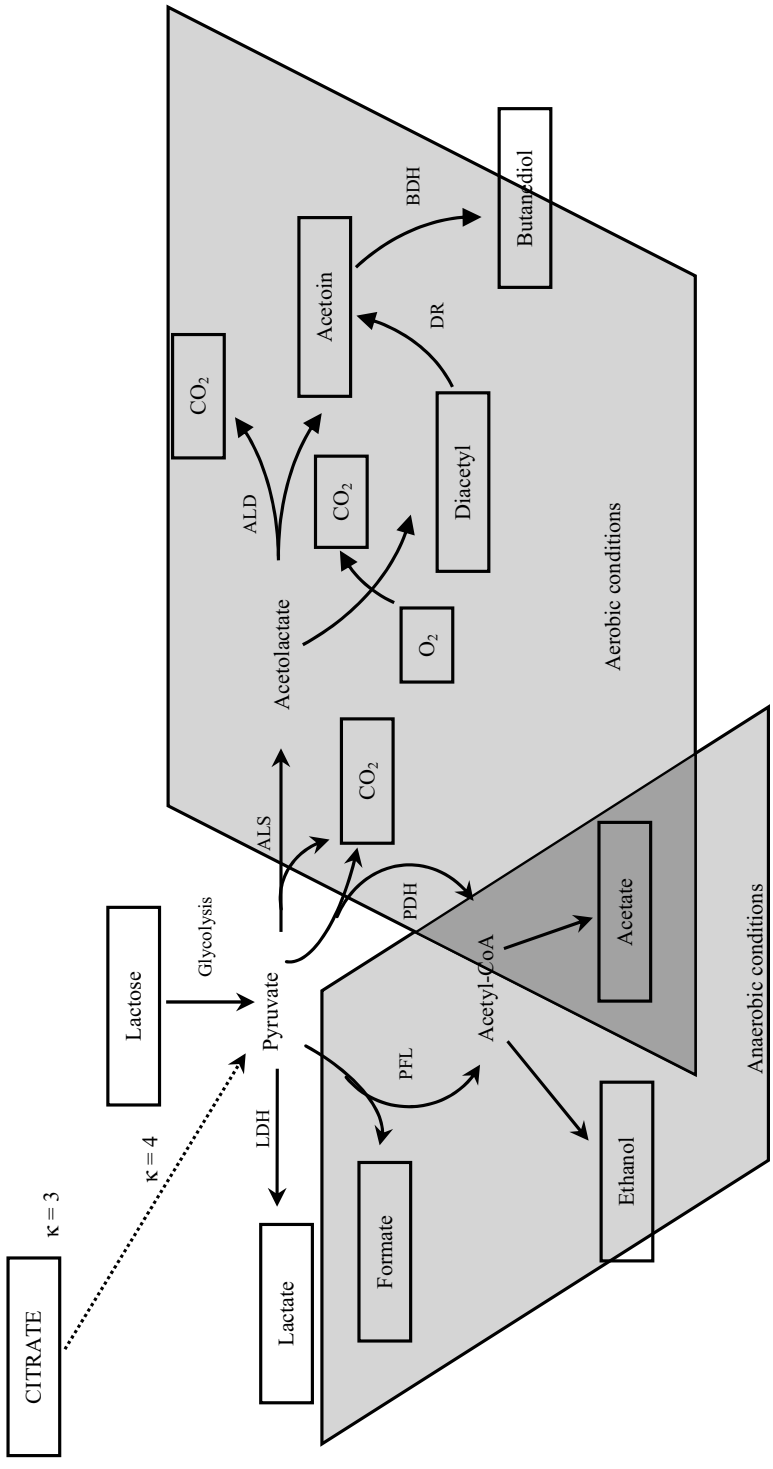


Fig. 2.5 Citrate catabolism in homofermentative lactic acid bacteria. The citrate and lactose levels in milk are 10 and 130 mM, respectively. Reproduced by permission of N.B. Jensen (personal communication).

2.3.3 *Formation of acetate, formate, acetaldehyde and ethanol*

Curic (1998) reported that under changed environmental conditions, such as the presence of air (Bassit *et al*., 1993; Boumerdassi *et al*., 1997), energy starvation (Brown & Collins, 1977; Snoep *et al*., 1992a; Sjöberg *et al*., 1995) and/or growth on sugars that are slowly degradable like maltose or galactose (Garrigues *et al*., 1997; Qian *et al*., 1997), pyruvate catabolism is redirected using other enzymes to yield acetate, acetaldehyde and ethanol. These compounds are formed via the common intermediate acetyl-CoA. Also, acetyl-CoA is required for the synthesis of fatty acids (Speckman & Collins, 1968; Anders & Jago, 1970 ; Magnusson *et al*., 1993), and it can be formed from pyruvate via two non-competitive alternative pathways: (a) catalysed by pyruvate dehydrogenase complex (PDC), which is active in the presence of oxygen (Broome *et al*., 1980), or (b) catalysed by pyruvate formate lyase (PFL), which is active under strictly anaerobic conditions (Yamada, 1987). The products that will be formed downstream of acetyl-CoA depend mainly on the intracellular redox state (Fig. 2.5). The formation of both acetaldehyde and ethanol is coupled with the regeneration of NAD^+ under anaerobic conditions. When the redox balance is maintained via other reactions (i.e. NADH oxidase, which is active under aerobic conditions), acetyl-CoA is degraded via acetate kinase, producing acetate in a reaction coupled with ATP formation.

Pyruvate dehydrogenase complex

The PDC is an NADH-dependent enzyme complex requiring coenzyme-A, thiamine pyrophosphate (TPP) (Broome *et al*., 1980; Hugenholtz, 1993), and lipoic acid as cofactors (Anders & Jago, 1970; Snoep *et al*., 1993b). The affinity of PDC for pyruvate is very high, i.e. K_m = 1 mM (Snoep *et al*., 1992b), and provides good conditions for the acetyl-CoA. However, high sensitivity of PDC for NADH inhibits its activity under anaerobic conditions when the ratio of $NADH/NAD^+$ is high (Snoep *et al*., 1992b), but the PDC reaches maximal activity under aerobic conditions and lactose (glucose)-limiting conditions (Broome *et al*., 1980).

The PDC has been characterised and extensively investigated in *Escherichia coli*, *E. faecalis* (Snoep *et al*., 1992c, 1993a) and *Lac. lactis* subsp. *lactis* biovar *diacetylactis* (Snoep *et al*., 1992b). It consists of four subunits – E1a, E1b, E2 and E3 – where dihydrolipoyl transacetylase (E2) and dihydrolipoyl dehydrogenase (E3) have lipoic acid as a cofactor (Snoep *et al*., 1993b). Since lactococci are not able to synthesise lipoic acid *de novo* (Collins & Bruhn, 1970), the availability of lipoic acid in the medium can be a growth-limiting factor when the cultures are grown under aerobic conditions (Snoep *et al*., 1992b) unless acetate is supplemented as a precursor of acetyl-CoA (Collins & Bruhn, 1970). This fact offers the possibility of additional manipulation of pyruvate flux by changing the composition of the defined medium by omission of lipoic acid.

Pyruvate formate lyase

Under anaerobic conditions, the pyruvate is catabolised to acetyl-CoA and formate via the pathway catalysed by pyruvate formate lyase (PFL). In *Lac. lactis* subsp. *lactis* biovar *diacetylactis*, expression of this enzyme is regulated at transcriptional level and is induced under anaerobic conditions (Arnau *et al*., 1997). The active form of this enzyme is present under strictly anaerobic conditions, and is irreversibly inactivated in the presence of oxygen (Yamada *et al*., 1985; Arnau *et al*., 1997). The activity of PFL is influenced by pH, and the optimal condition was reported to be 7.5 (Takahashi *et al*., 1982). As reported by Hugenholtz (1993), the formate is not produced at pH values lower than 6.0, which is in contrast to product formation as reported by Jensen (1999) (Figs 2.4 and 2.5). The affinity of the enzyme for pyruvate is found to be very high, with K_m = 1 mM (Garrigues *et al*., 1997), but it is strongly controlled by intermediates in the glycolytic pathway, e.g. dihydroxyacetone-phosphate (DHAP), and glyceraldehyde-3-phosphate (GAP) (Fig. 2.3) (Takahashi *et al*., 1982; Cocaign-Bousquet *et al*., 1996, 2002). The rapid flux through the glycolytic pathway, which results in high intracellular concentrations of both GAP and DHAP, inhibits PFL activity (Garrigues *et al*., 1997), and directs the pyruvate flux towards lactate formation. Alterations in the rate of glycolysis influence the activity of lactate dehydrogenase (LDH), so that less lactate and more formate, acetaldehyde and ethanol may be formed.

Acetolactate formation

During microbial growth under certain environmental conditions, pyruvate can accumulate in the cell. It has been postulated that accumulation of pyruvate is highly toxic to the cell, especially at low pH (Collins, 1972; Tsau & Montville *et al*., 1990), but no explanation of the toxic effect was given. In *Lac. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp., extra pyruvate originating from citrate is converted into α-acetolactate (α-AL) (Fig. 2.5). This compound is formed by condensation of two pyruvate molecules in a reaction catalysed by α-acetolactate synthase (α-ALS) with thiamine pyrophosphate (TPP) and Mg^{2+} as cofactors (Hugenholtz & Starrenburg, 1992). In the lactococci, two enzymes catalyse this process. One is the constitutively expressed catabolic enzyme (Cogan 1981; Hugenholtz & Starrenburg, 1992) encoded by *als*S (Marrug *et al*., 1994), which has low affinity for pyruvate with a K_m of 50 mM (Snoep *et al*., 1992b), and thus active only at high internal pyruvate concentrations.

In *Lac. lactis* subsp. *lactis* biovar *diacetylactis*, ALS activity shows a 20-fold variation between the strains (Monnet *et al*., 1994). The optimal pH is 6.0 (Snoep *et al*., 1992b), and activity rapidly decreases below pH 5.5 (Monnet *et al*., 1994). Oxygen has a positive effect on the specific activity of ALS (Bassit *et al*., 1993; Boumerdassi *et al*., 1997), which results in improved flux of pyruvate under aerobic conditions. It appears that the activity of ALS is not subject to feedback regulation by any of the downstream products at either the genetic or the enzymatic level (Goupil-Feuillerat *et al*., 1997).

The second enzyme is another acetolactate synthase present in *Lac. lactis* subsp. *lactis* biovar *diacetylactis*, which was identified and characterised (Godon *et al*., 1992). This enzyme is encoded by the *ilv*BN genes, and is involved in the biosynthesis of the branched-chain amino acids, isoleucine, leucine and valine. This enzyme catalyses anabolic reactions and has a much higher affinity for pyruvate than the catabolic enzyme, with K_m = 8.3 mM (Benson *et al*., 1996). Beside the difference in affinity for pyruvate, catabolic and anabolic ALS differ in other properties, which are the result of their physiological function. The catabolic ALS is constitutively expressed and not subjected to feedback control by branched-chain amino acids (leucine, isoleucine and valine) as is the anabolic enzyme (Marrug *et al*., 1994; Goupil *et al*., 1996). As a consequence of regulation of the biosynthesis, the *ilv*BN genes (and anabolic ALS) are expressed only in the absence of branched-chains amino acids (Godon *et al*., 1992), and in strains that have functionally active *ilv* genes. Bearing in mind that milk contains all three amino acids and that most dairy lactococcal strains are auxotrophic for isoleucine, leucine and valine (Hugenholtz *et al*., 1987; Godon *et al*., 1993), it is safe to assume that anabolic ALS are not involved in pyruvate degradation during the growth of these bacteria in milk.

Acetoin and diacetyl formation

Diacetyl is produced by *Lac*. *lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp. during citrate fermentation (Fig. 2.5). Until recently, two different metabolic pathways for diacetyl formation in citrate-fermenting lactococci have been considered. The direct synthesis of diacetyl from acetyl-CoA and active acetaldehyde via diacetyl synthase have been postulated (Speckman & Collins, 1973; Cogan, 1981; Kaneko *et al*., 1989; Verhue & Tjan, 1991). However, the enzyme diacetyl synthase has never been directly isolated and characterised in lactic acid bacteria. More recent studies of pyruvate and citrate metabolism in *Lac. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp. clearly show that diacetyl is formed by chemical oxidative decarboxylation of α-acetolactate (Hugenholtz & Starrenburg, 1992; Marrug *et al*., 1994; Phalip *et al*., 1994; Ramos *et al*., 1994, 1995; Platteeuw *et al*., 1995; Renault *et al*., 1995; Goupil *et al*., 1996).

2.3.4 *Production of exopolysaccharides*

Currently, yoghurt manufacturers often use methods such as increasing milk solids (e.g. the protein content up to 4.5 g 100 g^{-1}) and/or addition of stabilisers (e.g. 0.5–1.0 g 100 g^{-1}) to improve the texture, mouth-feel and viscosity and to minimise syneresis (separation) of the product (Laws & Marshall, 2001). The former approach is costly, while the latter has the disadvantage that all the added stabilisers must be declared with an E number in European Union (EU) countries. Furthermore, the use of stabilisers in yoghurt making is, in many countries, regulated, and often stabilisers may not be used at all. In recent years, lactic acid bacterial exopolysaccharides (EPS) have received increasing attention, mainly because of their potential use as 'natural' thickening agents to replace plant and/or microbial stabilisers (Cerning,

1995; Sikkema & Oba, 1998; Cerning & Marshall, 1999; Ricciardi & Clementi, 2000; Laws *et al*., 2001); the immunogenic and prebiotic properties of EPS materials should not be overlooked (Kitazawa *et al*., 1991; Schiffrin *et al*., 1995; Chabot *et al*., 2001; Jolly *et al*., 2002; Korakli *et al*., 2002; Zisu & Shah, 2003; Vaningelgem *et al*., 2004).

Structure and characterisation

Exopolysaccharides (EPS) are produced by lactic acid bacteria during the fermentation stage, and their *in situ* production in fermented milks is given 'generally recognised as safe' (GRAS) status, which is a very interesting alternative to conventional stabilisers (Cerning, 1990). In general, starter cultures are able to produce homo-EPS (Monsan *et al*., 2001) and hetero-EPS types (Degeest *et al*., 2001; de Vuyst *et al*., 2001), which can improve the texture of fermented milk products. The structure of the EPS produced by starter cultures has been heavily researched, and some detailed studies have been reported by Gruter *et al*. (1992, 1993), Robijn *et al*. (1995, 1996), Dueñas-Chasco *et al.* (1998), Faber *et al*. (1998, 2001), Laws *et al*. (2001), de Vuyst *et al*. (2001), and Pluvinet *et al*. (2004).

Chemical composition and biosynthesis

Homopolysaccharides are composed of one type of monosaccharide, which is either D-glucose or D-fructose; these fructans and glucans have a common feature in being synthesised by extracellular transglycosylases (glycansucrases) using sucrose as the glycosyl donor (Monsan *et al*., 2001). The heteropolysaccharides, which are most common among the yoghurt starter cultures, consist of a backbone of repeated subunits of three to eight monosaccharides. D-galactose, D-glucose and/or L-rhamnose are almost always present, but the ratios vary (Uemura *et al*., 1998; Urashima *et al*., 1999; Ricciardi & Clementi, 2000; Laws *et al*., 2001; Marshall *et al*., 2001; Ricciardi *et al*., 2002; de Vuyst *et al*., 2003). D-mannose is also found in some EPS as well as the acetylated aminosugars like *N*-acetylgalactosamine and *N*-acetylglucosamine, and glucuronic acid. Molecular weights of EPS have been reported in the range between 1×10^4 and 6×10^6. EPS quantities in fermented milk products are normally between 50 and 600 mg L^{-1} depending on the lactic acid bacteria cultures used and the fermentation conditions, such as pH, temperature of incubation, added supplements and presence of ions (Gancel & Novel, 1994; Mozzi *et al*., 1995, 1996; Bouzar *et al*., 1996; Grobben *et al*., 1996, 1998, 2000; Kimmel *et al.* 1998; Yang *et al*., 1999; Frengova *et al*., 2000; Looijesteijn *et al*., 2000; Zisu & Shah, 2003).

EPS are made by the polymerisation of repeating unit precursors formed in the cytoplasm. These are assembled at the membrane by the sequential addition of activated sugars (i.e. sugar nucleotides or nucleoside diphosphate sugars) to the growing repeating unit that is most probably anchored on a lipid carrier. After completion of a repeating unit, it seems to be exported through the cell membrane, becoming

polymerised into a final EPS. Hence, several enzymes and/or proteins are involved in the biosynthesis and secretion of hetero-EPSs.

However, EPS repeating units show few common features, which raises an important question about the relationship between EPS structure and texturising properties. It would be of interest to understand this relationship and to have the means chemically, enzymatically or genetically to modify the native biopolymers (de Vuyst *et al*., 2001, 2003); Jolly & Stingele, 2001; Boels *et al*., 2001; Jolly *et al*., 2002; Lamothe *et al*., 2002).

Influence of exopolysaccharides on texture

It is evident that EPSs, which are produced by the starter cultures during the fermentation stage, modify or significantly affect the texture properties of fermented milks (Cerning, 1990). Such products have been reported to obtain a higher viscosity and a lower degree of syneresis (whey separation) compared with products produced with non-EPS producing cultures (Cerning, 1990; Marshall & Rawson, 1999; Laws & Marshall, 2001; Duboc & Mollet, 2001; Hassan *et al*., 2001, 2002, 2003a, b, 2004; Ruas-Madiedo *et al*., 2002). Further, presence of EPS in fermented milk products often leads to a ropy character (Cerning, 1990), which is why EPS-producing starter cultures are sometimes known as 'ropy' strains. However, ropiness is not always correlated with the EPS concentration, making 'ropy' strains an imprecise terminology. For instance, products where the EPS is situated in capsules closely associated with the bacteria (capsular EPS) are found to be less ropy than products in which the EPS is distributed in the whole product (Hassan *et al*., 1996a, b).

The effects are complex, and many studies have shown that the texture of fermented milks is far from proportional to the EPS content (van Marle & Zoon, 1995). Faber *et al*. (1998) even found that two cultured skimmed milks with similar EPS concentration and identical repeating unit structure of the EPS differed in viscosity. The only difference observed between the two EPS types was the molecular mass. Furthermore, Hess *et al*. (1997) reported that EPS-containing yoghurts required less force to penetrate the undisturbed gel than did yoghurts without EPS, and similar observation was also reported by Skriver (1995). Hassan *et al*. (2003b) found lower values for storage and loss modulus from oscillation measurements for yoghurts with EPS than for yoghurts without EPS, which indicates that the initial gel stiffness is actually lower in products with EPS (see also Sebastiani & Zelger, 1998). These authors suggested that this lower gel stiffness results from the protein–protein interactions in the gel network being interrupted by the EPS molecules placed in between the proteins.

Role of exopolysaccharides in the microstructure of the gel

The microstructure of the gel of fermented milk products consists of: (a) protein matrices composed of casein micelle chains and clusters; (b) appendages of denatured β-lactoglobulin that forms a complex with κ-casein; (c) fat globules (if present in the milk base) embedded in the casein particles; (d) void spaces within the protein

matrices; (e) filaments formed by starter cultures producing EPS that are attached to the microbial cells and the protein matrices (Tamime & Marshall, 1997; Tamime and Robinson, 1999; Hassan *et al*., 2002, 2003a). Recent studies on the microstructure of yoghurt made with EPS-producing cultures showed that the gel consisted of compartmentalised protein aggregates around channels containing the ropy material, which hindered syneresis as well as the reformation of the gel after stirring (Hassan *et al*., 2003b). However, the latest developments in this field will be detailed in a planned future book (*Structure of Dairy Products*) within this Technical Series.

2.3.5 *Bacteriocins*

Even though the main preservative effect of lactic acid bacteria is due to their production of lactic acid with the concomitant lowering of the pH, it has been known for a long time that many lactic acid bacteria also produce other antimicrobial compounds known as bacteriocins (S. Wessels, B. Jelle & I. Nes, personal communication). These antimicrobial compounds are ribosomal synthesised peptides, and have attracted special attention from the scientific community, the food industry and the medical profession.

Bacteriocins are antimicrobial proteins or peptides that are only produced by bacteria and that kill or inhibit the growth of closely related species and some pathogens. Such compounds are distinctly different from antibiotics. The latter are substances that are produced by both prokaryotic and eukaryotic micro-organisms, and are inhibitory at low concentrations to other organisms (i.e. both to prokaryotes and eukaryotes). Bacteriocins from lactic acid bacteria have been studied extensively during the last 10 years (de Vuyst & Vandamme, 1994; McAuliffe *et al*., 2001; Twomey *et al*., 2002), and are now generally considered to be an important tool for preserving food naturally and for controlling bacterial infections in man and animals (see also Jack *et al*., 1995; Jack & Sahl, 1995; Sahl *et al*., 1995; Konings & Hilbers, 1996; Nes *et al*., 1996; Kleerebezem *et al*., 1997; Nissen-Meyer & Nes, 1997; Cleveland *et al*., 2001; Eijsink *et al*., 2002; see also Aktypis & Kalantzopoulos, 2003).

A large number of bacteriocins have been discovered and, within this diversity, some common features allow them to be placed into only a few classes. In particular, Klaenhammer (1993), Nettles and Barefoot (1993), Nes *et al*. (1996), Nes and Holo (2000), Sablon *et al.* (2000) and McAuliffe *et al*. (2001) have suggested the following categories: class I (<5 kDa), the lantibiotics; class II (<10–>5 kDa), the small heat-stable and non-lantibiotics; class III (>30 kDa), large and heat-labile bacteriocins (Table 2.2). Incidentally, the word lantibiotic is derived from *lan*thionine-containing an*tibiotic*.

Classification of bacteriocins

Bacteriocins in classes I and II are by far the most heavily studied because they are found most frequently in the lactic acid bacteria, and they have great potential for industrial applications. Class I and II bacteriocins are heat stable, hydrophobic, cationic, usually small in size (i.e. 20–60 residues long) and not too vulnerable to proteolysis.

Table 2.2 Classification of bacteriocins produced by lactic acid bacteria

Category	Grouping	Examples
Class I (heat-stable lantibiotics)	Type A	Nisin, lacticin 481 and 3147, cytolysin
	Type B	Mersacidin, actagardine, cinnamycin, ancovenin, duramycin B and C
Class II (heat-stable non-lantibiotics)	Subgroup (a) (two-peptide bacteriocins)	Lactococcin G, plantaricin S, lactacin F, plantaricin EF, plantaricin JK
	Subgroup (b) (strong antilisterial activity)	Pediocin PA-1, sakacin A and P, enterocin A, leucocin A-UAL 187
	Subgroup (c) (*sec*-dependent leaders)	Divergicin A, acidicin B, enterocin P Enterocin L50
	Subgroup (d) (without leaders)	Lactococcin A, lactococcin B, enterocin B
	Subgroup (e) (miscellaneous bacteriocins)	
Class III (large, heat-labile proteins)		Helveticin J, acidophilucin A, caseicin 80, lacticin A

Data compiled from McAuliffe *et al*. (1998).

Class I – characterisation, structural properties and mode of action

These bacteriocins contain either one or both of the modified amino acid residues (lanthionine and β-methyllanthionine). In addition, they have been shown to contain hydrated amino acid residues, such as 2,3-didehydroalanine and 2,3-didehydrobutyrine, both of which serve as precursors with cysteine to make the lanthionine and β-methyllanthionine residues. Furthermore, a number of other post-translationally modified amino acid residues are also found in lantibiotic products (Sahl *et al*., 1995; Konings & Hilbers, 1996). Lantibiotics can kill Gram-positive bacteria, but not Gram-negative; however, in combination with metal-chelating agents, lantibiotics can also inhibit the growth of, or kill, Gram-negative bacteria (Abee, 1995).

The lantibiotics are commonly divided into types A and B (Konings & Hilbers, 1996). Type A lantibiotics are elongated and screw-shaped peptides, which primarily act on the membranes of sensitive bacteria. The lantibiotics found amongst 'food grade' lactic acid bacteria are all type A (Table 2.2), and 'non-food grade' Gram-positive bacteria also produce this type of bacteriocin. Type B lantibiotics, which are not found in lactic acid bacteria, are smaller and more globular in structure and act more often as enzyme inhibitors; these will not be discussed further here.

The susceptibility of bacteria to lantibiotics varies considerably. Some lantibiotics such as nisin and lacticin 3147 (Table 2.2) are active against a broad variety of Gram-positive bacteria, including a few food spoilage and pathogenic micro-organisms. However, lacticin S has a narrow spectrum of activity, but it is mostly active against other lactic acid bacteria, while nisin is active against both vegetative bacteria and spore-formers.

The mechanism by which type A lantibiotics interact with and kill susceptible cells has only recently been determined (Abee, 1995); a number of questions over the mechanism of action still remain to be answered. It is well documented that

the prime target for type A lantibiotics is the cytoplasmic membrane, where they interfere with energy transduction and cause the membrane to become permeable to ions, amino acids and ATP (Abee, 1995; Sahl *et al*., 1995; Verheul *et al*., 1997). Studies have demonstrated that lantibiotic-treated cells and artificial vesicles dissipate the membrane potential, which suggests that pore formation allows influx of protons and other ions normally located outside the membrane. It is believed that no specific receptors in the cell membrane are required for the action of lantibiotics. Recently it has been suggested that some kind of 'docking molecule' is found in bacteria, which are very sensitive to nisin and the epidermis (Sahl, personal communication). Where the 'docking molecule' is absent, increased concentrations of nisin are required to kill susceptible bacteria.

At present, two lantibiotics are used or will be in use in food-related production. Nisin has been approved within the EU and in the USA for use in some food products, and has been on the market for many years. In addition, nisin is also used in some veterinary applications, in particular to prevent bacterial infections that cause mastitis in cows. Also, lacticin 3147 has been licensed for use against bacterial infections in the cow's udder. A number of new applications in various food products are reported to be under way.

Class II – characterisation, structural properties and mode of action

More than 50 class II bacteriocins, i.e. not modified, have been characterised in lactic acid bacteria in recent years. These bacteriocins usually consist of between 30 and 60 amino acid residues, and are usually larger than type A lantibiotics (Klaenhammer, 1993; Nes *et al*., 1996; McAuliffe *et al*., 2001). They are cationic and either amphiphilic or hydrophobic. Most of these bacteriocins are produced by lactic acid bacteria, which have been isolated from fermented food; although some have been isolated from fresh and chilled food.

In order to deal practically with such large numbers of bacteriocins, it has been proposed to divide class II bacteriocins into different subgroups (Table 2.2). It should be emphasised that many class II bacteriocins are included in subgroup (d) because they only contain one peptide, with no particular features that categorise them into the other subgroups.

For some time it was believed that one-peptide bacteriocins were the rule, with very few exceptions. Now it has been biochemically proven that two-peptide bacteriocins are the most common. For some of the two-peptide bacteriocins, both peptides are required for activity against bacteria. In some cases, the two peptides produce a more synergistic antimicrobial effect against pathogenic micro-organisms (Nes *et al*., 1996). By definition, a two-peptide bacteriocin has the two peptide genes located next to each other, and only one immunity gene is found next to the two pre-peptide genes.

An interesting feature of class II(b) bacteriocins is their strong antilisterial effect, which is found in a wide variety of lactic acid bacteria (*Pediococcus* spp., *Leuconostoc* spp., *Lactobacillus* spp. and *E. faecium*). All these peptides share a 35–70% sequence of identity, which is most pronounced in the N-terminal part of the bacteriocin.

The mode of action of some class II bacteriocins has been studied by Abee (1995) and Moll *et al*. (1996). The results so far show quite conclusively that they all act on the membrane of the susceptible bacteria, and lead to its permeabilisation. Varying abilities to permeabilise bacteria have been reported by Moll *et al*. (1996, 1997, 1998). Some bacteriocins cause leakage of cations, while others allow even larger molecules, such as ATP and amino acids, to pass through the membrane. Details of how the permeabilisation takes place are not known. However, some bacteriocins that are very potent to a narrow spectrum of bacteria may exploit some kind of receptor, but this has yet to be confirmed. NMR analysis of an antilisterial bacteriocin has shown that its C-terminal portion forms an α-helical structure; this portion of the molecule is most probably the membrane-interacting part of the bacteriocin (Fregeau Gallagher *et al*., 1997). The two-peptide lactococcin G has also been studied by circular dichroism. Here it has been shown that both peptides form α-helical structures in membrane-mimicking solvents and, in combination, the two peptides seem to interact so that an increased level of amphiphilic α-helical structures results (Hildeng-Hauge *et al.,* 1998). It is possible to suggest that lactococcin G forms membrane pores in a susceptible cell by a 'barrel-stave' mechanism.

Class III – characterisation, structural properties and mode of action

Class III bacteriocins are large in size and heat labile. Limited data are available on such bacteriocins produced by lactic acid bacteria, and for this reason they will not be reviewed here. Reviews by Klaenhammer (1993) and Nes *et al*. (1996) are recommended for further reading regarding their scientific and technical characteristics.

Application of bacteriocins in fermented milks

In recent years, there has been a growing interest in preservation of foods by lactic acid bacteria. The consumer pressure on the food industry to reduce the addition of chemical preservatives is one of the main reasons for this interest in biopreservation. The consumer wants 'natural' foods of high quality that are safe and processed to a minimum. Bacteriocins and bacteriocin-producing cultures are possible responses to this consumer request.

The deliberate use of lactic acid bacteria that produce bacteriocins is not common in the food industry today. However, a few cultures are already on the market, which were introduced as 'protective' cultures. Their main objective is to increase the microbiological safety of the food. The function of the cultures is to suppress undesirable and pathogenic bacteria, such as *Listeria monocytogenes* and *Staphylococcus aureus* in yoghurt (Benkerroum *et al*., 2002) or *Clostridium tyrobutyricum* in cheesemaking (Mathot *et al*., 2003).

Currently, nisin is the only bacteriocin approved as a food preservative. Many studies have been carried out on the use of nisin in cheesemaking including processed cheese, but very limited applications of nisin in milk are available (see the review by Tamime & Robinson, 1999). Benkerroum *et al*. (2002) added a *S. thermophilus* strain able to produce bacteriocins and *Lb. delbrueckii* subsp. *bulgaricus* to yoghurt

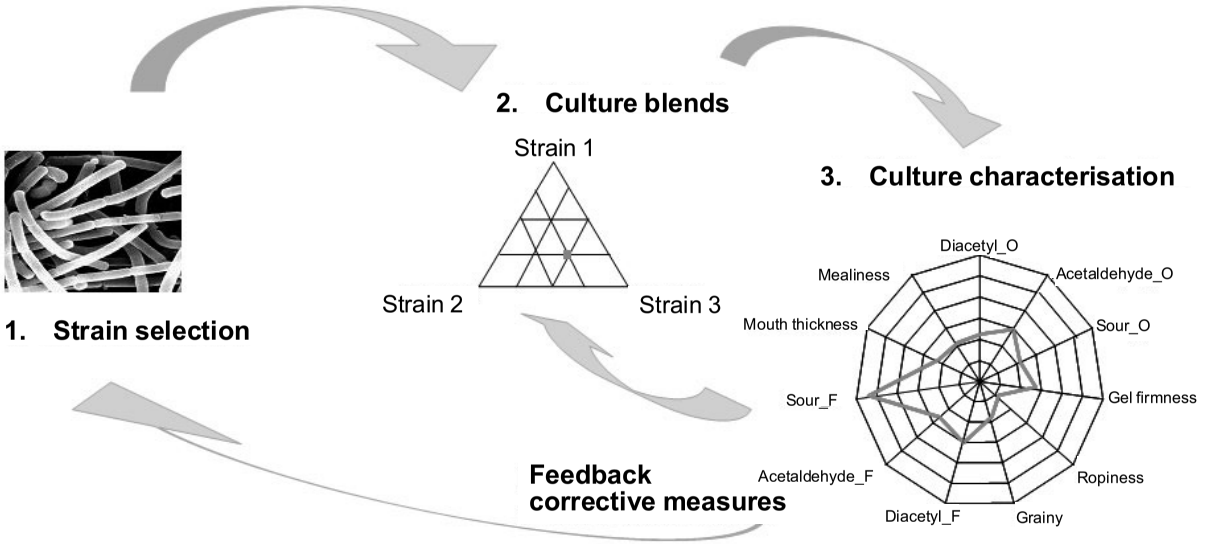


Fig. 2.6 Illustration of the main stages in starter culture development. By permission of Chr. Hansen A/S, Hørsholm, Denmark.

that was contaminated with *L. monocytogenes* and *S. aureus*. The results showed that *in situ* bacteriocin production was more active against *L. monocytogenes* than against *S. aureus*.

2.4 Development of starter cultures

Three main and important aspects have to be considered during the development stages of starter culture strains (Fig. 2.6). The first step is the choice or development of a single bacterial strain, the next step is blending the culture strains, and finally characterisation of the developed culture; Fig. 2.6 illustrates the development cycle. The triangle in the middle of Fig. 2.6 characterises three individual bacteria single strains, while the dot in the triangle indicates the 'primary' character(s) of the developed culture, which consists of mixing three bacterial strains in different ratios. Afterwards, the starter culture is grown in milk, and the fermentate is characterised by instrumental measurements and sensory profiling. The overall results and ratings are illustrated by a star or web chart, as shown in Fig. 2.6. If the bacterial blend does not provide the desired characteristics, for example, of a fermented milk product, the blend is either discarded or the mixture of the three strains is re-adjusted. This integration loop can be repeated several times, and the development of new strains or the blending of existing strains follows.

2.4.1 *Development of new bacterial strains*

At present, the development of new bacterial strains for the dairy industry has several options. Microbial evolution has created a huge variation in bacterial strains over the last several million years. However, finding the desired strain is not always an easy procedure because the approach is similar to looking for a needle in a haystack. Locating the right bacterial strain is often a matter of finding one strain among 100 000 other strains. Success depends on several aspects, for example the initial bacterial collection must be large and divergent enough to ensure that the strain is present. In addition, a screening procedure must identify the desired bacterial strain(s) based on a particular phenotype or physiological property.

Currently, there exist several different approaches to enhance the possibilities of finding the right strain and to speed up the process. One approach that can be employed is to increase the rate of mutations by using a mutagenesis method, i.e. by treating the bacteria strains with ultraviolet (UV) irradiation or a chemical mutagen so that the required variation can be generated. Instead of screening millions of strains to find the right one, the possibilities for picking one that meets predefined criteria are enhanced significantly. In other words, there are more needles in the haystack or the haystack has become smaller. The mutagenesis process can be repeated several times to enhance the chances even more. Classical mutagenesis can be seen as a kind of accelerated form of evolution; such a method is efficient, but is not directed at the genetic level. While the method is a great tool to obtain genetic variation, it lacks the possibilities for control. UV irradiation or a chemical mutagen can only be

used in limited quantities, so it is not possible to control exactly how many genes will be mutagenised.

However, a more controlled approach is to use genetic engineering, and the modern techniques available offer the right tools to mutagenise only the gene of interest (Sasaki, 1994; Heller *et al*., 1995; Klaenhammer, 1995; de Vos, 1996, 1999; Lick & Heller, 1998; de Vos *et al*., 1998; Henriksen *et al*., 1999; Lindgren, 1999; Mollet, 1999; Chaves *et al*., 2002; Hillier, 2002; Kondo & Johansen, 2002). Also, this direct approach means that it affects a specific gene; so that the desired mutant is well represented. A strain constructed using genetic engineering techniques is often considered a genetically modified organism (GMO), and the strain should be evaluated if marketing is considered. Furthermore, such a strain provides an important model because it will reveal a specific phenotype that can be used for screening and selection of other strains, either from a natural source or from a sample that has been mutagenised classically. No matter whether the strain has to be isolated from a natural sample or from a mutagenised or genetically engineered population, the chances of finding the desired strain are greatly enhanced if the strain has a phenotype or property that gives a selective growth advantage.

Despite all the different screening and selection methods applied to accelerate the evaluation process of strains, the bacterial population that needs to be screened to ensure that the desired mutant is available might still represent a rather large number. Manual screening of bacterial samples of this size presents a major bottleneck, but the modern automatic robotic equipment available today can speed up the screening process. The high-throughput screening (HTS) systems can handle more than 10 000 samples or 100 microtitre plates per day. When programmed with the screening and selection procedure, the system can run without supervision almost 24 h per day. Needless to say, when combined with efficient microbiological and genetic methods described above, the HTS system provides an even faster pathway for development of new bacterial strains.

To select the right strains, sets of screening criteria are employed, which have to reflect the required performance in the final fermented milk product, and also have to be fast, simple and easy to handle.

The basic criteria for screening new strains for the manufacture of fermented milks can include the acidification rate, phage sensitivity, texture properties (EPS producers), flavour and off-flavour properties, post-acidification, strain interaction and resistance to specific conditions (e.g. level of sugar in the milk base). Furthermore, other criteria, which have to be considered for production and preservation of bacterial strains, are ease of growing and concentration, stability during freezing and drying, and survival in storage.

2.4.2 *Blending of cultures*

Blending bacterial strains into a culture is the next step in the development cycle. The choice and the ratio of the strains in the culture are the key factors in this respect. However, the number of possible combinations of strains is overwhelming, even if relatively few strains are considered. Multivariate statistical analysis is a tool for

assessing the influences of strains and their ratios on the quality of the dairy product. In the case of fermented milk products, the fermentation time, texture, acidity, sharpness, astringency and post-acidification profiles are some of the factors that are considered (Fig. 2.7). Hence, mathematical modelling of cultures takes us one step further by predicting the performance of a culture before trial in the laboratory. These tools have speeded up the development process.

2.4.3 *Characterisation of cultures*

Different variables are considered to characterise a fermented milk starter culture.

Acidification rate

Speed of fermentation is a key variable in modern yoghurt production. Not only are the starter cultures required to acidify the milk in a short time, but they should also ensure that the time to reach the end pH does not vary substantially from batch to batch.

It is well established that the associative growth exists between strains of *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Join & Prajapati, 1998; Tamime & Robinson, 1999). However, the acidification rate of mixed cultures of these micro-organisms is faster than the summation of the acidification rate of each single strain. Consequently, both strains are carefully selected for their ability to acidify the milk quickly as single strains and in combination with each other. The analysis of the acidification performance of any one blend of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* is highly useful in reducing the batch-to-batch variation that must be expected when dealing with biological materials. Recently, the acidification rate of *Lactococcus* spp., *S. thermophilus* and *Lb. helveticus* during the course of milk fermentation have been reported by Cashon *et al*. (2002), and they concluded that the results obtained may be used to select starter culture for the manufacture of fermented milks.

Texture determination

The texture of yoghurt or any type of fermented milk product is important with regard to the quality of the product. Sensory evaluation of texture is often carried out as part of a full sensory profiling, which can also provide information on odour, flavour and texture of the yoghurt (Fig. 2.7). The most important attributes of texture of fermented milk products are assessed as follows: (a) gel firmness is evaluated visually by slowly placing a spoonful of yoghurt on the surface of an untouched yoghurt, and recording how long the structure is retained, i.e. the longer the time required, the greater the gel firmness of the yoghurt; (b) mouth thickness is assessed by eating the yoghurt at a high rate, i.e. the longer it takes to swallow the product, the higher the mouth thickness of the product; (c) ropiness is evaluated visually by taking a spoonful of yoghurt and assessing the length of the ropy ‘threads’ that hang from the spoon, i.e. the longer the threads, the higher the ropiness of the yoghurt.

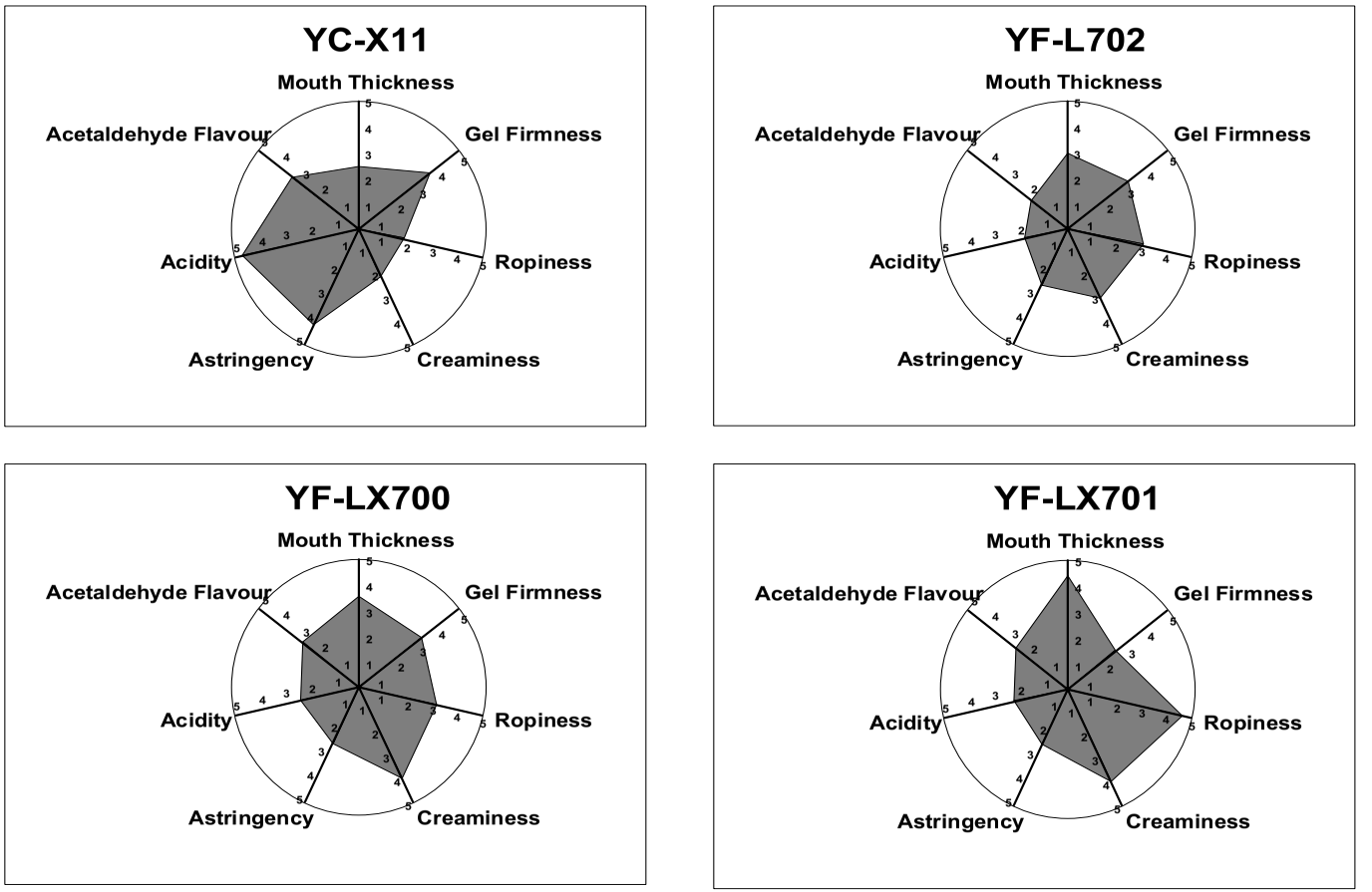


Fig. 2.7 Sensory profiling of four different blends of yoghurt starter cultures. It is evident that starter cultures YF-LX700 and YF-LX701 are similar, and they are bacteriophage unrelated to each other; while starter culture YC-X11 has a completely different flavour profile when compared with the other cultures. The results are average of three replicates. By permission of Chr. Hansen A/S, Hørsholm, Denmark.

For set-types of fermented milk products, gel firmness and mouth thickness are the most important parameters. Ropiness, as well as mouth thickness, is an important characteristic of stirred yoghurt. However, with the exception of a few products, for example viili, a high degree of ropiness is not desired in the product.

These sensory properties can all be correlated with specific instrumental measurements, which will not be described here.

Flavour assessment

Flavour can be described by sensory profiling (Fig. 2.7) and/or by a chemical analysis of volatiles. Even though a lot of information may be generated from the chemical analysis, sensory results are often valuable, and a typical example of the ratings of four yoghurts made with different types of starter cultures is illustrated by the star/web charts shown in Fig. 2.7.

Miscellaneous factors

The existence of bacteriophages (phages) in dairy starter cultures is well recognised, and it is a major problem in the cheese industry and to a lesser degree in the yoghurt industry. One of the measures practised by dairy starter culture companies is to blend phage-unrelated strains or phage-resistant strains. Hence, blends of starter cultures have to take into account this aspect before they are marketed.

Another factor that has to be considered when blending starter cultures is the compatibility between the bacterial strains used; otherwise the rate of acid development during the manufacture of fermented milk will be affected.

2.5 Production and preservation of commercial starter cultures

Historically, culture production was carried out in the dairies using liquid starters propagated either by the dairy or supplied by local culture producers. At the beginning of the 1960s, commercial starter culture companies developed the production technology to freeze-dry liquid cultures and produce concentrated frozen starter cultures for the direct inoculation of 500–1 000 L bulk starter tanks at the dairy (Jespersen, 1977). In the late 1970s, the production of freeze-dried concentrated cultures for the inoculation of bulk starter was followed by the introduction of super-concentrated frozen cultures for direct inoculation of the fermented milk and cheese milk. Today, commercial starter companies offer an extensive range of frozen and freeze-dried concentrated cultures for direct inoculation in the bulk starter, fermented milk or cheese tank.

Due to the demand placed on these cultures, the production procedures used by high-quality commercial starter companies have approached pharmaceutical standards over the last 10 years. Food-grade fermentation equipment and classified process areas are used in the factory, combined with good manufacturing practices (GMP) and an understanding of the critical control points. A typical production

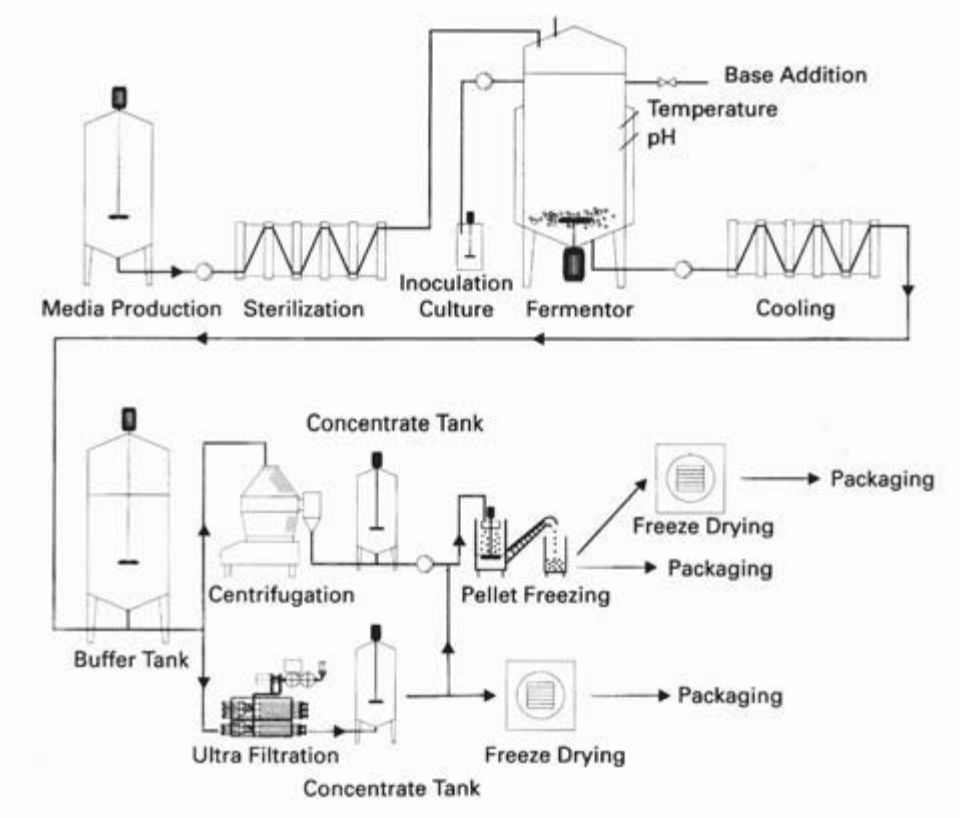


Fig. 2.8 Production of a typical commercial starter culture. Redrawn from Høier, E., Janzen, T., Henriksen, C.M., Rattray, F., Brockmann, E. & Johansen, E. (1999) The production, application and action of lactic cheese starter cultures. *Technology of Cheesemaking* (ed. B.A. Law), pp. 99–131, Sheffield Academic Press, Sheffield. By permission of Sheffield Academic Press, Blackwell Publishing, and Chr. Hansen A/S, Hørsholm, Denmark.

process is illustrated in Fig. 2.8, and consists of the following steps: (a) handling of inoculation material; (b) preparation of media; (c) propagation in fermenters under controlled pH conditions; (d) concentration of bacterial cells; (e) freezing; (f) drying; (g) packaging; (h) storage. The starter culture collections of the suppliers are the basis of all fermentations. Mixed or single strains used as inoculation material are prepared under aseptic conditions.

The parameters for the production of frozen and freeze-dried starter cultures are now well established. Growth media are composed of selected milk components and supplemented with various nutrients, such as yeast extract, vitamins and minerals. To produce high-quality products efficiently, a specific medium for each culture has to be developed and continuously optimised. Heat treatment of the medium is an ultra high temperature (UHT) process with subsequent aseptic transfer to the fermenters. After inoculation of the starter culture, growth is extended by maintaining the pH at 6.0–6.3 for mesophilic cultures and 5.5–6.0 for thermophilic cultures by the addition of alkali, such as NaOH or NH_4OH. Other critical variables such as

temperature, rate of agitation and headspace gases in the fermenters are optimised for each strain. These conditions produce cell suspensions, which are 5- to 10-fold more concentrated than a normally acidified bulk starter. After fermentation, which normally is a batch fermentation in vessels with a capacity of 5000–30 000 L, the contents are cooled and the biomass is concentrated by centrifugation or membrane ultrafiltration (UF). At the concentration step, a further 5- to 40-fold concentration of the cells occurs.

After concentration, the bacterial cells biomass is pelletised by 'raining' the concentrate into an agitated bath of liquid nitrogen. Cryoprotective agents (especially important for freeze-dried starter cultures) are added to the concentrated product before freezing to increase the survival rate of the micro-organisms. Cryoprotective agents include ascorbate in milk or a water base and monosodium glutamate. To prevent the formation of extracellular and intracellular ice crystals, polyols such as mannitol, glycerol and sorbitol, or disaccharides such as lactose and sucrose, can also be added (Champagne *et al*., 1991; Porubcan, 1991; see also review by Tamime, 2002).

After freezing or freeze-drying, the activity of the culture is maintained by storage under the specified conditions. Frozen cultures should be stored at –45°C and freeze-dried products should be stored at –18°C. Both culture types will retain their activity for at least 12 months. Freeze-dried cultures have the extra advantage that they can be despatched at room temperature without losing activity. The above section has been reproduced with permission of Sheffield Academic Press and Blackwell Publishing.

2.6 On-site production and use of starter cultures

2.6.1 *Background*

The fermentation process of any cultured dairy product (i.e. fermented milk or cheese) relies entirely on the purity and activity of the starter culture, provided that the milk or growth medium is free from any inhibitory agent(s), such as antibiotics or bacteriophage (phage). Since starter cultures are preserved in small quantities, the traditional method for the production of bulk starter cultures was a scale-up system of propagation in order to meet the required volume of any production line. The sequence of operations for bulk starter production is:

stock culture → mother culture → feeder or intermediate culture→ bulk culture

It is important to note that the culture inoculation is carried out under aseptic conditions to minimise contamination and the proliferation of phage, and growth is initiated in a sterile growth medium (Tamime, 1990). It is anticipated that starter cultures must contain the maximum of viable organisms (bulk starter cultures normally contain about 1×10^9 cfu mL^{-1}), must be highly active under production conditions in the dairy, free from contaminants and safe for human use.

Since the 1940s, different systems for production of bulk starter cultures have been developed, mainly for the preparation of the growth medium and the protection of the culture from phage:

- simple microbiological techniques;
- mechanically protected tanks including pH control (e.g. internal or external);
- the propagation of starter cultures in phage or bacteriophage inhibitory medium (PIM or BIM, respectively).

Illustrations and descriptions of the tanks have been published by Tamime (1990) and will not be reviewed in this chapter. The following are recommended for further reading regarding aspects of starter culture technology (Tamime & Robinson, 1985, 1999; Lewis, 1987; Bylund, 1995; Sandine, 1996; Tamime, 2002; Anon., 2003).

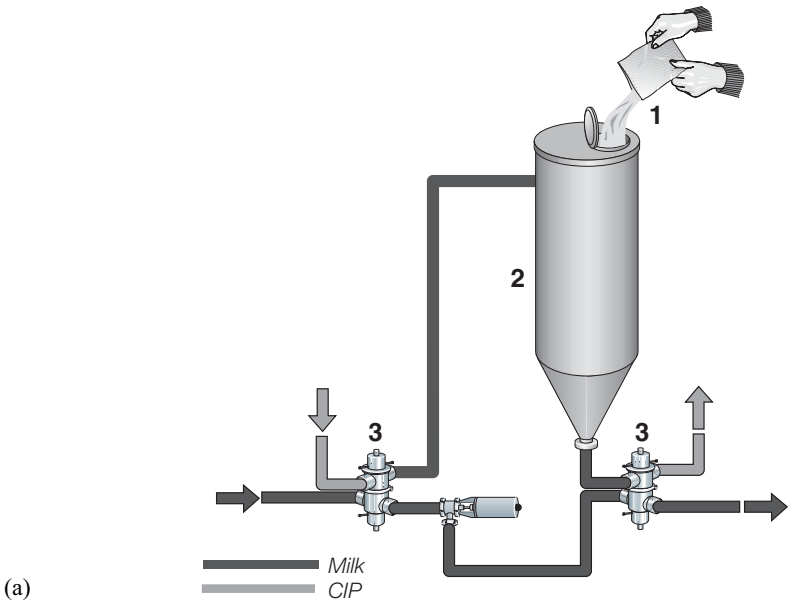
When using mechanically protected systems, the transfer or inoculation of 'liquid' cultures at every stage of the scale-up is carried out through, for example: (a) a chlorinated barrier, such as the Lewis system, or using sterile air to transfer the culture from one unit to another (Tetra Pak system); (b) a ring of flame (Jones system); (c) sterile filtered air (under positive pressure) using a high-efficiency particulate air (HEPA) filtration system. In the HEPA system, the manhole is opened and the starter culture is added to the tank; such systems may not pose any difficulty when using concentrated freeze-dried or frozen cultures to inoculate the bulk starter tank. More recent developments in starter culture technology whereby the culture can be aseptically inoculated into the bulk tank or directly inoculated into the processed milk base, for example, may include the following systems.

2.6.2 *In-line inoculation with freeze-dried or frozen concentrated culture*

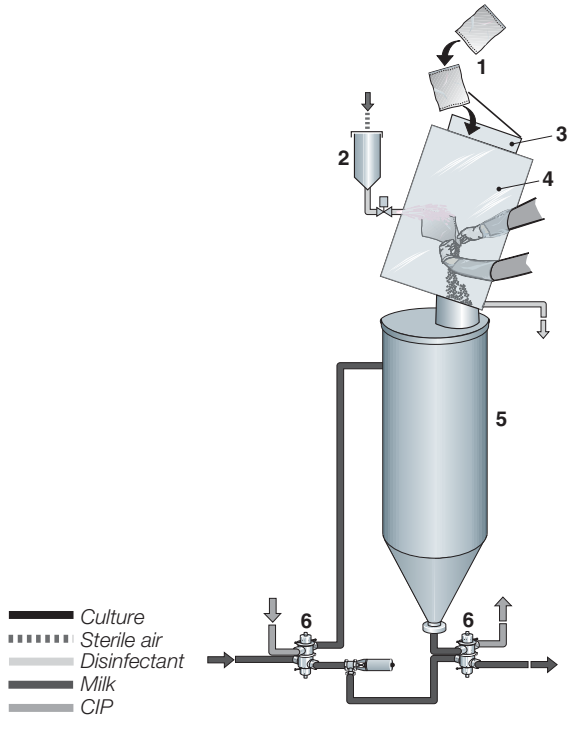
Tetra Pak AB in Sweden developed this method of inoculation in the early part of 2000, and the culture is inoculated directly into the processed milk base before entering the incubation tank (Fig. 2.9a). A stainless steel container (constructed as a bypass of the milk line) holds enough culture to inoculate one incubation tank, and is connected to the milk pipeline. When the operator decides to inoculate the incubation tank during the milk filling stage, he or she opens the valves for the bypass line, and the milk thaws or rehydrates the culture and transfers it to the tank. Afterwards, the container and the bypass line are cleaned and sanitised to be loaded again with culture ready for inoculating a second incubation tank. Such units could be built within a sterile air cabinet in order to minimise the risk of infection during the loading of the culture into the container.

Another technique to improve the hygienic aspect of the inoculation container is shown in Fig. 2.9(b), which consists of a specially designed box, connected over the opening unit of the inoculation container. The box is fitted with a sight glass on top to facilitate visual contact for the operator, armholes on the side that are equipped with rubber gloves, and spraying and draining devices for disinfectant solution. Thus, each type of culture sachet or package can be disinfected and opened under hygienic conditions inside the box. For opening the culture package(s), the operator uses a pair of scissors, and every action in such a box is done via the two gloves of the armholes. Alternatively, the box can be mounted either on top of the bulk starter culture tank or the incubation vessel (Fig. 2.10; Anon., 2003; Chapter 5).

The operating instructions for the inoculator's box are summarised as follows:



(a)



(b)

Fig. 2.9 In-line inoculation system using concentrated freeze-dried or frozen cultures. (a) – 1, Starter culture package; 2, Mixing container; 3, Mix-proof valves. (b) – 1, Starter culture package; 2, Disinfectant container; 3, Sight glass; 4, Inoculation 'box'; 5, Mixing container; 6, Mix-proof valves. By permission of Tetra Pak AB, Lund, Sweden.

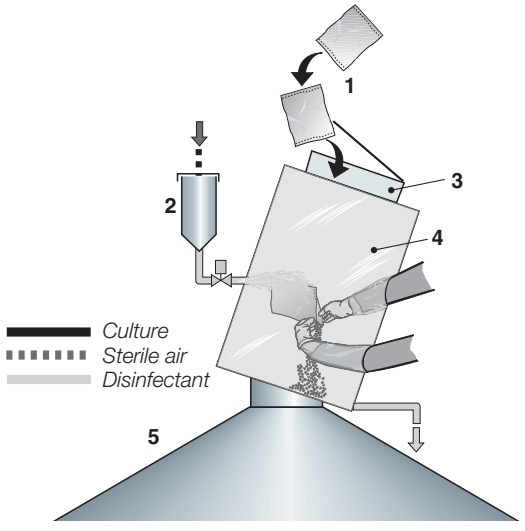


Fig. 2.10 Hygienic inoculation system fitted on top of a fermentation or bulk starter culture tank. 1, Starter culture package; 2, disinfectant container; 3, sight glass; 4, inoculation 'box'; 5, incubation or bulk starter culture tank. By permission of Tetra Pak AB, Lund, Sweden.

- Operate only with cleaned inoculator.
- Disconnect hose for sterile air supply from the disinfectant container.
- Manually fill the disinfectant unit with a sanitising solution, e.g. 2–3 L containing ~0.1 mL 100 mL^{-1} H_2O_2.
- Close the disinfectant pot and quickly connect the hose for sterile air supply.
- Open the upper lid, bring the closed starter culture package and a tool (e.g. pair of scissors or knife) into the inoculator.
- Close the upper lid and switch on the light.
- Use gloves on both hands and check that the lower lid is closed.
- Using a switch, activate the spray nozzles for the disinfectant solution to sanitise the starter culture package and the scissors.
- Spray as long as possible, so that all the surfaces are wet with the disinfectant solution.
- The sanitising agent must be emptied from the inoculator via the drainage valve, and make sure that it does not enter the incubation tank.
- Open the culture package, and carefully open the lower lid. (Note: before opening the lower lid, disconnect the starter tank from the sterile air supply and make sure that there is no over pressure in the tank.)
- The starter culture falls into the tank through the lower opening, and switch on the agitator.
- Close the lower lid, and remove the empty starter culture package and the scissors from the inoculator via the upper lid.
- Close the upper lid and switch off the light.
- Switch off the agitator after 5–10 min.

- Clean the inoculator together with the incubation tank after production; make sure that the lower lid of the inoculator is open during the cleaning-in-place (CIP) cycle.
- Close the lower lid of the inoculator after cleaning the tank.

2.6.3 *Automatic inoculation system*

The development of the automatic inoculation system (AISY) in starter culture technology was patented by Chr. Hansen A/S in Denmark in the 1990s in collaboration with Tetra Pak (Dairy & Beverages System) AB in Sweden. It uses DVS concentrated starter cultures by integrating and automating the thawing or rehydration, mixing and dosing operation from the starter culture pack right through to the fermented milk or cheese vats or tanks (Fig. 2.11).

The AISY method of inoculation can be summarised as follows (Tamime, 2002; Anon., 2003):

- Add the required amount of water to a buffer tank to make a 10 g 100 g^{-1} bacterial solution (e.g. 10 kg frozen DVS culture and 90 kg of water).

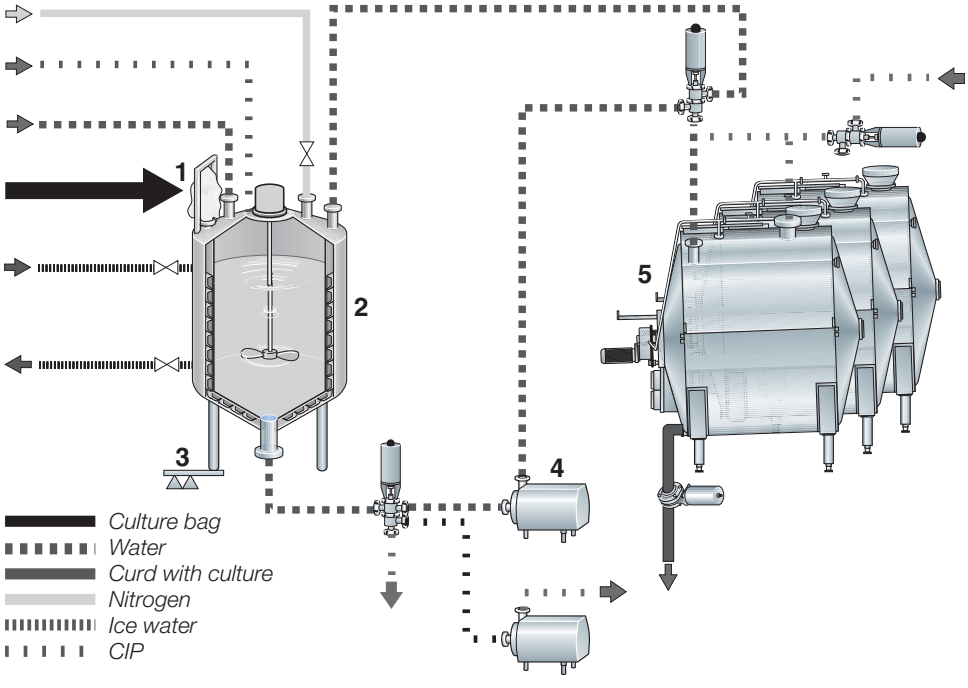


Fig. 2.11 An illustration of the automatic inoculation system (AISY). 1, Starter culture package; 2, mixing tank; 3, weighing cell; 4, feed pump; 5, fermented milk or cheesemaking tank. By permission of Tetra Pak AB, Lund, Sweden.

- It is recommended that: (a) the water should be pasteurised at 72°C for 15 s, followed by cooling to 14–18°C, (b) it should be good quality water and (c) an active carbon filter should be installed to remove chlorine if present at > 5 μg g^{-1}.
- Remove the culture from the freezer (i.e. at ≤–45°C) and empty it into the buffer tank with agitation; the agitators should operate at slow speed to minimise foam formation or the incorporation of air into the solution.
- The dissolved culture is stirred for 20 min before being metered into the processed milk or production tank; the activity of the dissolved culture can be maintained up to 24 h if it is cooled to < 10°C.
- The headspace of the buffer tank should be purged with nitrogen to minimise frothing or loss in microbial activity.

2.7 Future developments

It is evident that the technological aspects of fermented milk production are well established (see other chapters in this book), and the knowledge of how to use, produce and control starter cultures has greatly advanced over the past few decades. In the future, there will be further development and application of genetically modified starter cultures, which have the desired characteristics built in, but their use will need to be governed by national and international regulations to safeguard the consumer. Furthermore, the control of undesirable and pathogenic micro-organisms in fermented milks by using starter cultures that can naturally inhibit their growth (i.e. production of bacteriocins) will be greatly exploited in the coming years.

Similarly, the present and future strategies employed in the development of new fermented milks will involve the use of starter culture blends (with exopolysaccharide producers) to modify the perceived sensory and physico-chemical properties of these products. In order to achieve such developments, the producers of fermented milks will rely more and more on the progress achieved by the starter culture companies. As expected, the equipment manufacturers will constantly develop the necessary technologies to meet the requirements of adding starter cultures hygienically into the milk under industrial operation(s). In conclusion, there will be greater need for fermented milk producers to co-operate with starter culture companies and equipment manufacturers in the years to come.

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Section 2.3.1 is modified from Jensen (1999) with permission. Sections 2.3.2 and 2.3.3 are modified from Curic (1998) with permission.

Section 2.5 is adapted from Høier, E., Janzen, T., Henriksen, C.M., Rattray, F., Brockmann, E. & Johansen, E. (1999) The production, application and action of lactic cheese starter cultures. *Technology of Cheesemaking* (ed. B.A. Law), pp. 99–131, Sheffield Academic Press, Sheffield, with permission of Sheffield Academic Press and Blackwell Publishing.

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Fermented Milks
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3 Manufacture of Yoghurt

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3.1 Background

Communities in the Middle East and Asia are widely acknowledged as having introduced fermented milks such as yoghurt into their diet almost as soon as man began to domesticate animals. Some fermented milks did, of course, become popular with local populations in regions like Scandinavia and Russia (Koroleva, 1991), but it was thousands of years later that sections of the general public in Europe and North America began to take a serious interest in fermented milks. One of the reasons for this limited uptake was that natural yoghurt can taste extremely acidic to Western palates unless accompanied by another dish, and it was not until the various forms of sweetened and fruit-flavoured yoghurt went on sale that the market for yoghurt really expanded.

In some brands, a layer of fruit was placed in a retail carton and carefully covered with milk (around 150 mL) containing sufficient culture for the milk to coagulate within a few hours and 'trap' the fruit below. Such products – often referred to as Swiss-style yoghurts – acquired a limited popularity but, once manufacturers began to add a specified level of fruit into the white base and package the combined material into retail cartons suitable for lunchboxes or picnic baskets, the concept of stirred fruit yoghurt as a pleasant and nutritious snack had arrived.

Today, yoghurt remains a milk-based fermented milk that is presented to the consumer in either a gel form (set yoghurt) or as a viscous fluid (stirred yoghurt) but, as figures for consumption have risen (see Chapter 1), so manufacturers have expanded the market by introducing an ever wider range of fruit flavours and/or changing the image of the product, e.g. by raising the total solids and fat contents of a standard stirred yoghurt to give a product with a luxury image (Robinson, 2000a). Nevertheless, despite these and other innovations, the method of manufacture is still based on the system employed by nomadic herdsmen many centuries ago. For example, the majority of yoghurts consumed worldwide are manufactured with cultures of bacteria with growth optima of 37–45ºC, and this characteristic derives from the fact that the species in question, namely *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, evolved in the Middle East where the ambient temperature in the summer months is often well in excess of 35ºC. Similarly, the universal method of manufacturing a satisfactory yoghurt is based on the traditional process, and the principal stages in the production of both set and stirred yoghurts are summarised in Table 3.1 and Fig. 3.1.

Table 3.1 Outline of the important stages in the manufacture of natural set or stirred, fruit yoghurt.

Processing	Materials	Comments
Standardisation of fat, addition of skimmed milk powder or vacuum/membrane concentration	Full-cream or skimmed milk at 14–16 g 100 g^{-1} solids-non-fat, and milk fat at 0.1–5.0 g 100 g^{-1}	Sucrose (7–10 g 100 g^{-1}) and stabilisers may be added to the base for stirred, fruit yoghurt
Homogenisation at 15-20 MPa and 60–70ºC	Process milk	Reduces fat globules to < 2.0 μm and improves texture of end-product
Heat treatment at 80–85ºC for 30 min or 90–95ºC for 5–10 min	Process milk	Reduces bacterial load and oxygen content of milk; denatures whey proteins which interact with κ-casein and improve texture of end-product
Cooling to 30 or 42ºC and inoculation with culture (see text)	Inoculated milk	Set yoghurt will be packaged at this point
Incubation for 16 h at 27–30ºC or 3.5–4.5 h at 42ºC	Milk coagulated by lactic acid, and flavour/texture compounds released by culture	Incubation rooms for set yoghurt or in-tank incubation for stirred yoghurt
Cooling to 2–4ºC for set yoghurt in cartons, 15–20ºC for stirred yoghurt in tanks	Fruit purée (10–15 g 100 g^{-1} addition rate) or fruit flavours for stirred varieties	The coagulum must be handled carefully to avoid damage to the warm gel
Packaging of stirred, fruit yoghurt and cooling to 2–4ºC	Retail products – natural set yoghurt and stirred, fruit yoghurt	120–150 g individual cartons or 500 g family packs are normal

Adapted from Robinson (2000b).

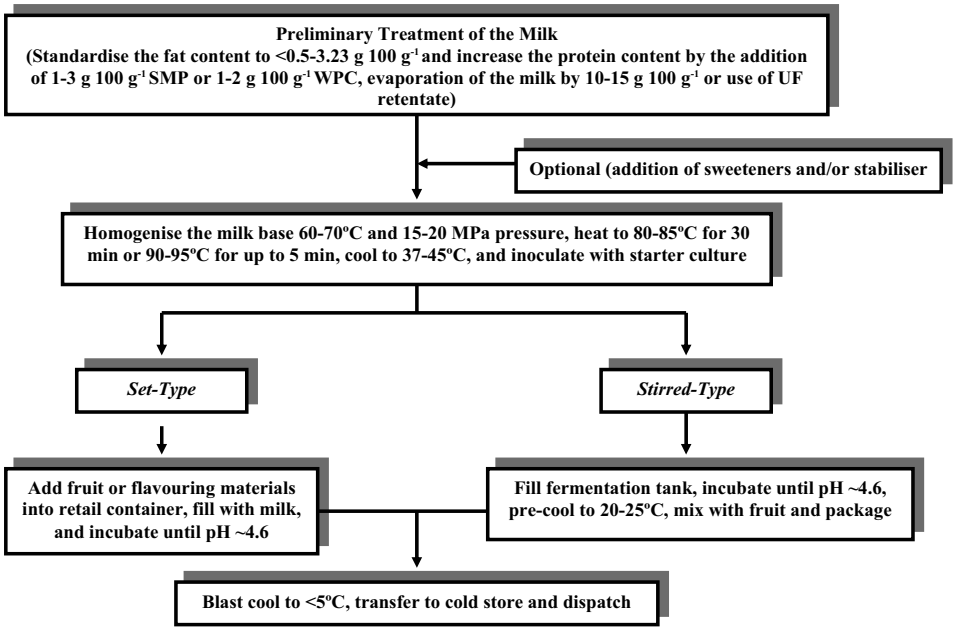

Fig. 3.1 Main processing stages during the manufacture of set and stirred yoghurt.

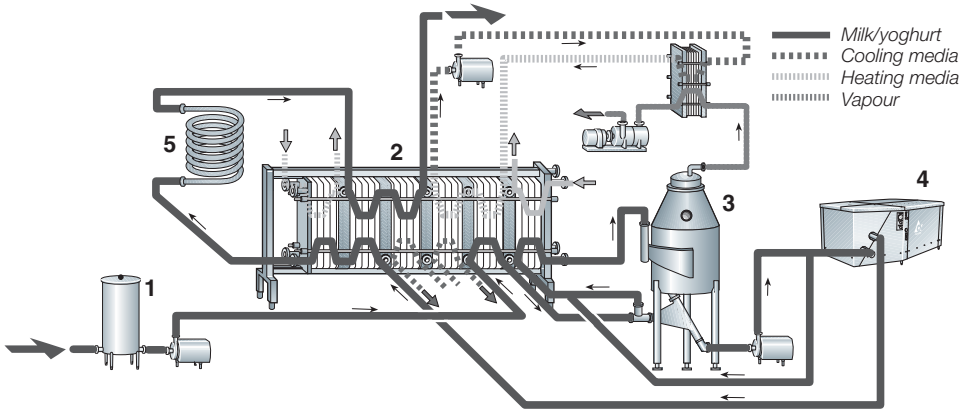

Fig. 3.2 Flow diagram of general pre-treatment of milk for the manufacture of set and stirred yoghurt. 1, Balance tank; 2, plate heat exchanger (PHE); 3, evaporator; 4, homogeniser; 5, holding tube. By permission of Tetra Pak AB, Lund, Sweden.

It is well established that the way the milk is handled or prepared, including the processing conditions used in yoghurt manufacture, greatly influence the gel texture, strength and stability (Lucey & Singh, 1997; Walstra, 1998; Tamime & Robinson, 1999; Jaros & Rohm, 2003a, b), and could be briefly summarised as:

- fortification level and material(s) used in the mix;
- stabiliser type and usage levels;
- fat content and homogenisation conditions;
- milk heat treatment conditions;
- starter culture (type, rate of acid development and production of exopolysaccharides – EPS);
- incubation temperature (influences growth of starter cultures, gel aggregation, bond strength);
- pH at breaking of the gel (stirred) and/or start of cooling (set);
- cooling conditions;
- post-manufacture handling of the product, e.g. physical abuse (vibration) and temperature fluctuations (i.e. if the product is not maintained at ≤ 5°C).

It is evident that production lines that produce set and stirred yoghurt and a combined processing plant have some stages in common (Figs 3.1 and 3.2), until the fermentation stage.

3.2 The basic requirements for making yoghurt

3.2.1 *Introduction*

In general, bovine milk (i.e. whole, partially skimmed or skimmed including powders and/or whey protein concentrates) and/or cream are used during the manufacture of

yoghurt. To ensure a high quality end-product, the milk should have a low bacterial count (i.e. maximum of 1.0×10^5 colony-forming-units (cfu) g^{-1}). Furthermore, the milk and other dairy ingredients should be free from taints, antibiotic compounds, sanitising agents and bacteriophages; somatic count should be $< 4.0 \times 10^5$ cells mL^{-1} (optimum $\leq 2.5 \times 10^5$ cells mL^{-1}) (Tamime & Robinson, 1999; Oliveira *et al*., 2002).

3.2.2 *Milk as the base material*

Fresh bovine milk is usually the base material for making yoghurt in the Western world, although ovine, caprine or buffalo milks can also be employed. The fat content of bovine milk tends, depending on breed of cow and diet, to be in the range 3.0–3.5 g 100 mL^{-1}, and this value has to be reduced by separation or supplemented with cream according to consumer taste and/or market demand; the fat content of most retail yoghurts lies in the range 1.0–4.5 g 100 mL^{-1}. However, the critical feature of the milk is the level of solids-non-fat (SNF), which, in bovine milk, varies from 8.5–9.0 g 100 mL^{-1} according to the season of the year, with around 4.5 g being lactose, 3.3 g being protein (2.6 g casein and 0.7 g whey proteins) and the balance being minerals.

The proteins, together with minerals such as calcium and phosphorus, give rise to the basic gel structure of yoghurt, but the groups from the Middle East who developed the earliest forms of yoghurt were well aware that the levels of protein present in liquid milk are not sufficient to produce an attractive end-product in terms of consistency or 'mouth-feel'. Hence, the first step in yoghurt making was always to raise the SNF content by heating the fresh milk in an open pan suspended over a fire (Tamime & Robinson, 1999). The main aim of this action was to evaporate off sufficient water to raise the total solids in the milk, but it is of note that the subsequent process of heating to > 90°C would have other desirable effects as well (see later).

3.2.3 *Standardisation of fat content and fortification of solids-non-fat content*

The fat content in yoghurt made in different parts of the world may range from as low as 0.1 g to as high as 3.5–5.0 g 100 g^{-1} in order to meet existing or proposed compositional standards. Therefore, it is necessary to standardise whole milk, and the methods employed for fat standardisation are as follows: (a) removal of all or part of the fat content; (b) mix whole milk with skimmed milk; (c) addition of cream to whole milk or skimmed milk; (d) a process that may combine some of these methods listed (e.g. the use of standardising centrifuges) (Tamime & Robinson, 1999).

Today, various levels of SNF are sought by manufacturers but, in general, the recognised categories (in g 100 mL^{-1}) are:

- Cheap/cooking yoghurt 12
- Standard base for stirred fruit yoghurt 13–14
- Top of the range, natural set yoghurt 17–18

On an industrial scale, the elevation of the SNF can be achieved by evaporation (EV) or ultrafiltration (UF); reverse osmosis (RO) is an optional process. The UF and EV processes remove water and hence raise the levels of both fat and SNF in the yoghurt base, but UF does allow some losses of lactose and minerals (Lankes *et al.*, 1998; Robinson *et al.*, 2002). In terms of product quality, either process is acceptable and, while the final choice may depend on the cost and/or availability of process plant, some data suggest that the more extensive heating linked with EV gives rise to a smoother coagulum. This improvement may result from the temperature profile of the EV process, for the incoming milk passes from a balance tank and through a plate heat exchanger before entering the evaporator. The temperature of milk entering the evaporator is 70°C and, by recirculation of the milk through the evaporator, the desired amount of water is removed (Anon., 2003); the temperature of the milk is slightly higher than during UF (50–55°C) (Figs 3.2, 3.3 and 3.4). The UF process (coupled with diafiltration) is used to produce a low-lactose (low-carbohydrate) yoghurt that is requested by some consumers.

The alternative route is to add skimmed milk powder (SMP) to the milk base, and systems of hoppers, high-speed blenders and in-tank mixing can be employed to ensure full and rapid incorporation of the milk powder (Robinson & Tamime,

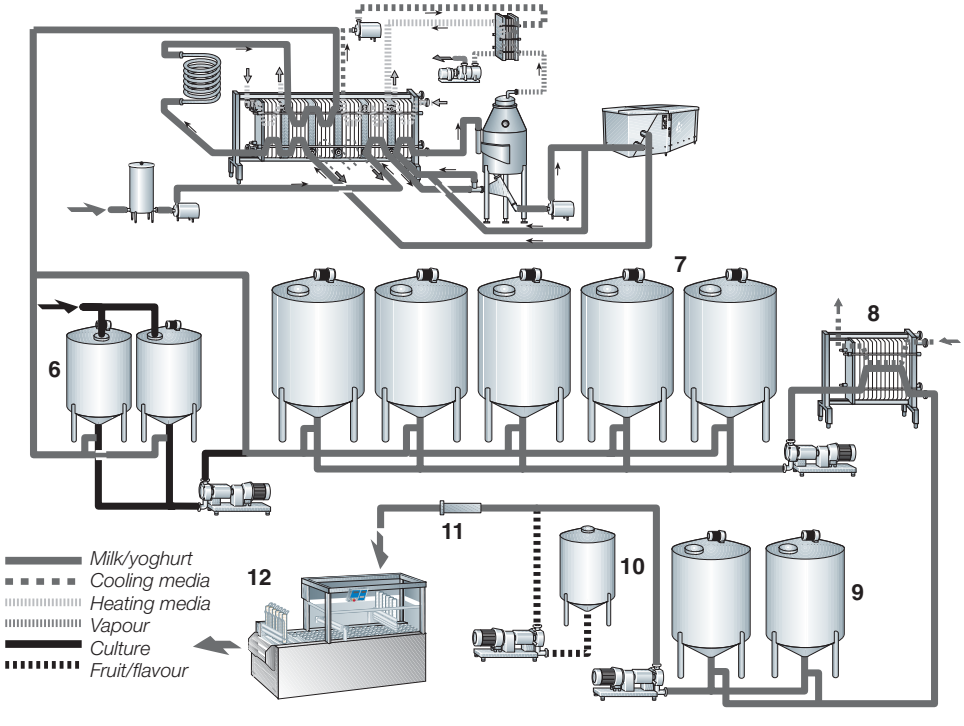

Fig. 3.3 Production line for stirred yoghurt. 1, Bulk starter culture tanks; 2, incubation/fermentation tanks; 3, plate cooler; 4, buffer tanks; 5, fruit/flavour tank; 6, in-line mixer; 7, filling machine. By permission of Tetra Pak AB, Lund, Sweden.

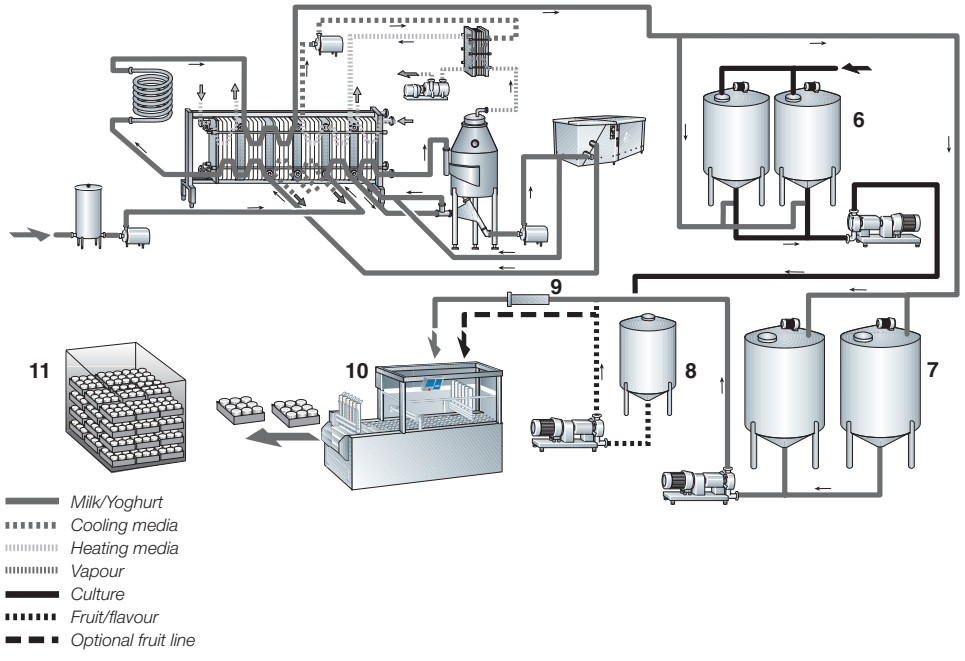

Fig. 3.4 Production line for set yoghurt. 1, Bulk starter culture tanks; 2, buffer tanks; 3, fruit/flavour tank; 4, in-line mixer; 5, filling machine; 6, incubation. By permission of Tetra Pak AB, Lund, Sweden.

1993; Fitzpatrick *et al*., 2001; Fitzpatrick & Cuthbert, 2004), or using mixing vessels under vacuum (Anon., 2003; see also Chapter 5). The advantages of this route are that: (a) it is adaptable for factories with low-volume throughputs; (b) sugar and/or stabilisers can be incorporated along with the milk powder for the production of stirred fruit varieties; (c) initial plant costs are lower than with EV or UF. On the negative side, the fluctuating price of milk powder can be a problem, which is why many high product volume–low profit margin manufacturers prefer to rely on EV or UF. Nevertheless, fortification of the milk solids with skimmed milk powder (in one study, twelve milk powder batches were produced at different intervals over one year) greatly influenced the physical characteristics of set and stirred yoghurts, and it is recommended that varying the total solids in the milk base throughout the year will produce yoghurts of more consistent gel strength, viscosity and drained whey (Cheng *et al*., 2002).

However, other sources of milk protein powders may be more economic, and whey proteins at usage rates of 0.7–2.0 g 100 mL^{-1} can be used to reduce any tendency for whey separation in the final product (Bhullar *et al*., 2002). Indeed, increasing the whey protein content and decreasing the casein to whey protein ratio increases gel firmness, so long as the mix is given a sufficiently high heat treatment to denature the whey proteins and cause them to associate with the casein micelles (Puvanenthiran *et al*., 2002; Augustin *et al*., 2003; Onwulata *et al*., 2004). An excessive level of whey proteins can, under some conditions, lead to a grainy texture (Lucey & Singh, 1997).

Caseinates are more efficient than whey proteins in increasing yoghurt consistency, and the physical structure of sodium caseinate, for example, is likely to be responsible for this effect (Guzmán-González *et al*., 2000). Whey protein isolates (WPI) added at a rate of 8 g 100 g^{-1} to the milk base decreased the viscosity of yoghurt (Patocka *et al*., 2004). High-protein powders derived from the UF retentates can be equally effective (Guzmán-González *et al*., 1999; Mistry, 2002). However, the quality of fat-free yoghurt containing 5 g 100 g^{-1} of protein from SMP and whey protein concentrates (WPC) at a ratio of 1.5 : 0.5 was similar to the control in terms of texture profile, syneresis and sensory properties (Antunes *et al*., 2004).

Furthermore, deaeration of the milk base may be needed if powders and other dry ingredients are used due to likely air incorporation during mixing; the presence of oxygen can affect the growth of the lactobacilli (Beshkova *et al.*, 2002).

Whatever system is employed, the 'bottom line' is that milk fat and proteins are expensive constituents, and an accurate measurement of fat and protein in the yoghurt base is essential. Modern infra-red systems of analysis allow these variables to be measured with sufficient rapidity and accuracy to enable routine on-line monitoring and adjustment to take place (Andersen *et al.*, 1993).

3.2.4 *Other ingredients*

It is generally accepted that natural set yoghurt should comprise nothing other than milk and the starter culture, but stirred fruit yoghurts are permitted in some countries to contain stabilisers, fruits, flavours, sweetening agents and preservatives (Table 3.2). The most active stabilisers are hydrocolloids chosen for their ability to absorb water, and gums of plant origin, e.g. guar gum and locust bean gum, provide a stirred product with a very favourable mouth-feel. Gelatin gives a distinct, shiny appearance to stirred yoghurts, and a gel-like structure that melts cleanly in the mouth (Fiszman *et al.*, 1999); it is also effective in controlling the migration of moisture. Low methoxyl pectins provide an alternative source of viscosity in stirred yoghurts, or stabilise the fruit preparation in Swiss-style yoghurts, and some sources of dietary fibre added for alleged nutritional reasons (e.g. polydextrose) may affect viscosity as well. Other types of stabiliser that have been used in yoghurt making include carboxymethyl cellulose, locust bean gum, alginates, carrageenan, starch and modified starch (Tamime & Robinson, 1999; Williams *et al*., 2003; Sagdiç *et al*., 2004).

Table 3.2 Some ingredients which, if permitted, can be used during the manufacture of yoghurt.

Sweeteners	Stabilisers	Colours	Preservatives
High fructose corn syrup, fructose, honey, aspartame and/or sucralose	Pectin, gelatin, agar-agar, starch (unmodified and modified), carrageenan, locust bean gum, guar gum, carboxymethylcellulose and/or whey protein concentrate	Annatto, brilliant blue FCF, turmeric, brilliant black PN, red 2G, caramel or carmine	Nisin, K- or Na-sorbate, sorbic acid, benzoate

Compiled from Tamime & Robinson (1999).

Sweetening agents, such as sucrose, high-fructose corn syrup or honey, are usually added to stirred yoghurts to mask the acidity for acid-conscious consumers and, perhaps, produce a firmer texture. However, since sugars increase the osmotic pressure of the milk base, the addition of excessive levels (> 10 g 100 mL^{-1}) prior to fermentation can inhibit starter activity (Tamime & Robinson, 1999); for this reason, sugars may be added with the fruit to stirred yoghurts just before filling. Some brands of fruit yoghurt employ artificial sweeteners (e.g. aspartame or sucralose) as a means of lowering the calorie content, but some people have expressed reservations about this practice (Robinson, 2000a). However, sugar and sweeteners can influence the microstructure of yoghurt; the former caused the casein micelles to form clusters, while aspartame caused the casein micelles to form double longitudinal polymers (Haque & Aryana, 2002).

The extent to which yoghurt should be employed as a vehicle for components that may have nutritional benefits is open to debate, but a list of materials that have been added to yoghurts is shown in Table 3.3.

3.3 Initial processing

Once the desired composition of milk in terms of fat, SNF and, if applicable, other ingredients has been achieved, the milk will usually be homogenised using pressures of 15–20 MPa at 70°C (Fig. 3.2). This stage helps to ensure the full dissolution of any dry ingredients, e.g. sugar or stabilisers, that may have been added, and to reduce the size of the fat globules to < 2 μm, so minimising the risk of coalescence during the fermentation stage, and achieving a uniform distribution of the fat globules in the protein matrix during the gelation process (Fig. 3.5). In addition, the consistency of the yoghurt base is enhanced by homogenisation, in that a portion of the casein and whey proteins become attached to the fat globule surfaces, so effectively increasing the number of structure-building components in the system (Walstra, 1998); native fat globule membranes do not interact with proteins in the same way (van Vliet &

Table 3.3 Some materials that could be, if permitted, or have been, added to yoghurt for alleged nutritional reasons.

Material	Product
Prebiotics	Inulin, oligosaccharides, lactulose
Dietary fibre	Polydextrose
Phytosterols	Soya derivatives
Dairy-derived ingredients	Lipids, proteins, peptides
Fatty acids	Omega-3, conjugated linoleic acid
Glucosamine	
Lactoferrin	
Lycopene	
Antioxidants	Vitamins A, C, E
Health promoters	Various B-group vitamins
Minerals	Calcium, magnesium, zinc, selenium, iron

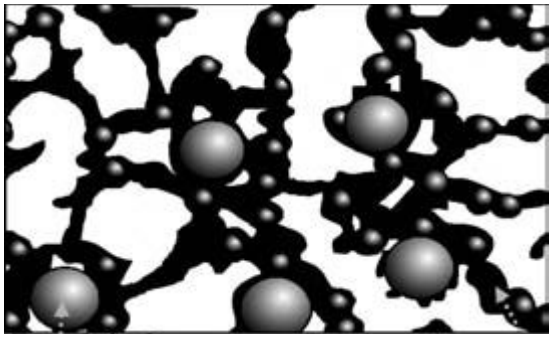

Fig. 3.5 Schematic illustration of an acid gel showing the homogenised fat globules embedded in the protein matrix.

Dentener-Kikkert, 1982; Lee & Sherbon, 2002). Also, the homogenisation of the milk reduces the incidence of whey separation and the product becomes whiter. It should be noted, however, that if the viscosity of a stirred yoghurt is dependent on a starch-based stabiliser, homogenisation at high temperature and pressure can have a negative impact. In addition, due to the release of lipase and disruption of the natural fat globule membranes, homogenised mixes should be heat treated without delay in order to reduce the risk of enzymatic rancidity developing.

Heat treatment follows, and at temperatures well above normal pasteurisation. One of two alternative systems is usually employed, and while one – the high temperature/short holding (HTSH) system – involves passing the milk through a plate heat exchanger with a holding tube of sufficient capacity to raise the temperature to 90–95°C for 5–10 min (Fig. 3.2), the other – the low temperature holding (LTH) system – necessitates heating the milk in a process vessel to 80–85°C with a holding time of 30 min. The HTSH system is the treatment of choice for large-scale operations, but the LTH approach is just as effective for the step that is essential to give a yoghurt with the desired textural properties. Although higher temperatures could be employed for the processing of the milk base, for example, ultra high temperature (UHT), yoghurt made from UHT-treated milk had lower viscosity and gel strength, but showed less syneresis, than yoghurt made by heating the milk using the conventional processes described above; the texture of the product could be improved by increasing the level of fortification of the SNF (Krasaekoopt *et al*., 2003).

In particular, heating over an extended period of time alters the physico-chemical properties of the caseins and denatures the whey proteins – > 80% of the β-lactoglobulin is denatured by a typical LTH treatment. As a result, β-lactoglobulin becomes attached to the κ-casein, so improving the texture (set yoghurt) or viscosity (stirred yoghurt) of the final product (Fig. 3.6; Mottar *et al.*, 1989). In particular, whey protein denaturation leads to gelation at a higher pH than in non-heated milk, and hence a shorter gelation time, and to increased cross-linking within gels (Lucey & Singh, 1997). This increased number of bonds between protein particles increases the rigidity of the network and its apparent water-holding capacity, although this

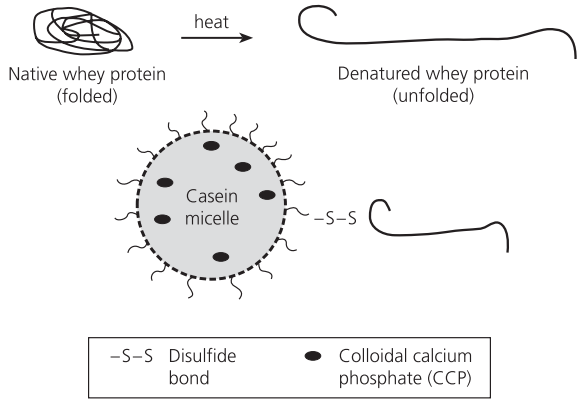

Fig. 3.6 Schematic diagram of the interaction of whey proteins with caseins due to milk heat treatment (not to scale as β-lactoglobulin is only 2 nm while the average size of casein micelles is ~ 150 nm).

depends on the incubation temperature used (Özer *et al.*, 1998; Bertrand-Harb *et al.*, 2003; Lee & Lucey, 2003; Croguennec *et al*., 2004).

Other essential actions of the heating stage are: (a) a partial breakdown of the whey proteins to amino acids that stimulate the activity of the starter culture; (b) an expulsion of oxygen from the milk that is beneficial for the growth of the micro-aerophilic starter bacteria; (c) a reduction in the indigenous microflora in the milk that might otherwise compete against the added lactic acid bacteria (see also de Brabandere & Baerdemaeker, 1999).

In some recent installations, homogenisation using aseptic plant follows the heating step on the grounds that later homogenisation may enhance further the whey protein/casein interactions (Walstra, 1998). However, the validity or otherwise of this hypothesis is open to debate, and most operators prefer to keep the heating stage as near to the fermentation step as possible in order to lower the risk of contamination by, for example, lactose-fermenting yeasts and moulds.

3.4 Fermentation

3.4.1 *Background*

After the heat treatment stage, the milk will be cooled to 42–43°C ready for the addition of the starter culture consisting of a 50 : 50 mixture of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. In general, the milk will be cooled in the plate heat exchanger and transferred at the desired temperature to an insulated fermentation vessel of 5000–10 000 L capacity, but smaller dairies may carry out the fermentation in the vessel previously employed to heat-treat the milk under the LHT regime. How the culture is added to the milk will depend on its physical form. For a liquid culture prepared in the dairy, the bulk culture will be held in tanks, and then pumped into the process milk at an addition rate of 2.0 mL 100 mL^{-1}; the addition rate for concentrated freeze-dried or frozen cultures purchased for direct

inoculation into the process vat is set by the culture supplier. However, the need to avoid contamination of the milk with undesirable bacteria, yeasts and moulds during inoculation is universal, and a number of systems have been developed to achieve this aim (Tamime, 2002; see also Chapter 2).

Once the milk has been inoculated, it will follow, as indicated in Table 3.1 (see also Figs 3.3 and 3.4), one of two routes: it will be filled into cartons for incubation as set yoghurt or it will be fermented in a bulk tank (stirred yoghurt). Once the cartons for set yoghurt have been filled, they will normally be heat-sealed with an aluminium foil lid and placed into holding trays containing up to 24 individual cartons (150 mL); for family cartons of 500 mL the tray size may be limited to six. The trays are then transferred to an incubation room at 42°C or placed on a conveyor belt that slowly runs through a tunnel operated at the same temperature and followed by blast cooling (Tamime & Robinson, 1999; Anon., 2003).

Stirred yoghurt is, by contrast, filled into cartons as the final retail product, and hence the base material is fermented in bulk. The types of tanks available for this stage have been described in detail by Tamime and Robinson (1999) and Anon. (2003); the essential features are temperature control during incubation, a facility for stirring the bulk yoghurt and a means of cooling the product once a preset pH has been reached.

Although 42°C is the typical fermentation temperature for yoghurt, using slightly lower incubation temperatures (e.g. 40°C rather than 45°C) will lead to slightly longer gelation times, but firmer and more viscous gels are formed that are less prone to whey syneresis (Fig. 3.7) or lumpy/grainy defects on stirring (Robinson, 1981; Lucey, 2002; Lee & Lucey, 2003). At a lower incubation temperature, there is an increase in the size of the casein particles due to a reduction in hydrophobic interactions, which, in turn, leads to an increased contact area between the casein

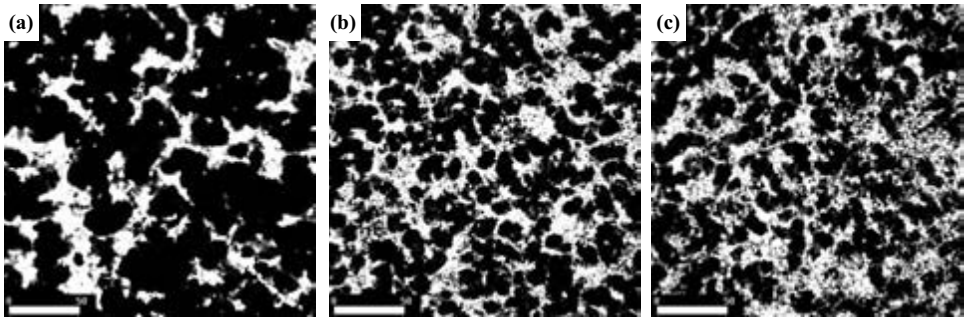

Fig. 3.7 Effect of incubation temperature on the microstructure of yoghurt gels prepared at (a) 34.3°C, (b) 40°C, (c) 45.7°C. Milk was heated at 82.5°C for 30 min. Reproduced with permission from Lee, W. & Lucey, J.A. (2003) *Journal of Texture Studies*, **34**, 515–536.

	Yoghurt sample		
Properties	a	b	c
Whey separation (s)	2.85	1.07	0.41
Permeability, porosity (10^{-13} m^2)	2.25	1.52	1.13
Firmness G′ (Pa)	136	204	246

particles (Lee & Lucey, 2003); a similar trend occurs when gels are cooled. A high incubation temperature also makes the gel network more prone to rearrangements, and these changes can lead to greater whey separation (Lucey, 2001; Mellema *et al.*, 2002). On the negative side, lower fermentation temperatures may result in a decreased production of flavour components by the starter cultures, but this effect may not be critical if the base will be flavoured at a later stage.

Overnight incubation at 30°C is used by some small-scale operations, but the reasons for employing this temperature are mainly practical rather than related to product quality. However, the use of lower incubation temperatures could allow gels to be made with lower SNF and/or stabiliser levels (Lee & Lucey, 2003).

3.4.2 *Microbiology of fermentation*

As the fermentation begins, the population of *S. thermophilus* develops rapidly and is responsible for the initial drop in pH (Robinson, 2000b), and small micro-colonies become visible in the gel (Fig. 3.8). However, over the next 2 hours, the synergistic influence of the streptococci encourages more rapid growth and metabolism in *Lb. delbrueckii* subsp. *bulgaricus* (Fig. 3.8) so that, after 4 hours, the populations of each starter organism may well exceed 2.0×10^7 cfu mL^{-1}.

The use of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* for the manufacture of yoghurt was, at least initially, historical in origin, in that they have frequently been isolated from natural yoghurt made by the indigenous peoples of the Middle East. However, there are good reasons for continuing with the tradition, for when growing in milk, the two organisms interact synergistically. This interaction depends on the fact that *S. thermophilus* grows more rapidly than *Lb. delbrueckii*

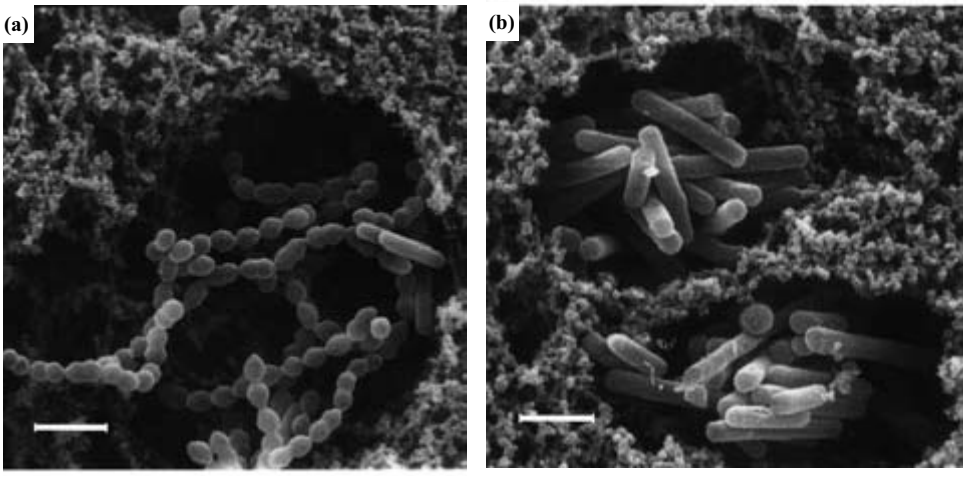

Fig. 3.8 Scanning electron micrographs of natural yoghurt showing 'void' spaces containing (a) a group of cocci (*S. thermophilus*), and (b) a colony of rods *(Lb. delbrueckii* subsp. *bulgaricus*). The matrix of coagulated milk proteins is also clearly visible. Bar = 2μm. Reproduced by permission of Drs M. Kalab and A.Y. Tamime (unpublished data).

subsp. *bulgaricus* in milk, and ferments lactose homofermentatively to give L(+) lactic acid as the principal product. In addition, carbon dioxide is liberated by the breakdown of urea in the milk by urease and, usually, formic acid (up to 40 μg mL^{-1}); all three metabolites stimulate the growth of *Lb. delbrueckii* subsp. *bulgaricus* (Robinson, 2000b).

Although some free amino acids occur naturally in the milk or are released during heat treatment, glutamic acid, histidine, cysteine, methionine, valine or leucine, for example, are not present at levels sufficient to support extensive growth of the culture. This situation is improved by the fact that *Lb. delbrueckii* subsp. *bulgaricus* can hydrolyse casein – especially β-casein – by means of a cell-wall-bound proteinase to release polypeptides and, by further enzymatic activity, free amino acids as well (Beshkova *et al*., 1998). In addition, *S. thermophilus* can readily hydrolyse peptides so that, again, the free amino acids that are essential for further development of both species become available. However, this anticipated pattern of activity is not universal and, in some mixed populations, the bulk of the free amino acids are produced by *Lb. delbrueckii* subsp. *bulgaricus*.

The practical result of the synergy is that both species grow rapidly and actively metabolise sufficient lactose to lactic acid to complete the fermentation of milk to yoghurt within 3–4 h – one species alone might take 12–16 h to produce the same level of acidity. The reality of this acid production is well illustrated in Fig. 3.8, for the micro-colonies of both species have generated sufficient lactic acid to cause the surrounding protein to contract and form a void space (Tamime *et al.*, 1984). In addition, metabolites liberated by the two species give yoghurt a flavour that is distinctly different from any other fermented milk. Acetaldehyde at levels up to 40 mg L^{-1} is the major component of the flavour profile, and the major pathway for its production by *Lb. delbrueckii* subsp. *bulgaricus* – and to a lesser extent, *S. thermophilus* – is the conversion of threonine to glycine by threonine aldolase (Zourari *et al.*, 1992; Marshall & Tamime, 1997; van Kranenburg *et al*., 2002).

Some strains of the two species can also produce appreciable levels of extracellular polysaccharide materials, such as glucans, or polymers involving glucose, galactose and rhamnose as the constituent sugars (Robinson, 1999; de Vuyst *et al.*, 2003; see also Chapter 2). The presence of these metabolites enhances considerably the viscosity and hence consumer appeal of the retail yoghurt, but a number of factors, such as the composition and structure of the polysaccharide, the amount produced and the acidity of the milk, all influence the properties of the final product (Laws & Marshall, 2001; Zoon, 2003). The exact mechanism(s) by which the polysaccharides influence the properties of the yoghurt varies with the strain of culture (Walstra, 1998). In some cases the material remains as a capsule around the bacterial cells; other types of polysaccharide form bridges between the cells and the surrounding protein (Robinson, 1988; Teggatz & Morris, 1990; Tamime & Robinson, 1999). In addition, some polysaccharides may disperse in the serum phase and impart viscosity.

It is not surprising, therefore, that *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are widely used for the manufacture of yoghurt throughout the world.

3.5 Coagulation of the milk

The result of this microbial activity is that the acidity of the milk will have risen to around 1.0–1.2 g 100 mL^{-1} lactic acid (around pH 4.2–4.3) after 3–4 h. At this acidity (which is probably the maximum level acceptable to consumers of stirred fruit yoghurt in the West) the milk proteins will have coagulated to form a firm gel (Lucey & Singh, 1997, 2003; see also O'Kennedy & Kelly, 2000; Laligant *et al*., 2003; Remeuf *et al*., 2003; Lucey *et al*., 2004; Panouillé *et al*., 2004; Ye *et al*., 2004).

Thus, the formation of lactic acid results in a reduction in surface charge (zeta potential) on a casein micelle from the originally high net negative charge at pH 6.7 to close to no net charge with the approach of the isoelectric point (pH 4.6) of casein (Fig. 3.9). This change in surface charge allows casein micelles to aggregate through hydrophobic and electrostatic bonds. Steric repulsion remains from the κ-casein macropeptide 'hairs', though these may 'curl up' somewhat as the pH drops. Aggregation of the casein particles results in a gel being formed by pH ~5.3 in the heat-treated milk, compared with ~5.0 in unheated milk (Fig. 3.9). This difference is because the main whey protein (β-lactoglobulin) has a high isoelectric point (pH ~5.3) and, as the majority of denatured whey proteins are associated with casein micelles during heating or during acidification of the milk, so aggregation and gelation is shifted to the higher pH value. It is these new casein–whey protein particles that initiate gelation, since they become unstable before unmodified casein micelles. It is relevant also that, at the temperatures used for the fermentation of yoghurt (i.e. ≥ 30°C), little dissociation of caseins from the micelles occurs during acidification of the milk (Lucey & Singh, 2003).

Insoluble or colloidal calcium phosphate (CCP) plays a key role in the stability of casein micelles, as it acts as a neutralising bridge between negatively charged phosphoseryl groups (Horne, 1999). Solubilisation of CCP occurs during acidifica-

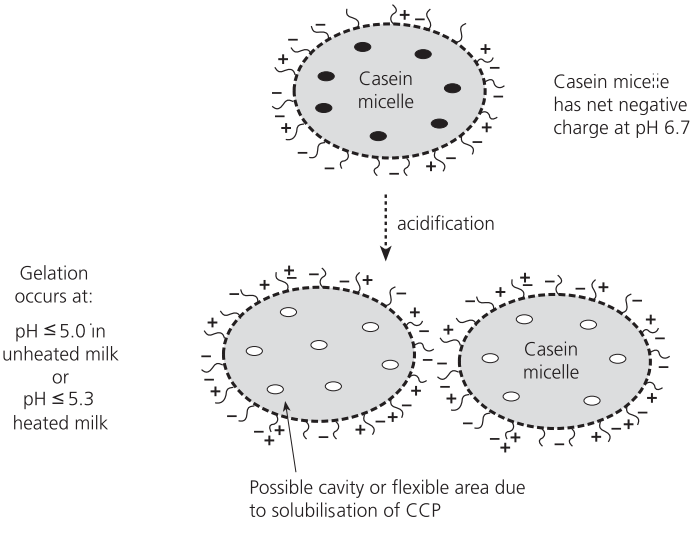

Fig. 3.9 Schematic diagram of some of the physical changes occurring to the casein micelles during acidification of milk.

tion, especially at pH ≤ 6, and this results in a concomitant increase in electrostatic repulsion between the exposed phosphoserine residues (Lucey, 2002). When milk is acidified slowly, nearly all the CCP is dissolved by pH 5.1 and, since unheated milk gels do not form until pH ≤ 5.0, the CCP-depleted casein particles that form the acid gel network are markedly different from the original casein micelles.

A very different situation occurs in acid gels made from heated milk, for the high gelation point (i.e. pH 5.3) means that some CCP continues to solubilise from within casein particles after the initial network has formed. This loss of CCP causes a loosening of the network and coincides with the appearance of whey on the gel surface. Conditions that encourage this loosening include low heat treatments of the milk, high incubation temperatures and very low starter inoculum levels. Below pH 5.0, gel firmness increases greatly, and is maximal at pH ~4.6 – the isoelectric pH of casein – and continues to increase with time. Furthermore, high heat treatment of the milk base leads to faster gelation and firmer gels, and this aspect is shown in Fig. 3.10 (Lee & Lucey, 2003).

The rate and extent of solubilisation of CCP during the fermentation process impacts on the texture of cultured products. For gels made at the same incubation temperature, the use of a low inoculum level (e.g. 0.5 mL 100 mL^{-1}) results in a weaker gel than the use of a high inoculum level (e.g. 4 mL 100 mL^{-1}) (see also Lee & Lucey, 2004). Probably, the slower acidification provides more time for dissolution of the CCP, especially during the early stages of gelation. Low incubation temperatures (e.g. 38°C) also result in longer fermentation times, but the firmness of the gels is higher and they are more stable than gels made at high incubation temperatures (e.g. 45°C). The improvements brought about by low temperature incubation are due to increased swelling of the protein molecules and stronger interactions between casein particles.

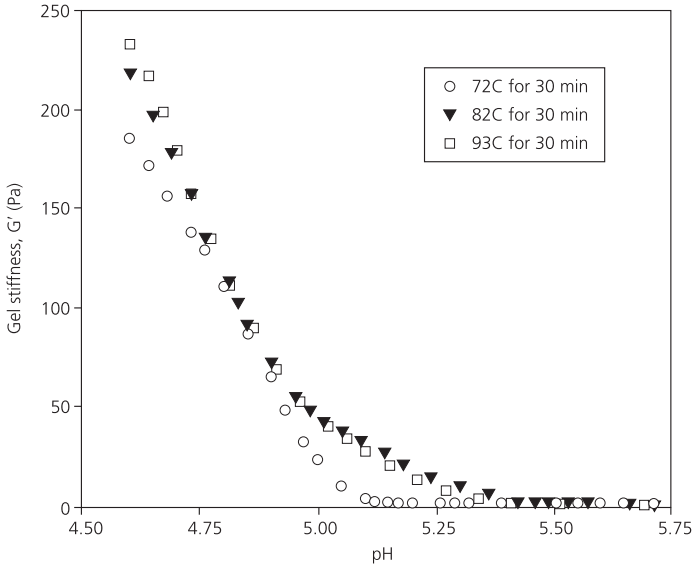

Fig. 3.10 Effect of different heating temperatures of milk for 30 min on the firmness of yoghurt gels. Reproduced with permission from Lee, W. & Lucey, J.A. (2003) *Journal of Texture Studies*, **34**, 515–536.

3.6 Final processing

3.6.1 *Cooling*

For set yoghurt fermented in retail cartons, cooling is usually achieved by blowing cold air through the incubation room or, if incubation is taking place in a tunnel system, running the conveyor belt into a section blasted with chilled air. Once cooled to 15–20°C, the trays of cartons can be stacked on pallets for storage at < 5°C prior to distribution along a chill chain. This cooling results in an increased firmness of the gel due to swelling of the casein particles as their hydrophobic interactions weaken, and an increase in contact area between particles. Contact between the particles through additional hydrogen bonds or disulphide cross-links between denatured whey protein and κ-casein (Özer *et al.*, 2002; Vasbinder *et al.*, 2003) leads to the improved texture, as may other types of bond (Lauber *et al.*, 2001).

In-tank cooling of the base for stirred yoghurt requires the circulation of chilled water (2°C) through the jacket/in-tank cooling system of the vessel, or pumping the warm yoghurt (42°C) through a plate or tubular cooler (Anon., 2003; Afonso *et al*., 2003). In the latter case, great care is necessary to avoid physical damage to the coagulum, and the system is probably best suited to products that are heavily stabilised. Thus, the rheological properties of yoghurt are dependent on shear rate and time, so that shearing at high temperature produces a smooth product, but one with low apparent viscosity. Once the yoghurt has been cooled to around 20°C, the metabolic activity of the culture almost ceases, and the base yoghurt can be mixed with fruit and/or flavouring.

3.6.2 *Fruit–yoghurt blending*

Commercially sterile fruits delivered to the yoghurt plant in drums or stainless steel tanks are the most widely used ingredients, and some typical features of these products are: (a) sucrose can be included at a level of 30.0–35.0 g 100 g^{-1}; (b) colouring and flavouring agents will be added to ensure consistency of colour and flavour; (c) stabilisers will be incorporated to protect the structure of the fruit, and these latter materials will also contribute to the viscosity of the retail yoghurt. The actual quality of the material in terms of fruit or sugar content can be varied to suit the type of yoghurt being produced, but this feature does not affect the method of blending with the base yoghurt.

The selected ratio of fruit to white base will depend on the quality of the processed fruit, the precise nature of the end-product and any local regulations, but the options for mixing the two components are either batch or continuous. The batch system involves simply metering the required volumes of fruit and yoghurt into a mixing tank, blending them and then pumping fruit yoghurt to the packaging machine. For small factories, this approach can be extremely convenient and, by having more than one mixing tank in operation, switching between flavours should not be too wasteful of time or product.

Larger plants that may need to handle up to 10 000 L of final product per hour need a process that is less labour intensive, and the static in-line mixer is often

the first choice. The actual mixing section consists of a hollow stainless steel tube (up to 6.0 cm in diameter and 1.0 m in length) with blades running throughout the length in the form of a spiral. At one end of the mixer, a T-junction allows fruit and yoghurt to be pumped into the mixing section in the desired ratio and, as the two streams pass between the blades, so a uniform yoghurt/fruit blend is produced. As there are no moving parts, the mixer is reliable and easy to clean.

Various dosing pumps are also available on the market (Tamime & Robinson, 1999), and each machine has features to suit a particular type of yoghurt.

3.6.3 *Packaging*

Basically a yoghurt manufacturer has two options for packaging his or her product: (a) preformed polypropylene cartons can be filled with yoghurt and then covered with an aluminium foil lid that is heat-sealed to the carton – snap-on lids are available for large cartons (500 mL); or (b) a filling machine can be purchased that takes a roll of film, thermoforms the cartons, fills them and then seals them with a foil lid.

The form–fill–seal option is, in reality, only suitable for large-scale operations, and the various types of machine differ mainly in the degree of asepsis being offered and the number of filling heads across the conveyor. Two advantages of this system are: (a) it is possible to form the cartons in 'nests' of four, each of which can be filled with a different flavour of yoghurt; this ease of purchase of different flavours appeals to some consumers; (b) the packaging material is thinner than that needed for preformed cartons and this saving of material, together with the production of cartons during filling, gives the yoghurt maker an economic benefit compared to producers using conventional cartons.

However, the attraction of employing preformed cartons is their versatility, in that not only can capacities range from 120 to 500 mL, but the available filling machines have maximum throughputs from 8000 cartons per hour up to 24 000. In other words, there is a machine for every scale of enterprise and, because the capital cost is usually lower than for a form–fill–seal machine, a yoghurt producer can afford to employ, for example, one low-capacity unit for family cartons of natural set yoghurt, and another for individual cartons of fruit/flavoured product. Details of a number of suitable machines are given in Tamime and Robinson (1999).

3.7 Post-production problems

Spoilage can occur through the activities of acid-tolerant yeasts, and species of *Saccharomyces* have been associated with gas formation and/or the 'doming' of cartons of fruit yoghurts (Fleet, 1990; Jordano *et al.*, 1991a). In this situation, the natural fruit sugars provide an abundance of substrates for fermentation but, as doming indicates a yeast count in the region of 10×10^4 cfu g^{-1} of yoghurt, it is likely that the source of the original contamination was either 'dirty' plant or unpasteurised fruit; in the absence of severe temperature abuse, e.g. storing the yoghurt at 15–20°C, casual air-borne yeasts rarely proliferate sufficiently to cause problems.

In natural yoghurt, lactose is the principal sugar available and, as few yeasts can ferment lactose, the major concern is species such as *Kluyveromyces marxianus* var. *lactis* or *K. marxianus* var. *marxianus*. Both of these lactose-utilising species grow readily on poorly cleaned surfaces in a dairy plant, and hence high standards of hygiene are essential if post-heat treatment contamination is not to occur; it is generally recommended that yoghurt for the retail market should have a yeast count of < 10 cfu g^{-1}. If the regulations permit, sorbic acid (usually added as potassium sorbate at a level up to 300 mg kg^{-1}) is effective in preventing the growth of yeasts in fruit yoghurts.

The same figure (< 10 cfu g^{-1}) applies also for moulds, for genera such as *Mucor, Rhizopus, Penicillium* or *Aspergillus* can grow readily at the yoghurt–air interface of an undisturbed carton (Jordano *et al.*, 1991b; Foschino *et al.*, 1993). Equally important is the fact that, unlike the situation with yeasts, just one spore of a fungus can spoil a carton of set yoghurt by growing over the surface of the product, and hence protection of the filling line from airborne contamination is essential.

Excessive acidity, as a result of continued starter activity during prolonged storage above 5°C, can also be a problem, because the acid-tolerant *Lb. delbrueckii* subsp. *bulgaricus* has the ability to generate lactic acid to levels of 1.7 g 100 mL^{-1} or even above, depending on the strain. Such a level is not only too harsh for the palates of most consumers (Sinha *et al.*, 1989) but, below pH 4.0, gel strength decreases due to excessive charge repulsion. As most yoghurts should have a pH value of 4.0–4.5, it is post-production acidification that, in general, tends to determine the shelf-life of commercial yoghurt. Some protection against the increase in lactic acid can be obtained by lowering the level of *Lb. delbrueckii* subsp. *bulgaricus* in the original inoculum but, as this practice can reduce the degree of synergism, such products tend to lack the characteristic flavour of yoghurt.

3.8 Conclusion

It is evident that a multitude of factors can influence the rheological properties of yoghurt including the mechanical handling of the coagulum. The latter aspect can reduce the viscosity of the product due to shear stress, but the phenomenon associated with improved firmness after 24 h of refrigeration is still not well established (Tamime & Robinson, 1999). Nevertheless, it is possible to suggest that future developments in yoghurt science and technology may include:

- Greater understanding of the physical gelation of milk and the behaviour of the coagulum after being subjected to shear stress;
- Developments in powders that are specifically designed to enhance certain physical characteristics of the yoghurt gel;
- Provision of a wider selection of starter cultures (see Chapter 2);
- Greater reliance on automation and mechanisation, especially in large centralised yoghurt factories, and improved on-line testing and monitoring of the product during manufacture (Tamime *et al*., 2001; see also Chapter 10);

- Novel or emerging techniques for altering the yoghurt texture may include enzymatic cross-linking of milk proteins (e.g. transglutaminase), the use of carbon dioxide treated milk and the use of high hydrostatic pressure (e.g. > 200 MPa) to cause denaturation of whey proteins or to prevent post-production acidification (IDF, 1998, 2003; de Ancos *et al*., 2000; van Hekken *et al*., 2000; Needs *et al*., 2000; Huppertz *et al*., 2002, 2004; Ahmed & Ramaswamy, 2003; Capellas & Needs, 2003; Gueimonde *et al*., 2003; Harte *et al*., 2003; Thiebaud *et al*., 2003).

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4 Properties of Yoghurt and their Appraisal

R.K. Robinson and P. Itsaranuwat

4.1 Background

The overall appeal of a yoghurt to a potential consumer may be defined in simple terms such as colour, flavour and mouth-feel but, for the manufacturer, these criteria are a reflection of the chemical, physical and microbiological characteristics of the product in question. Customers may buy a particular brand of yoghurt because they like it, but, in order to ensure consistency between batches, the factory manager has to be able to judge quality by a range of tests that are, as far as possible, objective. In addition, the product has to be safe for human consumption, conform to any regulations laid down by public health authorities and be capable of manufacture within existing constraints of price and available plant.

The definition and appraisal of product quality have, therefore, become a vital function of factory operation, and the properties of yoghurt that merit special examination can be considered under the headings shown below in Table 4.1. A routine examination of these features should enable the quality control manager to spot deviations from the norm well ahead of the stage when production schedules might be placed in jeopardy.

Table 4.1 Some properties expected by consumers of retail yoghurt, together with some of the factors that may influence them.

Consumer expectation	Factory analysis
Chemical composition	
Satisfying mouth-feel	Suitable fat and protein contents
Pleasant flavour (as appropriate)	Correct sugar, lactic acid and fruit contents; suitable starter culture
High nutritional value	Satisfactory level of protein and minerals
Physical properties	
Attractive mouth-feel and appearance	Acceptable consistency/viscosity and colour
Microbiology	
Safe product	Absence of pathogens
Sound appearance and no off-flavours	Absence of yeasts and moulds
Sensory characteristics	
Good appearance, colour and flavour	Good report from taste panel

4.2 Chemical composition

4.2.1 *Primary constituents*

Many countries have legal or non-legal standards covering the composition of yoghurt, and a selection of the existing proposals relating to solids-non-fat (SNF) are given in Table 4.2. The requirement of a value for SNF is not really essential, as the consistency or viscosity of a set or stirred yoghurt, respectively, with an SNF below the stipulated minimum would be unacceptable unless stabilisers – if permitted under local regulations – are employed to mask the weak coagulum. Nevertheless, calculating SNF values, e.g. from total solids minus fat, will give an indication of the likely protein (around 4.5–5.5 g 100 g^{-1}), lactose and mineral contents of the product and, as these components are a useful guide to the likely physical and nutritional properties of a yoghurt, the typical guideline values (g 100 g^{-1}) are of interest:

- Drinking yoghurt 11
- Standard fruit yoghurt 12–14
- Natural yoghurt (top quality) 15–17

The nutritional value of milk protein is self-evident, but it is important that the level in yoghurt is, due to the concentration of the milk solids, higher than in liquid milk, as is the concentration of minerals such as calcium and zinc. Furthermore, the low pH of yoghurt tends to make the latter more accessible for absorption through the intestinal wall. The metabolism of lactose as a source of energy in humans is also relevant but, as many yoghurts are sweetened with sucrose, the nutritional input from lactose is easily forgotten.

In addition, the protein and mineral components in yoghurt are essential for the formation of a satisfactory coagulum (see Chapter 3), while lactose at a level between 4.5 and 7.0 g 100 mL^{-1} – depending upon the degree of fortification of the milk solids – is essential for the successful growth of the starter culture.

A check on the level of total solids (SNF + fat) can be of value, of course, to monitor that concentration or fortification has been carried out correctly, and a modification of the standard gravimetric method for milk has been proposed as being suitable for yoghurt (Tamime & Robinson, 1999). However, now that infra-red detection

Table 4.2 Some of the standards proposed for the chemical composition (g 100 g^{-1}) of yoghurt in terms of minimum solids-not-fat.

Country/origin	Solids-not-fat
FAO/WHO	8.2
Kenya	8.5
Lebanon	8.2
New Zealand	8.5
South Africa	8.3–8.6
United Kingdom	8.5
USA	8.25–8.30

Adapted from Robinson *et al.* (2002).

systems can measure the individual components of a sample of yoghurt, i.e. protein, lactose and fat, the measurement of total solids has become less important (Anon., 2003). The readings are also more relevant for the manufacturer, as it is the actual level of protein that is going to govern the physical properties of the end-product.

In addition, retail products are usually designated as very low-, low-, medium- or full-fat, and the actual levels of fat are stipulated in some legal standards, e.g. a very low-fat yoghurt should have a fat content below 0.5 g 100 g^{-1}. Such designations are provided for the benefit of diet-conscious consumers, but it is important also that the level of fat does change the perceived mouth-feel of the product quite dramatically. Even quite small increases at the lower end of the range, e.g. from 0.8 g to 1.2 g 100 g^{-1}, give an improvement in sensory quality that is readily detectable on the palate. Attempts to mimic this quality using proteins (Barrantes *et al.*, 1994) or starches (Dubert & Robinson, 2002) have proved somewhat disappointing to date, because the presence of fat provides a 'luxury' mouth-feel that is quite unique (Monneuse *et al.*, 1991; Folkenberg & Martens, 2003).

The gravimetric methods of determining fat in yoghurt (e.g. the Rose Gottlieb method) are regarded as the most accurate but, for routine purposes, the normal Gerber method using 11.3 g of yoghurt in a milk butyrometer is totally acceptable. However, if a large number of samples is to be handled, an infra-red detection system using a homogenised sample of yoghurt (50 g) neutralised with sodium hydroxide (15 mL, 0.5 M NaOH at 40ºC) is much quicker (Anon., 2003; Ellen & Tudos, 2003). All these examinations should be performed on the natural yoghurt base, prior to the addition of fruit.

The pH of the final product is monitored principally in relation to consumer preference, and hence the selected end-point will vary with the type of yoghurt. In standard natural yoghurt, for example, a final pH of 4.2 might be acceptable to most tastes but, as the total solid and fat contents increase, so a pH of 3.7–3.8 does not seem over-acidic, e.g. Greek-style yoghurts or labneh. However, pH is important also with respect to public safety, for while pathogens such as *Listeria monocytogenes* die out rapidly in yoghurt at pH < 4.2 (Gohil *et al.*, 1995), mild yoghurts with pH values > 4.5 can allow the survival of salmonellae for up to 10 days (Al-Haddad & Robinson, 2003) or *Escherichia coli* 0157 for up to 7 days (Massa *et al.*, 1997).

The measurement of acidity is, therefore, an important feature of production and, although the relationship between titratable acidity and pH is not straightforward in yoghurt (Lück *et al.*, 1973), the direct electrometric determination of pH is extremely convenient. Thus, once a correlation has been established between pH and the desired characteristics of a particular type of yoghurt, then routine monitoring during manufacture can become normal practice.

4.2.2 *Secondary constituents*

The monitoring of other components, particularly non-dairy ingredients, is probably not important as a routine, but the introduction of national regulations covering levels of stabiliser, for example, or consumer resistance to the use of preservatives could necessitate additional analyses. Examples of some current regulations are given

Table 4.3 Some proposed or existing regulations concerning the introduction of non-dairy ingredients into yoghurt.

Country	Stabiliser (g 100 mL^{-1})	Fruit (g 100 g^{-1})	Preservatives (mg kg^{-1})
Denmark	Nil	10–15	Nil
France	—	Up to 30	—
United Kingdom	Starch, pectin and/or gelatin (all up to 1.0) Alginate, agar, edible gums and/or celluloses (all up to 0.5)	Up to 30	Sulphur dioxide (60) Benzoic acid (120) Sorbic acid (300)

Note: Colours and flavours – as applicable regulations.
Compiled from Tamime & Robinson (1999).

in Table 4.3. The frequency of such analyses is a company decision and, although standard texts, such as AOAC (1990), can provide appropriate methods, it is probably better if such analyses are subcontracted to an accredited laboratory.

Although yoghurt is primarily regarded as a useful source of protein and minerals, vitamins from the original milk or secreted by the starter culture bacteria are usually present in detectable quantities. For example, a small (100 g) carton of full-fat yoghurt may contain up to 140 international units (IU) of vitamin A (about 50% less in low-fat yoghurt), along with – depending on the starter culture – appreciable amounts of riboflavin, thiamine, folic acid and pantothenic acid (Tamime & Robinson, 1999; Lin & Young, 2000). Unless fortification is being considered (Ilic & Ashoor, 1988), few manufacturers will be concerned with monitoring their products for such components, but it is important that the presence of these minor components should be recognised.

4.3 Assessment of physical characteristics

4.3.1 *Physical nature of yoghurt*

Yoghurt is normally retailed in one of three physical states, namely set (gelled), stirred (medium to high viscosity) or fluid (drinking yoghurt) and, in practice, each manufacturer will adopt an agreed 'in-house' standard for viscosity (or consistency in the case of set yoghurt). The standard selected will, in large measure, be a reflection of the anticipated market, for a low-cost product will have very different characteristics from a top-of-the-range yoghurt. In the latter range, for example, a satisfactory mouth-feel can be attained through the incorporation of high levels of protein, whereas a low-budget product may rely on starch to provide viscosity. Nevertheless, the important physical features of the product have to be monitored as a normal part of quality control.

As was discussed in Chapter 3, the secretion of lactic acid by the starter bacteria brings about the gelation of the milk proteins, and the result is a network of protein strands linked by hydrogen bonds and disulphide links (Özer *et al.*, 2002; Vasbinder *et al.*, 2003; Bertrand-Harb *et al*., 2003). The fragile nature of these bonds means that mechanical disturbance, e.g. stirring, or the addition of liquid, e.g. a fruit purée,

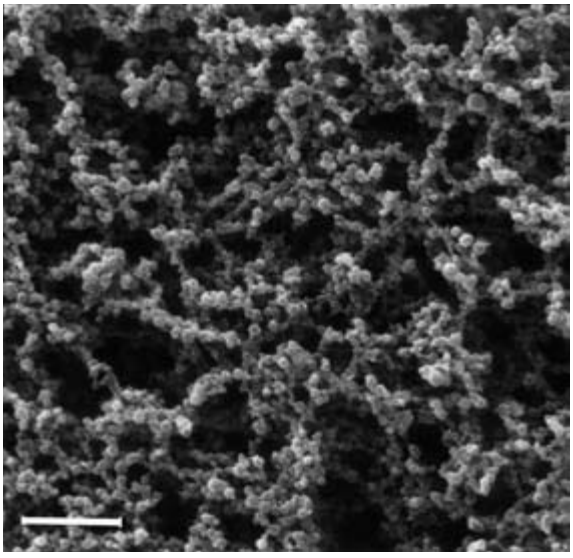

Fig. 4.1 Scanning electron micrograph of a typical yoghurt gel. Each strand consists of milk protein held together by hydrogen and other bonds, which are easily disrupted by mechanical agitation. Bar = 2 μm. Adapted from M. Kalab and A.Y. Tamime (personal communication; unpublished data).

leads to a loss of inter- or intra-strand bonding. Similarly, as the protein content of a yoghurt is reduced, so the number of strands available to interact is reduced. In both situations, the reduction in the degree of bonding leads to a loss of perceived viscosity or consistency, and it is for this reason that protein content and/or the mechanical stresses applied to the coagulum have such a major impact on product quality. This overriding importance of protein is evident even in the presence of exopolysaccharides of bacterial origin (Laws & Marshall, 2001; Hassan *et al.*, 2002, 2003a, b; Remeuf *et al*., 2003).

Obviously no manufacturers are likely to become involved with an electron microscopic examination of their products but, nonetheless, they should never lose sight of the fact that the yoghurt gel is a delicate structure (Fig. 4.1), and that it deserves to be handled accordingly.

4.3.2 *Physical characteristics of set yoghurt*

The surface of a set yoghurt should be smooth, shiny and free of visible whey, and exposing the inner surface with a spoon should reveal an equally homogeneous appearance. The nature of the acid gel is discussed in Chapter 3 and, given the fragile nature of the coagulum, it is curious that Shaker *et al*. (2001, 2002) claimed that increasing the incubation temperature resulted in improved rheological properties of set yoghurt, with maximum consistency observed at 48°C and minimum at 40°C. If this result is reproducible, then it could have profound implications for the dairy industry with respect to the production of set yoghurt.

If a chemical analysis has confirmed that the level of total solids is correct, then a casual examination of the physical properties may well suffice. However, if a more formal appraisal is required, then the simplicity of the penetrometer has much to recommend it; the more so, perhaps, as the measurements can be carried out using retail cartons of product. The weight of the spindle can be chosen in relation to the total solids of the product, e.g. a light spindle for examination of low cost–low solids yoghurt and, once standardised, the technique can discriminate, at a given temperature, between samples of different gel strengths (Jaros *et al*., 2002; Jaros & Rohm, 2003).

More precise rheological data can, if needed, be acquired through the use of a texture profile analyser (La Torre *et al.*, 2003) or with a dynamic rheometer (Özer *et al.*, 1998; Cayot *et al.*, 2003), but these approaches are not essential for routine work.

Rheological properties

Rheology is the study of the relation between forces exerted on a material and the ensuing deformation or flow of the material as a function of time (van Vliet, 1999). The magnitude of the force that has to be applied to a material in order to deform it (or make it flow) depends on the area over which the force is applied. The force divided by the area over which the force is applied is known as the *stress*. The unit of stress is the pascal ($Pa = N\ m^{-2}$). If the sample deforms as a result of a tangential stress, the deformation is known as the *shear strain*, or more simply as the *shear* (Sherman 1976; Prentice, 1992).

When a stress is applied to a mechanically non-homogeneous material such as yoghurt, the ensuing relative deformation may vary across the product. Thus, the change in distance between two points in the material relative to the original distance should be taken as a quantitative measure of the local deformation. This ratio is called the *strain* and, as strain is the ratio between two quantities with units of length, it is dimensionless.

Foods are usually neither liquids nor solids, but are viscoelastic materials. Viscoelastic materials reveal a combination of viscous (liquid) and elastic (solid) behaviour, and yoghurt is a typical example of a weak viscoelastic gel (Rohm & Kovac, 1994). Dynamic rheological measurements of yoghurt are of interest as they are directly related to its viscous and solid properties. A dynamic study involves subjecting the material to a small oscillating stress, and measuring the resulting stress in the material. At the steady state, the stress oscillates at the same frequency as the strain, but is out of phase, and hence stress can be expressed in terms of components in phase and out of phase with the strain. This enables two shear moduli to be defined, namely G' and G''. G' is the storage modulus and corresponds to the elastic character of the material, whereas G'' is the loss modulus, which is correlated to the viscous contribution of the material.

The elastic modulus (in-phase component) is associated with the storage and release of energy during a cyclic deformation, while the loss modulus (out-of-phase component) is associated with the energy lost as heat (van Vliet, 1999). The ratio

between the storage and loss moduli is called the loss tangent (G″/G′ = tan delta). This value represents information about the overall structure of the material under test. When the material behaves more like a solid (the deformation being essentially elastic and recoverable), the storage modulus (G′) exceeds the loss modulus (G″), and consequently tan delta is < 1.0. On the other hand, when the material is more like a liquid, then the viscous character dominates and tan delta is > 1.0.

Small amplitude shear stress oscillatory testing is an appropriate method for studying the time dependency of structure formation in food systems such as fermented dairy products (Clarke & Ross-Murphy, 1987; Özer *et al.*, 1998), and the viscoelastic properties of yoghurt can be evaluated using a controlled stress oscillatory rheometer (Fig. 4.2a).

Rheological measurements

In practice, samples of yoghurt are held at 20°C in a thermostatically controlled waterbath to standardise the temperature, and sub-samples of gel are removed and placed beneath a parallel plate probe (e.g. 10 mm radius and 1 mm gap setting) to determine the viscoelastic properties of the product using the storage modulus (G′) and loss modulus (G″) measured as functions of amplitude. Useful frequency and amplitude ranges are 0.25 Hz and 2×10^{-3} to 2×10^{-2} mNm, respectively (Özer *et al.*, 1998) and, if required, the loss tangent (tan delta) values can be calculated as well.

In a correctly made product, the data should indicate that the yoghurts are typical viscoelastic gels in which the elastic modulus predominates over the viscous modulus. The results may also show, for example, that yoghurts fermented at 42°C have substantially higher values for the storage and loss moduli than those incubated at 37°C, or the impact of fermentation with different starter cultures (see also Moreira *et al*., 2000; Haque *et al*., 2001).

4.3.3 *Stirred and drinking yoghurts*

The texture of yoghurt is an important variable in its quality, and stirred yoghurt, which can be regarded as a concentrated dispersion of gel 'pieces' in serum, should be homogeneous and fairly viscous. This viscosity, and the structure of the gel, is influenced by several factors, including incubation temperature, casein concentration, heat treatment of the milk, acidity and type of starter culture (Walstra, 1998); the temperature at which the measurements are made can also influence the recorded viscosity. To support this conclusion, Skokanova *et al.* (1998) compared the effect of the different culture conditions, such as incubation temperature, final pH and strains of lactic acid bacteria, on the final texture of stirred yoghurts, while the influence of the addition of proteolytic strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* to a commercial probiotic starter culture was investigated by Shihata and Shah (2002).

It has been claimed by some researchers that an improved product should be obtained when the incubation temperature is lower, since slow gelation provides the yoghurt with a finer structure and, in addition, the gel is more stable and less

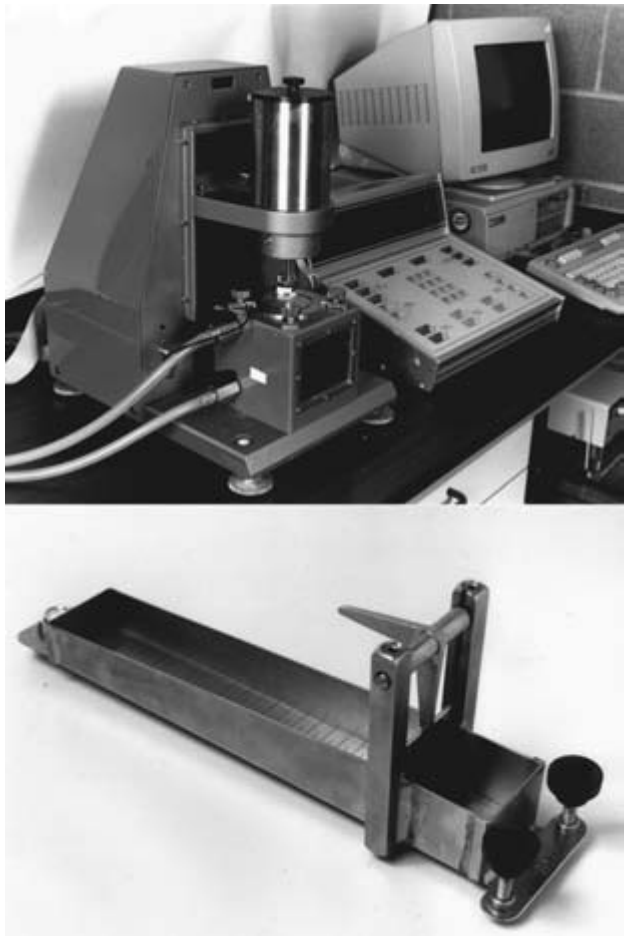

Fig. 4.2 (a) The dynamic rheometer (Rheo Tech International, Surrey, UK) is capable of discriminating between gels of different strengths, while (b) the Bostwick Consistometer (Christison Particle Technologies, Gateshead, UK) is a typical simple device that can be employed to obtain an objective comparison of the viscosities of samples of stirred fruit yoghurt.

prone to syneresis (Bianchi-Salvadori, 1998; Walstra, 1998; Beal *et al.*, 1999). Nevertheless, Sherman (1976) mentioned that lowering the incubation temperature from 43° to 32°C reduced the viscosity of yoghurt, as measured at 5°C, and the product was rated as 'thin'.

Some of the methods that are available to measure the viscosity of fluid and semi-fluid products have been discussed by Tamime and Robinson (1999), and the choice of method is really a matter of operator preference. Thus, in the present context, interest centres on making an objective comparison between samples, or between a sample and an 'expected result' representing product of acceptable quality, and a number of techniques can be employed for this purpose.

Ease of operation makes the rotational viscometer a popular choice for obtaining an objective measure of viscosity and, once the type of spindle and its speed of rotation have been established for a given product, then comparison between successive batches of white base should present few problems. For example, in a routine examination of some retail samples, the apparent viscosity of the yoghurt was determined with a Brookfield Viscometer (Model LVF, Brookfield Engineering Labs, Soughton, MA, USA) using a helipath adapter and a T-bar spindle (T-E size) at 6 revolutions per minute. Before taking a reading, the yoghurt sample was blended in a Kenwood kitchen mixer and cold-stored overnight (4°C). Next morning, 100 mL of sample was placed into a 250 mL beaker and stirred manually for 30 seconds before analysis. The readings obtained with the Brookfield were converted into centipoise units (cps) using a conversion factor provided by the manufacturer.

The recorded figures ranged from 31 400 cps for a 'good quality' product to 28 100 cps for a 'regular' brand, and these figures are probably fairly typical. It was of note, however, that the system did not discriminate between the brands as easily as the taste panel (data not shown), because the mouth-feel of the regular brand was rated as vastly inferior to that of the quality product. Obviously the sensation of mouth-feel is more complex than just texture or viscosity, but the result does tend to confirm that, while viscosity measurements from batch to batch within a factory can be useful to spot deviations from a norm, too wide an application of the technique could be misleading. It may be for this reason that some factories employ a simple torsion wire apparatus to monitor the viscosity of each batch.

Assessment of the viscosity of a retail product with fruit pieces poses problems in that the material under test is not homogeneous, and hence a rather more arbitrary system may have to be employed (Tamime & Robinson, 1999). The Bostwick Consistometer shown in Fig. 4.2(b) is one such system. In this case, the chamber at one end of the channel is filled with yoghurt and, once the door is raised, the time taken for the yoghurt to reach a certain point can be measured. The more viscous the product, the slower the flow rate, and even simple procedures that allow for repeatable measurements make it possible to stipulate that a particular variety of stirred (fruit) yoghurt should have a viscosity that falls within preset limits. If these limits have been set by sensory analysis, a routine check on the physical properties of the product should ensure that the retail yoghurt meets with consumer expectations for the brand in question; the physical nature of a drinking yoghurt could be similarly ascertained and routinely verified.

4.4 Colour

The surface of a natural set yoghurt made with homogenised milk should present a clean white appearance with a pleasant sheen, while flavoured (set or stirred) or stirred yoghurts containing fruit will have a matt surface of a colour that reflects the flavour/fruit. Precise standardisation employing an instrument like the Hunter Colourquest Spectrophotometer (Hunter Associate Laboratory, Reston, VA, USA) can be entertained for development purposes, but visual comparisons against a colour

standard offer a far more practical alternative. The specific standard can be objective, e.g. a reference colour with a British Standard specification, for example, or one selected in-house, but it is important that successive batches of product will be acceptable to consumers, i.e. in line with expectations based on previous purchases. The preferred depth of colour is usually judged in relation to the market and, while muted colours attract some groups of consumers, others expect the yoghurt to have a colour close to the original fruit, e.g. bright pink for strawberry.

Whether added colours should be natural, nature-identical or synthetic will depend on the image desired for the product but, while the origin of the colouring material may alter its dosage rate, monitoring for batch-to-batch variation will still be essential (Tamime & Robinson, 1999; Jaros & Rhom, 2001).

4.5 Microbiological analysis

The flavour of natural yoghurt depends on the production of aromatic compounds during fermentation, and the yoghurt bacteria, in particular *Lb. delbrueckii* subsp. *bulgaricus*, are responsible for the production of these volatile compounds, mainly acetaldehyde (Bianchi-Salvadori, 1998; Robinson, 2000; Robinson *et al*., 2002). Consequently, a microbiological examination of the finished product may include checks on the survival of the starter organisms, as well as for the presence of undesirable spoilage or pathogenic species.

Interest in the former examination stems from the following: (a) excessively low or high counts of the two species can lead to flavour defects in natural yoghurt; (b) high counts of *Lb. delbrueckii* subsp. *bulgaricus* can result in continued acid production during storage, leading to a product with an excessively acidic taste; (c) in countries such as France and Portugal, *Lb. delbrueckii* subsp. *bulgaricus* must be 'abundant' in any retail product sold under the name 'yoghurt' (Tamime & Robinson, 1999). However, whatever the precise concern, it is widely accepted that yoghurt must contain live bacteria of starter origin unless specifically designated as 'pasteurised' or 'heat-treated'.

The methods available for examining the starter flora of yoghurt have been discussed elsewhere (see Tamime & Robinson, 1999; IDF, 2003; Tharmaraj & Shah, 2003), and it might be reasonable to anticipate that both *Streptococcus thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* will be present in a retail product at counts of 1.0×10^6 to 1.0×10^8 cfu g^{-1} when monitored with the pour plate technique and selective media, such as M17 Agar for *S. thermophilus* and RCPB Agar for *Lb. delbrueckii* subsp. *bulgaricus* (Ghoddusi & Robinson, 1996). However, it is worth emphasising that many selective media are only truly restrictive under specific conditions and, in the presence of a varied microflora, may allow the growth of species other than those expected (Witthuhn *et al.*, 2004).

Given that yoghurt may be sold up to 21 days after manufacture and that the microflora should still be 'abundant and viable', survival of the starter bacteria in the retail product is important. A typical pattern of survival is shown in Table 4.4, and the initial (day 0) viable counts of *S. thermophilus* were significantly higher

Table 4.4 Viable counts (cfu mL^{-1}) of yoghurt starter cultures in natural yoghurt stored in a refrigerator at 4°C.

Time (h)	pH	*Streptococcus thermophilus*	*Lactobacillus delbrueckii* subsp. *bulgaricus*
0	4.40	1.3×10^8	3.9×10^7
7	4.40	3.0×10^8	3.4×10^8
14	4.43	2.5×10^8	3.6×10^8
21	4.40	1.6×10^8	3.3×10^8

The results are means of duplicate determinations.
Adapted from Robinson & Itsaranuwat (unpublished data).

than those of *Lb. delbrueckii* subsp. *bulgaricus* – 1.30×10^8 and 3.90×10^7 cfu mL^{-1}, respectively.

After 7 days of storage, an increase in numbers of *Lb. delbrueckii* subsp. *bulgaricus* was observed due to continued growth activity during this period and, at this point, the ratio between the species was approximately 1 : 1. This growth of *Lb. delbrueckii* subsp. *bulgaricus* indicates its adaptability to the environment, such as the micro-aerophilic and acidic conditions, created by *S. thermophilus* during the fermentation.

The final counts of *S. thermophilus* were slightly lower because this bacterium is not normally tolerant of an acidic medium. The better tolerance of *Lb. delbrueckii* subsp. *bulgaricus* to low pH is supported by Beal *et al.* (1989, 1999) who studied the influence of controlled pH and temperature on the growth and acidification of pure cultures of these two yoghurt bacteria. Overall, the figures in Table 4.4 are probably fairly typical, and it was reported that the viable populations of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* exceeded 10^7 cfu g^{-1} in 54% and 68%, respectively, of samples of Australian yoghurts (Rybka & Fleet, 1997).

It should be noted, however, that the survival of yoghurt bacteria in fruit/flavoured products is often lower than in natural yoghurt, and pH lower than those recorded in Table 4.4 would also lead to a decline in viability. Storage temperature can be important as well, and Wang *et al*. (2002) revealed that, when sucrose was added to soy milk drinks, the numbers of *S. thermophilus* declined substantially during storage of the product at 25°C; only a slight change was recorded at 5°C.

As indicated earlier, yoghurt with a pH below 4.3 should be a safe product from a public health point of view, and reported lapses tend to be very rare (Keceli & Robinson, 1997). Nevertheless, many retailers like the added security of occasional checks for specific pathogens like *Salmonella* spp. and *L. monocytogenes*, but such analyses are best contracted out to a reputable laboratory.

Spoilage organisms such as moulds or yeasts are a more familiar problem and, while moulds like *Alternaria* spp. or *Aspergillus* spp. can ruin the appearance of set yoghurt, conditions within a stirred yoghurt are rarely aerobic enough to allow detectable development. However, lactose-fermenting and non-lactose-fermenting species of *Saccharomyces* can grow in both set and stirred yoghurts, and the impact on flavour can be devastating. Strict standards of environmental and plant hygiene should help to minimise the risk of infections, but it is worth remembering the

obvious – prevention is better than cure – because eradicating a lactose-fermenting yeast from a factory can prove expensive.

4.6 Sensory properties and analysis

The sensory properties that may be anticipated by a consumer of yoghurt were indicated earlier, and clearly the expectations relating to natural set yoghurt will be different from those associated with a stirred fruit product. The taste of a natural yoghurt, for example, will be dominated by acidity (Ott *et al.*, 2000a), while the intensity of aroma and flavour will reflect the presence of components such as acetaldehyde (Ott *et al.*, 2000b). In a fruited product, recognition of sweetness and flavour notes from the fruit may be the desired reactions, while most tasters will also record a satisfying mouth-feel.

To ensure that a given recipe is meeting these criteria, most companies will organise regular taste panels to monitor the quality of a specific product type, and an array of such schemes has been discussed by Tamime and Robinson (1999). However, all schemes involve the assessment of a sample of yoghurt for selected sensory criteria, and an example of one such approach is set down below. This approach assumes that a panel of around ten tasters (with alternates) can be assembled from volunteers interested in the work, and that each taster has been screened to ensure that they can discriminate between good, fair and poor examples of the selected attributes.

4.6.1 *Sensory analysis of yoghurt*

For stirred products, bulk samples of natural or flavoured yoghurts should be mixed thoroughly and stored overnight at 4°C before serving. Next morning, sub-samples (about 50 g) should be served in clear glasses labelled with random three-digit codes to avoid identification. A score sheet of selected attributes (Fig. 4.3) is presented to each taster, and each participant uses a nine-point scale (9 for 'like extremely' down to 1 for 'dislike extremely'; Lawless & Heymann, 1999) to score each attribute. A space is provided on the form for additional comments.

In all sessions, mineral water and unsalted crackers for cleaning the palate between samples should be available, along with plastic spoons, white soft tissue and a spittoon. In addition, testing should be conducted using partitioned booths to avoid interaction between tasters, the panel should be convened at a set time each week and the number of samples under test should be limited to avoid fatigue.

By totalling the scores for each sample and comparing the result with the expected score for the yoghurt in question, the production team can assess how the batch under test compares with product from last week, last month or even last year.

Another typical scheme was proposed by Bergel in Germany in which a relative importance was assigned to certain attributes by incorporating a multiplying factor; again, tasters were provided with a clear guide as to what each number on the scale of 0–5 should mean (Table 4.5).

Schemes of this type work well as a simple method of comparing batches of yoghurt, and both are open to modification to suit a given product. For example, the appearance

Name: .....**Date:**.....
Choose the descriptor which, in your opinion, is most applicable to the characteristic being evaluated. Each sample should be judged individually, and not in comparison with others.

9 Like extremely
8 Like very much
7 Like moderately
6 Like slightly
5 Neither like nor dislike
4 Dislike slightly
3 Dislike moderately
2 Dislike very much
1 Dislike extremely

Properties	**Score**
Appearance	
Colour	
Aroma	
Flavour	
Mouth-feel	
Overall acceptability	

Comments:.....

Thank you very much

Fig. 4.3 A test score sheet of the type that could be employed to evaluate samples of yoghurt.

Table 4.5 The five-point scheme.

Characteristic	Maximum points	Multiplying factor	Total points	% of total
Flavour	5	5	25	50
Appearance	5	2	10	20
Consistency	5	2	10	20
Aroma	5	1	5	10
			50	100
Points	*Description of property*			
5	Very good, ideal			
4	Good			
3	Satisfactory, few mistakes			
2	Not very satisfactory, distinct mistakes			
1	Not satisfactory			
0	Bad, tainted			

Adapted from Tamime & Robinson (1999).

of natural set yoghurt is extremely important, and a manufacturer using the five-point scheme might wish to alter the multiplication factor for this attribute. Another potentially useful change involves specifying the nature of the 'comments' that are being sought and, in particular, asking tasters to identify particular faults. This approach was suggested by Nelson and Trout (1964), and they recommended that, after completing the sensory evaluation, panellists should be asked to look for specific defects, such as:

- Flavour defects: bitter, cheesy, coarse (sour), flat (insipid), undeveloped, metallic, yeasty, miscellaneous (unclean);
- Textural defects: curdy (granular), gassy, lumpy, ropy, thin body (low viscosity), wheying off (syneresis);
- Acidity defects: high, low.

The attraction of this additional feature is that the production team may be able to link a 'comment' to a possible cause and, hopefully, a possible remedy, and some examples of faults and potential solutions are given in Table 4.6.

Table 4.6 Some common defects of yoghurt that might be noted by a taste panel, and an indication of some possible causes and remedies.

Defect	Possible causes	Possible remedies
Syneresis	Low SNF content	Adjust formulation
	High incubation temperature	Reduce temperature to 42°C
	Low acidity (stirred yoghurt)	Ensure pH < 4.4
	Curd shrinkage (set yoghurt)	Check storage temperature
	Poor mechanical handling of the gel	Check stirring/pumping/filling temperature
Low viscosity	Low SNF content	Adjust formulation
	Excessive agitation	Improve mechanical handling of the gel Add permitted stabiliser(s) Change culture to 'viscous' type
Gas bubbles	Excessive agitation	Improve mechanical handling of the gel
	Contamination with yeasts	Eliminate source of infection
	Coliforms present	Improve plant hygiene
Granulation	Undissolved milk powder	Adjust processing conditions
	Agitation prior to cooling	Improve cooling and/or install sieve in pipeline
	High incubation temperature	Reduce temperature to 42°C
	Seasonal variation in the milk	Change starter cultures
Poor flavour	Insipid	Change starter cultures Extend incubation time
	Unclean	Check for coliforms
	Bitter	Change starter cultures
	Sour	Lower the inoculation rate Check the storage temperature
	Malty/yeasty	Suspect contamination and investigate source

Adapted from Robinson *et al*. (2002) and Anon. (2002).

4.6.2 *Attribute profiling of yoghurt*

A simple scheme, such as the one shown in Fig. 4.3, provides an easy method of confirming that a batch of yoghurt has the properties expected by a taste panel familiar with the product in question but, for development work, a rather more refined approach may be needed. The application of attribute profiling based on the quantitative descriptive analysis procedure of Stone *et al.* (1974) is one option, and Robinson (1988) used the technique to monitor the influence of three different starter cultures on the quality of natural stirred yoghurt.

In this study, three types of stirred yoghurt were produced under standard conditions and, after storage overnight at 4°C, individual cartons were given to a panel of assessors, all of whom ate yoghurt on a regular basis. During a discussion of the three products, a vocabulary of attributes emerged as being important, namely:

- Appearance – showing syneresis after stirring; smooth; irregular;
- Aroma – cheese-like;
- Taste – acidic; intense or mild;
- Mouth-feel – smooth; viscous; slimy;
- Aftertaste – sour.

This list of attributes was then entered onto a profiling sheet and, next to each characteristic, a 10-cm line was drawn with fixed end-points, but no intermediate markings. Each assessor was presented, on an individual basis, with a test sheet and one of the yoghurts selected at random, and asked to record a score for each attribute by marking a vertical line on the scale; the left-hand fixed point was taken as zero. The process was repeated for the remaining two yoghurts. The magnitude of each response was noted by measuring the distance between the vertical line and zero, and the average response for each attribute calculated for each yoghurt in turn. The entire trial was repeated one week later, and the results indicated that the selected attributes were appropriate for discriminating between the different samples.

The results were recorded on the spider diagram shown in Fig. 4.4, and it was of note that the attributes highlighted by the taste panel corresponded to those that might have been anticipated on the basis of previous culture behaviour. For example, culture A was known to be a culture that produced little, if any, exopolysaccharide material – hence the irregular nature of the stirred coagulum, but it was metabolically active in terms of acid and flavour production. By contrast, culture B was metabolically less active with respect to acidification, but it produced a quite viscous polysaccharide. The distinct nature of the polysaccharide can be seen by comparing the attributes of viscous and slimy for culture B and culture C, for the properties of the polysaccharide derived from culture C are close to those associated with xanthan gum (Robinson, 1999).

It is clear, therefore, that attribute profiling offers a comparatively objective method of comparing similar products, in this case natural yoghurts that differed only in respect of their starter cultures, and the approach was refined further by Muir and Hunter (1992) and La Torre *et al.* (2003) through the introduction of principal

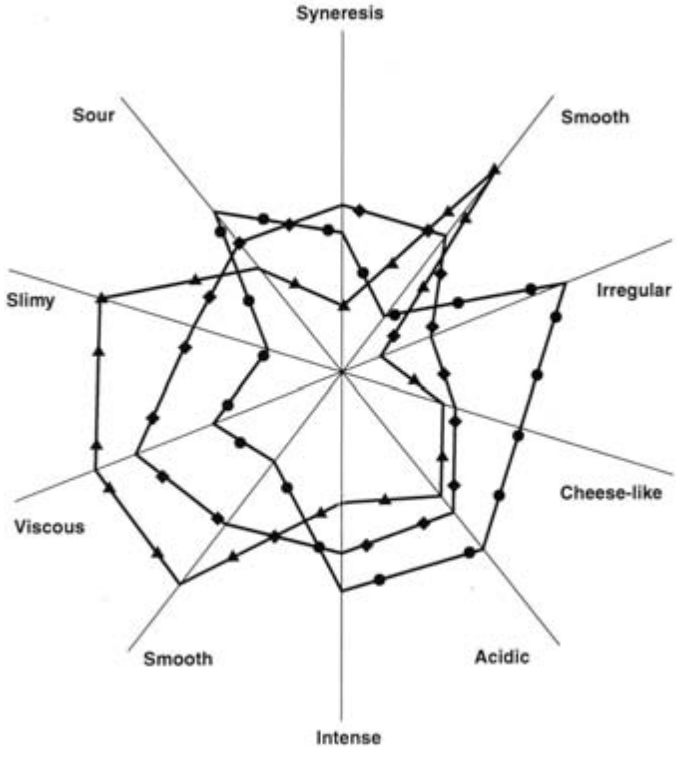

Fig. 4.4 The sensory profiles of three samples of natural stirred yoghurt made with three different starter cultures. By drawing the profiles on one spider diagram, the contrast between the attributes is easily discernible. Culture A (● – ●), Culture B (◆ – ◆), Culture C (▲ – ▲) – see text for details. After: Robinson (1988).

component analysis. This latter calculation determines which differences between selected attributes contribute significantly to any contrasts between products perceived by a taste panel. However, the technique is best employed with a taste panel specifically trained to handle dairy products.

4.7 Conclusion

It is evident from what has been written elsewhere in this book that the manufacture of yoghurt is a natural biological fermentation based on a raw material of potentially variable quality and employing a starter culture that may be temperamental. Occasional problems with the quality of the end-product are, therefore, inevitable but, if possible difficulties can be detected early, then solutions should be possible. Sometimes likely events are predictable, and one of the most obvious is the cyclical trend in protein content of bovine milk with seasons of the year. Similarly, in the UK at least, seasonal changes in feed quality in the spring and autumn have been cited as possible causes of granulation in yoghurt made from bovine milk (Robinson, 1981).

The application of the hazard analysis critical control point (HACCP) system to the process should provide a major constraint on the development of unexpected faults but, in the final analysis, what matters is the quality of the retail product. As a consequence, a routine appraisal of the end-product is essential, and it is for manufacturers to decide on the methods best suited for their needs.

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Fermented Milks
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5 Production of Drinking Products

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5.1 Introduction

In many countries a large amount of yoghurt and other fermented milk products are used as a base to manufacture drinking or beverage products that are consumed either from a glass or direct from the retail container. Such fermented milk drinks may have a large variation in both the chemical composition and viscosity. Consumers in different markets may prefer high viscous drinking (i.e. characterised as 'full bodied' and nice 'mouth-feel'), while others prefer a product that is thin or low in viscosity and sometimes salted.

These fermented drinking products are regionally adapted. For example, in Asia a diluted yoghurt-based drink, which is low in viscosity, is very popular, and often with an increased shelf-life that has been achieved by heating the fermented product followed by aseptic packaging. It should be noted, however, that this type of drink, where the yoghurt starter cultures have been inactivated after the fermentation stage, should not be designated as 'yoghurt'. The overall definition of yoghurt in many countries stipulates that the microflora of the product should consist of viable *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

In Europe, most of the commercial drinking yoghurts available on the market contain live micro-organisms, and the consistency of the gel is higher when compared with the low viscous products made in Asia. Nevertheless, in most Middle Eastern countries including Turkey and Pakistan, it is very popular to add a small amount of salt to the fermented drink. While the salt contributes a distinct salty taste to the product, it also helps to maintain the salt balance in the bodies of consumers living in hot climatic conditions.

Some drinking yoghurt products have a low fat content that appeals to diet-conscious consumers, but other products may contain probiotic bacteria or special sweeteners or flavours. Often, drinking yoghurt is dedicated to a certain consumer group, mainly the younger generation. An increased sugar content in the product, the addition of special combinations of different aromas and flavours, and the use of an attractive packaging container are some marketing aspects used to entice young people to consume such products. However, other targeted consumer groups are those looking for a healthy drink, for example, a product containing probiotic bacteria and prebiotic compounds. While drinking yoghurt is mainly consumed as a tasty, healthy and refreshing drink, it can also be poured over breakfast cereals or used in the preparation of salad dishes or soups.

5.2 Types of fermented drinking products

A number of different types of fermented drinking products are available on the market. Taking into account the physical characteristics of the product, these drinking products can be classified into the following categories: (a) viscous products; (b) diluted or beverage products; (c) carbonated products. Products in these categories may be fresh (containing live starter culture bacteria including probiotic bacteria, prebiotic compounds or omega-3) or extended shelf-life products with no live micro-organisms. Most fermented milks can be produced as a drinking variant by reducing the viscosity of the product, for example, by homogenising the fermentate. Factors such as the chemical composition of the milk, starter culture types, additives and process design will also contribute to the final consistency, taste and mouth-feel of the fermented drink product. It is common practice during the manufacture of these products to add stabiliser(s) in order to avoid sedimentation of the milk solids and whey separation in the package. The stabiliser may also improve the mouth-feel of the drink.

Fermented milk drinks are popular in many countries in Asia, and the product is known as laban drink (in most Arab countries), lassi (in India), dough and mast (in Iran and Iraq) and ayran (in Turkey and Lebanon). Some of these products are made from yoghurt that has been diluted with water up to 50 mL 100 mL^{-1}, and salt (~0.5 g 100 mL^{-1}) may be added. These are traditional and plain products, but on some occasions they may be flavoured with different fruits or aroma compounds (peach, lemon or strawberry) or with mint, or with spices such as cumin or chilli. The compositional quality of these products may be governed by existing legal standards, but the variation that may exist in the fat and solids-not-fat (SNF) content from different manufacturers, especially in Europe, is to meet consumer demand and/or local taste. In general, fermented milk drinks may have the same chemical composition as milk, or be lower in fat and protein content, especially the diluted products.

Buttermilk is another type of fermented drinking product. Originally, buttermilk was a byproduct of butter production, where mesophilic lactic acid bacteria were responsible for the fermentation. However, fermenting low-fat milk with mesophilic starter cultures may also produce buttermilk (see Chapter 7; Tamime & Marshall, 1997; Tamime *et al*., 2001).

5.3 Factors affecting product quality

Numerous factors have been identified and must be carefully controlled during the manufacturing processes of fermented milks in order to produce a high-quality drinking product with the required flavour, aroma, viscosity, consistency, appearance, freedom from sedimentation and a long shelf-life. These factors include:

- choice of milk and standardisation of the fat content;
- additives;

- deaeration;
- homogenisation;
- heat treatment;
- choice of culture;
- plant design.

The scientific aspects of the chemical and microbiological quality of raw milk, the effect of heat treatment and homogenisation on the main constituents of milk, the acid-gel formation in milk and the stability of the coagulum have been reviewed in Chapter 3; in this section, supplementary data will be reviewed in relation to the quality of drinking-types of fermented milk.

5.3.1 *Choice of milk*

Drinking yoghurt can be produced from milk of different species of mammals. The composition and quality of the milk will, to a large extent, affect the quality of yoghurt. Drinking yoghurt, that has been diluted with water, will have low fat and low dry-matter content and, as a consequence, the milk will be standardised/fortified to the desired fat and SNF levels in the product. Hence, the mouth-feel will be thin, but can be compensated for by the addition of different stabilisers. These can give the product a more full-bodied taste or mouth-feel. The level of milk protein in the fermentate plays an important role in the formation of the coagulum, and affects the stability and viscosity of the yoghurt drink. Furthermore, the higher the protein content in the milk, the greater the stability of the product. The lactose in the milk provides the energy source for the yoghurt starter organisms.

Fermented milk drinks can be made from recombined or reconstituted milk. Skimmed milk powder is the most widely used raw material. The milk powder can be of different variants depending on the degree of the heat treatment of the milk before drying. Low- or medium-heat spray-dried milk powder are often regarded as the best for production of fermented milk products, and the specifications of these powders have been reported by Tamime and Robinson (1999) and Anon. (2003).

5.3.2 *Additives*

Additives, such as stabilisers, sugar or sweeteners and fruit syrup or aroma, can be added either to the milk or to the coagulum, i.e. fermented milk. Other additives that can be used are vitamins, calcium, inulin, salt and special fatty acids such as omega-3.

Stabilisers

A fermented milk drink can be produced without the addition of any stabilisers. However, some sedimentation of the milk solids will occur in the product and has to be accepted, especially if the shelf-life of the product is more than 1 week. It is of interest to note that the aggregated proteins due to acidification are heavy and

Fig. 5.1 Stabilised (right) and unstabilised (left) drinking-type yoghurt. With permission of Danisco A/S, Brabrand, Denmark. Reproduced in colour as Plate 1, after page 110.

have the tendency to sedimentation, and cause syneresis or wheying off (Fig. 5.1). Therefore, the lower the level of SNF in the milk, the higher is the sedimentation risk. Sedimentation can be avoided by the addition of stabiliser(s), and they are always used for long-life fermented milk drinks that have been heated after the fermentation stage.

Many different types of stabiliser are available on the market; examples include pectin, sodium carboxymethyl cellulose (Na-CMC), guar gum, gelatin, starch (native or modified) and functional blends of these stabilisers. The sedimentation of the protein in drinking yoghurts can be prevented by adopting one of the following approaches:

Stabilisation of the product by increasing the viscosity

This approach is used in short shelf-life yoghurt drinks containing live micro-organisms, and an increase in viscosity can be achieved by using exopolysaccharide (EPS)-producing starter cultures or the addition of stabilisers. However, the disadvantage of this approach is a relative short period of protein stability, and a reduction in flavour release from any added fruit preparation or flavours.

Stearic stabilisation

The most common way to overcome the sedimentation of the proteins is by preventing such particles from aggregating together. This action is achieved by using stabilisers that have electrostatic interactions with the caseins, such as ester pectins, Na-CMC or soluble soybean polysaccharides (SSPS) (Fig. 5.2).

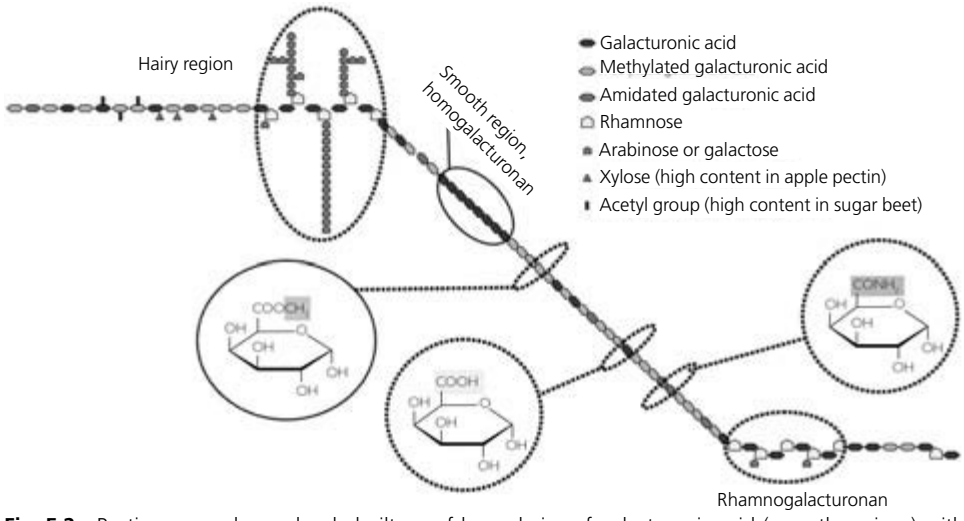

Fig. 5.2 Pectin, a complex molecule built up of long chains of galacturonic acid (smooth regions) with interruptions of amidated or methylated galacturonic acid sequences and branched regions. With permission of Danisco A/S, Brabrand, Denmark. Reproduced in colour as Plate 2, after page 110.

High ester pectin is one of the stabilisers most commonly used to stabilise acidified milk drinks. Pectin functions best in the pH range 3.7–4.3, and should be added to the fermented milk product before the final heat treatment. The purpose is to protect the proteins during the heating phase, and to prevent sedimentation and development of a sandy mouth-feel in the drink. Pectin is a hygroscopic powder, which makes it tricky to add directly to a liquid product without the formation of 'fish eyes', i.e. shiny, hard particles. One method that is used to avoid this fault in the product is to dry blend the pectin with sugar before addition. An alternative method is to use efficient high-speed mixers (see section 5.4.1), which will ensure wetting of the stabiliser powder before the particles start to form. In addition, pectin contains long chains of galacturonic acids (i.e. smooth regions), which are negatively charged and will bind effectively to the positively charged casein molecules. Furthermore, another part of the pectin molecule contains esterified galacturonic acids and branched chains of higher sugars (i.e. hairy regions), such as rhamnose, arabinose and galactose (Fig. 5.2). This unique structure of the pectin molecule (with both smooth and hairy regions) ensures that part of the molecule is bound to the casein while the other part prevents the casein particles from aggregating together (Fig. 5.3).

Sugar and sweeteners

Different types of sugar or sweetener may also be added to the milk base before the processing and fermentation stages. In this respect, it should be noted that too high a sugar content may negatively influence the growth of the lactic acid bacteria. Different bacteria have different tolerance levels for sugar. Most often, the recom-

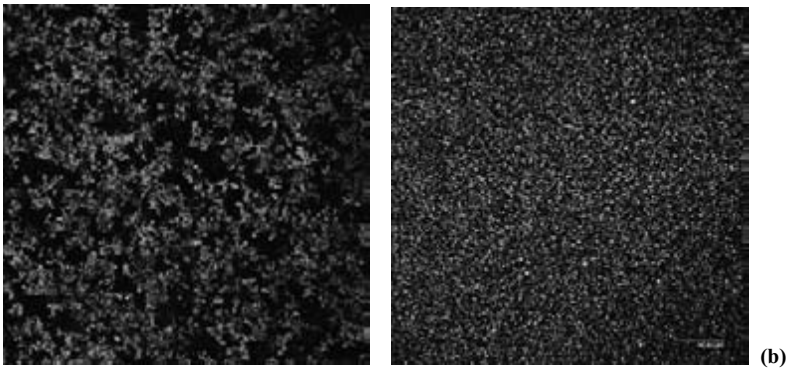

Fig. 5.3 Confocal laser scanning microscopy (CSLM) showing the quality of drinking yoghurt with (a) or without (b) high ester pectins. The stabiliser used was Grindsted® AMD 780. With permission of Danisco A/S, Brabrand, Denmark. Reproduced in colour as Plate 3, after page 110.

mendation is that the sugar content should be below 8–10 g 100 g^{-1} in the milk; however, some newly developed strains of the yoghurt bacteria have a higher tolerance to sugar. It is of interest to note that by adding high levels of sugar (especially sucrose), the rate of EPS production by the starter culture will increase and thus enhance the mouth-feel and increase the length of the 'slimy' strands.

An alternative approach is to add sugar after the fermentation stage and before the final heat treatment during the manufacture of long shelf-life drinking products. In this way, the activity of the starter culture will not be affected by the presence of sugar in the milk. However, the risk of microbial re-infection is eliminated because the product is heated after the addition of sugar. In commercial practice, it is most common to make a solution of sugar, stabiliser and water and heat-treat it separately before it is added to the fermented milk. When using pectin, the recommendation is to heat-treat the solution separately to 70–80°C. After addition to the fermented milk and vigorous mixing, the product is heat-treated before aseptic packaging.

Fruit syrup and/or aroma

Although some fermented milk drinks are consumed in their natural state, in Europe most of these products contain a fruit syrup or aroma. Fruit syrup, which may also contain small fruit particles, can be added to the fermentate. Fruit syrups are often an expensive ingredient and thus it is important not to overdose. One factor that influences the amount of fruit syrup in fermented drinks is the acidity level of the product. The higher the acidity of the fermented milk, the greater is the amount of fruit syrup used. In addition, by using 'mild' acidity starter cultures, which have been popular in recent years, it is possible to reduce the amount of fruit syrup added.

The fruit aroma, which is normally obtained in a concentrated form, will have a larger aromatic effect in less acidic products, and is added to long shelf-life products. The aroma compounds can be added in the buffer tank or in-line before the fermen-

tation stage if the aroma and/or colouring matter will stand the heat treatment. For some aromas and colours the heat treatment will have a negative effect, and these are added aseptically before the aseptic filling.

Miscellaneous additives

Inulin is a natural dietary fibre, which can be found in different types of fruits and vegetables, such as asparagus, onions and bananas. Inulin is considered as a functional ingredient as it stimulates the activity and growth of beneficial bacteria in the gut (Robinson, 1995). It is also reported to increase the absorption of calcium and magnesium. Moreover, inulin is regarded as a low-calorie product, which does not affect the blood-sugar level.

As mentioned elsewhere, ayran contains salt and, by tradition, the product is a fresh fermented milk drink that contains live lactic acid bacteria and has a rather limited shelf-life under refrigeration. In addition, prolonged shelf-life products are produced so that they can be stored and transported at ambient temperature. However, salt is corrosive to stainless steel equipment, and it is not recommended to add the salt before the final heat treatment in order to avoid the risk of corrosion of the heat exchanger. Salt is normally added aseptically, if possible, before packaging the product.

Another reason for adding salt after the heating of the fermentate is that it has a negative effect on the stability of the product. In theory, the pectin should first react with the proteins before the addition of the salt. The salt solution can be sterilised by passing through a sterile filter before aseptic filling of the product.

5.3.3 *Deaeration*

Raw milk contains a certain amount of air depending on milking conditions and the handling of the milk between the farm and the dairy factory. The milk may contain 6% total gas, on average, in the cooling tank at the farm, but it is not unusual for the milk to contain up to 10% air by the time it is received at the dairy. Also, during the reconstitution of milk powders, it is unavoidable that air will be included as the powder contains a lot of air.

The air content in the milk can be removed by deaeration, and the positive effects can be summarised as follows:

- Improves the skimming efficiency of the separator;
- Improves the homogenisation efficiency;
- Minimises fouling effects in the plate heat exchanger;
- Improves on-line fat standardisation of the milk;
- Reduces fermentation time;
- Enhances the stability of the product;
- Removes volatile off-flavours (i.e. deodorisation).

5.3.4 *Homogenisation*

Homogenisation is another important factor for production of a high-quality fermented milk product. The main objectives of homogenisation of the milk base are: (a) to decrease the size of the fat globules to avoid cream separation in the fermentation tank; (b) to improve the mouth-feel of the product, especially if any additives in powder forms have been used; (c) to improve the water-holding capacity of the milk proteins and reduce the tendency to syneresis. Another effect of homogenisation is the increased stability of the drinking product; a secondary homogenisation is normally carried out after the fermentation stage. For fermented milk products, full stream or total homogenisation of the milk is the most commonly used form.

The homogenisation pressure and temperature are dependent on a number of factors, and one aspect is the formulation of the milk base. The most common homogenisation pressure for milk intended for fermented milk products is ~20 MPa at a temperature range of 60–70°C. The homogeniser may be equipped with one homogenisation device (single-stage) or two (double-stage) connected in series. In both single- and double-stage homogenisation, the whole homogenisation pressure (P1) is used over the first device. In a single-stage homogeniser, the back pressure (P2) is created by the process, while in a double-stage homogeniser, the back pressure (P2) is created by the second stage of homogenisation. In this case, the back pressure can be chosen to achieve optimal homogenisation efficiency. Using modern homogenisers, the best results are obtained when the relation of P1/P2 is about 0.2.

From a product point of view, single-stage homogenisation of milk is often good enough for products with low- or medium-fat contents. However, it is common to install a double-stage homogeniser in the line, for the following reasons.

- Lower energy costs due to the lower pressure that is required compared to single-stage homogeniser to obtain the same homogenisation effect;
- Higher flexibility because the plate heat exchanger can also be used for other products, such as recombined products and desserts;
- Improved running conditions of the homogeniser as the second-stage pressure will reduce the noise and minimise vibrations in the outlet pipe.

As mentioned earlier, heat-treated fermented milk products should include a stabiliser in order to avoid sandiness and sedimentation. Different stabilisers have different effects on the process. Some of them should be added to the milk before the heat treatment and fermentation stages; other stabilisers should be added to the fermentate. In addition, stabilisers may also need different mechanical treatments, such as the application of high pressure to ensure the expected stabilising effect. For example, the pectin solution, which is recommended to be added to the fermentate, is homogenised before heating the product. Homogenisation will allow the pectin to interact properly with the proteins and will enhance stability during the post-fermentation heating stage.

Other stabilisers may be inactivated by homogenisation at very high temperature and, in the case of starch-based stabilisers, they should not be homogenised during

the swelling phase. This means that the homogenisation temperature (60–65ºC) has to be set below the swelling point of the starch to avoid the destruction of the partly swelled starch granules by the high pressure.

5.3.5 *Heat treatment*

The effects of heat treatment of the milk have been detailed in Chapter 3 and, as a consequence, a firm and stable coagulum can be formed in the incubation tanks. However, the degree of heat treatment applied to the milk base for the production of a yoghurt drink will be one of the main parameters to be considered, as it influences the temperature of heating required to heat the fermentate during the manufacture of a long-life drinking product. In general, it is safe to recommend that the higher the heat load of the milk base, the lower the heat treatment required of the fermentate in order to achieve the lowest microbial count in the drinking product.

5.3.6 *Choice of starter culture*

Commercial blends of yoghurt starter cultures are widely used in the industry and, for fermented drinking products, low viscosity culture strains are most often used. However, the mean particle size (diameter) of the gel fragments in an acidified milk drink can affect protein stability and sedimentation. A slow fermenting starter culture or incubation at low temperature will lead to the formation of small and equal mean particle sizes. This results in a more stable product compared to a drinking yoghurt made with a fast acid-producing starter culture (Table 5.1 and Fig. 5.4).

5.3.7 *Plant design*

One aspect that can affect the quality of the yoghurt in terms of texture is the design of the processing equipment, mainly the treatment of the milk and the handling of the coagulum. Modern plants are designed to satisfy the demands for high production levels, continuous operation and high quality. It is, however, important not to overlook other aspects, such as the design of the cleaning systems employed, minimising product losses in the processing equipment and ensuring high yields of product. In addition, product traceability is another factor that affects the design of the processing plant. These factors are detailed in the subsequent sections.

Table 5.1 Effect of fermentation temperature on the particle size of the gel and the extent of sedimentation.

	Temperature 32ºC	Temperature 42ºC
Sedimentation (%)	3.5	5.2
Particle size (μm) (D[*v*,0.5])	1.48	1.89

Note: D[*v*,0.5] is the volume (*v*) median diameter [D], and this value is sometimes shown as D_{50}.

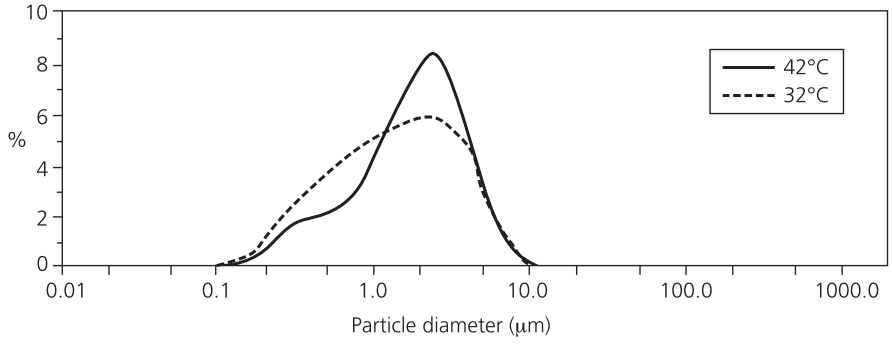

Fig. 5.4 Effect of the fermentation temperature on the particle diameter of the gel. With permission of Danisco A/S, Brabrand, Denmark.

5.4 Manufacturing technique for fresh fermented milk drinks – cold distribution

Fermented milk products are normally sensitive to mechanical treatment. For example, a process line for stirred-type yoghurt must be designed for careful handling of the coagulum in order not to reduce the viscosity of the product and stability of the gel after the fermentation stage. Hence, the production line must be designed with low product velocities in pipes, a low pressure drop in the plate heat exchanger for cooling the coagulum and the speed of the positive pump(s) maintained at low revolutions per minute (rpm). When fermented drinking products are produced, the plant design can be adapted slightly. The principal stages of the process for the manufacture of a stabilised drinking yoghurt are shown in Fig. 5.5.

5.4.1 *Powder mixing equipment*

It is important not to incorporate air during recombination of milk or mixing in other ingredients. To design equipment for the air-free addition of a liquid product is quite easy, but it is more difficult when different types of powder ingredients are added because some are hygroscopic, e.g. pectin, and others may contain a large volume of air. Such aspects can generate greater demands on the design of the equipment in order to minimise the incorporation of air into the milk or water.

There are a number of mixing units available on the market, and one unit that is specially designed for minimal air incorporation is the Tetra Almix (Fig. 5.6). The mixing unit is a complete frame-mounted module that comprises a mixing tank, a belt-driven mixing device placed beneath the mixing tank and a control panel. One model is designed for mixing under vacuum, and it is applied in the mixing tank in order to reduce the incorporation of air and to ensure optimal wetting of the powder. All the dry additives including the milk powder are automatically fed into the mixing tank by means of vacuum, and the amount that is delivered is measured by load cells of the powder silos. The efficacy of the module is the design of the mixing

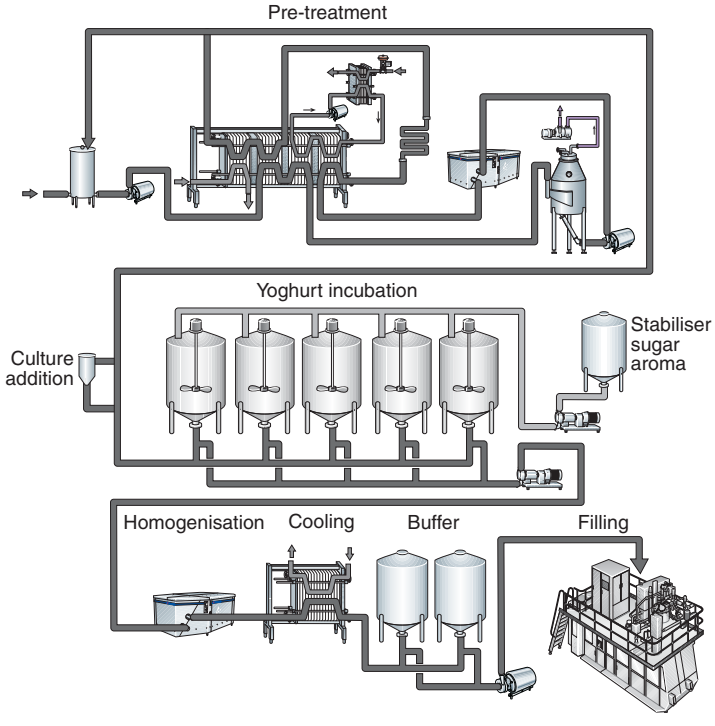

Fig. 5.5 Production of stabilised fresh drinking yoghurt for storage at 5°C. By permission of Tetra Pak AB, Lund, Sweden.

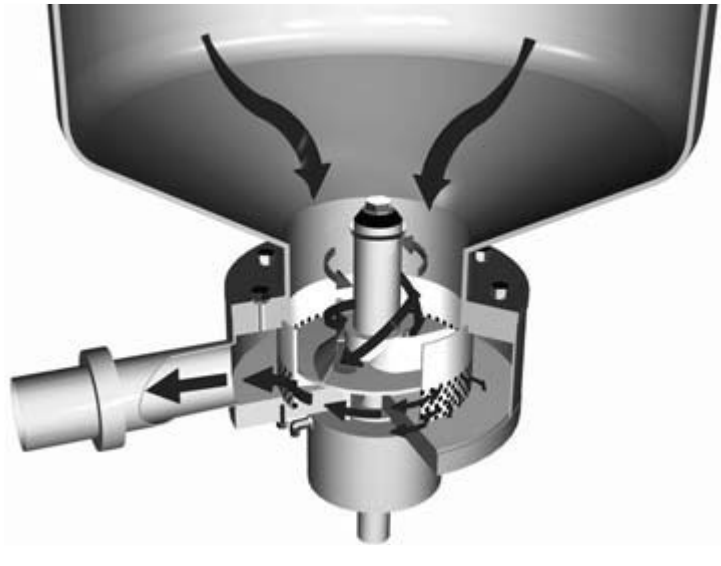

Fig. 5.6 High shear mixing unit (Tetra Almix) with rotor and stator positioned underneath a water/milk-filled vessel. By permission of Tetra Pak AB, Lund, Sweden.

unit, which consists of a rotor and a perforated stator (Fig. 5.6), and this module provides the following functions: (a) mixing, (b) pumping and (c) dispersing. The ingredients are sucked down into the mixing unit by the rotor and are pushed out through the holes in the perforated stator. On its way through the perforated stator, the product is subjected to shear by the impeller wings at the bottom of the rotor. The mixing unit is placed in the bottom of the mixing tank in order to ensure that the dried product(s) has to pass through the mixing unit at least once. Powder can be either manually added or automatically fed into the mixing tank, and liquids, such as oils, can be metered directly into the tank during mixing.

The mixing unit gives full dispersion and a very stable emulsion. The former aspect ensures that the mixed product is free from lumps and that there is no build-up of sediment in the tanks, a cause-and-effect sequence that can lead to clogged filters. A mix without lumps ensures effective heat treatment and a better quality product, which for post-fermentation heated products means a longer shelf-life.

The mixing unit can be operated in two ways; either with external circulation over a buffer tank (Fig. 5.7) or with internal circulation over the mixing tank. To reach maximum capacity, external circulation is recommended as it is also the most common mode of operation. In the case of external circulation, a preset amount of the main liquid is fed into a buffer tank and brought into circulation over the mixing tank. Powders and liquids, such as oils, are then added and mixed with the main liquid to a homogeneous product. A continuous flow to downstream equipment is achieved by using two or more buffer tanks.

However, in the case of internal circulation, a preset amount of the main liquid is fed directly into the mixing tank and brought into circulation over the mixing tank via an external pipe.

5.4.2 *Temperature for mixing powders*

The optimal temperature for dissolving of milk powder is 45–50°C. The rate at which the molecular equilibrium in the product is established depends on the mixing temperature. At a high temperature the molecules move faster and, therefore, reach equilibrium more quickly than at a lower temperature. In an efficient powder mixing unit, such as the Tetra Almix, the time for equilibrium to be established is short or minimised because the first part of the dissolution of the powder is made almost ‘instant’ when passing the mixer. The molecular equilibrium is then established fairly quickly at a temperature ranging between 45 and 50°C. Increasing the temperature to more than 50°C has not been shown to have any more positive effect on powder dissolution.

A second reason for a warm mixing temperature is that air incorporation will be less in warm milk than if mixing is performed at a low temperature.

The disadvantage of warm mixing is the risk of microbiological growth if the rehydrated product is held too long at such temperature before being heated. A safe microbial time lag at 45°C is 2 h, but such time is, of course, dependent on the microbiological quality of the ingredients that are used.

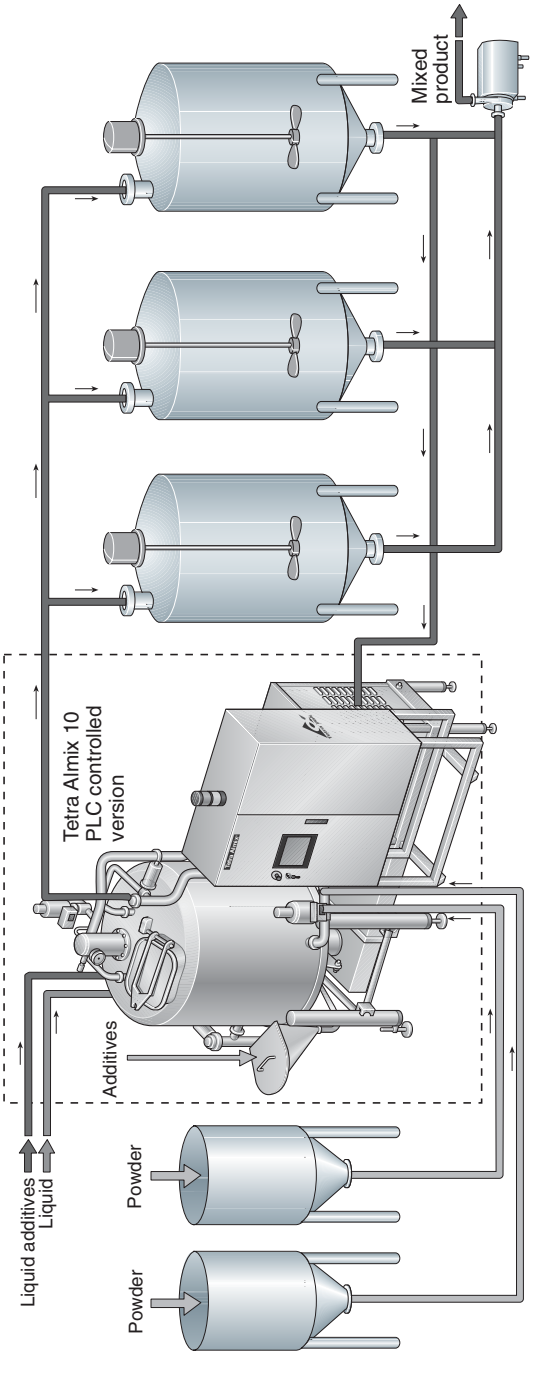

Fig. 5.7 Recombination plant with vacuum mixing. By permission of Tetra Pak AB, Lund, Sweden.

5.4.3 *Miscellaneous treatments of the milk base*

The standardised milk, stored in buffer or mixing tanks, is now ready for further treatment. If the milk contains a lot of air, it is recommended that a deaerator is installed beside the plate heat exchanger (PHE). From the balance tank, the milk is pumped to the PHE where it is pre-heated in the regeneration section to ~70°C, which is a common deaeration temperature. The milk enters the deaerator tangentially through a narrow path (Fig. 5.8); a vacuum is created in the deaerator by means of a vacuum pump.

The vacuum corresponds to a water evaporation point below the product inlet temperature. Vapour and gases rise to the top of the vessel, and the vapour condenses in a spiral condenser, mounted in the top of the deaerator, and drops back into the product. The incondensable gases are discharged through the gas outlet by the vacuum pump, and the deaerated product is pumped out through the bottom outlet of the vessel. The milk temperature drops by a few degrees during deaeration due to the vacuum treatment. Modern deaerators are designed with a very small temperature drop (~4°C) in order to minimise energy consumption.

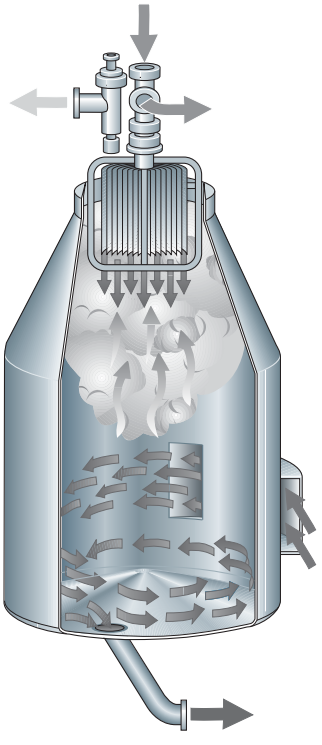

Fig. 5.8 Flow of milk and air in the vacuum deaerator with built-in condenser. By permission of Tetra Pak AB, Lund, Sweden.

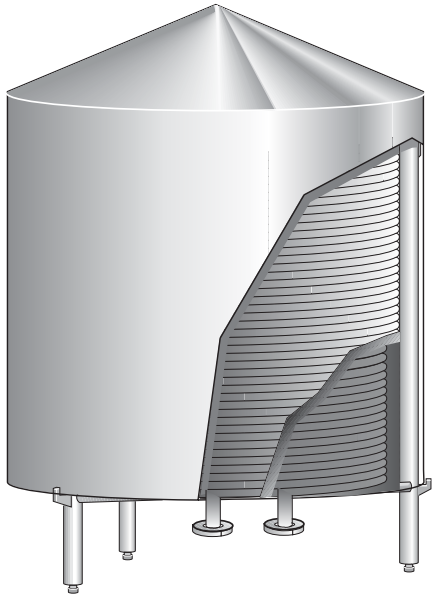

Fig. 5.9 Tubular holding unit. By permission of Tetra Pak AB, Lund, Sweden.

The deaerated milk is pumped to the homogeniser at ~65°C, and homogenised at ~20 MPa pressure in a single- or double-stage homogeniser before it is heated to 90–95°C in the PHE for 5 min. The tubular holding section (Fig. 5.9) offers a holding efficiency of 90–95%, which is appreciably higher than when one holding tank is integrated in a continuously operating plant. It is also equipped with a protective cover to maintain the temperature.

The heat-treated milk is first cooled in the regeneration section and later with water (indirectly) to the desired inoculation temperature (typically 20–30°C or 40–45°C for mesophilic and thermophilic starter cultures, respectively). The milk is then delivered to the incubation tanks.

However, the starter cultures can be added either in-line to the milk or direct to the incubation tank. Dairies using cultures from bulk starter tanks are most often dosing the culture direct into the milk stream during filling of the incubation tank. One method of inoculation is to use a positive pump operating on a timer. In this case the bulk starter is dosed during a relatively short period, e.g. 5–10 min. The amount of culture added has to be calculated based on the capacity of the pump and the dosing time. It is of course also possible to measure the amount with a flowmeter. Another way is to use a centrifugal pump and a flow-regulating valve together with a flowmeter.

At present, most fermented milk manufacturers use concentrated deep-frozen or freeze-dried starter cultures, which can be added direct to the incubation tank(s) in a hygienic way or in-line (Fig. 5.10a,b). The latter method is detailed in Chapter 2.

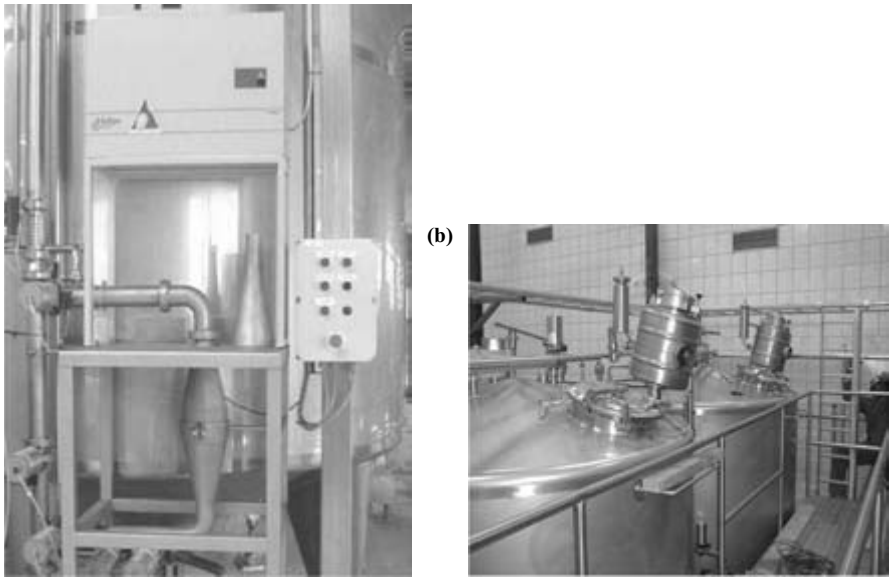

Fig. 5.10 (a,b) In-line inoculation of the processed milk with freeze-dried starter culture. By permission of Tetra Pak AB, Lund, Sweden.

5.4.4 *Incubation tanks*

The milk is fermented in insulated tanks, and the capacity of these tanks is dependent on factors such as processing plant capacity (L h^{-1}), process technology, production schedule and the growth rate of the starter cultures used.

Many dairies have a guideline that the processed milk base should not be held at fermentation temperature without the added culture for more than 2 h, in order to avoid bacteriological risks. However, it is quite common to have a 1 h filling time as this span gives the optimal production time schedule.

The tank size is also dependent on the gradient of the fermentation curve at pH ~4.4. If the curve is rather flat, minimal changes in pH occur with time and the cooling time of the coagulum can be relatively long. There will not be any significant difference in pH between the first and the last product being emptied from a tank. In such a case, the capacity of the fermentation tank may be 100 000 L, and the cooling time is up to 4 h. On the other hand, if the change in pH is significant at 4.4, the size of the fermentation tank must be small enough to be able to pump out and cool the product within, for example, 30 min.

The fermentation tank should be equipped with an agitator, and the type used is, to a large extent, dependent on the process technology. For example, a tank can be equipped with a normal propeller agitator (i.e. a type that is adapted for handling low-viscosity products) with the sole purpose of properly mixing the starter cultures into the processed milk, but not the coagulum at the end of the fermentation period. The risk with this technology, however, is that if the fermented coagulum is firm and it is not broken up or mixed it will be difficult to prime the pump for emptying.

In this case the tank can be equipped with a larger propeller agitator, designed to break up the coagulum in the lower part of the tank. Besides mixing the milk and starter cultures, the action of the propeller will reduce the viscosity or firmness of the end-product and enhance its flow to the pump. Once the product has reached the pump, there is no need to agitate the remaining coagulum in the tank unless whey separation has occurred.

A third alternative type of agitator is for mixing the milk and starter cultures and breaking up the whole coagulum in the incubation tank. For this purpose, a high-speed agitator, along with a propeller or scraped surface agitator, can be used as long as they are designed to handle the yoghurt coagulum.

For some fermented drinking products, a stabiliser solution is added to the fermentate, and this can be done either in the fermentation tanks or in the buffer tanks. In either case, the agitator should also be designed to mix the fermented product with a stabiliser solution.

With an increased demand on the shelf-life of fermented products, it is important to protect the process line from microbial contamination. For this reason, it is common practice to use specially designed tanks fitted with sanitary air supply units. Different levels of security can be obtained in the incubation tanks and, with an open manhole tank, the sanitised air is introduced above the product. Hence, the tank is equipped with a sanitary air filter and the airflow is maintained by means of a fan.

A higher level of security can be obtained by using a tank with a completely enclosed manhole. The tank is equipped with a sanitary air filter and the static air pressure is < 5 kPa, which can be maintained in the tank by means of an oil-free compressor (Tamime & Robinson, 1999; Anon., 2003). An even higher degree of security can be reached by using a pressurised tank with a completely enclosed manhole. The tank is equipped with a sanitary air filter and the static air pressure is < 0.1 MPa, which can be maintained in the tank by means of an oil-free compressor. Incidentally, the tank can be sterilised after cleaning with, for example, hot water at 120°C.

It should be noted that, in combination with these different levels of hygiene, other processing equipment and packaging machines should be designed with corresponding hygiene and security levels. However, prime risk areas for possible microbial contamination of the product are fruit handling and product packaging and, for this reason, buffer and mixing tanks should be fitted with sanitary air filtration units.

Tanks with an open manhole allow samples of the product to be taken manually from the top of the tank. Tanks with an enclosed manhole are fitted with a sampling valve on the side of the tank. There are a number of different types of sampling valve, and it is important that the design is suitable for sampling viscous products. Samples taken are normally checked for pH or acidity and by tasting the product.

5.4.5 *Cooling and miscellaneous treatments*

At the desired pH of the fermentate, i.e. ~4.4, the coagulum is cooled very quickly so as to minimise the pH differential between the product that first leaves the incubation tank and the last to be pumped out. A PHE is normally used to cool the coagulum and,

for drinking yoghurt containing live micro-organisms, the viscosity of the product can be set or achieved by certain mechanical treatments, such as homogenisation, pumping (i.e. using high pressure pumps), sieving and/or valves. Such treatments break up the protein network of the gel, and the viscosity of the drinking product is greatly reduced when compared with 'normal' yoghurt.

However, a drinking product with no added stabilisers may have the increased risk of whey separation and sedimentation of the milk solids during storage (Fig. 5.1). Often, a message to the consumer (e.g. 'shake before opening') is clearly displayed on the package so that the product becomes more homogenous before and during consumption. If pectin is added, the stabiliser is mixed with the fermentate in the incubation tank, and the fermentate is flavoured (optional), homogenised, cooled and packaged. The homogenisation effect enhances the interaction between the pectin and the proteins and minimises sedimentation during the storage period.

5.4.6 *Buffer tanks and fruit concentrate/aroma mixing*

The cooled product is transferred to one or more buffer tanks before it is pumped to the filling machine. The design of the buffer tanks is similar to the incubation tanks; however, the agitator type, if any, is designed for handling low viscosity products. The primary purpose of the agitator is to keep the product homogenous (if no stabiliser has been used) before mixing in the fruit concentrate/aroma compounds and packaging. This system of mixing is more flexible, any changeover of fruit flavour is easier and product loss is minimised. In addition, it is important to use an accurate system for the dosing of the fruit flavours/aroma compounds, and a typical system consists of two fruit concentrate tanks that can be used in parallel. The pipe and valve arrangements for each tank are designed to be cleaned and sterilised individually so that, during the changeover from one fruit flavour to another, production is not interrupted. Quite often, these buffer tanks (e.g. products and fruit flavours) are positioned on load cells, and their capacities can be measured by means of mass flowmeters.

Different types of fruit pump are available on the market, and they can be used to meter the fruit into the yoghurt. The most commonly used pumps are the positive lobe, progressing cavity or piston types. The mixing of the yoghurt and the fruit concentrate takes place in a static or dynamic mixer. In some mixing systems, a piston pump is used for the fruit, while a flowmeter in the yoghurt line will trigger the signal to the fruit pump when a certain amount of yoghurt has been transferred. Normally, the fruit pump makes one stroke to deliver the fruit concentrate to a given amount of yoghurt, and a dynamic mixer is required in order to achieve a homogenous distribution of the fruit in the fermentate. In addition, aroma concentrates can be added but, as the concentration is very high, the amount added is small. Hence, accurate equipment is required for in-line dosing of the aroma concentrate. However, for drinking products it is recommended that the fruit concentrate is added in-line before packaging (Fig. 5.11).

An alternative to in-line dosing of the fruit flavour to drinking yoghurt is to add the flavouring material to the buffer tank. This method of mixing is less flexible

Fig. 5.11 Concentrated aroma dosing system. By permission of Tetra Pak AB, Lund, Sweden.

than in-line dosing and, if more than one type of fruit is used per day, higher product losses can be expected.

5.4.7 *Packaging*

The drinking yoghurt is now ready for filling and, although the packaging container is mainly aimed to protect the product during storage, distribution and retailing, it may also contain an advertising message for the consumer purchasing the product. Many packaging containers for fermented drinking products are intended for direct consumption (with the exception of a few family-size containers), and must have an easy-to-pour port or opening. Some examples of packaging containers that are popular include high-density polyethylene (HDPE) and paperboard cartons, such as Tetra Top, Tetra Brik or Tetra Prisma (Tamime & Robinson, 1999).

5.5 Manufacturing techniques for long-life fermented milk drinks – ambient distribution

5.5.1 *Production stages*

The preparation of the milk base and the manufacturing stages of yoghurt and fresh drinking yoghurt (Fig. 5.5; sections 5.3, 5.4) are also suitable for the production of

long shelf-life drinking yoghurt (Fig. 5.12); only the process variables relevant to the latter product will be reviewed in this section.

Long-life fermented milk drinks can be based on products fermented with any type of lactic acid bacteria. However, the most common of these products is long-life yoghurt, which is made in a similar manner to that described in section 5.4, but the product is post-fermentation heat-treated and aseptically packaged. The quality and shelf-life of the product are dependent on the many factors listed in Table 5.2, and the manufacturing stages are shown in Fig. 5.12; however, stability of the protein is critical in order to minimise sedimentation and syneresis as the product is heated post-fermentation, and takes place at a pH lower than the isoelectric point of the caseins.

Post-fermentation heat treatment of the coagulum can extend the shelf-life of drinking yoghurt, and two types of commercial process are available on the market. First, a low heat treatment of the gel and the fruit flavours can be carried out at 60–70°C for a few seconds, which may not kill all the starter cultures and other micro-organisms that can be present in the product; however, by using aseptic packaging and storage at 5°C, the shelf-life of the product will be prolonged to 1–2 months. Second, a high heat treatment (e.g. 75–110°C for a few seconds) and aseptic packaging of the drinking yoghurt will produce a product with a shelf-life of several months at ambient temperature (Tamime & Robinson, 1999; Anon., 2003).

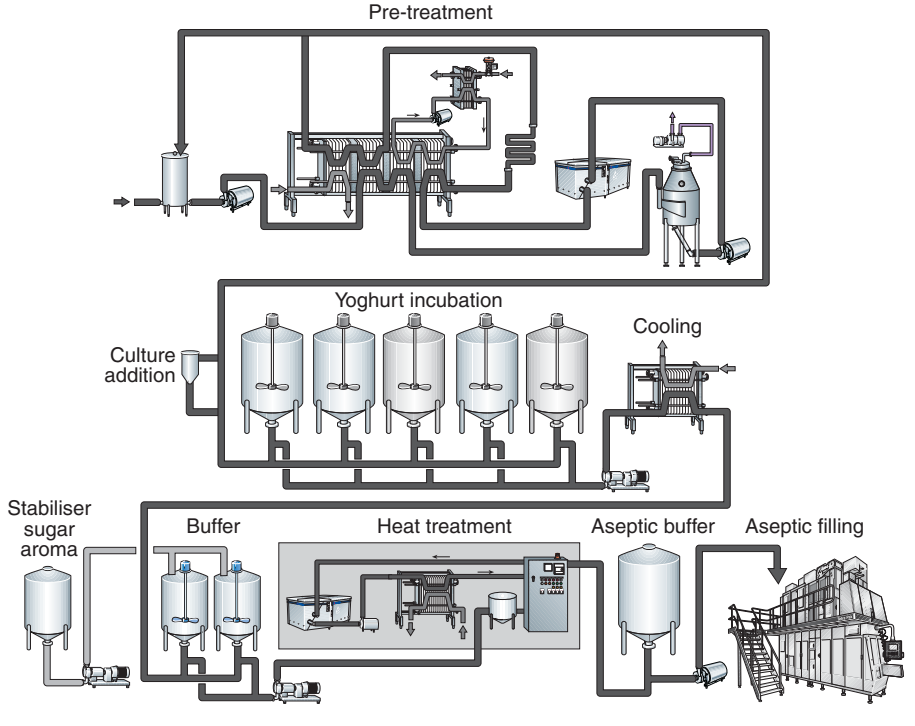

Fig. 5.12 Production of stabilised long-life drinking yoghurt for storage at ambient temperature. By permission of Tetra Pak AB, Lund, Sweden.

Table 5.2 A summary of factors that can affect the quality and the shelf-life of sterile drinking yoghurt.

Factors/aspects	Factors/aspects
Milk quality	Engineering
Milk composition	Process technology
Deaeration	Packaging
Homogenisation	Additives
Heat treatment	Cleaning-in-place (CIP) and sterilisation
Starter cultures	Storage conditions
Production equipment	Dairy personnel

The latter method of production, especially when using a high heat treatment (e.g. 110°C), ensures that the product is free from bacteria, yeast or moulds and can be designated as 'sterile'.

The temperature–time relationships that can be employed are dependent on factors such as the chemical and microbiological quality of the raw milk including the added ingredients, pre-treatment of the milk base (e.g. heating to 120°C for 2 min; at such time–temperature relationships a lower heat treatment of the drinking yoghurt is used), pH of the product, the presence of stabiliser (i.e. to stabilise the proteins and improve the mouth-feel effect of the product), quality of the final product and storage conditions. For example, the information generally available suggests that the lower the pH value of the product, the lower the heat treatment temperature required to reach the same microbiological status of drinking yoghurt.

Drinking yoghurt heated at 75°C may contain very few cfu mL^{-1} and it is most likely that the count consists of spore-forming bacteria, which do not grow well in an acidic environment and may not pose a risk regarding the shelf-life of the product.

5.5.2 *Preparation of stabiliser solution*

Gelatin may be used as a stabiliser, but the product will have a low viscosity at ambient temperature when compared with a parallel product stabilised with pectin (Fig. 5.13). If a long shelf-life yoghurt drink is distributed, retailed and consumed at higher temperatures (~20ºC), a gelatin-stabilised yoghurt will be perceived as 'thin' and 'runny' on consumption. Hence, pectin is used instead to enhance the viscosity of the product, as it has a higher gel strength at high temperature than gelatin. The effect of pectin on the viscosity of long shelf-life yoghurt stored at different temperatures is shown in Fig. 5.13. Some commercial examples of gelatin-free stabilisers are Grindsted® 719 (containing guar gum, xanthan gum and carrageenan) and Grindsted® NP (containing guar gum and pectin).

As mentioned elsewhere, the stabiliser mix is normally added to the fermentate before heat treatment of long shelf-life drinking yoghurt otherwise the galactomannans (e.g. guar gum, xanthan gum and carrageenan) would disrupt acid gel formation during the fermentation period. The most common method is to rehydrate the pectin at 70–80°C (i.e. easy to dissolve) in water at a rate of 5 g 100 mL^{-1} (i.e. easy to handle). Alternatively, the pectin may be added directly to the yoghurt, and it is recommended

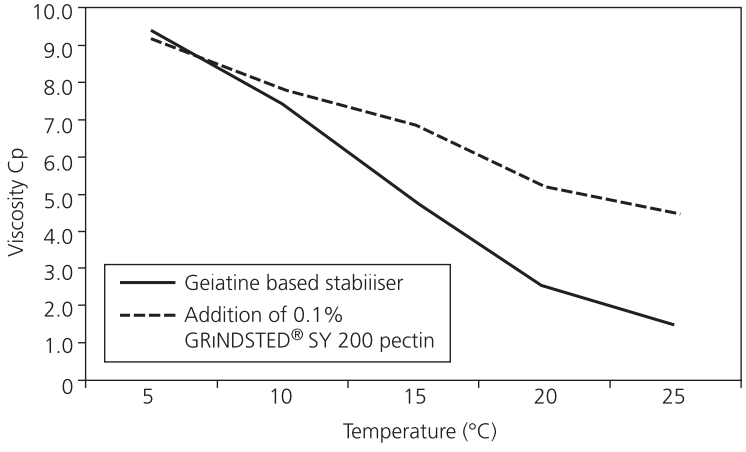

Fig. 5.13 Effect of pectin or gelatin on the viscosity of fermented milk drinks as influenced by different storage temperatures. With permission of Danisco A/S, Brabrand, Denmark.

to blend the stabiliser with the sugar at a ratio of 1 : 5. The Tetra Almix, for example, can be used to reconstitute the pectin because the pump wheel forces the stabiliser into the water in the mixing tank through a perforated stator; this physical action increases the wettability and reduces the formation of hard particles of pectin(s).

Another method of rehydrating the pectin is to prepare a saturated sugar solution (65 g 100 g^{-1}) into which the stabiliser is dispersed (up to 10 g 100 g^{-1} sugar solution). It is not recommended to prepare a higher concentration of pectin because the sugar–stabiliser solution will be very viscous for the processing equipment to handle. In low-pH drinking yoghurt the typical concentration rate of pectin addition is 0.3–0.4 g 100 g^{-1} of yoghurt, which is mainly dependent on the protein content in the product.

The stabiliser solution can be mixed with the yoghurt either in the incubation or the buffer tanks. Irrespective of where the pectin solution is added to the yoghurt base, it is important that the stabiliser is well mixed with the coagulum by means of an adapted agitator in order to make sure that the pectin makes proper contact with the protein(s). However, the water used for rehydrating the pectin will decrease the dry-matter content in the yoghurt base, and this effect has to be considered when fortifying the milk base before the production of fermented milks.

5.5.3 *Cooling*

Although the warm yoghurt could be pumped direct to the PHE for heat treatment, this method of production, especially when using large incubation tanks, is not recommended, for two reasons. First, a large-capacity PHE and packaging machine would need to be installed and this can increase the processing plant cost, and second, a long time would be required to empty the large incubation tanks, which can affect the level of acidity of the product (i.e. the first yoghurt leaving the tank will be high in pH compared with the product last leaving the tank). To avoid such differences in acidity and consistency of the product, it is most common to have an

intermediate cooling of the yoghurt. The cooler capacity is adapted to the cooling time needed for the tank volume.

The yoghurt is cooled in a heat exchanger to around 20°C and fed to an intermediate buffer tank. An incubation tank is normally emptied and cooled within 30 min in order to maintain uniform product quality. This time is, however, dependent on the starter culture used and its rate of acid development. Emptying and cooling of a tank can also take longer when using a mild acid starter culture or using a starter culture that requires a long fermentation time.

5.5.4 *Addition of sweetening agents and fruit juice concentrate*

The yoghurt drink may be sweetened (with sucrose, fructose, glucose, sucralose or glycerol), and the agent can be added direct to the yoghurt base separately or mixed with the stabiliser. Fruit juice concentrate is often used as a flavouring/aroma ingredient, and the amount added including the sweetening agent is dependent on factors such as: (a) the concentration level and the type of fruit used; (b) the anticipated consumer acceptability of sweetness – for example, in some countries a sweet product is preferred, while other consumers may prefer a more acidic drinking product; (c) the acidity of the yoghurt base, i.e. a smaller amount of fruit is added to a low acidity product compared with a high acidity yoghurt in order to obtain the desired flavour in the product.

Either the fruit concentrate can be added to the yoghurt base before the final heat treatment or an alternative is to heat treat the fruit concentrate separately and add it aseptically to the heat treated yoghurt drink before filling. However, some dairies are using a concentrated liquid aroma that can be natural, nature identical or artificial in order to enhance the added fruit flavour for the production of flavoured drinks. The aroma is dissolved in an alcoholic solution like ethanol.

Fruit concentrates and aromas can be mixed in-line with the yoghurt base before the final heat treatment. In some cases, the added aroma may be heat sensitive and is dosed aseptically into the product after the heat treatment stage. Different approaches can be used to dose the aroma solution into the product. For example, the aroma solution may be sterilised by filtration before adding it to the product aseptically (Fig. 5.11); or a sterilised aroma solution that has been packaged aseptically in a flexible container (e.g. the FlexDose™ system) can be used. In the latter approach, the sterilised aroma solution can be dosed into the product before packaging the product in cartons, and the dosing station consists of a peristaltic pump and weighing balance. A sterile flexible tube fitted with a special connecting device and a sterile needle are used to inject the solution aseptically from the dosing chamber (Fig. 5.14).

5.5.5 *Final treatments of the fermented milk drink*

As mentioned elsewhere, the yoghurt base (plus the added ingredients) is heated at temperatures ranging between 75 and 110°C for a few seconds. In combination with the heat treatment of the yoghurt drink, the product is also homogenised (upstream

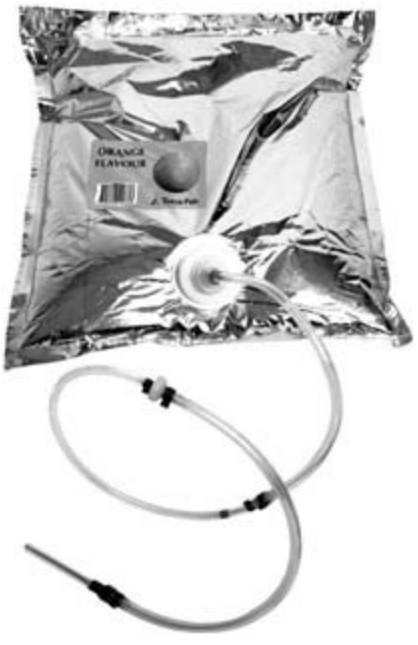

Fig. 5.14 Flavour dosing system. By permission of Tetra Pak AB, Lund, Sweden.

or downstream). Homogenisation will improve the stability of the product, for example, by enhancing the interactions of the pectin with the protein(s), and the recommended pressure is 20 MPa, which will give the optimal effect. If a lower homogenisation pressure is applied, sedimentation of the milk solids in the container can occur earlier during the storage period; however, the viscosity of the product will increase with decreased pressure.

Aseptic filling machines are normally used to package long-life drinking yoghurt (e.g. Tetra Brik® Aseptic cartons, 125–1000 mL), and detailed illustrations of such machines have been reported by Tamime and Robinson (1999).

5.6 Quality assessment

The shelf-life stability of fermented milk drinks can be determined using visual assessment, sensory profiling or analytical measurements. Although an indication can be obtained immediately after production, it is advisable to allow the product to rest under storage conditions for at least one day before analysis or assessment in order to ensure a proper equilibrium of the product constituents.

Although it is of interest of the manufacturer of fermented milk drinks to be able to predict the long-term shelf stability immediately after production, this can only be done using one or more of the following approaches.

5.6.1 *Stability*

The breakdown of a stabile protein system can be accelerated by exposure to mechanical stress during the manufacture of the product, for example centrifugation or increased storage temperature, and this can lead to sedimentation of the protein. A simple method to measure the sedimentation property is to weigh a small amount of product (e.g. 40–50 g) into a glass tube, and centrifuge the sample at 2800 *g* for 20 min at room temperature. After centrifugation, the supernatant is decanted, left upside down for 5 min and the sediment is weighed and recorded:

$$\% \text{ of sediment} = \text{weight of sediment} \times 100/\text{weight of sample}$$

In theory, the degree of sedimentation should be close to 0% in order for a drink to be physically stable throughout its shelf-life.

5.6.2 *Particle size*

As described earlier, the stability of low-pH fermented milk drinks is closely related to the level of stearic stability. This will ensure that the milk proteins will not aggregate, and the mean particle size is expected to be low. If fruit pulp is added to the product, this explains the presence of large particles (10–100 μm), which may be due to protein aggregates and may cause instability (Fig. 5.3).

The particle size of the product can be measured with a Malvern Mastersizer where light generated by a He-Ne laser is passed through the dispersed sample and is diffracted by the particles (Fig. 5.15). The diffraction angles, which are the intensities generated, are converted into a particle size measurement using the Mie

Fig. 5.15 The Malvern Mastersizer for measuring of particle size distribution. With permission of Danisco A/S, Brabrand, Denmark. Reproduced in colour as Plate 4, after page 110.

theory, which predicts all scattering by a particle of known diameter in response to light of a known wavelength (Rawle, 2001; Jones, 2003).

5.6.3 *Viscosity*

Viscosity measurements can be used to confirm the stability of the product. If the viscosity decreases continuously during measurement, it may be due to shear thinning caused by an unstable protein system. Furthermore, the viscosity measurement can be used to identify the optimal pectin concentration for a specific recipe and process. For example, the optimal concentration of pectin is when the viscosity of the product increases during storage, and the sedimentation percentage is very low or at a minimum level.

Basically, different types of rheometer can be used to measure the viscosity of fermented milk drinks when a constant shear is applied to the sample. For example, the Brookfield Viscometer uses a rotating spindle to transfer a constant shear rate to the sample, and the torque is recorded. To obtain a valid result, it is important to keep the sample volume, temperature, speed of rotation and the measuring time constant. In addition, this viscometer is provided with different types of spindle, which can be applied to a wide range of products, depending on their viscosity.

More advanced viscosity measurements and flow property readings of low-pH fermented milk drinks can be made with controlled stress and deformation rheometers (Fig. 5.16). The stress applied is so small that measurement is, in fact, performed on the undisturbed sample, i.e. within the linear viscoelastic region. The delay in displacement relative to the stress applied can be calculated into a film

Fig. 5.16 Stresstech CS rheometer for controlled stress viscosity measurement. With permission of Danisco A/S, Brabrand, Denmark and Reologica, Sweden. Reproduced in colour as Plate 5, after page 110.

rigidity (complex modulus) that can be split up into an elastic (G′) and viscous (G″) modulus. However, using the Bohlin VOR rheometer, a controlled deformation is applied and the force needed to do so (i.e. the stress) is measured.

Hence, the viscosity measurement can predict the stability of fermented milk drinks. If the viscosity remains unchanged at increasing shear rates, the low-pH product can be described as having Newtonian behaviour (Fig. 5.17a); fermented milk drinks that show approximate Newtonian behaviour are likely to be stable during the shelf-life of the product. Alternatively, if the viscosity decreases as the shear rate increases (Fig. 5.17b), the fermented milk drink can be described as having shear thinning behaviour, and it is likely to be unstable unless stabilised (see section 5.3.2).

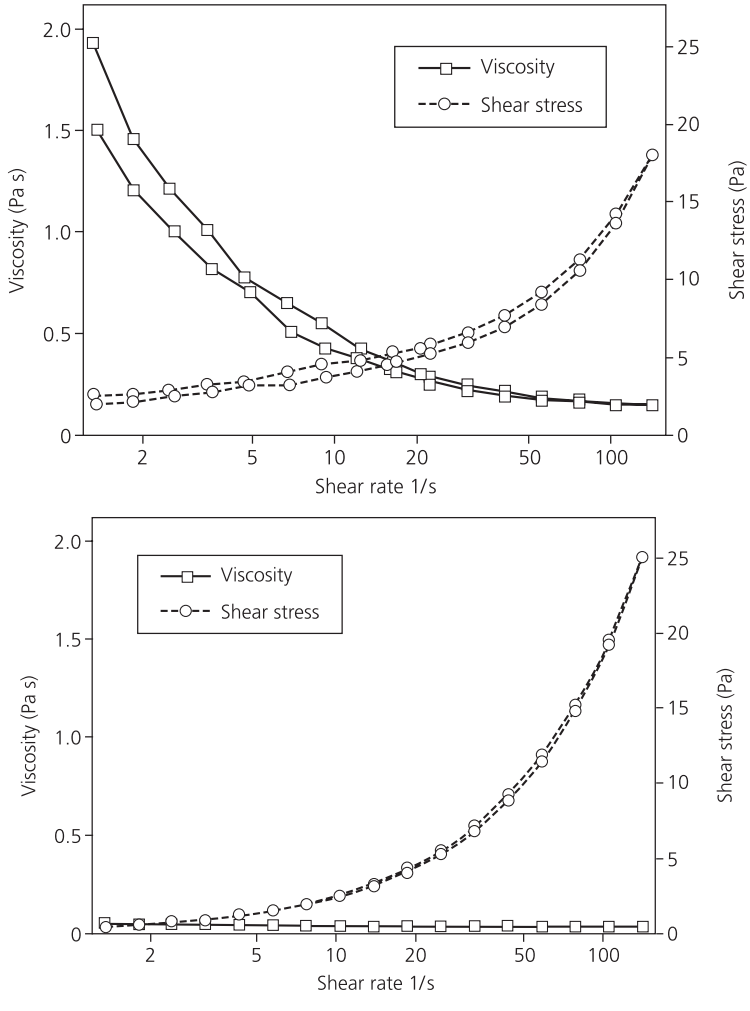

Fig. 5.17 Flow data of drinking yoghurt (a) with highly shear thinning properties and (b) with nearly Newtonian behaviour. With permission of Danisco A/S, Brabrand, Denmark.

5.6.4 *Zeta potential*

During the manufacture of long shelf-life fermented milk drinks, the casein gel is heated and homogenised after the fermentation period, which results in the formation of casein aggregates. These aggregates are unstable and will sediment. Nevertheless, the added pectin will bind to these aggregates, and the acidic groups in pectin will give the particles an increased negative charge. The resulting electrostatic repulsion decreases the tendency to sedimentation.

Zeta potential is a physical property that is related to the surface charge of any particle in suspension or emulsion. It is highly variable and dependent on, for example, pH, temperature or composition of the solution, and the zeta potential of a product is measured by micro-electrophoresis. The sample is placed in an electrophoresis chamber consisting of two electrode compartments and a connecting chamber. A voltage is applied between the two electrodes and produces a uniform electric field in the connecting chamber. The charged particles respond by moving towards one or other electrode, depending on the particle charge. To avoid electro-osmosis the measurements are made in the stationary layer. The speed of the particles is directly proportional to the magnitude of the particle charge or the zeta potential. The zeta potential in most colloidal dairy systems is quite low, and stearic stabilisation by the milk proteins is a dominant factor.

5.6.5 *Sensory profiling*

Sensory profiling of fermented milk drinking products by a trained sensory panel combined with a well-designed statistical model to evaluate the data (e.g. two-factor ANOVA) is a very strong tool to illustrate how consumers perceive the finished product. A typical scheme for sensory assessment is shown in Table 5.3 and, as a consequence, the results can be illustrated with a star diagram comparing different samples (Fig. 5.18).

5.7 Miscellaneous fermented milk drinks

5.7.1 *Background*

The relevant published data on drinking yoghurt and other related dairy beverages were reviewed by Tamime and Robinson (1999), and Mann (2002, 2004) has published an update of the technological and scientific aspects of these products. The role of pectin in fermented milk drinks and beverages is as follows: (a) pectin increases viscosity and cloudiness of the product; (b) it stabilises the protein; (c) possible use in dietary fibre-enriched products (Endress & Mattes, 2001). Drinking yoghurt can be fortified with iron and vitamins, for example by microencapsulation of ferric ammonium sulphate and vitamin C with polyglycerol monostearate as a coating material; the highest efficiencies of microencapsulation of iron and vitamin C were 73% and 95%, respectively (Kim *et al*., 2003).

Table 5.3 A sensory scheme for the evaluation of milk-juice drinks, which can be used for fermented milk drinks.

Instruction/attribute	Explanation	Rating scale 1 → 8
Pour approximately 10 ml of sample into the mouth and distribute		
Viscosity (immediate observation)	Work the sample with the tongue; if the sample is viscous and difficult to move, it is thick = 8 or is perceived as thin = 1	Thin → Thick
Notice immediately after swallowing		
Flouriness	Press the tongue against the palate; if the sample feels floury = floury = 8 or completely without particles = smooth = 1	Smooth → Floury
Pour approximately 10 ml of sample into the mouth		
Structure	Press the tongue against the palate; if the sample is difficult to disintegrate with the tongue = long = 8 or is easy to disintegrate = short = 1	Short → Long
Watery note	Work the sample with the tongue, but ignore the viscosity; if the sample demonstrates any resistance at once = watery = 8 or feels soft = creamy = 1	Creamy → Watery
Please rinse your mouth! Pour 10 ml of sample into the mouth, distribute and swallow		
Clean eating	Notice the mouth and throat after swallowing; if nothing is felt in the mouth and throat = clean eating = 8 or a remnant of the sample in the mouth or throat = sticky = 1	Sticky → Clean
Desiccation	Evaluate the throat, oral cavity and teeth 10–15 s after swallowing the sample; if drying out is felt = desiccating = 8 or no effect is felt = 1	Nothing → Desiccating

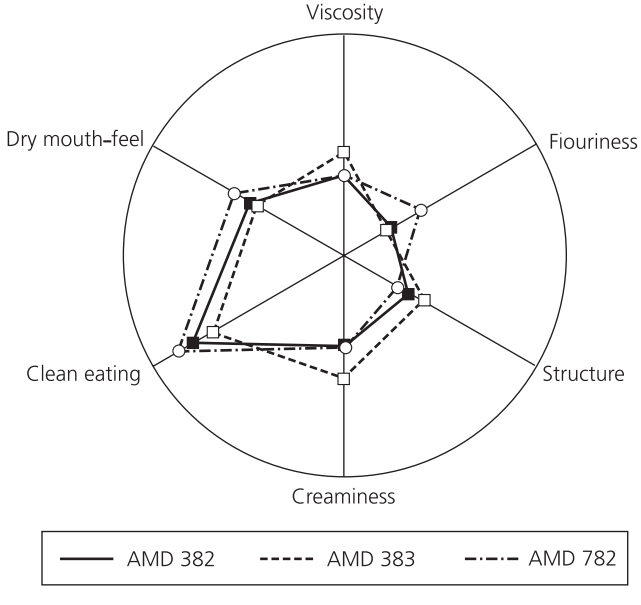

Fig 5.18 Star plots for the effects of three different types of pectin on the perceived texture attribute of a milk-juice drink. The product contained 1.7 g 100 g^{-1} milk solids-not-fat (SNF), pH 3.9 and pectin dosage rate at 0.4 g 100 g^{-1}. With permission of Danisco A/S, Brabrand, Denmark.

In some countries (e.g. in the Middle East and Brazil), the most popular method for the production of drinking yoghurt is to mix natural yoghurt with water, cheese whey or the whey obtained during the production of strained/concentrated yoghurt (M. Ghandour, personal communication; de Almeida *et al.*, 2001, 2002; see also Minkova, 2001; Oliveira *et al.*, 2002; Penna *et al.*, 2003). Some well-known examples of diluted drinking yoghurt follow.

5.7.2 *Ayran*

This is a Turkish fermented drinking yoghurt, with a chemical composition (g 100 g^{-1}) of total solids 9.5, SNF min. 8.0, full-fat 1.5 and salt max. 1.0 (Akin & Rice, 1994). Ayran is a fresh product that has a limited shelf-life, and the traditional method for the manufacture of full-fat ayran is very similar to yoghurt making. The fat in the milk is standardised and the SNF fortified (3.0 and 9.0 g 100 g^{-1}, respectively), and the milk is then heated to 85ºC for 30 min or 95ºC for 5–10 min, pre-cooled to 60ºC and homogenised at ~15 MPa of pressure, cooled to 40–45ºC, inoculated with a yoghurt starter culture at a rate of 1–4 mL 100 mL^{-1} and incubated in bulk to pH 4.5–4.7. The fermentate is cooled to 10ºC, mixed well with drinking water at a rate of 30–35 mL 100 mL^{-1} and salt (1.0 g 100 g^{-1}), packaged and stored at 5ºC (Akin & Rice, 1994).

Alternatively, ayran can be produced by pre-heating the standardised milk to 60–70°C for homogenisation upstream at 15–20 MPa. Then the milk is heated to 90–95°C for 5 min, cooled to 40–45°C, inoculated with a yoghurt starter culture and fermented to the desired pH. The fermentate is cooled and mixed well with 30–50 mL 100 mL^{-1} of drinking water and salt (0.5–1.0 g 100 g^{-1}) before packaging and storage at 5°C.

Ayran has a short shelf-life, and a long-life product for ambient distribution has recently been developed; the manufacturing stages are identical to those for the production of long-life yoghurt drink. The addition of salt should be as late as possible in the process to minimise the risk of corrosion of the equipment. In addition, salt may have a negative influence on the effect of the stabiliser, and the amount of added water and salt can affect the rheological properties of ayran (Kökosy & Kiliç, 2003). The yoghurt (pH < 4.2) and stabiliser (e.g. pectin) are thoroughly mixed before final homogenisation and heat treatment. The salt solution is sterilised by filtration, for example using Tetra Aldose™, and added aseptically to the product before aseptic filling. Alternatively, the sterile salt solution in a bag may be added aseptically to the ayran in a similar manner to aroma dosing using the FlexDose™ (Tetra Pak Arom Pak AB; Fig. 5.14; see also Fig. 5.11).

5.7.3 *Dough*

This is an Iranian fermented and diluted yoghurt drink, which is manufactured in a similar process to ayran. The product may be salted, but no sugar or flavouring ingredients are added. Tradition and the current consumer preference in Iran avoid the need to stabilise the product, and sedimentation of the SNF in retail containers and whey separation is expected. The consumer shakes the product before con-

sumption. Recently, dough has been carbonated to produce a fizzy variant of the traditional product.

5.7.4 *Carbonated yoghurt drinks*

Drinking yoghurt (plain and sweetened and flavoured types) can be carbonated to affect the mouth-feel of the product. However, carbonated yoghurt can also be manufactured in a dry form (Tamime & Robinson, 1999). The latter type is not commercially widely available and will not be reviewed. Advances in the scientific field are limited and recipes of carbonated yoghurt technology are scarce. Recently, sweetened low-fat and flavoured probiotic yoghurt (i.e. *Lactobacillus acidophilus* and *Bifidobacterium longum*) was made using cream, skimmed milk powder, sugar, stabiliser (a blend of modified starch, carrageenan and pectin) and skimmed milk. The cooled product was carbonated by incorporating carbon dioxide (to achieve 0.08–0.09 kg cm^{-2} of pressure in the product, by adding 1 g CO_2 kg^{-1} of product), and stored up to 45 days at 4ºC. Such treatment did not affect the sensory properties and consumer acceptability of the product (Karagül-Yüceer *et al.*, 1999; see also Karagül-Yüceer *et al.*, 2001; Gueimonde *et al*., 2002; Taylor & Ogden, 2002; Wright *et al*., 2003).

In principle, the production line of carbonated long-life drinking yoghurt is, in many ways, similar to that normally used for the manufacture of long-life drinking yoghurt. The only difference is the addition of a gas injection unit, which is located before the packaging equipment. One such example is the continuous in-line carbonation module; it consists of a specially designed mixer that creates micro-bubbles and ensures total dissolution of the gas in the yoghurt. The CO_2 gas is injected into the cold yoghurt after the final heat treatment of the product, and the gas level is ~1 g CO_2 kg^{-1}. The product may be packed aseptically in PET plastic bottles or in paperboard cartons. It is important to use a packaging material that is impermeable to CO_2 otherwise the gas will diffuse through the package very quickly.

Although long-life carbonated drinking yoghurt can be stored at ambient temperature for several months, extended storage at high temperatures can cause the solubility of the gas in the product to decrease, and some gas will form a headspace in the package. Depending on the amount of gas added and the storage temperature, there is also a risk that the shape of the package may bulge due to increasing gas pressure. If this should occur, the product is re-cooled and the package shaken in order to reincorporate the free carbon dioxide into the yoghurt. Fresh drinking yoghurt can also be carbonated.

5.8 Conclusion

Over the past couple of decades, many different varieties of stirred-type yoghurt have been launched on markets worldwide, and fermented milk drinks, especially yoghurt-based products, have been well received by consumers.

Drinking yoghurts have been classified in different ways, such as the viscosity of the product, storage temperature and/or viability of the starter culture. Most of these

products have to be stored, distributed and retailed at < 5ºC, but a post-fermentation heat treatment helps to prolong the shelf-life; in some countries, they may not be designated as 'yoghurt', since the application of heat inactivates the starter culture bacteria as well as other contaminants (e.g. yeasts and moulds). As a consequence, the long shelf-life products should be designated as yoghurt-based drinks.

It is evident that the technology for the manufacture of fermented milk drinks is very similar to the production of stirred yoghurt. The technical aspects (e.g. processing equipment, mechanical handling of the coagulum and plant design) and the scientific guidelines (e.g. standardisation of the fat, fortification of the SNF and starter cultures) that are commonly used for the production of stirred yoghurt may, in part, also be applicable for the production of fermented milk drinks. However, the rheological property of stirred yoghurt (i.e. viscosity) is one of the important aspects regarding the quality of the product. The low viscosity of fermented milk drinks is a desirable feature; however, some precautionary measures have to be considered to stabilise the protein, i.e. prevent sedimentation or syneresis.

In a production line specially designed for the manufacture of fermented milk drinks, it is possible to control the viscosity of a product containing a high viable count of the yoghurt organisms by, for example, post-fermentation homogenisation of the coagulum. In addition, the use of stabilisers will enhance the stability of the fermented milk drink (fresh and heated), improve the mouth-feel of the product and prevent sedimentation of the protein.

Future developments in the production of fermented milk drinks will focus on product formulation, automation of the process, blends of different starter cultures, including the use of probiotic micro-organisms, and new stabiliser blends. In addition, these products are normally consumed direct from the packaging container, and future developments in package design should not be overlooked.

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Fermented Milks
Edited by Dr Adnan Tamime

6 Production of Concentrated Products

B. Özer

6.1 Introduction

Converting milk into a fermented milk product by lactic acid fermentation is one of the oldest methods employed for the preservation of milk (Atamer *et al*., 1988; Tamime & Robinson, 1988). Although yoghurt has many desirable properties and longer keeping quality if it is stored and retailed at < 5ºC, it still tends to spoil within a very short period of time at ambient temperature. This fact stimulated efforts to develop simple and efficient techniques in order to extend the keeping quality of yoghurt even further.

One method developed was to leave plain yoghurt hanging in the animal skin for some length of time (Tamime & Robinson, 1999). Since some of the milk serum was absorbed by the animal skin into which yoghurt was poured, and some of the whey passing through the skin would have been lost by evaporation, the resulting product was concentrated, strained, acidic and had a longer storage time due to the high level of lactic acid (Özer, 1999; Tamime & Robinson, 1999). The final characteristics of the resulting concentrated product were remarkably different from the initial yoghurt. For example, higher total solids (~ 25 g 100 g^{-1}) and acidity (lactic acid > 2.0 g 100 g^{-1}) made this product more resistant to microbiological deterioration. As a consequence, the nomadic people were attracted to manufacture such a fermented milk product, milk being their main source of nourishment (Tamime & Robinson, 1999).

Today, evaporation and drying techniques are still being applied in order to extend the lifetime of fermented milks. Many types of concentrated fermented milks are made in many countries and, although these products have different local names, in practice they are similar (Table 6.1). Torba or süzme (concentration by means of animal skin or cloth bag) and kurut (dried yoghurt) in Turkey, tan or than (concentration by pressing) in Armenia, leben zeer (concentration in earthenware vessels) in Egypt, and labneh or lebneh (concentration by means of a cloth bag) in most Arab countries are some of the well-known traditional concentrated fermented milk products. Additionally, chakka and shrikhand in India, skyr in Iceland and ymer in Denmark also have a place among such types of fermented milks (El-Gendy, 1983; Abou-Donia, 1984; Tamime & Robinson, 1988). The production and quality control aspects of skyr and ymer are detailed in Chapter 7, and further reading regarding these products (Tamime *et al.*, 2001; Tamime & Robinson, in press) is recommended.

Table 6.1 Synonyms for concentrated/strained yoghurt in different countries.

Traditional names	Origins
Labneh, labaneh, lebneh, labna	Eastern Mediterranean
Laban zeer, leben zeer	Egypt, Sudan
Torba, süzme	Turkey
Tan, than	Armenia
Stragisto, sakoulas, tzatziki	Greece
Syuzma	Russia
Mastou, mast	Iraq, Iran
Basa, zimme, kisela, mleko-slano	Yugoslavia, Bulgaria
Itiitu	Ethiopia
Greek-style	United Kingdom
Chakka, shrikhand	India
Ymer	Denmark
Skyr	Iceland

Data compiled from Azimov (1982), Tamime & Crawford (1984), Tamime & Robinson (1988, 1999), Kassaye *et al.* (1991), Kurmann et al. (1992), Akin & Rice (1994) and Özer (1997).

Labneh or strained yoghurt, a fermented milk product popular in the Middle East, has been defined as a semi-solid product derived from yoghurt by straining away part of its water and water-soluble components, mainly lactose and salts, using a cloth bag (Dagher & Ali-Ghariebeh, 1985; El-Samragy *et al*., 1988a; Yamani & Abu-Jauber, 1994; Özer, 1997; Nergiz & Seckin, 1998; Anon., 1999). Different types of milk such as cow's, buffalo's, sheep's and goat's milks can be used in the manufacture of labneh (Tamime *et al*., 1991a; Özer, 1997; Mehaia & El-Khadragy, 1999). Although cow's milk is widely used, sheep's and goat's milks are usually preferred for the production of labneh in hot climatic regions where cattle breeding is not favoured. Labneh should have a milky white colour when made from goat's milk to slightly yellow when made from recombined or cow's milk; it has a soft and smooth body, good spreadability and no wheying off (Mahdi, 1990).

In the Middle East, labneh is a daily staple food in most Arab households and is usually consumed as a sandwich spread, particularly for local breakfast meals. In Turkey it is used in the preparation of soups, as a yoghurt drink (ayran) or as a dressing; in the UK this product is marketed under the name of Greek-style yoghurt. Labneh is currently only produced as a plain product. However, considering the high market share of fruited and flavoured yoghurts in the USA and Europe, it may well be possible to produce sweetened and/or fruit-flavoured labneh to increase the popularity of concentrated yoghurt in these countries. The thick consistency of labneh makes it a good carrier for a variety of fruits and vegetables (Salji, 1991).

6.2 Processing techniques

Today, the production of plain yoghurt is carried out under controlled conditions using low-fat or whole milk, which is inoculated with yoghurt starter cultures consisting of a blend of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus*

thermophilus, and the labneh is still made by the traditional method using a cloth bag. However, in some parts of the Middle East (i.e. in Lebanon, Saudi Arabia and Egypt), UK and Australia, the mechanical centrifugation of skimmed milk yoghurt (Kharazzi, 1984; Dagher & Ali-Ghariebeh, 1985; Kehagias *et al*., 1992; Robinson & Tamime, 1993) or direct reconstitution/recombination techniques (Tamime & Robinson, 1999) are currently being used to make labneh. Recently, the use of membrane technology including ultrafiltration has been studied extensively (El-Samragy & Zall, 1988; Tamime *et al*., 1989a, 1991b; Özer *et al*., 1997a, 1998a, b, 1999a), but industrial application of this technique for the production of concentrated yoghurt is rather limited. At present, processing practices for concentrated yoghurt vary with locality.

6.2.1 *Traditional method (cloth bag)*

The traditional method of making concentrated yoghurt (i.e. home, rural or small scale) is achieved by straining cold natural yoghurt using a cloth bag (or parzin in Turkey), animal skin or earthenware vessel (Tamime, 1977; Tamime & Robinson, 1978; Hamad & Al-Sheik, 1989; Atamer *et al.*, 1993). The basic principle of using the traditional cloth bag method is to extract water from plain yoghurt until the desired total solids level has been reached (Fig. 6.1). The main drawbacks of this method are the unhygienic condition(s) during the straining stage, and the process is labour intensive (Tamime *et al*., 1989a, 1991b; El-Samragy, 1997; Özer, 1997). In addition, the yield of labneh is reduced due to adherence of yoghurt to the cloth bag

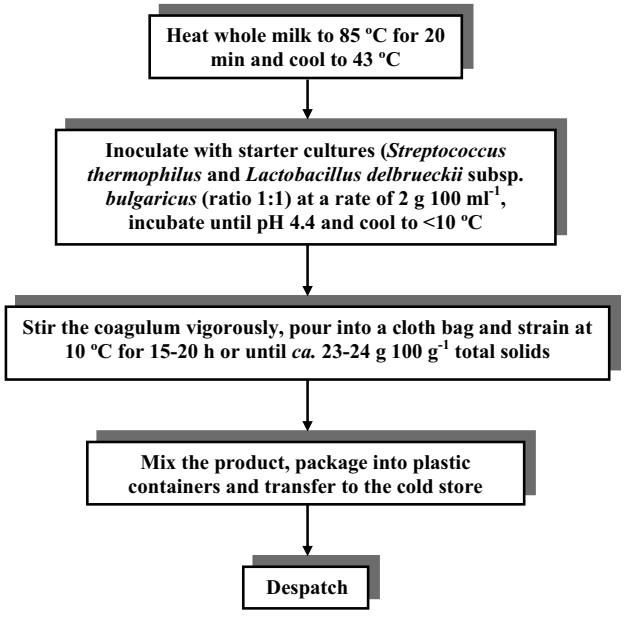


Fig. 6.1 Generalised scheme illustrating the different stages of the production of traditional labneh.

during drainage. However, despite these disadvantages, such method of production is still preferred in some Middle Eastern countries as the investment in mechanised system(s) of production is rather high. The duration of drainage for yoghurt in cloth bags takes about 15–20 h at <10ºC. The whey separation can be achieved either by gravity drainage (small scale production) or by pressing (large scale production, i.e. by piling 25-kg bags on top of each other); however, the drainage time can be shortened up to 6 h by applying pressure of 2 kg kg^{-1} of yoghurt (Abou Donia *et al*., 1992).

A number of factors can interfere with the drainage period and the quality of the final product after drainage, such as total solids content of the milk base for the yoghurt and drainage temperature. There is a strong relationship between the yield of labneh and the total solids content of the initial plain yoghurt; the yield increases as the level of total solids of the yoghurt increases (Hamad & Al-Sheik, 1989), and the optimum starting total solids level for a good-quality labneh is reported to be 16 g 100 g^{-1} (Tamime & Robinson, 1978; Gilles & Lawrence, 1981; Jensen & Nielsen, 1982; Hamad & Al-Sheik, 1989). The yield of labneh is also strongly dependent on the type of milk used in the manufacture and, according to Giannoukou *et al*. (1992), the highest yield of strained yoghurt was for sheep's milk followed by goat's and cow's milks. Shaker *et al*. (2002) reported that a decrease in curd pH at the draining stage led to a significant increase in viscosity and a decrease in yield of the product. In some countries, due to an insufficient number of cold rooms in a factory, it is common practice to concentrate the yoghurt at ambient temperature. In general, higher drainage temperature leads to a lower yield (Hamad & Al-Sheik, 1989) and, as a consequence, the labneh is more acidic and the structure of the product is weak (Özer, 1997).

The effects of various strains of starter culture bacteria used for the manufacture of labneh on the drainage period and on the quality of the final product were studied in detail (see Tamime & Robinson, 1999). The studies showed that ropy and/or exopolysaccharide (EPS)-producing strains prolonged the drainage period up to 47 h compared to 14 h using normal yoghurt starter cultures.

A traditional labneh should contain total solids 24–25 g 100 g^{-1}, protein 8–9 g, fat 10 g, lactose 4.5–5.0 g and minerals 1 g 100 g^{-1} (Özer, 1997). In general, the cloth-bag filtrate contains almost exclusively lactose and minerals. The retention of fat and protein is high, but slight losses of crude protein (0.2–0.3 g 100 g^{-1}) may occur; additionally, some amino acids and peptides liberated by the starter culture bacteria may pass through the cloth bag into the filtrate (Özer, 1997). The overall chemical composition of plain yoghurt and traditional labneh is given in Table 6.2. Labneh contains double the protein content and three times more fat than yoghurt. The reduction in lactose could be an important advantage for people who suffer from lactose intolerance. Moreover, labneh can be digested more easily than standard milk, and its consumption is recommended for people who suffer from digestive problems (Mahdi, 1990).

Apart from plain concentrated yoghurt, other yoghurt-derived concentrated foods are also popular in some countries. For example, shrikhand is a whole-milk product native to India, which deserves special attention since this product shows some dis-

Table 6.2 Compositional quality (g 100 g^{-1}) of traditional labneh and natural yoghurt.

Product	Total solids	Protein	Lactose	Fat	Ash	Calories[1]	Source
Labneh	23.3	8.0	5.1	9.2	0.79	ND	Özer *et al.* (1997a)
	25.5	10.5	4.0	10.2	1.80	161	Hofi (1990)
	26.1	10.3	3.6	10.0	0.71	ND	Hofi (1988)
	25.3	9.1	3.7	11.9	0.60	ND	Tamime *et al*. (1991c)
	22.3	7.7	4.5	9.3	0.88	ND	Tamime *et al*. (1991c)
	24.3	8.3	4.3	11.1	0.69	ND	Tamime *et al.* (1989a)
Yoghurt	11.6	3.8	4.3	3.0	0.95	59.0	Hofi (1990)
	13.9	4.8	4.5	3.8	0.86	73.7	Rasic & Kurmann (1978)
	12.0	3.3	4.8	3.1	0.92	62.0	Rasic & Kurmann (1978)

1 kcal per g of yoghurt.
ND Not determined.

similarities to plain concentrated yoghurt. Shrikhand is a semi-soft sweetish-sour milk product prepared from lactic fermented curd called dahi (Jagannath *et al*., 2001). The curd is partly strained through a cloth bag to remove the whey, producing a solid mass called chakka. This product is white to pale yellow in colour, semi-solid with a good texture and uniform in consistency. Chakka may be made by draining off the whey from buffalo's, cow's, skimmed, recombined or standardised milk fermentate (Atreja & Deodhar, 1987). Alternatively, partial or full replacement of cow's and buffalo's skimmed milk with sweet cream buttermilk can be employed during the production of chakka (Karthikeyan *et al*., 2000, 2001), or chakka can be fortified with sour whey concentrate up to 5 g 100 g^{-1} (Giram *et al*., 2001) resulting in an end-product with good textural and sensory properties. The product should be mould-free, and show no signs of fat or water seepage; however, the addition of gelatin (excluding sodium alginate) or Gelodan® and nisin to the fermented concentrate enhances the quality and shelf-life of the product (Sarkar & Misra, 2002). Chakka is mixed with the required amount of sugar, flavouring agents such as saffron, nuts and cardamom to produce shrikhand (Fig. 6.2). Shrikhand made from buffalo's milk with initial total solids of 15 g 100 g^{-1} was reported to be superior to that made from cow's or goat's milk (Karthikeyan *et al*., 1999; see also Agnihorti & Pal, 1996). In addition, the yield of shrikhand was greater with buffalo's milk followed by cow's and goat's milks (Subramonian *et al*., 1995). Shrikhand contains most of the total solids of dahi including the protein, fat and only part of the whey, which contains some lactose, lactic acid and water-soluble vitamins (Boghra & Mathur, 2000).

The added flavour(s), aromatic spices, dry fruits and nuts increase the calorific values of the product, and shrikhand should meet the following specification (g 100 g^{-1}):

- total solids > 58
- milk fat (on dry basis) > 8.5
- milk protein (on dry basis) > 9
- titratable acidity (as lactic acid) < 1.4
- sugar (i.e. sucrose – on dry basis) < 72.5
- total ash (on dry basis) < 0.9

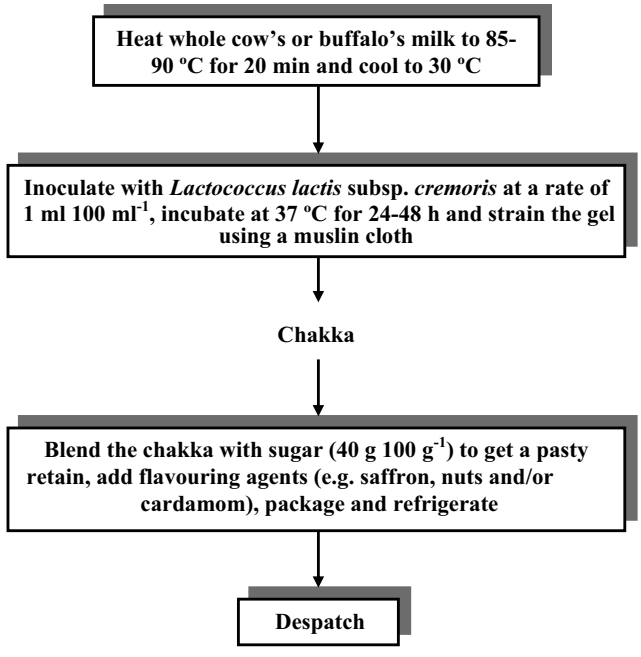


Fig. 6.2 Schematic illustration of the manufacturing stages of traditional shrikhand.

In order to reduce fat losses in the whey during preparation of chakka or shrikhand, the milk has to be homogenised before the heat treatment and the fermentation stages (Desai *et al.,* 1985; Patel & Chakraborty, 1988). Traditionally, shrikhand is packed in earthenware containers and, in large-scale production, in plastic or plastic-coated cups; the recommended storage temperature for shrikhand is < 10°C (Patel *et al*., 1993; see also Jain *et al.*, 2001).

6.2.2 *Centrifugation method (mechanical separation)*

Different mechanical separators are employed in the manufacture of concentrated yoghurt (Kharazzi, 1984; Dagher & Ali-Ghariebeh, 1985; Robinson & Tamime, 1993; Tamime, 1993, 2003; Tamime *et al.*, 2001). Nozzle or quark (low-fat curd cheese) separators have been used successfully for the manufacture of labneh, with physical and organoleptic properties of the resulting product similar to traditional labneh (Fig. 6.3) (Salji *et al*., 1983, 1987; Tamime & Robinson, 1999). The production of labneh made by centrifugation is described as a two-step procedure (Salji, 1991; Özer, 1997). First, fermented skimmed milk is stirred vigorously, heated up to 55–60°C and cooled to 40°C. Next, any large clots or clumps are removed by passing the fermentate through a metal sieve; it is concentrated to about 18 g 100 g^{-1} solids, cooled to below 15°C and blended with any source of fat or cream to provide the desired level in the finished product (Mortensen, 1995).

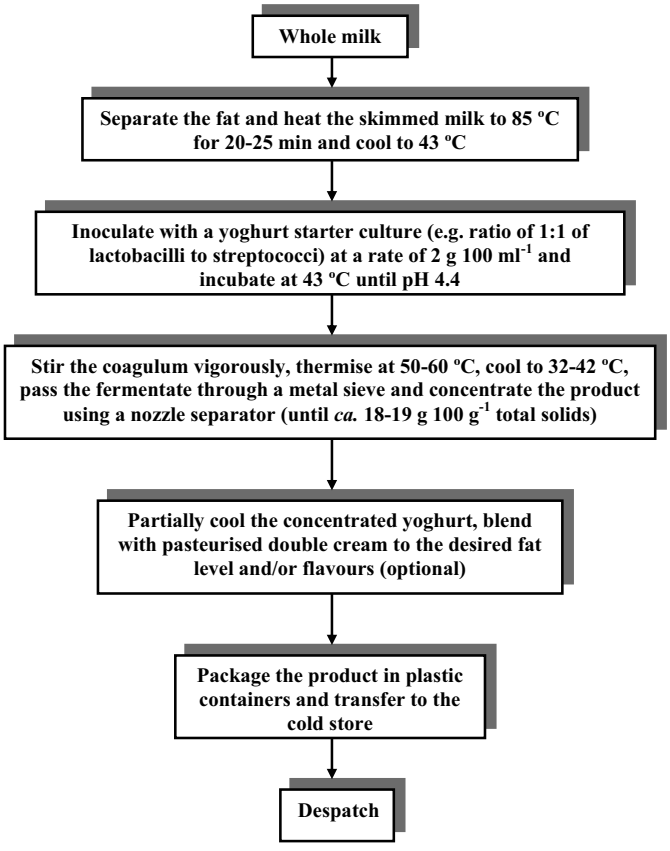
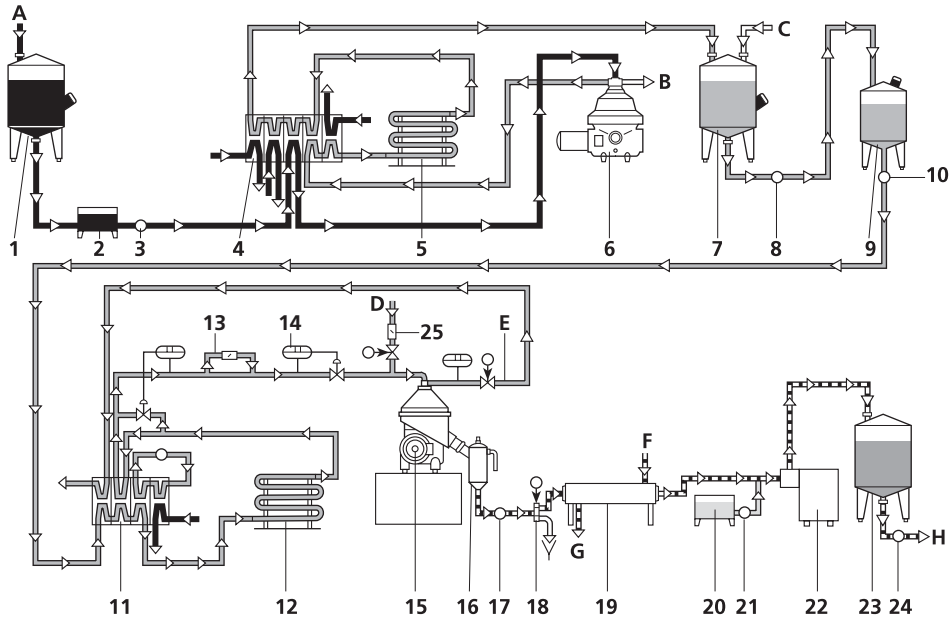


Fig. 6.3 Production of concentrated yoghurt using the nozzle separator method.

If fermented whole milk is used in the manufacture of labneh, the fat globules may block the nozzle opening. With the recent developments in the design of separators, it has become possible to use whole milk in the production of concentrated yoghurt (Lehmann *et al*., 1991). Figure 6.4 shows a layout for the production of concentrated milk products with equipment from GEA-Westfalia Separator AG. Basically, the process includes mechanical agitation of the fermented milk, separation of the required level of the liquid phase by means of a nozzle separator (Fig. 6.5) and blending the concentrated product with pasteurised cream. In this process, the feed control valve ensures a constant flow of fermented milk to the separator, which is necessary to obtain a uniform composition of the concentrated product at the outlet (Lehmann *et al*., 1991).

In order to improve the yield of concentrated yoghurt, a thermisation method was developed by Tetra Pak Dairy & Beverages Systems AB (Fig. 6.6). According to this method, the fermented and homogeneous skimmed milk is heat-treated at 55–58°C for 0–3 min. The thermisation stage will release some whey from the milk gel to facilitate mechanical separation, and may inactivate most of the yoghurt bacteria. Thermisation time–temperature relationship should be selected carefully in order



1 Raw milk tank with agitator
2 Balance tank
3 Centrifugal pump
4 Plate heat exchanger
5 Holding tube
6 Skimming separator
7 Renneting tank for cheese milk with agitator
8 Centrifugal pump
9 Balance tank with agitator
10 Centrifugal pump
11 Plate heat exchanger
12 Holding tube
13 Double strainer (reversible)
14 Feed control
15 Soft cheese separator
16 Soft cheese hopper
17 Positive displacement pump
18 Reversing valve
19 Soft cheese cooler
20 Cream tank
21 Mono pump
22 Soft cheese mixer
23 Soft cheese silo
24 Positive displacement pump
25 Tubular strainer
A Raw milk feed
B Cream discharge
C Culture and rennet inoculation
D Water inlet
E Whey discharge
F Ice water inlet
G Ice water outlet
H To packing

Fig. 6.4 Illustration of a typical thermo-soft cheese plant that can be used for the production of concentrated yoghurt. By permission of GEA-Westfalia Separator AG, Oelde, Germany.

to avoid running into problems such as hardening of the protein particles formed by high heat treatment. To improve the shelf-life and yield of the concentrated yoghurt even further, following mechanical separation the product is heat-treated at 70°C. In this way, yeasts and moulds are inactivated as well as yoghurt bacteria (Nilsson, 2000; Tamime *et al.*, 2001).

There is a strong relationship between the pH at the end of the incubation period and the yield and firmness of strained yoghurt obtained by centrifugation. A pH range of 4.1–4.3 for the end of incubation gives the highest yield and the firmest body (Kehagias *et al.*, 1992). In another process for the production of labneh, yoghurt is blended with 25–100 mL 100 mL^{-1} brine (3–12 g salt 100 g^{-1}), and the mixture is concentrated by means of a centrifugal separator (Kharazzi, 1984).

Other fermented dairy products that have been concentrated by using mechanical separators are skyr (in Iceland) and shrikhand (in India) (Mahdi, 1990). Shrikhand is now being produced on an industrial scale using the process line (Fig. 6.7) that was developed by the National Dairy Development Board of India (NDDB) (Gupta, 2000). In this process, fermented skimmed milk, which is made with classical yoghurt

Fig. 6.5 Production line for thermo-soft cheese showing a nozzle separator type KDB 30. By permission of GEA-Westfalia Separator AG, Oelde, Germany.

starter cultures, is centrifuged in a continuous quark separator to produce chakka. Cream, ground sugar and flavours are added to the concentrated fermentate, and the mixture is passed through a scraped surface heat exchanger to extend the shelf-life of the product, followed by packaging, cooling and despatch. Additionally, basket centrifuges and planetary mixers used by bakeries are also employed in the manufacture of shrikhand. Today, the volume of shrikhand manufactured by the dairy sector exceeds that of processed cheese sold in India (Gupta, 2000).

6.2.3 *Recombination/reconstitution method*

While information on the subject of recombined fermented milks in the literature is rather limited, there appears to be little doubt that labneh of satisfactory quality can be produced from recombined milk (El-Shibiny *et al*., 1979; Gilles & Lawrence, 1981; Özer, 1997; Tamime, 2003). The production of recombined concentrated yoghurt involves reconstitution of skimmed milk powder in water, and mixing it with anhydrous milk fat, stabiliser (optional) and salt (optional) (Tamime & Robinson, 1999). The concentrated yoghurt-making procedure is similar to the production of plain stirred-type yoghurt (an alternative procedure is similar to set-type yoghurt – see Chapter 3). Following the incubation stage, the product is packaged at ambient temperature and stored at 5°C until consumed. Replacement of milk fat with vegetable oil (e.g. sunflower oil) in the production of recombined labneh was reported by Hefnawy *et al*. (1992) and Taha *et al.* (1997), and they recommended that sunflower oil should be used to reduce the cholesterol level and decrease the production cost of labneh.

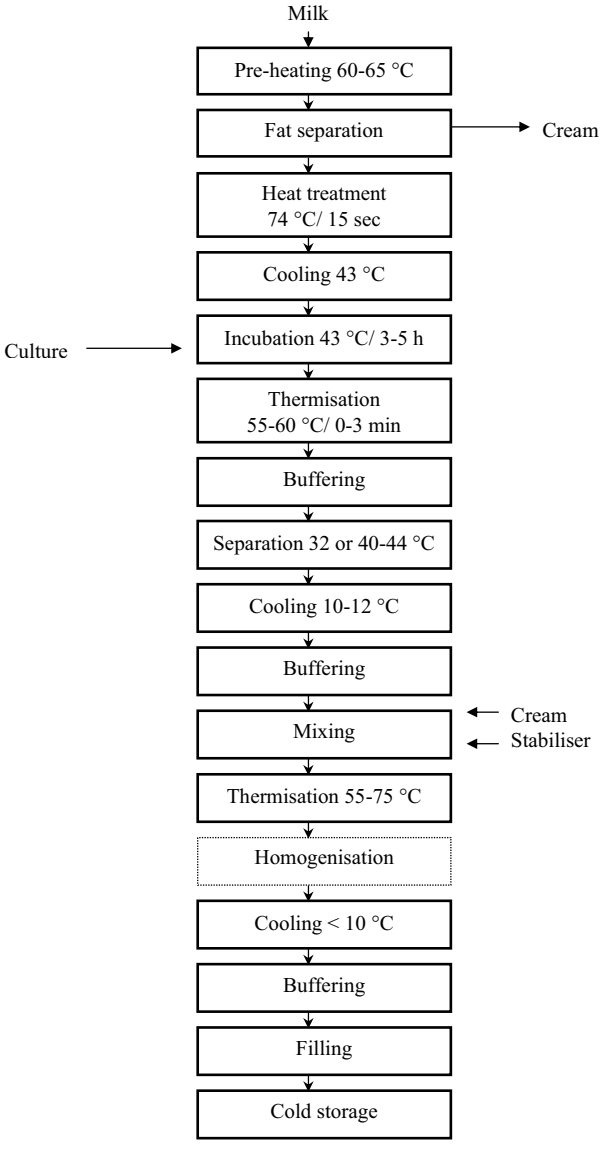


Fig. 6.6 Production of concentrated yoghurt with improved shelf-life. By permission of Tetra Pak AB, Lund, Sweden.

In order to eliminate the drainage stage during the manufacture of labneh, direct reconstitution of whole-milk powder to the same level of total solids in labneh has been successfully employed (Gilles, 1978). A typical composition (g 100 g^{-1}) of labneh made using such a method of production consisted of 23.5–24.0 total solids, 6.5–7.0 protein, 8.5–9.0 lactose, 7.0–7.5 fat, 1.3–1.6 ash (Özer, 1997). The major drawbacks to producing labneh by the direct reconstitution method are the risk of after-acidification during storage, and excessive whey separation (Özer, 1997). It

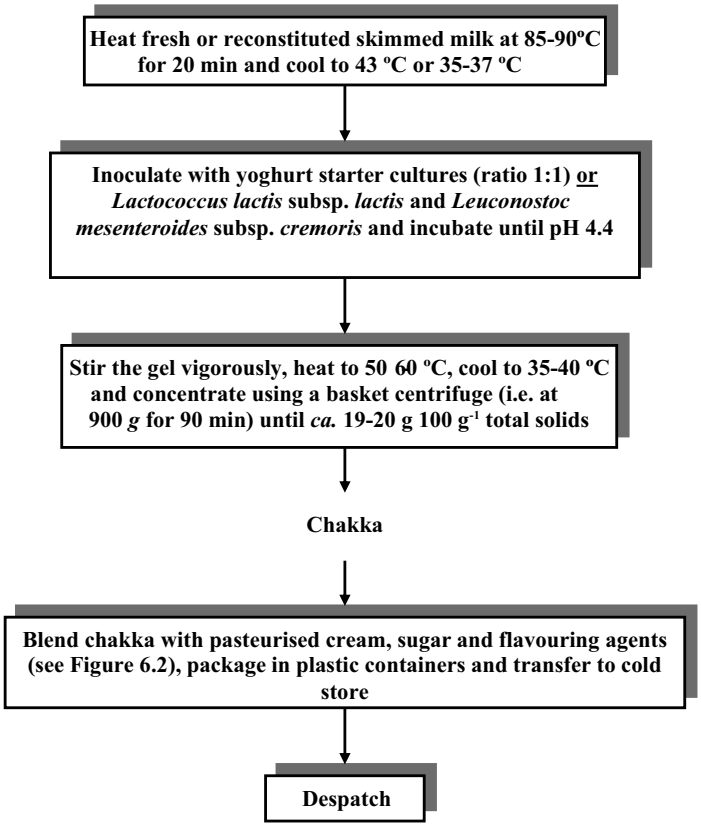


Fig. 6.7 Production line for shrikhand using basket centrifuge.

should, therefore, be borne in mind that the lactose content of the milk powder should be reduced to 2.0–4.0 g 100 g^{-1} in the finished product in order to avoid flavour and texture deficiencies (Gilles & Lawrence, 1981). For this reason, standard milk powder, which gives 10–12 g 100 g^{-1} lactose in labneh produced from whole-milk powder directly by the reconstitution method, should be replaced with ultrafiltered whole-milk powder. The reconstitution temperature is also a crucial parameter for the elimination of textural defects such as sandiness and excessive whey separation.

The incorporation of buttermilk or whey protein concentrate (WPC) has also been used successfully in the production of labneh or chakka (El-Samragy *et al*., 1988b; Mahfouz *et al*., 1992; Karthikeyan *et al*., 1996). It is possible to produce strained yoghurt made from skimmed milk powder fortified with buttermilk without any major problem such as the development of unacceptable flavour and texture defects (El-Samragy *et al*., 1988b).

6.2.4 *Membrane methods*

Membrane techniques (especially ultrafiltration; UF) have been successfully used in the yoghurt industry for the last 20–25 years, but their application to the production of concentrated yoghurt is rather limited (Özer *et al*., 1997b).

Two different systems of ultrafiltration have been developed for the manufacture of concentrated yoghurt: first, the fermentation of UF-retentate (Fig. 6.8) that has the solids content desired in labneh, about 24 g 100 g^{-1} (Veinoglou *et al*., 1978; Abd-El Salam & El-Alamy, 1982; El-Samragy & Zall, 1988; Hofi, 1988; Tamime *et al*., 1989a, b; Tamime, 1993, 2003; Özer *et al*., 1997a, b, c, 1998a, b, 1999a, b; Özer, 2001); second, the ultrafiltration of yoghurt at 40–50°C until the target level of total solids in the product is reached (Tamime *et al*., 1989b, 1991b, c; Tamime, 1993, 2003; Özer *et al*., 1997a, 1998a, 1999a; Tamime *et al.*, 2001) (Figure 6.9). Although the final chemical compositions of both products are similar to each other, the physical and organoleptic properties are considerably different (Özer, 1997). The concentration of milk by ultrafiltration before labneh making carries a risk of bitterness in the final product since the calcium content will be higher. The main advantages of the ultrafiltration technique as compared with other conventional methods are higher yield (increase by 10%), shortening of processing time (e.g. by 25%) and reduced whey-ing off (Hofi, 1988; Tamime *et al*., 1989a, 1991b; Özer *et al.*, 1997a, 1998a, 1999a). In addition, the volumes of milk, starter cultures and salt (optional) are reduced by

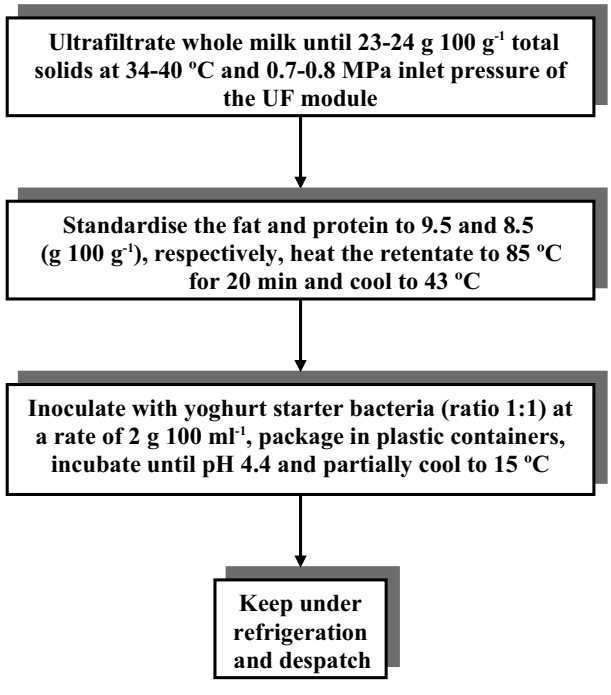


Fig. 6.8 Production of labneh from UF whole-milk retentates.

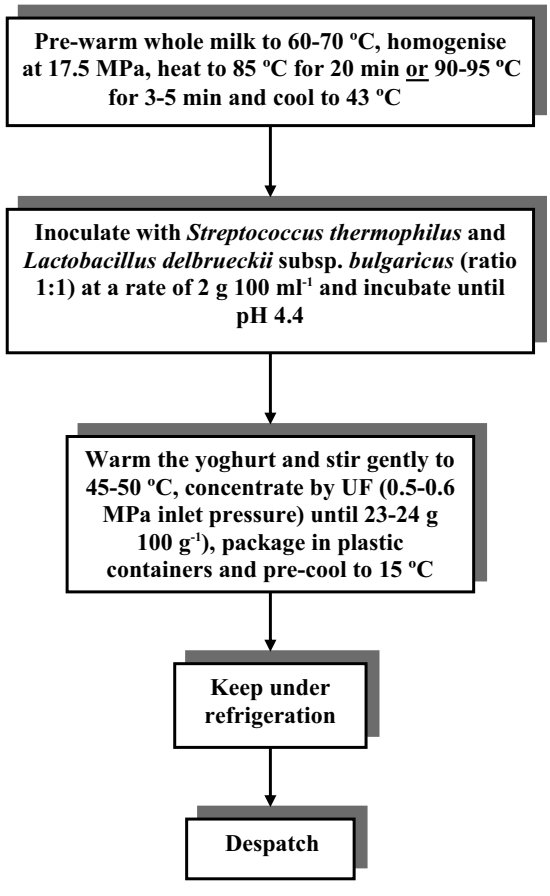


Fig. 6.9 Production of UF labneh from warm yoghurt.

around 10%, 80% and 50%, respectively. It was reported that the labneh produced using ultrafiltration had a smoother body and texture and was more palatable in taste than that manufactured by the traditional method (Özer, 1997).

The chemical composition of UF labneh is shown in Table 6.3. As the ultrafiltration and traditional methods of concentration are selective, fat and protein are increased in labneh at the expense of lactose and minerals, with the slow-draining cloth-bag system giving the best retention of fat (Özer & Robinson, 1999). For a good quality UF labneh, the standardisation of UF milk with respect to fat and protein content is of critical importance. Milk with 8.5 g protein 100 g^{-1} and 9.5 g fat 100 g^{-1} gives a product that from the standpoint of composition and organoleptic qualities is similar to that manufactured traditionally, and that can be made without special precautions for the control of acidity and drainage (Veinoglou *et al*., 1978).

Milk can be concentrated by ultrafiltration to the desired total solids level without any difficulty, but the efficiency of UF plant in concentrating warm yoghurt is largely dependent on operational temperature (Tamime *et al*., 1989a, 1991c). At low

Table 6.3 Chemical composition (g 100 g^{-1}) of ultrafiltered concentrated yoghurt and ultrafiltered permeate.

Product	Total solids	Protein	Lactose	Fat	Ash	pH	Source
Retentate	20.1[1]	6.36	4.03	8.75	0.96	4.42	Tamime *et al*. (1989a)
	22.7[1]	9.00	3.35	9.00	1.35	ND	Abu-Jdayil & Mohameed (2002)
	22.4[1]	8.24	ND	10.70	ND	4.05	Veinoglou *et al*. (1978)
	22.6[1]	8.30	4.71	9.00	0.81	4.14	Özer (1997)
	24.3[2]	7.98	3.44	12.20	0.68	4.23	Tamime *et al*. (1991c)
	22.3[2]	8.40	5.00	8.80	0.91	4.21	Özer (1997)
	21.8[2]	7.42	4.09	9.60	0.71	4.28	Tamime *et al*. (1989a)
Permeate	5.4[3]	0.28	4.68	—	0.42	ND	Tamime *et al*. (1989a)
	5.3[3]	0.37	4.61	—	0.30	ND	Özer (1997)
	5.7[4]	0.18	4.84	—	0.70	ND	Tamime *et al*. (1989a)
	5.5[4]	0.35	4.51	—	0.36	ND	Özer (1997)

[1] Concentrated yoghurt made from ultrafiltered cow's milk.
[2] Concentrated yoghurt made from ultrafiltered warm yoghurt.
[3] Ultrafiltered permeate of concentrated yoghurt made from ultrafiltered cow's milk.
[4] Ultrafiltered permeate of concentrated yoghurt made from ultrafiltered warm yoghurt.
ND Not determined.

processing temperatures (30–35°C), the permeability of the aqueous phase across the membrane is rather slow and, therefore, warm yoghurt should be ultrafiltered at relatively higher temperatures (45–50°C). Temperatures > 50°C cause an increase in the fouling rate of the UF membrane, which may affect the processing conditions in large-scale operations (Attia *et al*., 1991a, b). In general, with the increase in processing temperature, the flux rate and the firmness of the labneh increases, but at > 50°C the total viable count of yoghurt starter organisms declines.

The operational pressure is another factor that can determine the physical quality of UF labneh. The inlet pressure of the UF plant should not exceed 0.7–0.8 MPa, and the difference between the inlet and outlet pressures should be around 0.25–0.30 MPa in order to avoid detrimental effects of shearing on the physical properties of UF labneh (Tamime *et al*., 1991c; Özer, 1997). The production of shrikhand and chakka by ultrafiltration shows similarities with UF labneh. Skimmed milk is heated at 85–90°C for 10–20 min, cooled, incubated with selected starter culture at 21–22°C for 16–18 h to produce a curd at pH 4.5–4.6, agitated, heated to 60–65°C, cooled to 50°C and concentrated by ultrafiltration to produce chakka. Cream (70 g fat 100 g^{-1}) and sugar are added to the concentrated fermentate, followed by kneading at 25–26°C and cooling to 10–12°C to produce shrikhand (Sharma, 1998).

6.3 Microbiology of labneh

Classical yoghurt starter culture bacteria (*S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) are usually employed in the production of labneh. Different cultures including *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (currently known as *Lactococcus lactis* subsp. *lactis*$^{(cit+)}$) and *Leuconostoc mesenteroides* subsp. *cremoris* (at 1 : 1 : 1 ratio) have been recom-

mended. Blends of different lactic acid bacteria, for example *Lac. lactis* subsp. *lactis* and *Lac. lactis* subsp. *cremoris* and yoghurt bacteria or *Lac. lactis* subsp. *lactis* and *Lac. lactis* subsp. *lactis* biovar *diacetylactis* have been used in the manufacture of chakka and shrikhand (Tamime & Robinson, 1988; Karthikeyan *et al*., 1998). Incorporation of probiotic bacteria in the manufacture of dietetic shrikhand was also reported by Subramonian *et al*. (1997).

The number of viable lactic acid bacteria in labneh and shrikhand is on average higher than in regular yoghurt. There is a tendency for these bacteria to be retained in the curd during the concentration process of traditional labneh and shrikhand rather than being drained away with the whey (Salji, 1991). However, the number and growth characteristics of the starter organisms in labneh are largely dependent on the method of concentration and the pH of the product (Özer & Robinson, 1999). Overall, the growth trend of yoghurt starter bacteria in UF concentrated milk is similar to those in unconcentrated milk. During the concentration of warm yoghurt by ultrafiltration, the counts of *S. thermophilus* are expected to continue to increase, since the operational temperature of UF membrane is usually selected as 40–45°C in order to increase the flux rate of the membrane. This temperature range is suitable for the optimum growth of the yoghurt starter bacteria. A similar, albeit more limited, rise in the counts of *Lb. delbrueckii* subsp. *bulgaricus* is also observed, with the more even temperature profile of the traditional system being more conducive to growth (Özer, 1997; Özer & Robinson, 1999).

The ratio of 1 : 1 between *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* is extensively quoted in the literature as the optimum ratio for a good-quality yoghurt (Tamime & Robinson, 1999). Such a ratio is maintained in labneh provided it is made from yoghurt with a similar ratio (Salji, 1991). This property is important in establishing the characteristic aroma and flavour of labneh, and extending the shelf-life of the product to 3 weeks without impairing its quality.

The counts of yoghurt starter bacteria vary depending on the methods of manufacture of labneh. The number of *S. thermophilus* in labneh made from UF concentrated milk is expected to be higher than in labneh produced either traditionally or from UF concentrated warm yoghurt (Özer & Robinson, 1999). This is probably due to higher concentrations of stimulatory factors, such as whey proteins that are retained in the UF milk (Hickey *et al*., 1983). This effect has been confirmed to some extent by calculations of maximum generation times (G_{max}) for the mixed cultures (Özer & Robinson, 1999).

A strong relationship between *S. thermophilus* and acidity is evident in both unconcentrated and UF concentrated milks (Sinha, 1991; Yamani & Ibrahim, 1996; Özer, 1997). In contrast, *Lb. delbrueckii* subsp. *bulgaricus* is not markedly affected by the acidity in milks with lower total solids; however, when the total solids of milk is elevated to 23–24 g 100 g^{-1} by ultrafiltration before labneh making, the effect of acidity on the growth of *Lb. delbrueckii* subsp. *bulgaricus* becomes relatively more important (Özer & Robinson, 1999). This contrast could perhaps be attributed to the more prolonged exposure of the cells of *Lb. delbrueckii* subsp. *bulgaricus* to levels of acidity during the longer incubation periods used with the concentrated milk.

Overall, labneh contains about 1–3 × 10^9 cfu g^{-1} of each of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. Hydrolysis of the lactose in milk by β-galactosidase (EC 3.2.1.23; lactase) prior to production of labneh can cause an increase in the microbial counts, due to the presence of glucose. Nevertheless, labneh made from lactose-hydrolysed milk may be too sweet, which is not characteristic for this product, and an ideal medium to support growth of moulds (Tamime, 1977, 1978; Tamime & Robinson, 1978; Hofi, 1990).

Although classical non-viscous strains of yoghurt starter cultures are usually used in the manufacture of labneh, different strains of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* and species of other genera can also be employed (Abou-Donia *et al*., 1992; Amer *et al*., 1997). In general, ropy or viscous strains are not suitable for the production of labneh since the release of water from the yoghurt becomes difficult (Tamime & Robinson, 1978).

Different combinations of strains of *Enterococcus faecalis* in combination with a certain strain of *Lb. delbrueckii* subsp. *bulgaricus* were used successfully by El-Samragy *et al.* (1988a) to produce a labneh-like product. Similarly, when *Propionibacterium freudenreichii* subsp. *shermanii* was used in combination with yoghurt starter cultures, the keeping quality of labneh was improved (Sharal *et al*., 1996), and the vitamin B_{12} and folic acid contents increased in labneh by 210% and 25%, respectively (Khattab, 1991).

Due to their attributed therapeutic effects, probiotic cultures (e.g. *Bifidobacterium bifidum* and *Lactobacillus acidophilus*) can be incorporated into labneh making (Mahdi, 1990). However, comparing labneh with yoghurt and other fermented milks, higher acidity in the product may limit the growth of these probiotic bacteria, and the counts of *B. bifidum* and *Lb. acidophilus* may well be below the threshold level for a suggested therapeutic minimum (10^6–10^7 cfu g^{-1}) (Yilmaztekin *et al*., 2004). If the probiotic strains are to be used in the manufacture of labneh in combination with yoghurt starter cultures, the pH of the final product should be > 4.2.

The major contaminants of labneh are yeasts (Yamani & Abu-Jauber, 1994; Mihyar *et al*., 1997). Although higher acidity in labneh makes this product more resistant to microbiological deterioration than plain yoghurt, in some countries additives such as potassium sorbate and sodium benzoate are used to control the growth of yeasts. However, since the use of additives in the production of labneh is often avoided due to legal restrictions and public concern, necessary hygiene measures should be observed during the manufacture and storage of labneh. This is more critical for traditional labneh and shrikhand, which are more readily subject to microbial contamination from the air during gravity drainage (Jagannath *et al*., 2001). Therefore, mechanisation in the production of concentrated yoghurt would help to extend the keeping quality of this product.

6.4 Organoleptic properties of labneh

Besides its nutritional aspects and longer shelf-life, labneh is also very much appreciated by consumers for its sensory properties. The sensory properties of labneh are

largely determined by the method of manufacture. Overall, the traditional labneh is characterised as slightly creamy in colour and lumpy in texture (Hamad & Al-Sheik, 1989). Short body and lumpiness in traditional labneh make it difficult to spread. However, by increasing the initial total solids of the plain yoghurt used in the production of labneh, the texture of labneh becomes smoother and its spreadability improves. With respect to flavour, 16 g 100 g^{-1} total solids in the milk base gives rise to a product that is more appealing to the consumer (Abd-El Salam & El-Alamy, 1982). Regarding the texture and appearance, UF labneh made from UF warm yoghurt is superior to labneh produced using either the traditional method or UF concentrated milk, because it is smoother and has a creamy texture (Özer, 1997). It was noted that labneh made by direct reconstitution/recombination or from concentrated milk by ultrafiltration had a crumbly structure, coarse texture and wheying off after the coagulum was broken (Özer, 1997). However, these faults were not associated with the labneh made from the concentration of warm yoghurt by ultrafiltration. Al-Kanhal (1993) observed that traditional labneh made from fresh milk had the best organoleptic scores compared with a similar product made from recombined milk or cultured buttermilk concentrated by a nozzle separator.

The concentration of acetaldehyde, a major aroma compound of yoghurt, is dependent on both production methods and processing conditions (Table 6.4). It may well be possible that some acetaldehyde escapes into the filtrate during gravity drainage of traditional labneh and/or is volatilised. In case of the production of labneh by ultrafiltration of warm yoghurt, the processing temperature should be selected carefully in order to minimise the loss of acetaldehyde by evaporation. The level of acetaldehyde in labneh can also be increased by incorporating aroma-producing starter culture combinations. The deterioration in sensory quality and changes in physico-chemical parameters of cloth-bag labneh are largely governed by the growth of yeast and moulds in the product (Al-Kadamany *et al*., 2002).

Since the organoleptic preferences of consumers vary from one society to another, slight modifications in the manufacturing process of labneh may be introduced to meet consumer demand. For example, a rancid-like flavour is preferred by people of the eastern Mediterranean (e.g. Lebanon, Syria, Turkey and Greece), and this

Table 6.4 Sensory properties of labnehs produced using different methods of manufacture and level of acetaldehyde recorded in the same products (average total solids 23 g 100 g–[1]).

Product	Appearance[1]	Body & texture[1]	Aroma & flavour[2]	Acetaldehyde (mg kg^{-1})
Traditional labneh	2.38	2.09	4.76	9.90
Direct reconstitution labneh	3.12	2.98	6.24	12.34
Ultrafiltered labneh from warm yoghurt	4.30	4.16	7.00	22.80
Ultrafiltered labneh from concentrated milk	2.96	2.90	6.60	15.50

[1] Appearance and body & texture were scored out of 5 points.

[2] Aroma & flavour were scored out of 10 points.

From Özer (1997).

rancid-like flavour can be deliberately developed by ultrafiltering raw milk, and then stopping the lipase activity at some point by a further thermisation step instead of heat processing before ultrafiltration (El-Samragy & Zall, 1988). Similarly, in western societies, less acidic and sweeter yoghurt-type fermented milk products are more desirable and, by adding fruit and/or sweeteners, labneh can be converted into an appealing product for western consumers. In recent years, labneh garnished with some herbs and spices has attracted consumer interest in Turkey.

6.5 Rheology and microstructure of labneh

The recent increase in the popularity of labneh in Europe has led to more interest in the structure of this product, especially in relation to milk from different species of mammals and different concentration techniques (Tamime *et al*., 1989b, 1991a, b; Özer *et al*., 1997a, b, 1998a, 1999b; Abu-Jdayil & Mohameed, 2002). Much of the acceptability of labneh is dependent on its rheological/textural characteristics, which, in turn, seem to be heavily dependent on processing conditions. As mentioned elsewhere, various methods of production of labneh are available, and all these methods lead to products having different physical characteristics.

Özer *et al.* (1997a, b, 1998a, 1999b) evaluated different types of labneh made by: (a) the traditional method; (b) concentrating whole milk by ultrafiltration; (c) concentrating whole yoghurt (16 g SNF 100 g^{-1}) by ultrafiltration; (d) reconstituting whole-milk powder to give a milk base for fermentation of 23 g total solids 100 g^{-1}, using stress-controlled dynamic rheological testing. The same authors stipulate that labneh has a typical viscoelastic gel, and has evidence of small frequency dependency within the linear viscoelastic region (Fig.6.10). Labneh has an inhomogeneous particulate structure, which is characteristic for plain yoghurt as well (Benezech & Maingonnat, 1994). Also, Özer *et al.* (1999b) postulated that the differences in the overall gel strengths of the labnehs produced by different techniques at low amplitude suggest that, although the type of protein–protein interactions in each case may be similar, there are differences in the degree of interaction (Fig. 6.11). Subsequent breakdown at higher amplitude suggests that the overall domains of the protein may have been reduced and that different methods of manufacture may be producing materials that have different space occupancy in the gel material (Özer *et al.*, 1999b).

A strong dependence of complex modulus (G*) and loss tangent (tan δ = loss modulus/storage modulus) of concentrated yoghurt on the protein concentration was evident (Figs 6.12 and 6.13) (Özer *et al*., 1997a, 1997b). The minimum protein concentration at which a gel could be formed was 3 g 100 g^{-1}, which is presumably due to the lower number of protein contacts in the larger serum volume (Özer *et al*., 1999b). The total length of the stress-carrying strands per unit volume is a decisive factor for the gel strength of concentrated yoghurt, and the nature and position of the strands in the network also determine the rheological properties of the gel (Bremer *et al*., 1990). The dependence of G* on the protein concentration was non-linear, indicating that the number of stress-carrying strands was not proportional to volume fractions of casein particles, and so the network formed was very heterogeneous. It is

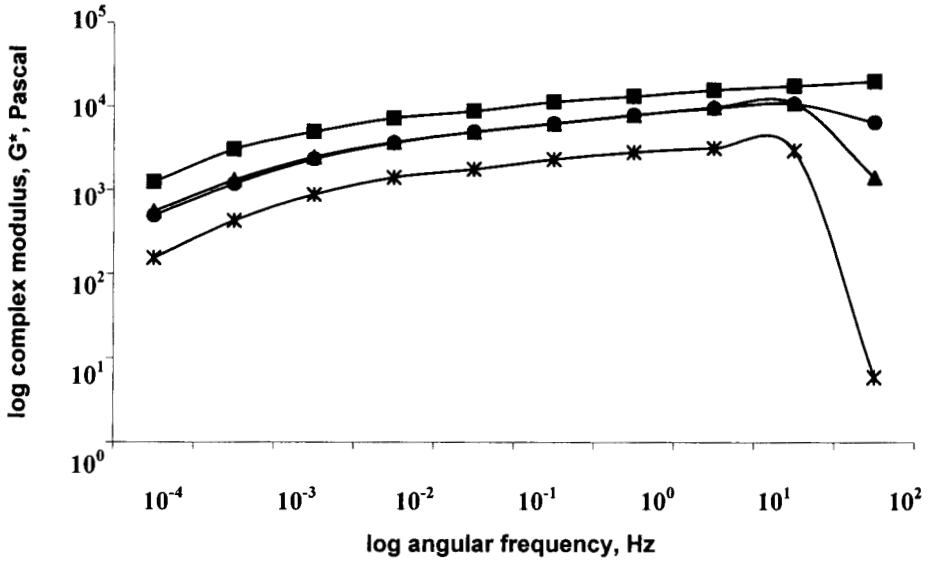


Fig. 6.10 Complex modulus of different types of labneh as a function of frequency sweep. Total solids contents of all the labnehs averaged 23 g 100 g^{-1}. Traditional method (■), ultrafiltration of warm yoghurt (●), fermentation of UF retentate (▲) and direct reconstitution of whole milk powder (*). Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

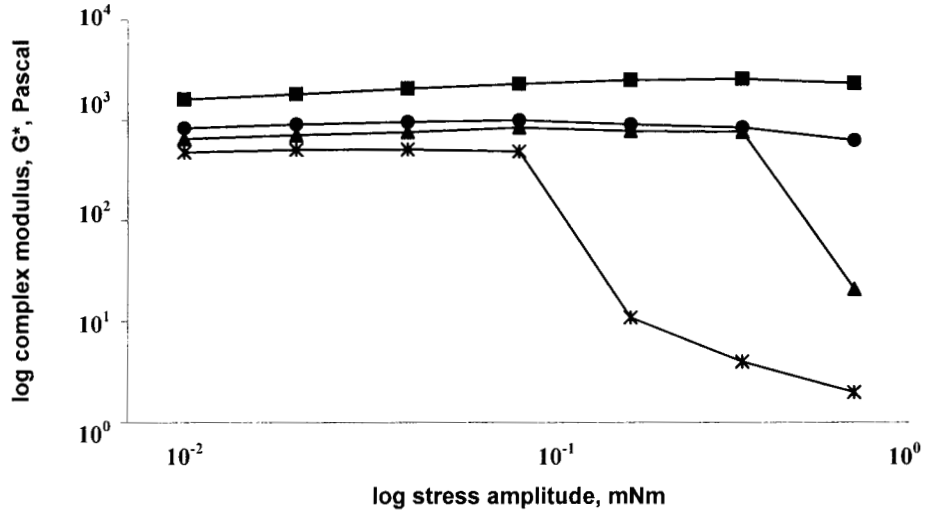


Fig. 6.11 Complex modulus (G*) of different types of labneh as a function of amplitude sweep. Total solids contents of all the labnehs averaged 23 g 100 g^{-1}. Traditional method (■), ultrafiltration of warm yoghurt (●), fermentation of UF retentate (▲) and direct reconstitution of whole milk powder (*). Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

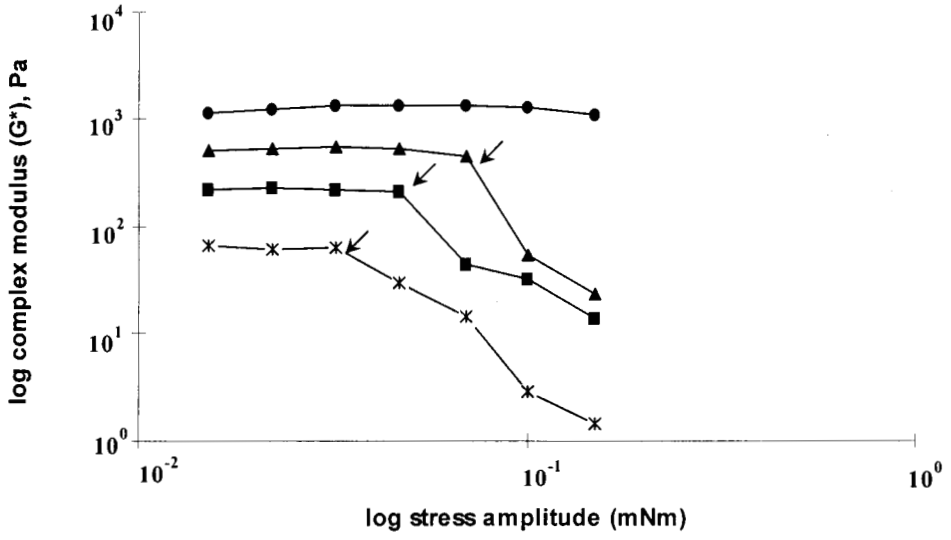


Fig. 6.12 Effect of the protein concentration on the complex moduli (G*). Protein levels (g 100 g^{-1}): * = 3, ■ = 5, ▲ = 7, ● = 9. Arrows indicate the complex modulus (G*) after the gel structure degraded (i.e. outside the linear viscoelastic region). Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

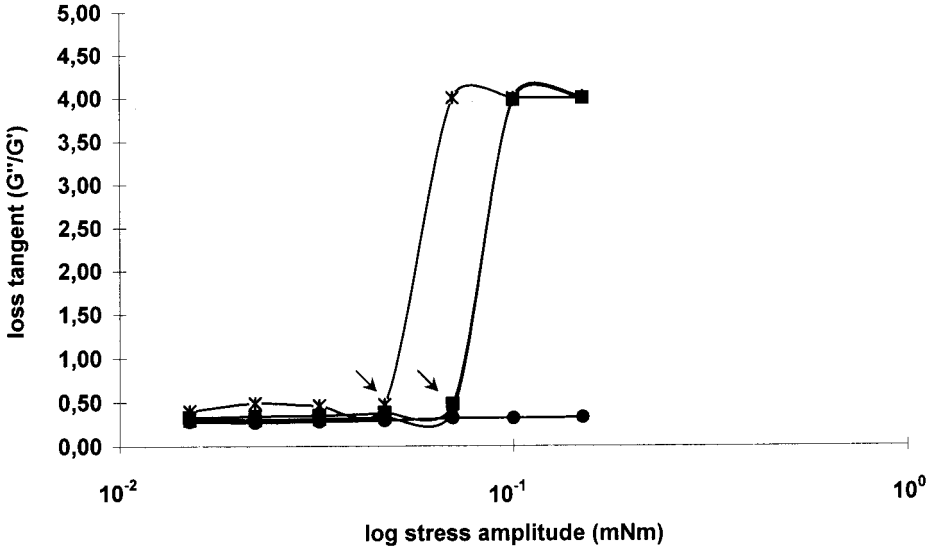


Fig. 6.13 Effect of the protein concentration on the loss tangent (tan δ = G″/G′). Protein levels (g 100 g^{-1}): * = 3, ■ = 5, ▲ = 7, ● = 9. Arrows indicate the complex modulus (G*) after the gel structure degraded (i.e. outside the linear viscoelastic region). Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

fair to assume that the application of ultrafiltration to warm yoghurt at low pressure (i.e. 0.3–0.4 MPa) may stimulate an increase in the number of smaller conglomerates and, therefore, the number of stress-carrying effective bonds. A similar effect can be observed when UF labneh is passed through a lactic curd homogeniser with a suitable structuriser head, as proposed by Tamime *et al*. (1989a). However, one should bear in mind that both UF labneh and/or homogenisation of the concentrate decrease the firmness of the product compared with both traditional labneh and UF labneh made from milk concentrated by ultrafiltration before fermentation. Labneh made from concentrated UF whole milk in the retail container (a process similar to set yoghurt) has a tendency to crack and crumble, and is considerably less elastic than the UF labneh manufactured by concentrating warm yoghurt (Tamime *et al*., 1989b). According to Abu-Jdayil and Mohameed (2002), a traditional labneh exhibits a shear thinning and thixotropic behaviour regardless of the storage time. Depending on the decrease in the pH, structural rearrangements in labneh continue during the whole period of storage (Abu-Jdayil & Mohameed 2002), and this is even more pronounced for labneh manufactured from milk concentrated by ultrafiltration before fermentation (Özer *et al*., 1998b). The type of milk used in the production of concentrated yoghurt is another factor determining the firmness of the final product. In general, cow's labneh gives the firmest body, followed by sheep's and goat's labneh (Tamime *et al*., 1991a).

The microstructure of labneh made by different concentration techniques from cow's, goat's and sheep's milk has been studied by Tamime *et al*. (1989b, 1991a) and Özer *et al*. (1999b). Both groups of researchers drew similar conclusions that the protein matrix of labneh is composed of casein particle chains and clusters. A more compact structure was noted in the traditional labneh (Fig. 6.14a). Labneh made

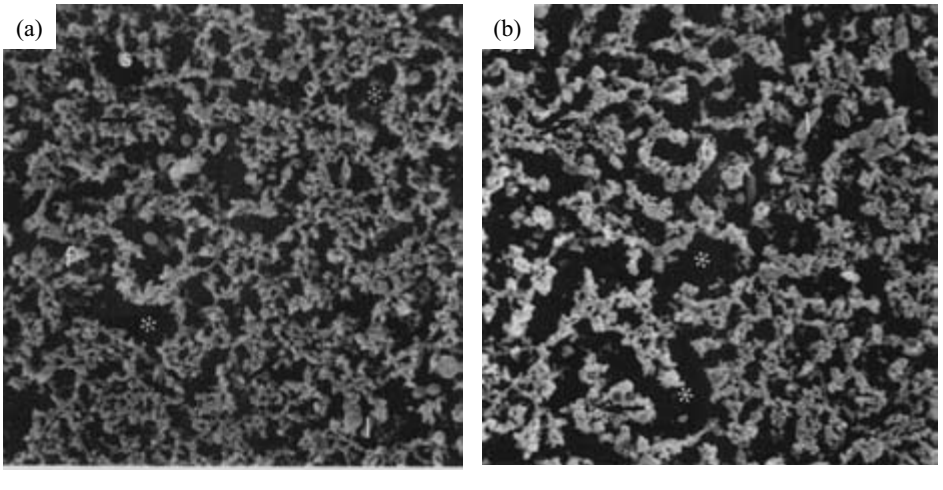


Fig. 6.14 Scanning electron microscopy micrographs of traditional labneh (a) and UF labneh made from concentrated yoghurt (b). l, lactobacilli; s, streptococci; f, fat globules; * = void spaces. × 2500. Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

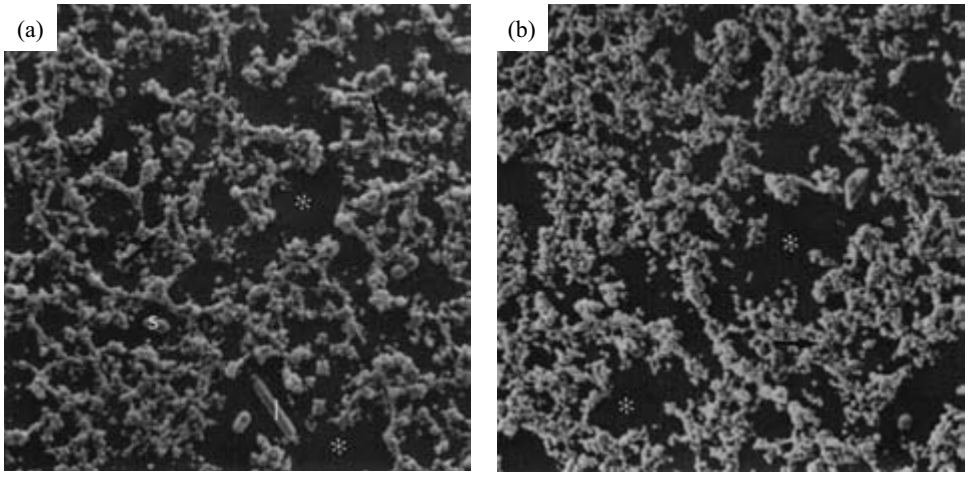


Fig. 6.15 Scanning electron microscopy micrographs of UF retentate labneh (a) and direct reconstituted labneh (b). l, lactobacilli; s, streptococci; f, fat globules; * = void spaces. × 2500. Reproduced with permission from Özer, B.H., Stenning, R., Grandison, A.S. & Robinson, R.K. (1999b) *Journal of Dairy Science*, **82**, 682–689.

from milk concentrated by ultrafiltration before fermentation and from reconstituted skimmed milk powder had close microstructural properties to each other, and the void spaces and the protein structure of these labnehs were relatively evenly distributed (Fig. 6.15a,b). Scanning electron microscopy (SEM) revealed that labneh generally displayed continuity of structure, but to a lesser extent in the labneh made by concentrating warm yoghurt by ultrafiltration (Fig. 6.14b). The slight discontinuity in labneh manufactured from membrane-treated warm yoghurt is probably linked to the detrimental effect of ultrafiltration on the delicate gel structure. The casein clusters were thicker in the membrane-treated labneh, perhaps as a result of pressure forcing the casein aggregates to come together during the early stages of the membrane processes. Small, thread-like structures were visible between the strands in the labneh made by ultrafiltration of warm yoghurt, which may be the result of stretching and shearing of casein aggregates during the later stages of ultrafiltration when the viscosity is increased. Tamime *et al*. (1989b, 1991a) showed that smoothing of concentrated yoghurts by a curd texturiser somewhat reduced the dimensions of the casein particle chains and clusters in labneh. As revealed by transmission electron microscopy (TEM), the protein particles were adsorbed onto the minute fat globules, leading to their integration into the labneh gel matrix (Fig. 6.16a,b).

6.6 Concluding remarks

The application of appropriate technologies to produce concentrated yoghurt is essential for the product to have physical and sensory properties acceptable to consumers. Any technology used in the manufacture of concentrated yoghurt should

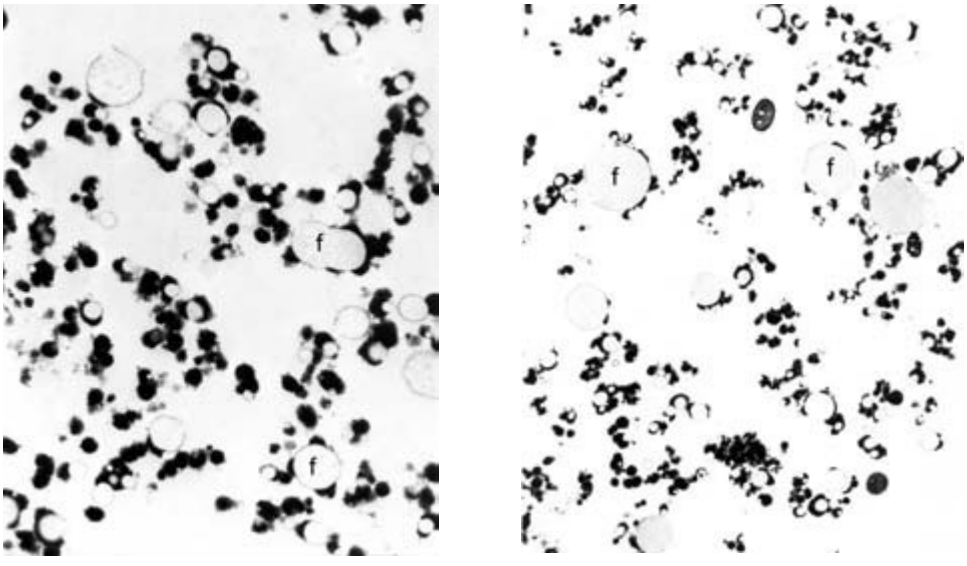


Fig. 6.16 Transmission electron microscopy images of traditional labneh (a) and UF labneh made from concentrated yoghurt (b). Black arrows indicate casein micelles. f, fat globules. × 8000.

provide a balance between the proteins and other milk components, keeping in mind that the optimum protein level should be around 9–10 g 100 g^{-1}. In this context, ultrafiltration of warm fermented milk seems to be a particularly promising technique in the manufacture of good-quality concentrated yoghurt. Considering the detrimental effect of membrane treatment on delicate gels, and changes in the processing variables (e.g. pH at the beginning of concentration, transmembrane pressure and operating temperature), ultrafiltration can produce a material that has a less damaged structure and a better texture. Texturising should be employed to smooth traditional labneh; however, in the method of concentrating warm fermented milk by ultrafiltration, the process of smoothing may not be necessary if the UF labneh is cooled immediately after concentration by scraped surface cooler rather than cooling slowly in bulk (Tamime *et al*., 1989b). Finally, various modifications (such as sweetening, flavouring, adjusting the fat level) can be made to turn labneh into a more appealing product for the consumer.

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Fermented Milks
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7 Nordic/Scandinavian Fermented Milk Products

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7.1 Historical aspects

7.1.1 *Introduction*

The Scandinavian or Nordic countries are situated in the northern part of Europe close to and even north of the Arctic Circle. The summer season is short and the temperature is rather low most of the year; this fact has influenced dietary habits and food production methods in the region. In earlier times, domestic food production was mainly limited to animal foods, especially dairy products, and today the production and/or consumption of milk and fermented milks is still higher in Scandinavia than in most other parts of the world (Table 7.1). Among dairy products, fermented milks are common in northern Scandinavia with an average daily intake above 100 g per person, which is double the consumption in most regions of Europe (Table 7.2). In contrast, consumption in southern and western Scandinavia is lower and close to

Table 7.1 Annual consumption (kg per person) of milk drinks and fermented milks including yoghurt in Nordic countries in 2002.

Product	Norway	Denmark	Sweden	Iceland	Finland
Milk drinks	101	95	108	140	137
Fermented milks	20	41	34	35	41
Total	121	136	142	175	178

Average total consumption in 15 EU countries was 95 kg per person.
Data compiled from IDF (2003).

Table 7.2 Reported daily consumption (g per person) of yoghurt and other fermented milks by women in Northern Europe.

Country	Region	Consumption
Norway[1]	North and west	36
	South and east	37
Denmark[1]	Copenhagen	54
	Åarhus	58
Sweden[1]	Malmö (south)	65
	Umeå (north)	115
Finland[2]	Average of all females and males	100

Average total consumption in 27 regions of EU 10 countries was 50 g per person.
[1] Adapted from Hjartaker *et al*. (2002).
[2] Data from Valio (personal communication).

other parts of Europe. Based on these data, the annual retail value of all fermented milks in the Nordic countries is estimated to be about a billion euros.

The present high intake of fermented milks can be explained by a successful transformation of traditional products into modern variants. In contrast to most other countries, in Finland and Sweden consumption of fermented milks of traditional origin accounts for roughly 50% of the total. The other half is made up of yoghurt first introduced around 90 years ago.

In this chapter, a review of the old traditional Nordic fermented milk products will be given, together with data about the modern, industrially produced variants.

7.1.2 *Historical background*

Fermented milk products have a long history in the Scandinavian countries. In Roman times, described by Tacitus (Fleichman, 1915), curdled milk was an important food in Northern Europe together with wild fruit and fresh game. The popularity of fermented milks also has a long tradition, dating back to the Vikings. According to Icelandic tales, fermented milks were appropriate for men and boys, while sweet milk was only suitable for small children and sick people (Einarsson & Helgason, 2001).

From research in linguistic geography, Larsson (1988) has identified three different areas for fermented milks in Scandinavia. Tätmjölk was the preferred type in most of Norway and the northern parts of Sweden (i.e. three quarters of the area). The same preference applied in southern and western Finland (Forsén, 1966). In Iceland, Denmark, southern Norway and the remaining parts of Sweden, ordinary sour milk was popular and similar to those in the rest of Northern Europe (Larsson, 1988). In Iceland sour milk was traditionally sold in two forms – sour milk and skyr; the latter is similar to the product found today. It is probable that both these products existed in Scandinavia when the Vikings settled in Iceland, but sour milk later disappeared from Iceland and skyr from the rest of the Nordic countries, for reasons that remain obscure (Magnússon, 1988). All these products were made traditionally and often from a starter consisting of a selected batch of fermented milk with favourable characteristics. In addition, spontaneously soured milk or curdled milk was used to inoculate fresh milk, even though the product was thought to be of lower quality. Likewise, buttermilk was a byproduct and was considered of secondary importance compared to the main product, butter. Its quality was entirely dependent on how the process of buttermaking was optimised.

7.2 Traditional Scandinavian products

7.2.1 *Classification*

Traditional fermented milks in the Nordic countries are fermented at a low temperature (17–22°C), and mesophilic lactic acid bacteria mostly complete the fermentation.

In contrast to curdled milk, most of the other products were produced by specific methods similar to those illustrated in Chapter 1. These methods all aimed to ensure a specific fermentation, and some traditional examples may include the use of a vessel containing 'favoured' starter culture bacteria or the addition of fresh milk to a vessel already partly filled with fermented milk. Repeating such cycles of inoculation and/or fermentation of fresh milk may have helped in the natural selection of a suitable starter culture with favourable and desirable properties. Consequently, this approach was adopted for a starter culture to be used for an extended period of time (Arenander, 1911; Fleichman, 1915; Narvhus, 2003).

In some instances, heating of the milk before the fermentation stage was used to produce traditional fermented milks that could be stored for a long period of time. Using such methods of production, the available oxygen present was reduced by storage in closed containers, leading to a reduced growth of yeast and moulds (Arenander, 1911).

Some of the mesophilic starter cultures used (e.g. specific strains of *Lactococcus* spp.) could produce exopolysaccharides (EPS), and the fermented milk product was characterised as 'ropy'. This gave rise to two different groups of fermented milks (Table 7.3) and, furthermore, these products could be distinguished by their texture, being viscous, gelled or paste-like. In this way, the traditional products tätmjölk, filmjölk, filbunke (two varieties), sour milk and skyr could be properly differentiated (Table 7.3). Complementary to those products, spontaneously fermented milks such as curdled milk were also produced. Beside the different lactic acid bacteria present in the starter culture, yeast and moulds were also present in all traditional Nordic fermented milks.

7.2.2 *Tätmjölk*

Tätmjölk is made using whole milk or skimmed milk fermented with a starter including EPS-forming lactococci. Tätmjölk is the most common and traditional fermented milk in the northern part of Scandinavia, i.e. in Finland, Norway and Sweden (Forsén, 1966; Larsson, 1988). Synonyms of tätmjölk in different Scandinavian languages

Table 7.3 Traditional products – a scheme of classification.

Mesophilic LAB	Texture	Type of milk	Product
With EPS strains	Viscous	Whole or skimmed	Tätmjölk
	Gel	Whole	Filbunke
Without EPS strains	Viscous	Whole or skimmed	Surmjölk
	Gel	Whole	Filbunke
	Concentrated	Skimmed	Skyr*
	Fluid	Skimmed	Buttermilk
Spontaneous fermentation	Viscous	Whole	Curdled milk

LAB, lactic acid bacteria (i.e. starter cultures).
EPS, exopolysaccharides.
* Thermophilic starter cultures and yeast are used.

are shown in Table 7.4, and the product is characterised by being extremely viscous in texture, having a very mild acid taste and low syneresis (Gisler, 1749).

The milk is inoculated with tätte, a starter culture consisting of tätmjölk that is selected for its good quality and used for several days. If the tätte fails to ferment the milk, it is replaced by a newly selected tätmjölk. If one's tätmjölk failed, it was the traditional practice to obtain an active one from a neighbouring farm. The starter culture was preserved from one season to another by soaking a piece of linen cloth in the tätte and then drying it. When emigrants left Scandinavia for the United States, they took with them the cloth containing the dried tätte, which was then used as a starter culture in the new country. In its dried form it could be sent by post anywhere in the world.

In the absence of a starter culture, leaves of the butterwort plant (*Pinguicula vulgaris*) is added to the milk and incubated at 17–20°C until the milk has coagulated (Gisler, 1749). The process is repeated many times until suitable fermented milk is produced. The suitability of this traditional process has been studied for many years and confirmed in recent studies (Nilsson & Nilsson, 1958; Alm & Larsson, 1983; Haug, 1996). The latter author was able to demonstrate that the addition of such leaves to sterile milk resulted in a starter with different strains of *Lactococcus* spp. including the ESP-producing strains, and such starter culture could be used to produce a quality tätmjölk (Haug, 1996).

Traditionally, tätmjölk was produced by either adding a small proportion of the starter culture to the milk or adding milk to already fermented tätmjölk. The fermentation took place in wooden barrels or other vessels usually kept in a cellar at 17–20°C (Fig. 7.1). The milk coagulated the following morning or a day later, and could be stored for several days or even months. Tätmjölk with long extended shelf-life was produced and stored in tight barrels, which decreased the availability of oxygen. Due to contamination with yeast, part of the lactose was fermented to ethanol. According to Olsen-Sopp (1912), tätmjölk produced in this way could be stored for several months, and 60% of the lactose content was fermented to lactic acid, ethanol and carbon dioxide.

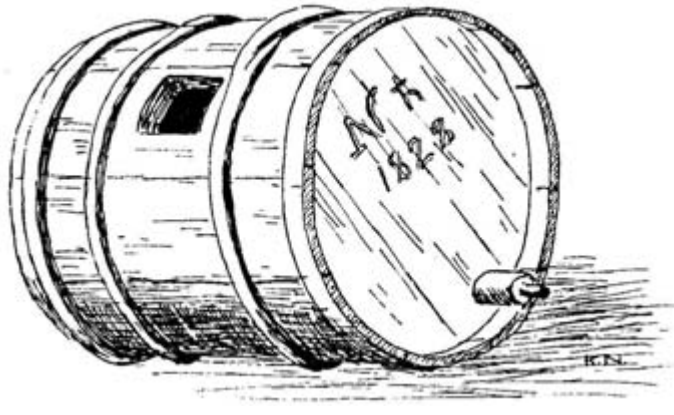

Fig. 7.1 A wooden barrel that was used to store traditional tätmjölk.

Table 7.4 Traditional and modern names of fermented milk products in Nordic countries.

Danish		Finnish		Icelandic		Norwegian		Swedish	
Traditional	Modern	Traditional	Modern	Traditional	Modern	Traditional	Modern	Traditional	Modern
			Långfil[1] Pitkäpiimä Viili[1,5]			Tettemelk[3] Tjukkmelk[3]	Tjukkmelk	Tätmjölk[4] Långmjölk[4] Tjockmjölk[4] Fil[4]	Långfil[1]
Tykmælk	Tykmælk	Piimä	Piimä			Surmelk	Kulturmelk	Surmjölk Filbunke[6]	Filmjölk[1] Filbunke[6]
				Skyr	Skyr[1]				
	Ymer[1]							Lactofil[7]	Lactofil[4]
Kærnemælk[8]	Kærnemælk[8]	Kirnupiimä[8,9] Kärnmjölk[8,9]	Kärnmjölk[8]			Kernemelk[8]	Kernemelk[8]	Kärnmjölk[8]	Kärnmjölk[8]

[1] The names of fermented milks used in the text.
[2] Swedish and Finnish names for långfil.
[3] Norwegian synonyms of the traditional product equivalent to långfil.
[4] Swedish synonyms of the traditional product equivalent to långfil.
[5] Name of a set-type product similar to långfil.
[6] Filbunke is the Swedish name of a set-type product similar to filmjölk or långfil.
[7] The name of ymer when it was manufactured in Sweden.
[8] Nordic names of buttermilk and cultured buttermilk.
[9] Finnish and Swedish names of buttermilk.

7.2.3 *Surmjölk*

This Nordic fermented milk (whole or skimmed) is similar to tätmjölk, but the starter culture does not contain EPS-producing lactococcal strains (Fleichman, 1915). In the Nordic countries, surmjölk was the dominant type of traditional fermented milk in the southern and western parts including the Faroe Islands (Larsson, 1988). Synonyms of the product are shown in Table 7.4, and it is characterised by a mild acid taste but more flavour than tätmjölk.

The milk is traditionally inoculated with a starter culture consisting of a good-quality surmjölk, and fermented at 18–22°C overnight. Afterwards, the fermentate could then be stored at low temperature for several days or even months. If it was to be stored for long periods, the milk was heat treated, fermented and stored in tight barrels in the same manner as tätmjölk. Usually, the fermented milk is stirred before consumption to break up the coagulum and to remix the free whey into the gel.

7.2.4 *Filbunke*

Filbunke is a variant product of either tätmjölk or surmjölk. Each variant is made using whole milk to which a starter culture is added, either tätmjölk or surmjölk. The inoculated milk is fermented in portion-sized bowls originally in wood and, during the fermentation stage, the fat globules rise to the surface forming a cream layer. The gelled milk (fresh or stored) is eaten with a spoon. Stored filbunke has a white layer of mould (*Geotrichum candidum*) on the surface, and may also contain lactose-fermenting yeasts.

Up until the 1950s, filbunke was produced at home by the traditional method. Due to urbanisation and the modern lifestyle, home production of filbunke is nowadays very limited.

7.2.5 *Buttermilk*

As long as butter has been produced, the resultant buttermilk has been utilised; however, buttermilk has never been produced as a product on its own. Traditionally, butter was made by churning milk or cream (Fleichman, 1915) but, when fat separators were developed in the late 1800s along with an improved process for fermenting the cream, this became the preferred method of producing butter and cultured buttermilk. At this time, buttermilk became widely available in southern Scandinavia, the northwestern part of continental Europe and in parts of the USA because of the eating habits of European immigrants. Traditionally, some buttermilk was consumed by people but most was used as pig feed (Pedersen, 1942). The availability of buttermilk was high in buttermaking regions. For example, in Denmark 100 years ago, the annual buttermilk production was ~40 kg per person; it had a fat content below 0.5 g 100 g^{-1} and a specific taste due to the presence of carbon dioxide and aroma compounds (e.g. diacetyl) formed by the starter culture used to ferment the cream. Fleichman (1915) characterised buttermilk as a healthy drink that was refreshing, particularly in summer, and could be used in cooking in

the same way as sour cream. The colour of buttermilk could be slightly yellowish due to the addition of colouring matter during butter production, and very low in viscosity due to the mechanical treatment, the churning of the cream.

7.2.6 *Skyr*

Traditionally, skyr was made with skimmed milk heated to 90–100°C, cooled to 40°C, mixed with rennet and starter culture (mostly thermophilic lactic acid bacteria) or good-quality skyr, fermented and concentrated by removing some of the whey using the cloth bag filtration method. The milk solids are concentrated five-fold and the pH of the product is below 4.0, which is the ideal condition for the thermophilic lactic acid bacteria, mesophilic acid-tolerant bacteria, and moulds and yeasts (Simonarson, 1978; Magnússon, 1988). The skyr whey is used in the production of pickle-fermented products, such as blood and liver sausages (Hilmarsdóttir & Arnadóttir, 1989).

7.2.7 *The microflora of starter cultures*

The exact identity of the bacteria that are active during the production of traditional fermented milk products is not known. Tätmjölk, surmjölk, filbunke and buttermilk are produced at 17–22°C, which is the optimal growth temperature for selected strains of mesophilic lactic acid bacteria. Most probably, the *Lactococcus* and *Leuconostoc* strains dominated the starter culture (including some yeast and moulds) in some of these products. However, skyr's milk is fermented at a higher temperature (40°C) followed by fermentation at a lower temperature. This might stimulate an initial growth of thermophilic lactic acid bacteria followed by mesophilic micro-organisms, such as yeasts and moulds, because of the low pH of the product. At present, commercially available starter cultures are used, mainly the DL- and/or L-type (see Chapter 2). The DL-type consists of a diversity of strains belonging to *Lactococcus lactis* subsp. *lactis*, subsp. *cremoris* and subsp. *lactis* biovar *diacetylactis*, and *Leuconostoc mesenteroides* subsp. *cremoris*. However, the L-type contains *Lac*. *lactis* subsp. *cremoris*, usually an EPS-producing strain, together with *Leu*. *mesenteroides* subsp. *cremoris* (Pettersson, 1988). Partial characterisations of these mesophilic lactic acid bacteria are shown in Table 7.5.

Even today, the exact composition of the starter cultures based on the strain(s) level is not always known because they are traditional cultures grown in a specified way to keep the balance between the different strains. *Lac*. *lactis* subsp. *lactis* and subsp. *cremoris* are the main acid-producing strains in the starter culture, while *Lac. lactis* subsp. *lactis* biovar *diacetylactis* and *Leu*. *mesenteroides* subsp. *cremoris* ferment the citric acid present in the milk; diacetyl and carbon dioxide are the two flavour components of special importance in these products (Jönsson & Pettersson, 1977; Pettersson, 1988). Both in the commercially available cultures and in the final products, *Lac*. *lactis* subsp. *lactis* and subsp. *cremoris* are the dominant micro-organisms. Various proportions, 2–25%, of *Lac*. *lactis* subsp. *lactis* biovar *diacetylactis* and *Leu*. *mesenteroides* subsp. *cremoris* are also present, giving rise to products with

Table 7.5 Starter culture bacteria used in traditional Scandinavian fermented milk products.

Traditional		Modern	
Product	Micro-organisms[1]	Product	Micro-organisms[1]
Tätmjölk	Mesophilic lactic starter cultures (e.g. *Lactococcus* spp. and EPS producing *Leuconostoc* spp.)	Långfil	L-type mesophilic starter cultures consisting of *Lac. lactis* biovar *longi*[2] and *Leu. mesenteroides* subsp. *cremoris*
Surmjölk	Similar to those present in tätmjölk, but using non-EPS producing micro-organisms	Filmjölk	DL-type mesophilic starter cultures consisting of *Lac. lactis* subsp. *lactis*, *Lac. lactis* subsp. *cremoris*, *Lac. lactis* subsp. *lactis* biovar *diacetylactis*, *Leu. mesenteroides* subsp. *cremoris*
Buttermilk	The micro-organisms present in the starter culture are similar to those used for the production of surmjölk	Kärnmjölk/ cultured buttermilk	DL-type mesophilic starter cultures (see above)
Filbunke	The micro-organisms present in the starter culture are similar to those used for the production of surmjölk	Viili	*Lac. lactis* subsp. *cremoris* biovar *longum*, *Leu. mesenteroides* subsp. *cremoris* and *G. candidum*
		Filbunke	DL-type mesophilic starter cultures (see above)
Skyr	*S. thermophilus*, *Lactobacillus* spp., yeasts and moulds	Skyr	Undefined starter culture containing *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus* and yeasts (e.g. *Saccharomyces, Torulopsis, Candida* spp.)
		Ymer[3]	DL-type mesophilic starter cultures (see above)

[1] For further details see Tamime & Marshall (1997) and Tamime (2002).
[2] This is a variant of *Lac. lactis* subsp. *cremoris* capable of producing EPS.
[3] This product was developed in 1937.

different sensory profiles. The different organisms in the DL- and L-type culture, and the proportion of different species and subspecies, can be enumerated using Nickels–Leesment agar or modified agar, respectively (von Nickels & Leesment, 1964; IDF, 1997). Both the DL- and the L-type starter cultures are usually developed for use in cheese production, and the DL-type is also used for cultured butter production. Thus, adjustments of the production conditions for optimal quality of the fermented milks are needed and usually performed by the individual dairy company. Chapter 2 provides more information on lactic micro-organisms present in fermented milks together with the technology for the production of starter cultures. Currently, starter cultures are usually purchased from starter culture manufacturers in concentrated freeze-dried or frozen form for the production of bulk starter or for direct-to-vat inoculation (DVI) or direct-to-vat set (DVS).

7.2.8 *Safety of traditional Scandinavian fermented milks*

The production of traditional Nordic fermented milks (fresh, concentrated and long shelf-life) involves the heat treatment of the milk before fermentation and the addition of starter cultures. Spontaneous fermentation is not normally used during

the manufacture of the traditional products; however, curdled milk is produced by spontaneous fermentation, and is considered to have an inferior quality (Fleichman, 1915). The combined effects of heat treatment of the milk and low pH ensure the microbiological safety of these products (Ryser, 1998; Kruse, 1999; Gran, 2002). However, without heat treatment of the milk, several micro-organisms originating from raw milk are able to grow during the fermentation period. Some examples of undesirable micro-organisms are *Enterobacteria*, *Enterococcus* and several species of yeast and mould able to ferment lactose or oxidise lactose to lactic acid. Even pathogenic strains such as *Escherichia coli* and *Staphylococcus* spp. may flourish. In addition, the microbiological quality of the raw milk is extremely important and, hence, using milk from healthy cows without udder inflammation and good milking procedure will help to minimise faecal contamination of the milk; these precautionary measures will help to reduce the count of pathogenic bacteria in the milk. By adding a high proportion of a starter fermentate to milk and establishing a standard of acceptable rate of pH decrease, the microbiological safety of these traditional Nordic products may be acceptable without heat treatment of the milk.

7.3 Some aspects of commercially available products

Almost all current Scandinavian fermented milks have a traditional counterpart. They are generally fermented by *Lactococcus* spp. and have an aroma formed by using specific strains (Marshall, 1982). The compositional and microbiological quality of raw milk for the manufacture of Nordic fermented milks, including cultured buttermilk, is similar to those applied to yoghurt (see Chapter 1 and Tamime & Robinson, 1999). Since the lactococcal species are very sensitive to several commonly used antibiotics, it is important that the milk should be free from such substances because a slight increase in the fermentation time will provide the ideal conditions for contaminating micro-organisms to grow.

The dry matter concentration of the raw milk is of specific importance during the manufacture of Nordic fermented milks because the milk is not fortified as in the case of yoghurt making. The natural variation in the protein content of Scandinavian milk may result in different texture and, in Sweden, a variation between 3.32 and 3.45 g 100 g^{-1} can occur in spring and late summer, respectively (Lindmark-Månsson *et al*., 2003). Interestingly, consumption of fermented milk is highest in summer, which might be partly explained by a better texture of the product.

Infection of mesophilic cultures by bacteriophages (phages) is common and has a large economic impact on cheese production (Cogan *et al*., 1991). In cases where whey is processed for the production of powder and later used in fermented milk making, awareness of the risk of phages surviving in the powder should not be overlooked as they are heat resistant (Everson, 1991). When EPS-producing cultures are attacked by bacteriophages, a dramatic effect on texture can usually be observed (U. Svensson, unpublished data).

The treatment of the raw milk is similar to that for yoghurt making, including homogenisation and deaeration of the milk. However, the milk solids-not-fat (SNF)

content is, in general, not fortified, and that is why the quality of Nordic fermented milk products correlates with the dry matter content of the milk.

Currently, mesophilic lactic acid bacteria are incubated at 17–23°C and the milk is fermented directly after inoculation. Except in set-type products, the coagulum of the fermented milk is broken and pumped through a cooler to prevent further acidification and to reduce whey separation. Post-fermentation heat treatment of these products will reduce the viable counts of lactic acid bacteria, and it is not used in the production of Nordic fermented milks.

7.4 Specific characteristics/technology of Scandinavian fermented milk products

7.4.1 *Långfil*

Långfil is the modern variant of the traditional tätmjölk and it is produced in Norway and Sweden. It has a very mild and slightly acidic taste, high viscosity and ropy consistency. It is poured or pressed out of the package onto a plate and eaten as it is with a spoon (Fig. 7.2). Due to the production of EPS, the product is very stable and has a low tendency to wheying off. To date, no flavoured products exist.

The milk is standardised to a fat content of 3 g 100 g^{-1}, homogenised at 15–20 MPa, deaerated and heat-treated at 95°C for 20 s to 5 min (Puhan, 1988). The milk is cooled to 17–23°C, inoculated with 1 g 100 g^{-1} of starter culture DL-type with ropy strains of *Lac*. *lactis* subsp. *cremoris*, packed and incubated for 20–24 h or to a pH below 4.6. The containers are cooled to a temperature below 8°C, and the product has a shelf-life of 14 days.

Fig. 7.2 A photograph showing the texture of långfil.

7.4.2 *Viili*

Viili is the modern variant of the traditional product filbunke. It is very popular in Finland with an annual consumption of 4.5 kg per person. A flavoured variant with a fruit preparation at the bottom is also available together with a variant including the probiotic bacteria *Lactobacillus rhamnosus* GG. It has a lower fat content, is made from homogenised milk and does not have any mould added (Leporanta, 2003).

On top of the traditional viili is a cream layer with white mould (*G. candidum*) growing on the surface that gives the product a velvety appearance. Viili has a mild acid and aromatic taste, and the consistency is thick and slightly ropy but easily spoonable. Normally, viili is eaten with a tablespoon so that the gel of the product can be cut into portions. If viili is mixed or eaten with a teaspoon, it becomes very ropy in texture, similar to långfil. In addition, when a spoonful of viili is cut from the package and placed onto a plate, it should retain its shape without collapsing or losing its strength, and show no sign of syneresis within 2 min (Fig. 7.3).

Traditionally, viili is made from non-fortified and fat standardised milk. In addition, the milk is not homogenised or deaerated, but heat-treated at 95°C for 5 min (Puhan, 1988; Leporanta, 2003), cooled to 20°C and inoculated with 3–6 mL 100 mL^{-1} of a specific starter culture (L-type) containing ropy strains of *Lac. lactis*. subsp. *cremoris*. A mould, *Geotrichum candidum*, is added with the starter culture. The inoculated milk is packaged, placed on trays, palletised and transferred to a ripening tunnel. The containers are incubated at 20°C for 18–24 h or to a pH below 4.6, cooled to < 6°C, and the product has a shelf-life of at least 14 days. The growth of the mould is limited due to the restricted amount of oxygen in the headspace of the sealed cup. If the lid is broken or improperly sealed, mould will continue

Fig. 7.3 An illustration of the typical texture of viili. Reproduced in colour as Plate 6, after page 110.

to grow to form a thick irregular layer on the surface, giving the product a strong 'mouldy' flavour.

7.4.3 *Filmjölk*

This fermented milk is the modern variant of the traditional surmjölk (Table 7.4). Filmjölk is the most common fermented milk in Sweden with an annual consumption > 10 kg per person. Its popularity, however, is limited in the other Nordic countries. Flavoured variants are available together with added probiotic microflora, such as *Bifidobacterium lactis* and many species of lactobacilli (*Lb. acidophilus, casei* and *reuteri*). In certain filmjölk, a specific variant of a probiotic strain (*Lactococcus lactis* subsp. *lactis* L1A), which is isolated from traditional tätte, is also added to the milk with the starter cultures (Grahn *et al*., 1994).

Filmjölk is a spoonable, semi-solid product, which is made from non-fortified milk and the fat content standardised to different levels. The product is normally consumed as a breakfast meal mixed with cereals or fruits, or as a quick lunch meal during the summer months. The taste of filmjölk is mild, slightly acidic but more aromatic than långfil due to diacetyl and carbon dioxide from the starter culture (Jönsson & Pettersson, 1977). Diacetyl and carbon dioxide are produced from citrate metabolism of *Lac. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp. (see Chapter 2, Fig. 2.5).

The fat standardised milk is homogenised at 15–20 MPa, deaerated and heat-treated at 95°C for 5 min (Puhan, 1988), cooled to 17–24°C and fermented with a DL- or L-starter culture in a tank for 17–24 h to pH 4.3 after cooling. The coagulum is broken gently, cooled to 8–12°C in a plate heat exchanger, and stored at 8°C. Filmjölk will have a shelf-life of 10–14 days at refrigeration temperature (Anon., 1987).

7.4.4 *Cultured buttermilk*

Cultured buttermilk is the modern variant of the traditional fermented milk product. It differs quite a lot from one country to another depending on the different traditional methods used for butter production. Usually, the texture of cultured buttermilk is rather thin and it is often used as a refreshing drink. It should also have an aromatic flavour depending on the diacetyl content, and should be without acetaldehyde.

In Denmark and the Netherlands, large volumes of cultured buttermilk are produced compared to other European countries. The milk is standardised to a fat content < 1 g 100 g^{-1} (the SNF content is not fortified), homogenised at 20 MPa, heat-treated at 90°C for 20 s to 5 min and cooled to 18–22ºC (Rønkilde-Poulsen *et al*., 1976; Driessen & Puhan, 1988; Puhan, 1988). The milk is fermented with a DL-type starter culture for 16–20 h or to pH 4.5, stirred, cooled to 4–9ºC and packaged.

Cultured buttermilk might, however, be made in a different way depending on the country of origin (Tamime & Marshall, 1997). For example, Australian cultured buttermilk is made from milk standardised to 1.5–2.5 g 100 g^{-1} fat, and the SNF is fortified by the addition of skimmed milk powder or by evaporation of the milk

base (Puhan, 1988). The milk is homogenised at 10 MPa, heat-treated in a batch process at 80–85ºC for 5–15 min, cooled to 27–32°C, inoculated with a starter culture (without aroma-forming strains) at a rate of 3–4 mL 100 mL^{-1}, incubated for 5–16 h to pH 4.4–4.5, cooled and packaged. If the product is stored at 4°C, it has a shelf-life of 28 days. However, the fermentate may be homogenised at low pressure before cooling to produce cultured buttermilk with a smooth consistency and without granulation (Tamime *et al*., 2001).

7.4.5 *Skyr*

Skyr is an Icelandic fermented milk, the modern variant of the traditional and home-made product with the same name. Its properties are close to quark or concentrated yoghurt, and sometimes it is categorised as being a concentrated, homogenous, fresh cheese product (Hilmarsdóttir & Arnadóttir, 1989).

Skyr is made from skimmed milk with added rennet and fermented with a specific mixed starter culture consisting of different undefined strains of thermophilic lactic acid bacteria including *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus helveticus* and lactose-fermenting yeasts (Simonarson, 1978; Gudmundsson, 1987; Magnússon, 1988). This blend of different micro-organisms seems to produce a better quality product than that made with a yoghurt starter culture, contrary to what was suggested by Pétursson (1939). The skimmed milk is heat-treated to 85° or 95°C for 30 or 15 min, respectively, cooled to 39–43°C and inoculated with the starter culture at a rate of 0.025–0.1 mL 100 mL^{-1} together with 0.005–0.01 mL 100 mL^{-1} of rennet. The milk is fermented for 4–6 h (i.e. to pH ~ 5.2), stirred and cooled in a plate heat exchanger to 12–20°C. The fermentate is further incubated for 12–18 h, cooled to 5–6°C and concentrated by removing the whey using the cloth-bag method (Magnússon, 1988).

The current mechanical method for the manufacture of skyr includes the following. After the secondary fermentation period at 18°C (pH 4.1–4.2), the fermentate is heated to 67°C for 15 s, cooled to 30°C and concentrated to 17.5 g 100 g^{-1} total solids. In addition, the skyr whey is ultrafiltered to the same total solids content of the product, heated to 80°C for 5 min, homogenised, cooled to 10°C and mixed with the skyr in order to increase the yield of the product (Gudmundsson, 1987; Wolpert, 1988). Optionally skyr may be blended with fruit flavours and/or cream, packaged and finally cooled in the refrigerated store. Incidentally, by applying the ultrafiltration process to the whey, the yield of skyr can be increased to 1 kg from 3.8 kg skimmed milk compared with 1 kg from 5 kg skimmed milk without ultrafiltration.

The heat treatment of the fermentate helps to inactivate the yeast in the product, but not thermophilic lactic acid bacteria, which results in an extended shelf-life of the skyr. However, due to the two-stage fermentation, the product tastes different from concentrated yoghurt or Greek-style yoghurt, as it has a rich and mild flavour that it is still favoured by Icelanders.

7.4.6 *Ymer*

Ymer is a product type similar to concentrated yoghurt, developed in Denmark in 1937. Traditionally, it was made from milk that had been concentrated by partial whey separation after the fermentation stage. At present, ymer is made from UF milk (Sørensen, 1974; Nielsen, 1976). Like skyr, it is a concentrated homogenous product, which can be similar to fresh cheese; however, ymer is not concentrated to the same level of total solids as skyr. Ymer is eaten like a fermented milk product, and has fat and protein contents of 3.5 and 5.6 g 100 g^{-1}, respectively. Ylette is a variant of ymer, containing half the amount of fat but with the same protein content. Lactofil is the Swedish name given to ymer manufactured in the south of Sweden.

The stages in the modern manufacture of ymer are as follows. Skimmed milk is pre-heated to 50°C, ultrafiltered to a specified protein content, blended with cream, homogenised and heated to 85–90°C for 5 min. The milk base is cooled to 20–22°C and fermented with a DL-type starter culture for 18 h (pH 4.5). After heavy stirring and cooling in a plate heat exchanger to 12°C, the fermentate is packaged and transferred to a cold store for final cooling to 5°C. Another mechanical method for ymer production, widely used before the UF process, is the use of quark separators (Puhan, 1973; Tamime *et al*., 2001).

7.5 Conclusions and future perspectives

Traditional Scandinavian fermented milks have been successfully developed into modern variants and, although similar in some of their characteristics, they are not identical to the originals. They have been adapted to full-scale production with a daily output at some large factories in excess of 100 000 kg. Simultaneously the products have been modified to achieve consumer acceptability. The fat content varies from as low as 0.4 g to 3.5 g 100 g^{-1}, and variants with even higher protein and fat content are available. During the last ten years, fruit-flavoured products have become very popular, with fruit at the bottom of the carton as exemplified by some variants of viili, and with fruit mixed into the fermented milk as with flavoured variants of filmjölk. In contrast to fruit-flavoured yoghurt consumers, Scandinavian consumers prefer flavoured filmjölk made with fruit pulp rather than pieces. Finally, Nordic fermented milks have proved to be well suited to carrying probiotic bacteria. As the pH remains stable during the storage period (4.2–4.4), the survival rate of lactobacilli and/or bifidobacteria has been excellent, allowing a shelf-life of up to 3 weeks when handled appropriately.

According to IDF statistical records, consumption of Nordic fermented milks has increased in Denmark, Finland and Sweden but decreased in Iceland and Norway over the last 50 years. However, during the last decade, overall consumption has decreased and has been partly overtaken by consumption of fruit-flavoured yoghurt and yoghurt drinks. In order to retain the important nutritional and health benefits of Nordic fermented milk products, present-day products with added value should be improved and adjusted in taste to meet consumer demand.

As reported elsewhere, the texture and taste of yoghurt has been modified during the last few decades, partly by including starter organisms that are EPS producers (Marshall & Rawson, 1999; Ruas-Madiedo *et al*., 2002). In contrast, starter culture types used in the production of Nordic fermented milks range between two extremes: first, with no EPS as in filmjölk, and second, a high EPS ropiness as in långfil and viili. Although mesophilic starter cultures are mainly developed for cheese and butter production, any future development of cultures specific for fermented milk products would offer interesting possibilities. For example, by taking advantage of the development of the yoghurt starter cultures with a variety of EPS-producing strains, specific starters of filmjölk could be developed with similar properties. Much is known about the genes responsible for EPS production and its chemical composition (van Kranenburg *et al*., 1999; Yang *et al*., 1999; Hugenholtz, 2000). Furthermore, it is now possible to study the appearance of exopolysaccharides and their importance to texture via scanning electron microscopy (Fig. 7.4; Hassan *et al*., 2003).

Another possibility might be in the development of drinks based on Scandinavian products. It might be possible, for example, to make use of the traditional starter cultures that produce large amounts of carbon dioxide for new drinks, keeping the refreshing characteristics of the traditional products that make them popular during the summer season (Fleichman, 1915; Bertelsen, 1983). Selection of packaging materials with an efficient gas barrier might result in new possibilities.

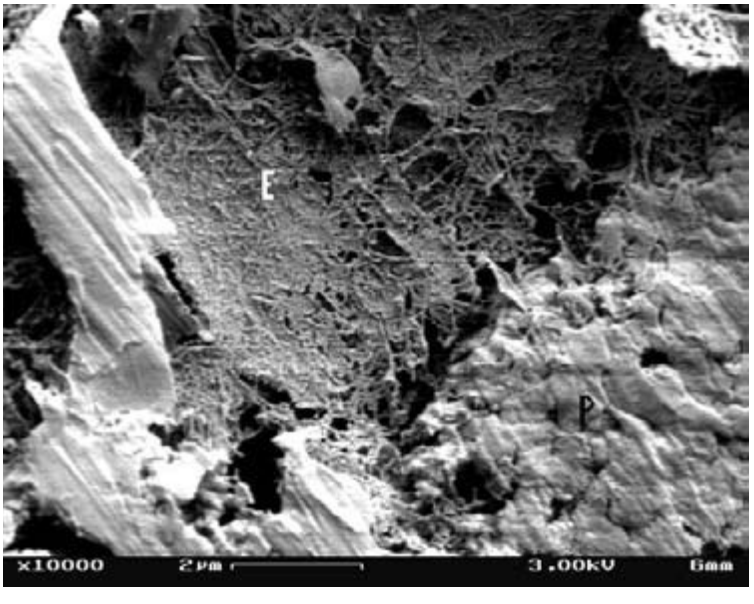

Fig. 7.4 The microstructure of an exopolysaccharide and its importance to the texture of a Nordic fermented milk product. Reproduced with permission from Hassan, A., Frank, J. & Elsoda, M. (2003) *International Dairy Journal*, **13**, 755–762.

Nordic fermented milks have been used successfully as 'functional' foods, mainly as carriers of probiotics such as lactobacillus and bifidobacteria. Little attention has been given to the health aspects of products such as långfil and its starter organisms. However, it might be possible to develop products with improved levels of some B vitamins, which are produced by the starters during the fermentation period. At present, fermented milks are considered second to milk as a source of some vitamins, e.g. B_{12} (Alm, 1982; Arkbåge *et al*., 2003). Furthermore, it might also be possible to use starters to improve the immune system, such as certain strains of *Lactococcus* spp. that have been shown to enhance the antigen-specific antibody production, and other metabolites might have a beneficial impact on the gut flora. Another area of interest is the use of certain species as delivery vehicles for vaccines (Grahn *et al*., 1994; Mercenier *et al*., 2000; Mori *et al*., 2000; Kimoto *et al*., 2004).

Improvement of the starters used in the production of Nordic fermented milks should be given priority. Knowledge of the genome of lactic acid bacteria can be used to select and to use the right strains to improve the products of today. Gene technology might be used to further improve the products in the future. *Lactococcus* spp. might be well suited to this technology as the bacteria are safe, the genome is already known and the bacteria do not survive in the intestine.

Modern science and technology offer many opportunities to improve and develop present products. By taking advantage of these opportunities, it will become possible to market Nordic fermented milks not only in the Scandinavian countries but in other markets as well.

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8 Production of Kefir, Koumiss and Other Related Products

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8.1 Introduction

Fermented milk products, which are made with certain strains of lactic acid bacteria and yeasts, are classified as yeast-lactic fermentations (see Chapter 1), and some typical examples are kefir and the related products, koumiss and acidophilus-yeast milk (Kurmann *et al*., 1992). The sensory attributes are the result of the production of lactic acid, carbon dioxide, alcohol and other flavouring compounds that are formed during the fermentation stage of the milk (Honer, 1993; Kroger, 1993; Simova *et al*., 2002). In addition, these products are also known as alcoholic milk beverages. However, the exact microflora of these products is not well defined as it depends on the origin of the starter culture, conditions of growth, processing of the milk and the type of milk used (e.g. cow, goat or sheep) (Kolakowski, 2001).

These products originated in central Asia between the Caucasus Mountains and Mongolia, and are very popular in many countries (e.g. the former USSR, Poland, Czech Republic, Slovakia, Hungary, Bulgaria and some Scandinavian countries) (Duncan, 1986; Libudzisz & Piatkiewicz, 1990; Dzwolak & Ziajka, 2000). Although many of these fermentations involve specific microfloras, there is a considerable degree of similarity in respect of the technological aspects, and there are differences in the type of milk used as a growth medium. Traditionally, kefir and koumiss are homemade, but the former product has been commercialised in many countries. The popularity of the alcoholic milk beverages has helped to increase their consumption, and also to promote their reputation as being good for health (Cevikbas *et al*., 1994; Farnworth, 1999; Garrote *et al*., 2000; Farnworth & Mainville, 2003). This chapter attempts to review the latest scientific, microbiological and technological developments of manufacture of these products.

8.2 Kefir

The sensory characteristics of kefir can be described as follows: (a) the colour is white or yellowish; (b) the aroma is balanced and yeasty; (c) the taste is acidic, but pleasant and refreshing; and (d) the texture is rather thick, but not gluey, with an elastic consistency. Lactic acid, volatile acids, diacetyl, carbon dioxide and ethanol are the main compounds influencing the sensory properties of kefir (Rea *et al*., 1996; Libudzisz & Piatkiewicz, 1990; Güzel-Seydim *et al.*, 2000a). During the 1990s, kefir attracted research interest, in part because of its probiotic properties.

Kefir has variously been described as a 'dairy champagne', 'the champagne of cultured dairy products' and 'yoghurt of the 21st century' (Kemp, 1984; Mann, 1989; Gorski, 1994). The Internet (http://www.medicinalfoodnews.com/vol102/issue1/kefir.htm, http://www.food-info.net/dutch/topics/to.php?c=kefir) provides some additional information, and even enables kefir grains to be exchanged among kefir enthusiasts worldwide.

8.2.1 *Historical background*

The origins of fermented milk beverages are very old, and date back to the domestication of certain mammals such as the cow, goat and sheep. It is safe to assume that the first types of product were made accidentally by the fermentation of milk, which was stored at ambient temperature (~20–30°C depending on the region). Due to variations in the climatic and environmental conditions in different parts of the world, specific strains of micro-organism became dominant in these products and, as a consequence, specific types of fermentations evolved and became distinct in a given region.

The production of fermented milks was known to the ancient Greeks and Romans. The Greek historian Herodotos (485–425 BC) reported that a refreshing drink produced from mare's milk was popular among the Ghets tribes. According to Caucasian legend, Mahomet (ca. 570–632) gave Mahomet's grains (also known as mushrooms) together with the secret kefir recipe to the inhabitants of the region (Koroleva, 1991). Thus, kefir is one of the oldest fermented milk beverages, and the technology of production and the use of a specific starter culture have developed through the ages. Its origin can be traced back to the Caucasus region where it has been produced by a traditional method in bags made from animal hides, or in oak barrels or earthenware pots. As these containers were used continuously (i.e. after some kefir being consumed, a new batch of fresh milk was added), the micro-organisms tended to form a thin layer and later clusters on the surfaces of the containers. This process of microbial film formation was helped by the warm conditions of the Caucasus.

Motaghi *et al*. (1997) suggested that kefir grains can be produced by using the same traditional method of handling the milk. They used a goatskin bag (4 L capacity), which was washed several times with sterile water, and filled with pasteurised milk and the intestinal flora from a sheep. It was kept at 24–26°C for 48 h and shaken every hour. When the milk coagulated, 75% of the fermentate was replaced with fresh milk, and this procedure was repeated for 12 weeks. Gradually, a polysaccharide layer appeared on the surface of the hide. The layer was removed aseptically from the hide and propagated in pasteurised cow's milk where grains were formed (0.5–3.2 cm in diameter), and added several times to fresh cow's milk.

8.2.2 *Kefir grains*

Appearance and chemical composition

Kefir grains range in size from 0.3 to 2.0 cm or more in diameter, and are characterised by forming an irregular, folded or uneven surface; the grains resemble cauliflower florets in shape and colour (Fig. 8.1). They are elastic and white or slightly yellow

Fig. 8.1 Structural form of typical kefir grains.

in colour, and have a characteristic smell. Kefir grains have a specific structure and biological function. When the grains are seeded in milk, they grow and pass their properties to the following generation(s) of newly formed grains (Güzel-Seydim, 2000b; Saloff-Coste, 2002; Simova *et al*., 2002). Furthermore, the microflora of kefir grains is remarkably stable, retaining its activity for years if preserved and incubated under appropriate cultural and physiological conditions.

The dry mass of the fresh grains amounts to 10–16 g 100 g^{-1}, which consists of about 30 g 100 g^{-1} protein and 25–50 g 100 g^{-1} carbohydrate (Libudzisz & Piatkiewicz, 1990). The chemical composition (g 100 g^{-1}) of kefir grains originating from Russia, Yugoslavia and Bulgaria contained approximately moisture 90, protein 3.2, fat 0.3, non-protein soluble nitrogen 5.8 and ash 0.7 (Ottogalli *et al*., 1973). Similar values have been found in grains originating from Sweden (Bottazzi *et al*., 1994), while the grains obtained from households in Argentina contained (g 100 g^{-1}) moisture 83, polysaccharides 9–10 and protein 4.5 (Abraham & De Antoni, 1999; Garrote *et al*., 2001). The grains are made of a conglomerate of microbial cells and their metabolites, coagulated milk proteins and carbohydrates. The latter include various mucous substances produced by the bacteria, mainly polysaccharides, which are known as kefiran (la Riviere *et al*., 1967; Marshall *et al*., 1984; Toba *et al*., 1986; Takizawa *et al*., 1998; see Production of mucopolysaccharides, below). According to LieBing *et al.* (1998), the surface of the kefir grains is richly colonised by bacteria and yeasts, which are mainly the autolysing type that cannot pass through the matrix of the kefiran.

Microflora of kefir grains

Kefir grains have a complex microbiological composition, and they consist of a blend of lactic acid bacteria ~ 83–90%, yeasts ~ 10–17%, acetic acid bacteria and possibly

a mould. According to Polish Standards (Anon., 2002), the microscopic observation of the grains should consist of 80% lactobacilli, 12% yeasts and 8% lactococci. The following are recommended for further reading regarding aspects of kefir grains and the product (Bottazzi & Bianchi, 1980; Molska et al., 1980; Engel, 1984; Glaeser et al., 1986; Glaeser & Hangst, 1987; Molska, 1988; Barnett et al., 1990; Libudzisz & Piatkiewicz, 1990; Rohm & Lehner, 1990; Rohm et al., 1992; Angulo et al., 1993; Yoshida & Toyoshima, 1994; Pintado et al., 1996; Saloff-Coste, 1996; Tamime & Marshall, 1997; ChinWen et al., 1999; Drake et al., 1999; Kuo & Lin, 1999; Lin et al., 1999; Tamime et al., 1999; Jukić et al., 2001; Verachtert, 2002; Loretan et al., 1998, 2003; Witthuhn et al., 2004; Liutkevičius & Šarkinas, 2004). Figure 8.2 is an electron micrograph showing the typical microflora present in the grains.

Lactic acid bacteria

Bacterial isolates from kefir and the grains are shown in Table 8.1. *Lactococcus* spp. (especially *Lactococcus lactis* subsp. *lacti*s), *Lactobacillus* spp., *Streptococcus thermophilus* and *Leuconostoc* spp. constitute the main species of the lactic acid bacteria (LAB) group; however, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus casei* subsp. *pseudoplantarum*, *Lactobacillus brevis* and *Lactobacillus kefir* have been found in kefir grains. Rea *et al*. (1996) reported that the *Lactococcus* spp. is located on the surface of the kefir grains, while

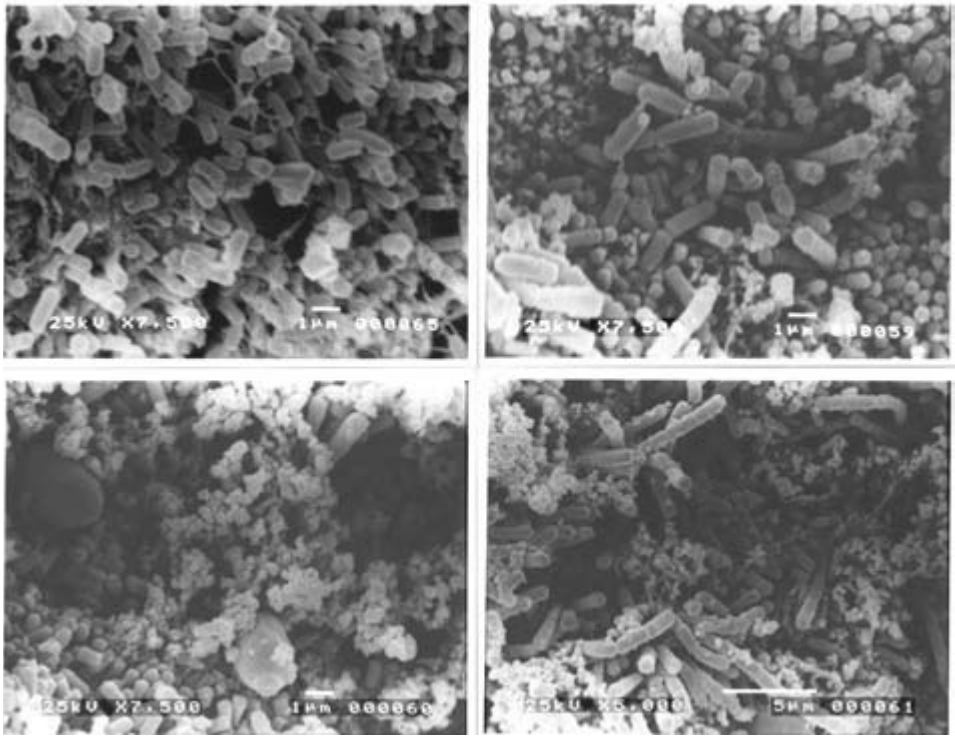

Fig. 8.2 Scanning electron micrograph showing a variety of micro-organisms of kefir grains.

Table 8.1 Reported microflora of kefir grains product and culture formulation.

Micro-organisms	Products[a]/references[b]															
	G 1	G 2	G 3	G 4	G 5	G 6	G 7	G 8	G 9	G 10	K 11	K 12	C 13	C 14	C 15	C 16
Lactic acid bacteria																
Lactococcus lactis subsp. *lactis*	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Lactococcus lactis subsp. *cremoris*				X		X				X			X			X
Lactococcus lactis subsp. *lactis* biovar *diacetylactis*				X		X				X			X			X
Streptococcus thermophilus	X	X								X				X	X	X
Enterococcus durans				X					X							
Lactobacillus acidophilus		X				X			X	X	X				X	X
Lactobacillus brevis	X	X		X	X	X	X		X	X	X	X				
Lactobacillus casei						X			X		X	X				
Lactobacillus paracasei subsp. *pseudoplantarum*	X															
Lactobacillus rhamnosus		X		X						X						
Lactobacillus paracasei subsp. *tolurans*		X								X						
Lactobacillus delbrueckii subsp. *bulgaricus*	X			X		X				X				X	X	
Lactobacillus cellobiosus										X						
Lactobacillus fermentum		X								X						
Lactobacillus gasseri		X								X						
Lactobacillus helveticus	X			X						X				X		
Lactobacillus kefir			X	X		X	X	X	X	X	X	X	X			
Lactobacillus kefiranofaciens			X				X			X						
Lactobacillus kefirgranum			X				X			X						

Lactobacillus paracasei subsp. *alactosus*										X							
Lactobacillus paracasei subsp. *paracasei*										X							
Lactobacillus paracasei subsp. *pseudoplantarum*										X							
Lactobacillus parakefir			X			X	X	X		X							
Lactobacillus plantarum								X		X				X			
Lactobacillus viridescens		X								X							
Leuconostoc spp.		X													X		
Leuconostoc mesenteroides subsp. *mesenteroides*				X				X	X		X	X					
Leuconostoc mesenteroides subsp. *cremoris*													X				X
Leuconostoc mesenteroides subsp. *dextranicum*				X		X				X							
Bifidobacterium animalis subsp. *animalis*[c]																	X
Acetic acid bacteria																	
Unidentified acetic acid bacteria									X		X						
Acetobacter spp.		X						X									
Acetobacter aceti				X						X		X					
Acetobacter rasens				X						X							
Yeasts																	
Candida colliculosa																	X
Candida friedrichii		X								X			X	X			
Candida kefyr		X		X	X	X			X	X	X	X					
Candida holmii		X								X							
Candida inconspicua	X									X							
Candida maris	X																
Candida pseudotropicalis						X			X	X	X						

(Continued.)

Micro-organisms	Products[a]/references[b]														
Candida tenuis						X			X						
Candida valida									X						
Cryptococcus kefyr									X						
Debaryomyces hasenii															X
Kluyveromyces marxianus var. *fragilis*				X	X				X		X				
Kluyveromyces marxianus var. *lactis*		X		X					X	X	X				
Kluyveromyces marxianus var. *marxianus*	X						X		X			X			X
Mycotorula lactis									X						
Mycotorula lactosa									X						
Pichia fermentans		X							X						
Saccharomyces spp.							X								
Saccharomyces cerevisiae	X	X	X	X	X	X		X	X	X	X		X	X	
Saccharomyces dairensis									X						
Saccharomyces exiguus								X	X						
Saccharomyces unisporus		X							X			X			
Torulaspora delbrueckii		X	X					X	X						
Zygosaccharomyces florentinus									X						
Moulds															
Geotrichum candidum						X			X						

[a] G = grain; K = kefir; C = culture formulation.

[b] 1, Simova *et al*. (2002); 2, Angulo *et al*. (1993); 3, Takizawa *et al*. (1998); 4, Koroleva (1988b); 5, Marshall (1982); 6, Gjengedal & Evavoll (1985); 7, Garrote *et al.* (1997); 8, Garrote *et al*. (2001); 9, Duncan (1986); 10, Tamime & Marshall (1997); 11, Marshall (1993); 12, Assadi *et al*. (2000); 13, Wszolek *et al*. (2001); 14, Beshkova *et al*. (2002); 15, Duitschaever *et al*. (1987); 16, Chr. Hansen/Bio-kefir culture.

[c] Masco *et al*. (2004).

the lactobacilli and the yeasts are present in the deeper layers of the grain. Electron micrographs very rarely give evidence of lactococci on the surface of the grains as they are loosely bound to the grains during growth, and are easily washed off during sample preparation.

Yeasts

Many species of yeasts (e.g. *Kluyveromyces marxianus* var. *lactis*, *Saccharomyces cerevisiae*, *Candida inconspicua* and *Candida maris*) have been found in kefir grains and the product (Table 8.1; Wyder *et al*., 1997). *Kluyveromyces* spp. (i.e. lactose-fermenting) are permanently present, and are responsible for the yeasty aroma in the product (Engel *et al*., 1986; Seiler & Kümmerle, 1997). However, Angulo *et al*. (1993) and Simova *et al*. (2002) reported that lactose-negative yeasts were also present (see also Kwak *et al*., 1996), but the count was reduced dramatically in the kefir made with the starter culture fermentate rather than the kefir grains. Furthermore, some scientists consider lactose-negative yeasts to be contaminating organisms (Krusch, 1984; Glaeser *et al*., 1986).

Wyder *et al*. (1999) isolated a new yeast species (*Saccharomyces turicensis*) from different kefir grains, which metabolises D-glucose, D-galactose and sucrose, but does not ferment lactose. In addition, Franzetti *et al*. (1998) have isolated *Hansenula yalbensis* from kefir.

Acetic acid bacteria

Other microbial species present in kefir grains include *Acetobacter aceti* and *Acetobacter rasens*. Angulo *et al*. (1993) considered them to be contaminants, while Koroleva (1988a) reported that their presence is desirable (see Associative growth, below).

Moulds

A similar ambiguity exists with regard to *Geotrichum candidum*, which is referred to as a contaminating organism (Koroleva, 1988b; Molska 1988; Demarigny *et al*., 2000) or as part of the kefir microflora that does not affect the quality of the product (Garrote *et al*., 1997, 1998; Tamime & Marshall, 1997; Berger *et al*., 1999).

It is evident that the microbial flora of kefir grains varies from one country of origin to another (Table 8.1) and, as a consequence, the sensory profile of the products made with different kefir grains will be different. Limited data are available comparing the sensory characteristics of kefir made with kefir grains from different origins, but one of us (H.S.G.) has evaluated four different commercially available Russian kefir products containing different fat contents, and the results are shown in Fig. 8.3. Kefir made from milk containing 1.0 g fat 100 g^{-1} was characterised by having a typical buttermilk odour and had the highest level of carbon dioxide. Three of the kefir products had a distinctive blue cheese flavour and odour, which could be associated with the metabolic activity of certain micro-organisms present in the kefir grains that are capable of hydrolysing the fat and casein in milk (Law, 1999; see also section 8.2.3). The yeast flavour, which was evident in one of the

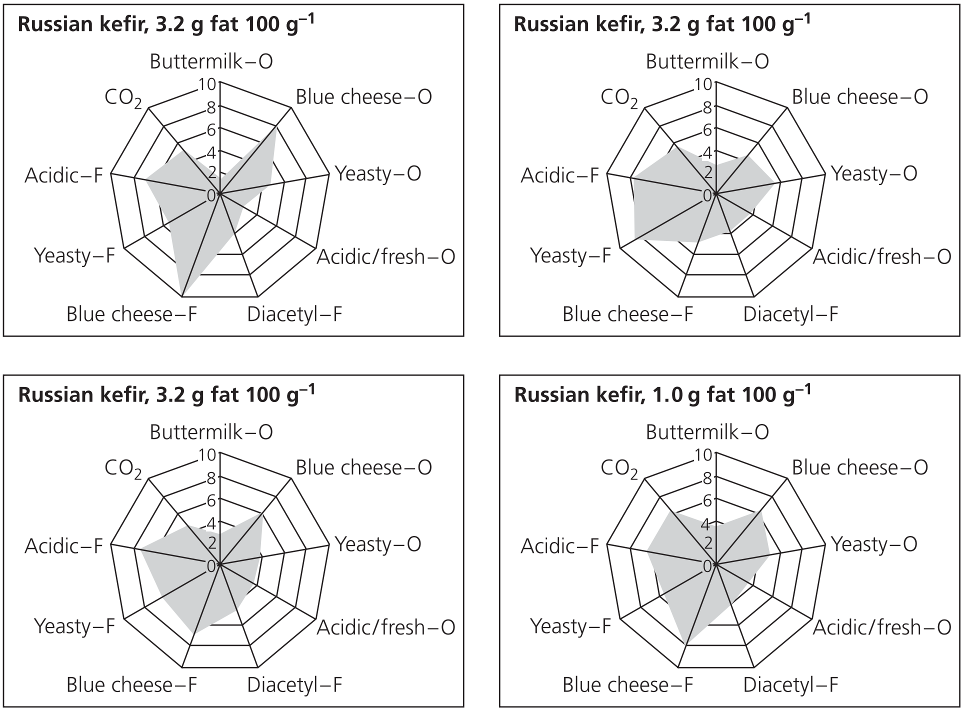

Fig. 8.3 Star plots of the effect of different kefir grains on some perceived sensory attributes of commercially available Russian kefirs. By permission of Chr. Hansen A/S, Denmark.

kefir products, could be associated with the strain of yeast(s) present in the kefir grains or the high viable count in the product (Fig. 8.3).

Associative growth

The lactococci tend to grow faster in milk than do the yeasts (Koroleva, 1988a; Rea *et al*., 1996) and hydrolyse lactose to produce lactic acid, providing a suitable environment for yeasts to grow (Seiler, 2003). In addition, the yeasts synthesise B-group vitamins, hydrolyse milk proteins, catabolise oxygen to produce carbon dioxide and produce alcohol (Koroleva, 1988a; Barnett *et al*., 1990; Linossier & Dousset, 1994; Jakobsen & Narvhus, 1996; Gadaga *et al*., 2001; Wouters *et al*., 2002). Also, the production of vitamin B_{12} by *Acetobacter* spp. is reported to stimulate the growth of other organisms present in the kefir grains (Rea *et al*., 1996).

During growth in milk, the proportions of the different microfloras of the grains will differ from those present in the product. The proportion of the organisms present in *kefir* may be 80% lactococci including *Leuconostoc* spp., 10–15% yeasts and 5–10% lactobacilli, while the *kefir grains* contain 65–80% lactobacilli, 10–15% yeasts and 5–25% lactococci including *Leuconostoc* spp. (Molska, 1988; Molska *et al*., 2003). Fermentation at higher temperatures will enhance the growth of the lactobacilli, while the Lactococcus and yeast counts will be reduced. Recently,

Simova *et al*. (2002) reported that changes in the microbial population in the product were also influenced by the type of inoculum used (e.g. kefir grains vs. starter culture fermentate).

The populations of *Lac. lactis* subsp. *lactis* and *S. thermophilus* tend to increase from 52–65% in the kefir grain to 79–86% in the product, whereas the lactobacilli decrease by 13–23%. This is the reason that some researchers investigating the grains refer to the lactobacilli as the dominating flora constituting 65–80% of the total count of LAB (Marshall, 1993; Takizawa *et al*., 1998). The latter authors reported that the homofermentative lactobacilli were the predominant micro-organism constituting about 90% of the lactobacilli, while the heterofermentative lactobacilli (*Lb. kefir* and *Lactobacillus parakefir*, ~ 10%) were the minor population in the grain.

A minimum count of 1×10^5 cfu g^{-1} of yeast should be present in the kefir grains to achieve the desired sensory properties in the product (Stam *et al*., 1998; Simova *et al*., 2002). However, the *Kluyveromyces* spp. are permanently present in the kefir grains and the product, but the count of the dominant lactose-negative yeasts present in the kefir grains tend to decrease dramatically in the product made with a kefir starter culture. If *K. marxianus* spp. and *Candida kefir* are present in the kefir grains, they tend to migrate to the inoculated milk and dominate the kefir (~ 80–100%) (Wyder *et al*., 1997) but, if these yeast species are not present, the lactose-negative species (*Saccharomyces unisporus*) will become predominant, and the total yeast count diminishes.

Commercially developed kefir starter cultures

Starter culture companies (e.g. Chr. Hansen A/S and Danisco Biolacta Spółka z o.o.) have made great efforts to develop kefir starter cultures that do not produce grains during the manufacture of kefir and, as a consequence, the characteristics of the product are different from a traditional product (see Production of kefir grains and starter cultures, below). These developed kefir starter cultures will make production of the product less laborious, and will ensure a longer shelf-life of the kefir. Basically, the choice and blends of starter organisms are based on similar criteria to those discussed in Chapter 2, and will not be reviewed again. Nevertheless, the choice of yeast strains is important in order to contribute to a typical flavour to kefir, but excessive production of CO_2 is undesirable as it may cause blowing of the packaging container, and high levels of ethanol may not be accepted in some countries where a levy by Customs and Excise has to be paid by the manufacturer.

Chr. Hansen A/S has recently developed and sensory profiled three freeze-dried kefir starter cultures containing different yeast species. The cultures were used as direct-to-vat set (DVS) inocula, grown in milk containing 2 g fat 100 g^{-1}, fermented at two different temperatures (30°C and 35°C), and the kefir products were evaluated after 5 and 12 days of storage at 8°C. The developed cultures are known as LAF-3 (containing *Debaryomyces hansenii*, non-lactose fermenting), LAF-4 (containing *Kluyveromyces marxianus* var. *marxianus*, lactose fermenting) and LAF-7 (containing *Candida colliculosa*, non-lactose fermenting); these cultures also contain a blend of LAB (mesophilic organisms CH-N22™) and EPS-producing *S. thermophilus*. In

addition to sensory profiling, the acetaldehyde content was measured by headspace gas chromatography (HSGC) and the presence of CO_2 as reported by the taste panel was included with the sensory results to enhance the characterisation of the kefir cultures. The results can be summarised as follows:

- Fermenting the milk at 30°C, all the kefir products (i.e. 5 days old) had similar odour, flavour and taste attributes including acetaldehyde content, CO_2, mouth thickness, gel firmness and ropiness (Fig. 8.4a). In general, all the products were mild in taste and lacked the typical kefir aroma (yeasty taste and flavour). Increasing the fermentation temperature to 35°C affected most of the attributes (Fig. 8.4a), and the product characteristics were primarily influenced by the strain of yeast present in the starter culture. The creamy flavour score increased in all three different kefir products at 35°C, while the 'sourish' taste was lower compared with parallel products fermented at 30ºC; these perceptions were closely related to the texture profile of the product. For example, an increase in mouth thickness character (Fig. 8.4a) led the tasters to perceive the product to be less acidic and creamier than a product with similar pH, but to give a lower mouth thickness score. In addition, culture LAF-4 fermented at 35°C developed a high kefir odour and taste (i.e. yeasty) due to the metabolic activity of *Kluyveromyces* species, and the fermentation temperature of the milk also influenced texture attributes.
- After 12 days of storage at 8°C, the sensory profiles of the kefirs were different when compared with fresher products, and it became apparent that the activity of the yeast had influenced the flavour attribute (Fig. 8.4b). In brief, the characteristics of each culture were: (a) LAF-3 kefirs (i.e. fermented at 30ºC and 35°C) were mild but 'sourish' in taste, and gave the highest score for the diacetyl odour, (b) irrespective of the fermentation temperature, LAF-4 kefir products had a distinctive kefir odour and taste, but fermenting the milk at 35°C resulted in slightly creamier flavour and amounts of acetaldehyde and CO_2 similar to those in LAF-7 kefir (Fig. 8.4b) and (c) the perceived sensory attributes of LAF-7 kefir products were moderate with regard to the kefir flavour and odour characters and CO_2 level compared with culture LAF-4, but had the highest acetaldehyde content (i.e. enhanced the 'fruity' note in the product) when the milk was fermented at 35°C.

8.2.3 *Biochemistry of fermentation*

Microbial metabolism

Marshall and Tamime (1997) reported that the mechanisms responsible for the production of flavour compounds are the result of metabolism (i.e. the catabolism of carbohydrate and nitrogen utilisation), which is necessary for the micro-organisms to grow efficiently and to achieve maximum populations. The biochemistry of organic acids and flavour production by LAB when grown in milk have been detailed in Chapter 2 and will not be reviewed in this chapter.

However, yeast metabolism in kefir is not well established, and may result in different types of degradation of the milk components, all of which can potentially

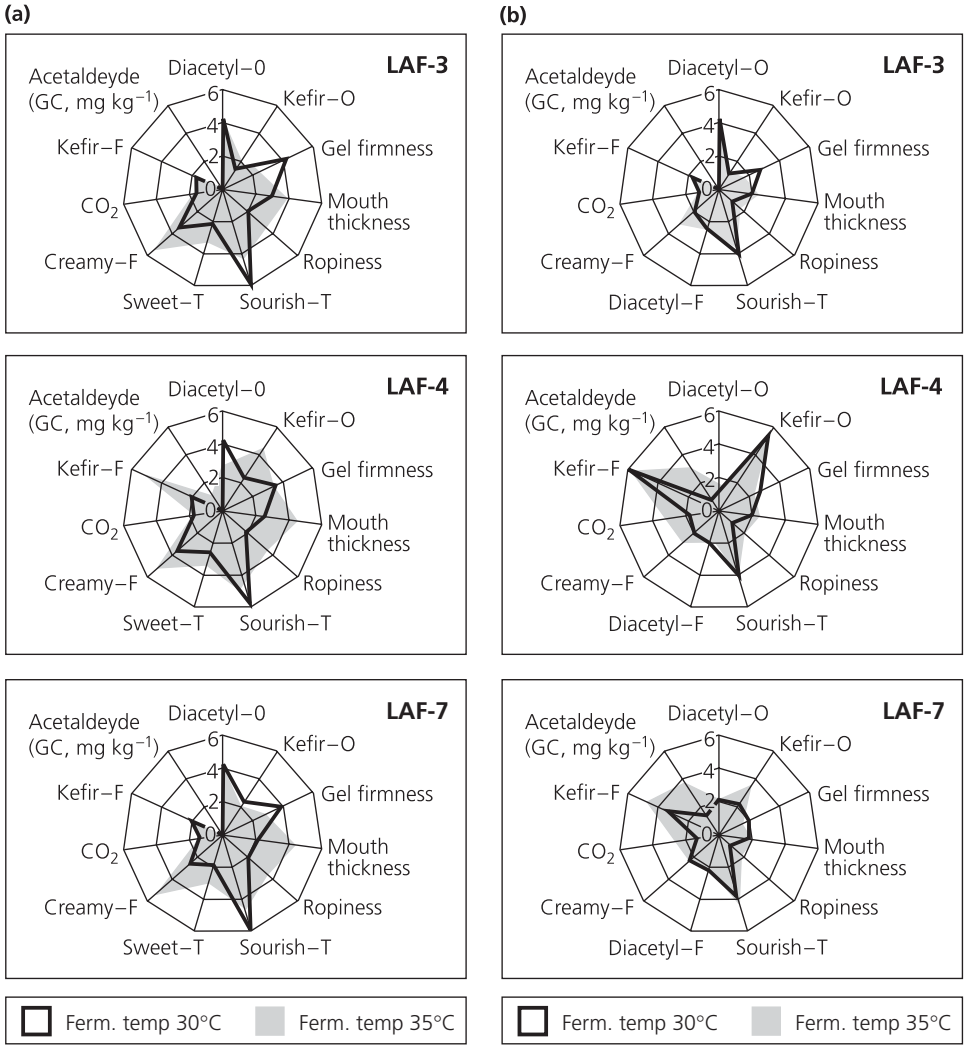

Fig. 8.4 Star plots of sensory profiling of commercially developed kefir starter cultures. (a) Product stored for 5 days at 8°C, and (b) product stored for 12 days at 8°C. By permission of Chr. Hansen A/S, Denmark.

contribute to the flavour of the product. The extent of milk constituent hydrolysis by the yeasts present in the kefir grains and/or the starter culture are as follows.

Fat/lipids hydrolysis

Lipolysis of the fat component in milk results in the formation of free fatty acids, which can be precursors of flavour compounds to be formed in kefir. These compounds may include methyl ketones, alcohols, lactones and esters. Lipolysis and decarboxylation of free fatty acids leads to the formation of methylketones, especially 2-nonanone and 2-heptanone, which are known as blue cheese flavours (Tomasini

et al., 1995). Yeast and mould fermentations generate methylketones (Marshall & Tamime, 1997), and some *Lactobacillus* strains (e.g. *Lb. helveticus*) can also produce large amounts of 2-nonanone and 2-heptanone (Drake *et al*., 1999).

Protein hydrolysis

The yeasts are able to degrade casein to small peptides and free amino acids; the latter are converted to alcohols, aldehydes, volatile acids, esters and sulphur-containing compounds (especially methionine, which is the precursor for volatile aroma compounds). Examples of sulphur compounds are dimethyldisulfide and dimethyltrisulfide, which are regarded as essential components for a cheesy flavour (Berger *et al*., 1999; van Kranenburg *et al*., 2002). Incidentally, *Geotrichum candidum* is also responsible for the production of these sulphur-containing compounds, which have been also found in Russian kefir (Skov Guldager, unpublished data).

Branched-chain amino acids are precursors of aroma compounds, such as isobutyrate, isovalerate, 3-methylbutanal, 2-methylbutanal and 2-methylpropanal (van Kranenburg *et al*., 2002). However, Kahala *et al*. (1993) examined the peptide content of a number of Finnish milk products, and found a higher rate of proteolysis and greater number of peptides in kefir than in yoghurt. No comment was made on the contribution to flavour. In addition, the presence of yeasts in food or dairy products contributes to the flavour of the final product by the synthesis of a variety of chemical compounds, and Stam *et al.* (1998) have given an overview of the role of yeast in flavour formation (see also Law & Haandrikman, 1997; Guzel-Seydim *et al*., 2003; Paraskevopoulou *et al*., 2003a). Recently, one of us (Wszolek, unpublished data) has measured the extent of proteolysis in kefir and yoghurt made from the same milk, and it is evident that the highest degree of protein hydrolysis was in kefir (Table 8.2).

Although kefir owes its flavour characteristics to the metabolism of LAB and yeast, ethanol has little impact on flavour but may contribute to the aroma attribute (Marshall & Tamime, 1997). Beshkova *et al*. (2003) reported that the content of carbonyl compounds produced by a kefir starter culture was greater than that produced by kefir grains, including the amounts of ethanol and CO_2 (3.98 μg kg^{-1} and 1.8 g kg^{-1}, respectively).

Metabolic pathways of yeast fermentation

A wide range of yeast species have been identified in kefir, and up-to-date names including the classification and differentiation of yeasts have been reviewed by Kreger-van Rij (1984), Barnett *et al*. (1990); Kurtzman and Fell (1998), Hansen and Jakobsen (2004) and Ng (2004). According to Walker (1998), yeasts can metabolise a wide range of organic substrates, which will provide the cells with essential carbon and energy; for historical reasons, glucose metabolism of *S. cerevisiae* is the best-understood metabolic process due to its importance in traditional industrial fermentations. Hence, this review of yeast metabolism will focus only on the genera that have been identified in kefir grains. Much of the relevant information has been extensively reviewed by Walker (1998), but the following reviews are rec-

Table 8.2 HPLC[1] measurement of low-density peptides present in milk, kefir and yoghurt.

Sample	Number of peaks	Number of peaks of amino acids and other low-molecular-weight substances (RT[2] 0–8 min)	Number of peaks of peptides and dissolved protein in 10% TCA[3] (<3 kDa)[4] (RT 8–73 min)	Number of peaks of peptides and undissolved proteins in 10% TCA (>3 kDa) (RT 73–110 min)	Ratio of area of peaks of >3 kDa to <3 kDa	Number of hydrophilic peptides (RT 8–35 min)	Number of hydrophobic peptides (RT 35–73 min)	Ratio of area of peaks of hydrophilic to hydrophobic peptides
Milk	50	9 (7.46)[5]	23 (2.64)	18 (90.70)	34.36	12 (1.84)	11 (0.63)	0.34
Kefir	84	10 (39.10)	61 (35.00)	13 (25.95)	0.74	22 (17.32)	39 (17.68)	1.02
Yoghurt	67	12 (55.49)	40 (22.30)	15 (22.22)	1.00	17 (14.72)	23 (7.57)	0.51

[1] HPLC = high performance liquid chromatography.
[2] TCA = trichloroacetic acid.
[3] RT = retention time.
[4] kDa = × 1000 Daltons.
[5] Data in parentheses is area of peaks (%).
From M. Wszolek (unpublished data).

ommended for further reading regarding metabolism by yeasts (Bacila *et al*., 1978; Berry *et al*., 1987; Rose & Harrison, 1989; Verachtert & DeMot, 1990; Broach *et al*., 1991; Jones *et al*., 1992; Wheals *et al*., 1995; Smith, 1995; Bramble & Marzluf, 1996; Fell, 1997; Nissen, 1999; see also http://biochemie.web.med.uni-muenchen.de/Yeast_Biology/03_Metabolism.htm).

According to Walker (1998) the process of metabolism involves biochemical assimilation (i.e. in anaerobic pathways, energy is consumed and the reductive processes lead to the biosynthesis of new cellular material) and dissimilation (i.e. the catabolic pathways are oxidative processes, which remove electrons from intermediates and use them to generate energy) of nutrients by the yeast cell. Similar to other micro-organisms, the reductive and oxidative processes in yeasts are mediated by dehydrogenase enzymes, which predominantly use NADP and NAD, respectively, as redox cofactors.

Most yeasts metabolise sugars as their main carbon source for energy and, for example, *S. cerevisiae* metabolises glucose via the glycolysis cycle to yield 2 pyruvate + 2 ATP + 2 NADH + H^+ (Walker, 1998). This scheme requires ten steps of phosphorylation to produce pyruvate from glucose and, in the alcoholic fermentation of sugars, the yeasts re-oxidise NADH to NAD in the terminal step from pyruvate (i.e. pyruvate decarboxylase; PDC) to acetaldehyde, which is later catalysed by alcohol dehydrogenase (ADH) to ethanol as follows (see also Fig. 8.5):

$$C_6H_{12}O_6 + 2\,P_i + 2\,ADP \rightarrow 2\,C_2H_5OH + 2\,CO_2 + 2\,ATP + 2\,H_2O$$

In addition, certain yeasts can catabolise pyruvate in the mitochondrial matrix where it is oxidatively decarboxylated to acetyl CoA by pyruvate dehydrogenase (PD). *Saccharomyces* (i.e. aerobic and anaerobic fermentation) and *Kluyveromyces* (i.e. anaerobic fermentation) species are able to metabolise a wide range of sugars

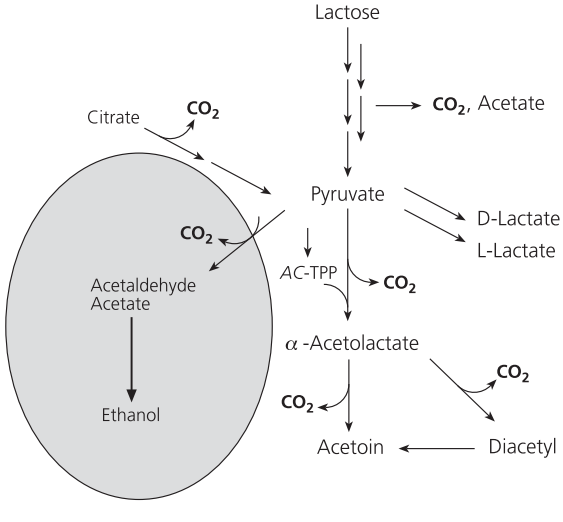

Fig. 8.5 Metabolic pathways of yeast for the production of ethanol. By permission of Chr. Hansen A/S, Denmark.

besides glucose, such as lactose, galactose, melibiose and fructose. However, the principal mode of sugar catabolism by *Candida*, *Pichia* and *Torulopsis* species is anaerobic fermentation. Since milk does not contain non-carbohydrate substrates, the metabolism of such compounds by yeasts will not be reviewed – see Walker (1998) for further detail.

Candida, *Saccharomyces* and *Kluyveromyces* species possess very limited extracellular proteolytic activity, while *Candida* and *Torulopsis* species possess extracellular lipase activity (Walker, 1998) and, if present in kefir grains, they can contribute to the flavour of the product because of their hydrolytic activity on the milk constituents.

Production of exopolysaccharides

The microfloras of the kefir grains are held together in a matrix of protein and polysaccharide (la Riviere *et al*., 1967; Ottogalli *et al*., 1973; Kandler & Kunath, 1983; Marshall *et al*., 1984; Mukai *et al*., 1988, 1990; Toba *et al*., 1990; Yokoi *et al*., 1990; Bottazzi *et al*., 1994; Takizawa *et al*., 1994, 1998; Abraham & De Antoni, 1999; Rimada & Abraham, 2001; Frengova *et al*., 2002; Liu *et al*., 2002). Some species of LAB are known to produce extracellular polysaccharides (EPS) material, which contributes to the texture of the grain and the fermentate (refer to Chapter 2 for further details). The EPS produced by the kefir micro-organisms is commonly known as kefiran, which is water-soluble and consists of branched glucogalactan containing equal amounts of D-glucose and D-galactose (Micheli *et al*., 1999; Mitsue *et al*., 1999). Figure 8.6 illustrates the EPS microstructure of kefir grains, and the EPS-producing LAB has been identified as *Lb*. *kefir* and *Lactobacillus kefiranofaciens* (Toba *et al*., 1986, 1987, 1990, 1991; Fujisawa *et al*., 1988; Arihara *et al*., 1990; Mukai *et al*., 1990; Yokoi *et al*., 1990, 1991; Yokoi & Watanabe, 1992; Cheirsilp *et al*., 2003a, b). Incidentally, the kefiran material has been reported to possess anti-cancer activity (Murofushi *et al*., 1983). Recently, Vancanneyt *et al*. (2004) isolated *Lactobacillus kefirgranum* from kefir grains and kefir and, based on its characteristics (e.g. morphotypes, phenotypic features and SDS-PAGE profiles of whole-cell proteins), found it to be closely related to *Lb*. *kefiranofaciens* (i.e. sharing 100% 16S rDNA); hence, it is proposed that the strain should be reclassified as *Lb*. *kefiranofaciens* subsp. *kefirgranum*.

Although the EPS produced by the LAB can affect the rheological properties of the product, e.g. improve the texture and mouth-feel, they can also exhibit advantageous biological properties, such as immunostimulatory, antimutagenic, antitumour and anti-ulcer activities, and act as a prebiotic compound (Oda *et al*., 1983; Nagaoka *et al*., 1994; Yoon *et al*., 1999; WonHo *et al*., 2003; see also Chapter 2). Recently, Rimada and Abraham (2003) developed an efficient quantitative method to determine accurately the amount of EPS production by kefir grains in milk and deproteinised whey.

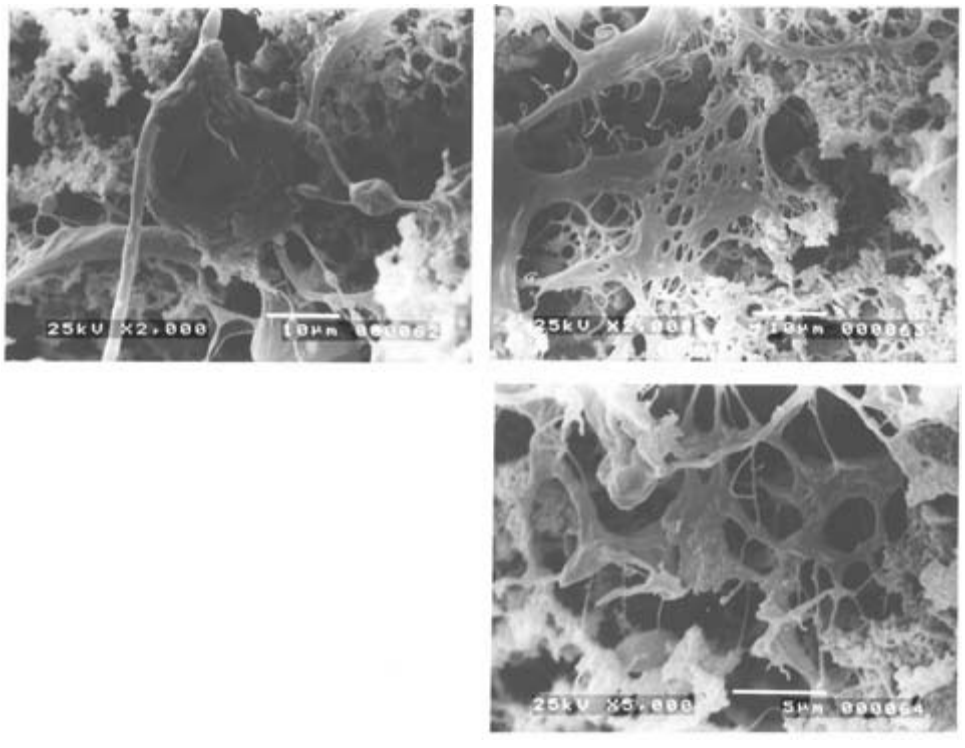

Fig. 8.6 Scanning electron micrograph showing exopolysaccharide (EPS) production by the kefir microflora.

8.2.4 *Production systems*

Traditionally, kefir was produced in animal skin bags as an ongoing fermentation in which the fresh milk (cow or goat) was seeded with kefir grains and, as the product was consumed, fresh milk was added. The historical development of the industrial production of set and stirred kefir in the former USSR has been reviewed by Koroleva (1991). The current methods available for the production of kefir are: (a) the traditional method based on the use of kefir grains and (b) the commercial or industrial process using direct-to-vat inoculation (DVI) or direct-to-vat set (DVS) kefir starter culture. The main changes, which took place, for example in Poland, two decades ago, in the industrial production of kefir were: (a) extending the shelf-life of the product beyond 3 days, (b) the replacement of the retail glass bottles with semi-rigid cartons or plastic cups and (c) the replacement of the kefir grains with DVI kefir starter culture blends. Glass bottles were sealed with an aluminium cap, which allowed some of the CO_2 produced to escape, but the shelf-life of the product was short. However, the use of cartons or plastic containers did not improve the shelf-life of the product, but resulted in blown packages due to the entrapment of the gas and, as a consequence, consumer complaints increased because of concerns about the freshness and safety of the product. Despite the fact that some manufacturers tried to educate consumers by informing them that the blowing of the container was caused

by the kefir grains, the response to the campaign was negative and, with increasing market competition together with the domination of the multiple retailers in the country, the industry was forced to produce kefir that has a shelf-life of at least 2 weeks. As a consequence, some manufacturers started to blend the kefir microflora with mesophilic LAB and yoghurt organisms. The taste of such fermented milks was slightly similar to that of kefir, but the beverage bore no resemblance to the original product, and it could not be named 'kefir' under Polish food legislation.

Kefir is also manufactured from goat's and sheep's milk, and characterisation of the quality of these products compared with cow's milk kefir has been reported by Pieczonka and Pasionek (1995), Wszolek *et al*. (2001), Bozanic *et al*. (2003) and Wójtowski *et al*. (2003). In addition, cow's milk fortified with cheese whey (Paraskevopoulou *et al*. (2003b) or with ultrafiltered skimmed milk (Tratnik & Kršev, 1991) has been used for the manufacture of kefir.

While the technological aspects of kefir production have been reviewed by Özer and Özer (2000), Robinson *et al*. (2002), Anon. (2003), and LiLi *et al*. (2003) details of manufacture may differ from one plant to another or between countries, but certainly many of these processes have much in common. Therefore, it is entirely appropriate to review in detail the steps in kefir production.

Production of kefir grains and starter cultures

The preparation stages for the production of kefir grains and a mother or bulk starter culture are shown in Fig. 8.7. The kefir grains are normally supplied in 50-g portions, and are preserved in an isotonic salt solution. Propagating the kefir grains is done on a daily basis in skimmed milk, which is heated to 95°C for 30 min, cooled to 18–20°C, inoculated with kefir grains and incubated for 20–24 h (0.78 mL lactic acid 100 mL^{-1}), and ripened for 7–8 h at 10–12°C to facilitate the growth of the yeasts. The grains are strained using a sterile sieve, and the kefir 'wash' (i.e. fermentate without the kefir grains or mother starter culture) is inoculated into freshly prepared

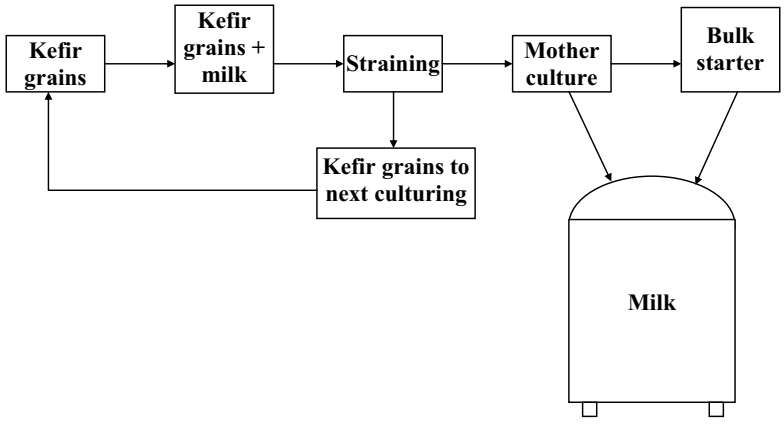

Fig. 8.7 Production stages for kefir grain preparation and the manufacture of kefir.

milk at a rate of 1–3 mL 100 mL^{-1} for low-volume stirred kefir production or, for large-volume production, it is used at a rate of 3–5 mL 100 mL^{-1} (depending on its activity) to prepare the bulk starter culture. However, as the mass density of the grains increases every time they are grown in milk, low activity grains are removed once or twice a week and discarded.

To maintain a good and consistent quality kefir, a standard ratio of kefir grains to milk has to be maintained. The use of a high proportion of grains (e.g. 1 : 20; Koroleva, 1988a) accelerates the acidification rate, but the starter culture contains relatively low counts of lactococci, *Leuconostoc* spp. and yeasts, but higher counts of lactobacilli, and loses its activity very quickly (Garrote *et al*., 1998). By increasing the proportion of milk from 20 : 1 to 50 : 1 (Koroleva, 1988a), the activity, growth and balance of the micro-organisms in the kefir grains is maintained (see also Kramkowska *et al*., 1986; Molska, 1988; Fil'chakova & Koroleva, 1997). Other proportions of kefir grains to milk that have been used for the production of kefir are: 20–50 g L^{-1} (Marshall & Cole, 1985; Merin & Rosenthal, 1986; Mann, 1989; Hosono *et al*., 1990); 20–100 g L^{-1} (Koroleva, 1988a); and 50–100 g L^{-1} (Marshall *et al*., 1984; Neve, 1992), but a low rate of addition of kefir grains is recommended during the production of kefir.

One of the most important aspects in preparing a kefir starter culture is the handling of the kefir grains. Molska *et al*. (1980), Koroleva (1988a, 1988b), Polish Standards (Anon., 2002) and starter culture suppliers do not recommend rinsing the kefir grains if they are transferred daily to freshly prepared milk. Alternatively, the grains can be delicately rinsed with pasteurised milk or sterile water, and stored at < 6°C or frozen. Rinsing the kefir grains, which is a common practice in the industry, can lower the counts of lactococci and yeasts and the biomass of the grains (Schoevers & Britz, 2003), and prolonged storage can alter the equilibrium between the various micro-organisms.

At present, freeze-dried kefir starter cultures (i.e. the blend based on the original microflora present in the kefir grain, with special care to use yeast strains that do not produce too much CO_2) are used by most kefir manufacturers. Only very few dairy plants still propagate kefir grains to be used as a starter culture. In such cases, the fermented beverage is now promoted as a 'special' product, i.e. traditional kefir of exceptional quality and health properties.

Danisco Biolacta Spółka z o.o. in Poland has developed different DVI or DVS freeze-dried kefir cultures: (a) the M-type is known as a mother culture, which is grown twice for the production of the intermediate and bulk starter cultures, respectively; (b) the S-D culture (i.e. semi-direct) is used for the production of the bulk starter; and (c) the D-culture (DVI or DVS) is added directly to the processed milk for the manufacture of kefir (Fig. 8.8 and Robinson *et al*., 2002). Incidentally, when these cultures are grown in milk, no kefir grains are produced. Currently, many starter culture companies offer dairy manufacturers freeze-dried kefir cultures containing, in part, some of the micro-organisms present in traditional kefir grains. The production of freeze-dried cultures consists of the following stages.

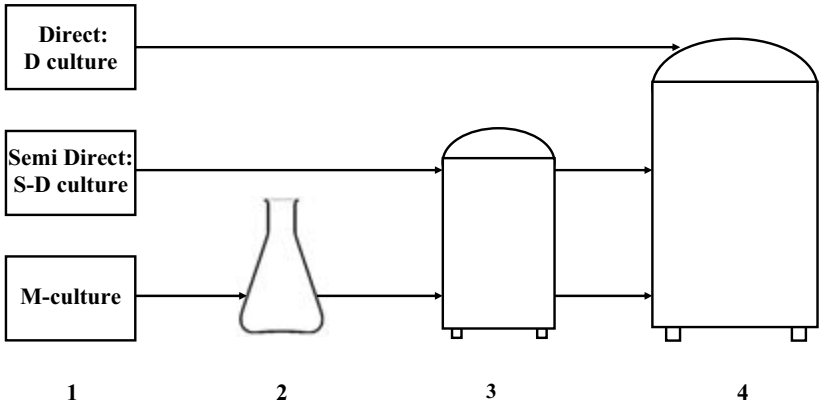

Fig. 8.8 Flow diagram for the industrial production of kefir using different types of cultures. 1, Lyophilised starter culture; 2, mother culture; 3, bulk starter culture; 4, kefir.

(1) Propagation of the kefir grains – this is carried out in an automated and strictly controlled process, which requires 7 × 1-day growth cycles. The grains are grown in milk, separated by centrifugation after the fermentation stage, re-seeded into fresh milk and, after the seventh cycle, the biomass of the grains has increased by 100–120%.
(2) A portion of the grains is retained for the production of more batches in the future, while the rest is homogenised, mixed with a cryoprotective compound, freeze-dried, pulverised and stored at 4°C.
(3) The dried culture is standardised in relation to its application (see subsequent section), packed in a modified atmosphere container and stored ready for despatch to customers.

The microflora of any freeze-dried culture varies depending on their designated application and, in a similar manner to kefir grains, the proportion of the different micro-organisms differ from those in the mother or the bulk starter culture, and in the kefir itself. However, to improve the properties of kefir produced commercially, the DVI/DVS cultures are blended with adjunct probiotic strains, such as *Lb. acidophilus* and *Bifidobacterium* spp. (see also Molokeev *et al*., 1998a, b; Rada, 1997; Petsas *et al*., 2002; Masco *et al*., 2004).

Manufacturing stages of commercial kefir

The manufacturing stages of set and stirred kefir production are shown in Fig. 8.9. The fat content in the milk is standardised (g 100 g^{-1}) to 0.1–3.3 in Russia or to 1.5, 2.0 or 3.1 in Poland (Muir *et al*., 1999). However, the most popular kefir contains 1.5 g fat 100 g^{-1} of milk (Koroleva, 1991; Dzwolak & Ziajka, 2000). The milk is warmed to ~ 65°C, homogenised at ~ 15 MPa, heated at 95°C for 5 min, cooled to 19°C in summer or to 22°C in winter and inoculated with the starter culture at a rate of 3–4 mL 100 mL^{-1} using a bulk starter culture. For set-type kefir, the inoculated

milk is packaged in glass bottles (traditional method) or semi-rigid containers, fermented for 10–12 h at 19–22°C (to an acidity of 0.7 mL 100 mL^{-1}), cooled to 9°C and ripened for 1–3 days; the product is then cooled slowly and stored at 6°C before despatch for distribution and retailing (see also Kilic *et al*. 1999).

However, stirred kefir is fermented in tanks for the same duration as that used for the manufacture of set kefir, but the fermentate is cooled to 15°C, packaged and ripened at 9°C for only 15 h and stored at 6°C. In addition, traditionally kefir was made in a glass container and, when stirred kefir was made, the viscosity was low. To overcome this problem, a luxury kefir was developed in Poland in which the milk solids content is fortified with skimmed milk powder by 2–3 g 100 g^{-1}.

An alternative approach for the production of commercially made set or stirred kefir is to use a DVI freeze-dried kefir starter; the inoculation rate is one sachet 300/500/1000 L^{-1} (Wszolek *et al*., 2001; Robinson *et al*., 2002). The manufacturing stages are similar to those shown in Fig. 8.9, with the following differences.

(1) Set kefir: the inoculated and packaged milk is fermented to pH 4.6–4.7, cooled to < 10°C and ripened for 15–20 h.
(2) Stirred kefir: the fermentate is cooled to 15–20°C (i.e. higher temperature), packaged, followed by slowly cooling to < 10°C, and ripened for 15–20 h. By filling the kefir when it is warm, the packaging seal collapses and, when the CO_2 is released during the ripening stage, the seal regains its original form rather than blowing up the container (see also Irigoyen *et al*., 2003).

Concluding remarks

In summary, the quality of traditional kefir is mainly influenced by the micro-organisms present in the grains, and the processing conditions (Marshall, 1982; Gjengedal & Evavoll, 1985; Koroleva, 1988a, b, 1991; Beshkova *et al*., 2002; Robinson *et al*., 2002; Irigoyen *et al*., 2003). For example, the use of a lyophilised kefir starter culture reduces the yeast count and eventually the yield of ethanol in the product (Brialy *et al*., 1995; JeRuei *et al*., 1999). Although scientists and culture companies have attempted to develop a kefir-like drink by blending different LAB (mesophilic and thermophilic types, e.g. CH-N22™, and ABT – *Lactobacillus acidophilus*, *Bifidobacterium* spp. and *Streptococcus thermophilus*), probiotic microfloras and yeasts (Duitschaever *et al*., 1987, 1988; Penido *et al*., 2001; Beshkova *et al*., 2002, 2003; Sotelo *et al*., 2002), the success when compared with traditional kefir has been limited but, for industrial production, these developments have been greatly welcomed. However, a probiotic yeast (*Saccharomyces boulardii*) may be used as an adjunct culture in a kefir starter culture blend in the future to enhance the health aspects associated with the product (Lourens-Hattingh & Viljoen (2001).

8.2.5 *Quality appraisal*

Systematic and effective quality control of any fermented milk product is necessary to ensure high consistent quality of the final product, and it is important that the raw

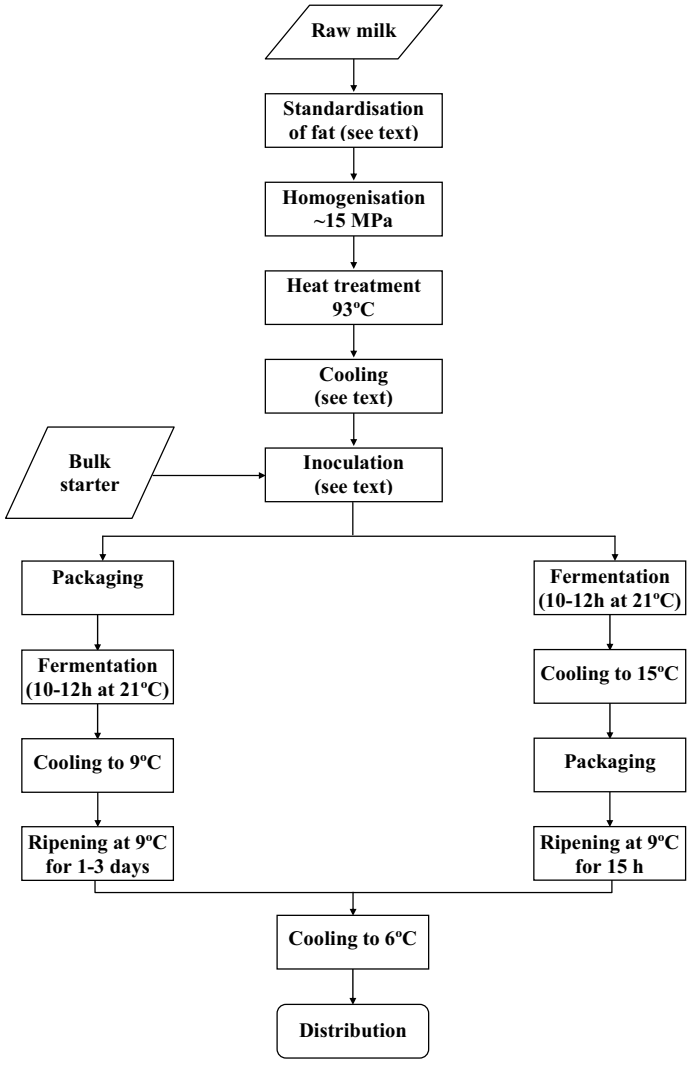

Fig. 8.9 Generalised scheme for the industrial production of kefir using kefir 'wash' or fermentate as a starter culture.

milk, starter culture or kefir grain and any other added ingredients be monitored. Traditionally, casual sampling was used in quality control, but this approach does not guarantee total safety of every packed container as the probability of detecting contaminated containers is rather low, i.e. only a few per cent. The hazard analysis critical control point (HACCP) system is highly recommended if the 'due diligence' concept and the ever-increasing quality requirements of retailers are to be fulfilled. The identification of control points (CPs) or critical control points (CCPs) during the production of kefir (e.g. standardisation of the fat and the milk solids in the milk base, heat treatment of the milk, the fermentation stages and packaging, storage

of freeze-dried starter cultures and the production of the bulk starter, and monitoring of packaging materials and containers) will help to ensure that the product is manufactured under good hygienic conditions. Although kefir may be considered a safe fermented milk beverage due to its low pH, the product also possesses some antibacterial activity (Garg, 1989; Cevikbas *et al*., 1994; Atanassova *et al*., 1999). However, final assessment of the product (e.g. compositional quality, starter cultures enumeration, examination for pathogens and sensory properties) can help the manufacturer to ensure the safety of the product.

Chemical composition and microbiological quality

The chemical composition and microbiological quality of kefir in Poland as described by Anon. (2002) and based on the FAO/WHO *Proposed Draft Standard A-11 for Fermented Milks* are as follows:

- Protein content not less than 2.7 g 100 g^{-1};
- Fat level (i.e. g 100 g^{-1}) is based on the manufacturer's declaration on the label, but not more than 10 g 100 g^{-1} (average commercial product ranges between 1.5 and 2.0 g fat 100 g^{-1});
- Titratable acidity not less than 0.6 mL 100 mL^{-1};
- Bacterial count in 1 g, not specified;
- Yeast count not less than 10^2 cfu g^{-1}.

The typical microflora of the kefir grains used for fermenting the milk should consist of *Lactobacillus kefir*, *Lactococcus* spp., *Leuconostoc* spp., *Acetobacter* spp., lactose-fermenting yeasts (e.g. *K. marxianus* spp.) and yeasts without the ability to metabolise lactose (*S. unisporus*, *S. cerevisiae* and *S. exigus*). All these organisms exist in a symbiotic equilibrium, and have been identified in the latest Codex Alimentarius Commission (CODEX STAN 243–2003; see also http://www.codexalimentarius.net and http://www.codexalimentarius.net/search/advancedsearch.do).

Nutritional value

The chemical composition of kefir provides a useful indication of the nutritional value of the product. According to Hallé *et al*. (1994) and Bottazzi *et al*. (1994), a typical compositional analysis (g 100 g^{-1}) of kefir consists of protein 3.0–3.4, fat 1.5 and lactose 2.0–3.5 (after the fermentation stage). However, the lactic acid content may range between 0.6 and 1.0 mL 100 mL^{-1} lactic acid, and the alcohol level may be 0.0–0.1 g 100 mL^{-1} in kefir made with a starter culture fermentate, or 0.03–1.8 g 100 mL^{-1} in kefir made with kefir grains.

Kneifel and Mayer (1991) reported that the vitamins in kefir made using grains and milk from different species of mammals increased by > 20% as follows: thiamine (B_1) – only in ewe's milk kefir; pyridoxine (B_6) – in kefir made with ewe's, goat's and mare's milk; folic acid – in all kefir products except mare's milk kefir; and the orotic acid content was reduced in all the kefir products throughout the fer-

mentation stage. Khamnaeva *et al*. (2000) reported that application of mechanical vibration and the injection of atmospheric oxygen into the growth medium during the fermentation of kefir with grains caused an increase in biosynthesis of riboflavin (B_2) and ascorbic acid when compared with the control sample.

Therapeutic properties

The antibacterial activity of kefir has been reported by many researchers (Garg, 1989; Serot *et al*., 1990; Cevikbas *et al*., 1994; Zacconi *et al*., 1995, 2003; Atanassova *et al*., 1999; Gulmez & Guven, 2003a, b, c; Santos *et al*., 2003; Yoon *et al*., 2003). In addition, kefir has been used for the treatment of tuberculosis, cancer, gastrointestinal tract disorders and a variety of other diseases (Sezginer, 1980; Kocak & Gursel, 1981; Ötleş & Çağindi, 2003). Other claimed beneficial effects for humans, associated with the consumption of kefir and/or the grains, include:

(1) Improvement of the digestion of the milk proteins, hydrolysis of lactose, treatment of severe intestinal infections and the correction of dysbiosis in children (Sukhov *et al*., 1986; de Vrese *et al*., 1992; Murashova *et al*., 1994; Safronova *et al*., 2001; Hertzler & Clancy, 2003);
(2) Some anti-tumour activity (Shiomi *et al*., 1982; Murofushi *et al*., 1983; Furukawa *et al*., 1990, 1991; Cevikbas *et al*., 1994);
(3) Some kefir micro-organisms can bind mutagenic substances, such as indole and imidazole (Hosono *et al*., 1990; Miyamoto *et al*., 1991; Tamai *et al*., 1995, 1996); and
(4) Kefir and sphingomyelin obtained from kefir lipids may stimulate the immune system in young, but not old, rats (Furukawa *et al*., 1991; Osada *et al*., 1994; Thoreux & Schmucker, 2001; see also Nagira *et al*., 2003; Teruya *et al*., 2003).

Nevertheless, there is not enough scientific evidence to confirm all these hypotheses or therapeutic properties, and more clinical studies are required to substantiate such claims.

The dietetic properties of kefir are, in part, related to protein breakdown, as the proteolytic activity of lactic acid bacteria and yeasts facilitates the formation of low-molecular-weight peptides and amino acids. The content of these substances in kefir is higher than in yoghurt produced from the same type of milk (Table 8.2).

Sensory properties

The traditional sensory properties of kefir made with kefir grains have been reported by Duitschaever *et al*. (1987), Assadi *et al*. (2000) and Wszolek *et al*. (2001). A product made with a kefir starter culture can have different characteristics when compared with traditional kefir, and also depending on the starter culture preparation and method of manufacture. The taste of kefir depends on the lactic acid content in the final product, which is usually around 0.8–0.9 mL 100 mL^{-1}; most of it is L(+),

and D(–) lactic acid constitutes only a few per cent of overall acid content in the product. Among the volatile acids that can be found in kefir, acetic acid dominates along with ethanol and aldehydes, but formic, orotic and propionic acids can also be present (Muir *et al*., 1999; Robinson *et al*., 2002; Beshkova *et al*., 2003). Diacetyl and acetaldehyde are the main aroma-forming compounds in kefir, but their levels are influenced by the type of starter culture used and method of production. The alcohol content in kefir is dependent on the type of yeast present in the starter culture or the kefir grain, and the extent of the ripening period (e.g. length of fermentation time at 10–12ºC); kefir may contain 0.1–1.0 g 100 mL^{-1} alcohol (Molska, 1988; Robinson *et al*., 2002).

Set-type kefir (i.e. made in the retail container) is characterised by having an acidic, milky taste and slight yeasty flavour, and the texture of the coagulum should be uniform (i.e. similar to cream), but with some visible CO_2 bubbles and no excessive effervescence. Stirred kefir has similar organoleptic properties, but as its coagulum is broken during the manufacture of the product, the viscosity of the fermentate will be low; it can be improved by fortification of the solids-not-fat (SNF) in the milk base to 11 g 100 g^{-1} using skimmed milk powder.

Product faults

The main common faults of kefir are attributed to taste (buttermilk-like) and aroma (very yeasty). The latter fault may be caused by *S. cerevisiae* as it ferments strongly and multiplies quickly in the absence of oxygen; often this fault is accompanied by a vinegar-like or solvent-like aroma (Seiler, 2003). Excessive acetic acid production in kefir is due to intense growth of *Acetobacter* spp. In addition, the presence of *Dekkera* spp. in kefir grains or starter culture may result in an acetic acid aroma. Most of the atypical yeasts in kefir do not produce acetic acid and only acidify the milk to pH 4.5. The yeasts, which produce distinct pseudo-mycelia on agar media and pellicles on the surface of the liquid media, are most likely to be *Pichia membranofaciens*, *Yarrowia lipolitica*, *Galactomyces geotrichum*, *Issatchenkia occidentalis* or *Issatchenkia orientalis*. Other yeast species, which have been reported by many researchers to cause faults in kefir, could be *Candida rugosa*, *Candida tenuis, Debaryomyces hansenii*, *Pichia fermentans*, *Torulaspora delbrueckii* and *Zygosaccharomyces florentinus* (Seiler, 2003). A bitter taste in kefir can be caused by moulds (e.g. *G. candidum*) and/or the activity of some atypical yeasts present in the product.

8.3 Koumiss

8.3.1 *Introduction*

Koumiss (also known as koumyss, kumiss, kumys, kumyz, kimiz or coomys) is a fermented milk drink first mentioned in the 5th century BC as a preferred drink of the gods. In the 7th century, koumiss became an everyday drink of the Mongolian tribes where, today, the product is known as airag, arrag, irag, chige or chigo. It is

consumed in the countries of the Caucasus region – Kazakhstan, Azerbaijan, Turkey (kumyz) – and in China (ma tung) (Tooner, 1994). Traditional koumiss is produced from mare's milk, and in Mongolia it is also produced from camel's milk. In Europe and North America, a koumiss-like product is made from full or skimmed cow's milk (see also Mann, 1989; di Cagno *et al*., 2004).

8.3.2 *Microflora of koumiss*

The microflora of koumiss is not well defined, but it consists mainly of:

- Lactobacilli (*Lb. delbrueckii* subsp. *bulgaricus* and *Lb. acidophilus*), lactose-fermenting yeasts (*Saccharomyces* spp., *K. marxianus* var. *marxianus* and *Candida koumiss*);
- Non-lactose-fermenting yeast *Saccharomyces cartilaginosus*;
- Non-carbohydrate-fermenting yeasts (*Mycoderma* spp.) (Koroleva, 1991; Varnam & Sutherland, 1994; Oberman & Libudzisz, 1998).

Montanari *et al*. (1996, 1997) and Montanari and Grazia (1997) examined 94 samples of traditional koumiss in Kazakhstan and identified *S. unisporus* (galactose-fermenting yeast) as the principal micro-organisms responsible for the alcoholic fermentation of the product. Incidentally, the non-lactose-fermenting yeasts give rise to a slower and not so homogenous alcoholic fermentation compared to *S. cerevisiae.* In the slower fermentation, a wider range of metabolites, such as glycerol, succinic acid and acetic acid, are produced (Montanari & Grazia, 1997).

Recently, Ishii *et al*. (1997) and Ishii (2003) have identified the lactic acid bacteria and yeasts from locally made chigo (i.e. koumiss made from mare's milk in inner Mongolia and China), and found 43 strains of LAB, including *Lactobacillus rhamnosus, Lb. paracasei* subsp. *paracasei*, *Lb. paracasei* subsp. *tolerans* and *Lb. curvatus*. Overall, the LAB counts averaged 1.6×10^7 cfu mL^{-1}. Moreover, 20 yeasts (lactose-fermenting strains) were identified, and these included *K. marxianus* subsp. *lactis* and *C. kefir*, and their counts ranged between 3.9 and 8.0×10^6 cfu mL^{-1}. The alcohol levels in the samples varied between 0.5 and 2.2 g 100 mL^{-1} (see also Watabe *et al*., 1998; Ishii, 1999; Fang *et al*., 2002; LinLin & MingSheng, 2003).

8.3.3 *Production systems*

Traditional process

Mongolians use mare's milk during the summer season (i.e. from July until October) to make traditional koumiss. Milking starts early in the morning and takes place every 2 h; altogether, the mares are milked six times a day. Three to five litres of milk can be obtained from one mare and, only after the last milking, the foals are returned to the mares to suckle. In the evening, the fresh milk (i.e. unheated) is added to already fermented milk in wooden pots to provide an ongoing fermentation. Traditionally, milk was fermented in horse-hide bags known as chöchuur, tursuks or burduks, which contained the koumiss microflora from the previous season (Tamime

& Marshall, 1997). However, at present, only wooden containers are used, which also contain the microflora from the previous season (Fig. 8.10). When fresh milk is added to the fermentate, the mixture is stirred vigorously for 1 h using a special implement (Fig. 8.11) to introduce air, which will create good growth conditions for the yeasts. The amount of yeast present in the milk determines the final content of alcohol in the product and, the more alcohol is produced, the better the quality of the koumiss; according to Mongolian experience, the best koumiss is produced when the ambient temperature is not too high. Airag (Mongolian koumiss) containing a high alcohol content can be distilled to obtain a homemade spirit.

Industrialised processes

The compositional quality of mare's milk is close to human milk (Table 8.3; see also Doreau *et al*., 1990; Pagliarini *et al*., 1993) and the physiological value of koumiss is higher than kefir. The protein content of mare's milk is very low compared

Fig. 8.10 Wooden container for traditional production of koumiss.

Fig. 8.11 Wooden mixing paddle that is used traditionally during the manufacture of koumiss.

to cow's milk and, as a consequence, the fermentate does not coagulate (see also Bonomi *et al*., 1994 regarding the thermal sensitivity of mare's milk protein). Due to the limited availability of mare's milk between November and June, the technology of koumiss making has been adapted to use cow's milk. However, the protein and lactose contents of mare's milk are different from cow's milk (Table 8.3), and these levels have to be adjusted to 'mimic' the former type of milk. One approach is to dilute skimmed milk with water to reduce the casein content, and add whey or whey protein concentrate to increase the protein content; also, glucose, sucrose or lactose hydrolysed by β-D-galactosidase is added (e.g. to a level of 6.7 g L^{-1} to increase the carbohydrate content of the milk base (Seiler, 2003). Addition of the latter ingredient helps the yeast (*S. cerevisiae*) to grow and become well established in the product.

Similar to kefir, there are problems associated with the production of gas and packaging of koumiss. When opening the glass bottle, up to a third of the contents can foam and effervesce and, if the product is packaged in a carton, the container may explode. Such problems can be minimised by gas flushing the containers with nitrogen before filling, or replacing the headspace of the container with nitrogen after

Table 8.3 Chemical composition (g 100 g^{-1}) of milk of some selected species of mammals.

Species	Water	Fat	Protein	Carbohydrates	Ash
Mare[1]	~90.9	1.1–1.7	2.0–2.1	6.4–6.7	0.34–0.47
Human[2]	87.0	4.5	1.1	7.1	0.3
Cow[3]	87.4	3.9	3.3	4.7	0.7

[1] From Berlin (1962), Doreau *et al*. (1990) and Pagliarini *et al*. (1993).
[2] From Lentner (1981).
[3] From Tamime & Robinson (1999).

filling the product; an alternative approach is to use cartons fitted with an integrated high-pressure vent (Seiler, 2003). Other reported methods to modify cow's milk composition for the manufacture of koumiss have been reviewed by Tamime and Marshall (1997), and may include the following:

- Addition of 2.5 g sucrose 100 g^{-1} to skimmed milk.
- Blending different powders (e.g. whole milk, skimmed milk and cheese whey).
- Blending 5 parts whole cow's milk with 8 parts ultrafiltered cheese whey (i.e. 2-fold concentration of the protein) or blending cow's milk with clarified whey in a ratio of 1 : 1 and sweetening with sucrose (2.5 g 100 g^{-1}).
- Recently, Kücükcetin *et al*. (2003) have successfully adapted cow's milk to match mare's milk using different membrane filtration technologies (microfiltration, MF, and nanofiltration, NF); the following cultures were used to ferment the modified milk: *K. marxianus* var. *lactis*, *Lb*. *delbrueckii* subsp. *bulgaricus* and *Lb*. *acidophilus*.

The classical method for the industrial production of koumiss using mare's milk was first reported by Berlin (1962), but commercial starter cultures (*Lb*. *delbrueckii* subsp. *bulgaricus* and *Torulopsis* spp.) were grown separately in skimmed milk (2 L and 1 L, respectively). After growth, which normally takes 3–4 days, the lactic acid and yeast cultures are mixed together for the production of a bulk starter culture (e.g. the acidity is lactic acid 1.0–1.3 mL 100 mL^{-1}). Koumiss is made by addition of the bulk starter culture at a rate of ~ 30 mL 100 mL^{-1} to the processed milk, thorough agitation to enhance yeast growth and incubation at 26–28°C for 50–60 min or until the acidity reaches 0.55 mL lactic acid 100 mL^{-1}. Afterwards, the fermentate is homogenised, cooled to 20°C, packaged, incubated for a further 1.5–2 h at 18–20°C and stored at 4–6°C for 12–24 h before despatch (see also Seiler, 2003).

8.3.4 *Compositional quality and nutritional properties*

Little data has been published regarding the compositional quality of koumiss, but Berlin (1962) and Lozovich (1995) have classified the product into three categories based on the extent of fermentation (e.g. flavour intensity, level of acidity and the amount of alcohol produced) (Table 8.4). It is of interest to note that only traditionally made koumiss may contain > 3.0 g 100 mL^{-1} alcohol, but the extent of protein hydrolysis is ten-fold higher when compared to kefir. In addition, the peptone level (0.2–1.0 g 100 g^{-1}) in koumiss is greater than that present in kefir (0.05–0.12 g 100 g^{-1}) (Seiler, 2003).

Apart from the increased level of nitrogenous compounds in koumiss including the free essential amino acids, which enhances the nutritional properties of the product, the starter culture microflora exhibits bacteriocidal and bacteriostatic properties against many pathogens, such as *Escherichia coli*, *Bacillus*. *cereus* and *Mycobacterium* spp. Similar to kefir, the product has been used in treatment and/or convalescence of tuberculosis, but more clinical studies are required to confirm such

Table 8.4 Classification of koumiss[1] based on extent of fermentation.

Flavour/taste/odour	Berlin (1962)		Lozovich (1995)	
	Acidity (mL 100 mL^{-1})	Alcohol (g 100 mL^{-1})	Acidity (mL 100 mL^{-1})	Alcohol (g 100 mL^{-1})
Weak/sweet-sour/yeasty	0.54–0.72	0.7–1.0	0.63–0.72	1.0
Medium/boldly-sour/ yeasty	0.73–0.90	1.1–1.8	0.73–0.90	1.5
Strong/hardy milk acid/ yeasty	0.91–1.08	1.8–2.5	0.91–1.08	3.0

[1] A typical koumiss may have counts of bacteria (5.0×10^7 cfu mL^{-1}) and yeasts (1.43×10^7 cfu mL^{-1}).

claims. Furthermore, some of the yeast species present in koumiss also exhibit an antibiotic activity against *Staphylococcus aureus* or *B. cereus* (Seiler 2003). Also, by blending *Lb. acidophilus* and/or *Lb. rhamnosus* with the starter culture, the probiotic properties of koumiss will be improved. Ham *et al.* (1999a) reported that feeding *Lb. plantarum* and *C. kefir* to broiler chickens increased their counts in the faeces and reduced the count of coliforms and *E. coli*.

8.4 Miscellaneous products

Yeast-lactic fermentation-related products are made in many countries, and some examples are as follows.

Acidophilus-yeast and *acidophiline* or *acidophilin* products were developed in the former USSR for the treatment of certain intestinal disorders, but little is known regarding the technology of these fermented milk beverages (Koroleva, 1991; Tamime & Marshall, 1997). Basically, *Lb. acidophilus* and *S. lactis* are the main microflora of the starter culture, but sometimes a blend of *Lb. acidophilus*, *Lactococcus* spp. and kefir yeast or kefir starter culture have been used for the production of acidophiline (see also Sharma & Gandhi, 1981, 1983; Subramanian & Shankar, 1985).

Buttermilk plant is a starter culture, which is used in Northern Ireland to ferment milk that is later diluted to be used in breadmaking. It has been referred to as a descendant of kefir stock because the microflora (LAB, acetic acid bacteria and yeasts) are similar to those of a kefir grain (Thompson *et al.*, 1990; Rea *et al.*, 1996).

Calpis^{TM} is a Japanese fermented milk product, which is prepared from skimmed milk using a starter culture containing *Lb. helveticus* and *S. cerevisiae*. Of interest in this product is the possible role of two peptides (Val-Pro-Pro and Ile-Pro-Pro) that are not decomposed by digestive enzymes, and which inhibit angiotensin I-converting enzyme (ACE) (Nakamura *et al.*, 1995a, b; Masuda *et al.*, 1996; Takano, 2000). Recently, a placebo-controlled clinical study on the effect of Calpis consumption in hypertensive humans showed a decrease in systolic and diastolic blood pressure, but no effect on pulse rate, body weight or blood serum variables (Hata *et al.*, 1996).

Korean alcoholic fermented milk products have been developed as kefir/koumiss hybrids. Hong *et al*. (1996) fermented skimmed milk with a mixed culture (*Lac*. *lactis* subsp. *lactis* TA29 and *S*. *exiguous*) isolated from raw milk and kefir, respectively. The product exhibited inhibitory effects against some pathogens. Another koumiss-like product was made from goat's milk fermented with *Lb*. *plantarum*, *C*. *kefir* (both isolated from Mongolian koumiss) and a commercial strain of *Lb*. *delbrueckii* subsp. *bulgaricus*, and the optimum sensory properties were noted after 2 days of culturing (Ham *et al*., 1999b; WonHo *et al*., 2003).

Bulgaros is a Mexican fermented milk made with a microbial grain, and Ulloa and Lappe (1993) identified the microflora of the grain, which consisted of homo- and heterofermentative lactobacilli, streptococci and yeasts; they concluded that such grains are very similar to kefir grains. A similar product is made with kefir-like grains by Brazilian dairy farmers; no traditional name has been reported. Recently, Mesquiari *et al*. (2000) have developed a starter culture consisting of yoghurt organisms and *S*. *cerevisiae* for a simplified process for the industrial production of a kefir-like product. They also recommended that the base milk should be fortified with skimmed milk powder and the addition of gelatin to reduce syneresis and improve the sensory properties of the product.

Awasi is a traditional fermented milk made in Zimbabwe; the starter organisms have been identified by Gadaga (2000) and Gadaga *et al*. (2001) and consisted of lactic acid bacteria and yeasts. A similar product (*rob*) is made in Sudan, and the predominant microflora consists of *Lb*. *fermentum*, *Lb*. *acidophilus*, *Lactococcus* spp., *S*. *thermophilus* and *C*. *kefir* (Abdelgadir *et al*., 2001).

Togwa is a Tanzanian fermented maize-sorghum gruel, which is made with LAB (*Lb. brevis, Lb. cellobiosus, Lb. fermentum, Lb. plantarum* and *Pediococcus pentosaceus)* and *yeasts (S. cerevisiae, Candida pelliculosa, Candida tropicalis* and *I. orientalis*) (Mugula et al., 2003).

Skyr is an Icelandic concentrated fermented milk similar to strained/concentrated yoghurt and the starter culture contains yeasts; for further details refer to Chapter 7.

8.5 Future developments

It is clear that yeast-lactic fermented milk beverages are widely produced in many countries, but the industrial development has been limited, possibly due to the development of CO_2 in the product by the yeast, and the presence of alcohol up to 4 g 100 mL^{-1}. In the United Kingdom such product is subject to Customs and Excise levy of £45.00 100 L^{-1} of product. It is evident that future development of these products will continue and the success will depend, most likely, on developing blends of micro-organism to replace the kefir grains and minimise the level of CO_2 in the product. To retain the yeasty flavour in the product, microencapsulation of the yeast could be an option (Nelson & Bishop, 1998). Other developments could encompass: (a) batch wise fermentation with Ca-alginate immobilised starter cultures for kefir production (Gobbetti & Rossi, 1994); (b) developments of the starter

culture, for example a blend of tea fungi and kefir grains (Lenova *et al*., 1997) or the use of multi-starter for continuous production of kefir (Rossi & Gobbetti, 1992); (c) application of high pressure technology (Jankowska *et al*., 2001); and (d) application of membrane technologies for modification, for example, of cow's milk to be similar to mare's milk and, hence, koumiss production could be all year round rather than seasonal.

In addition, the human health benefits attributed to consumption of kefir and similar products are increasing its popularity worldwide, but more clinical studies are required to substantiate these claims.

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Fermented Milks
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9 Miscellaneous Fermented Milk Products

S. Lyck, L.-E. Nilsson and A.Y. Tamime

9.1 Introduction

During the first decades of the last century, sales of fermented dairy products in Europe and North America were limited to consumers who had acquired a taste for natural yoghurt on holiday in the Middle East or around the Mediterranean. However, the retail market for yoghurt expanded dramatically during the 1960s with the arrival of stirred fruit yoghurts. Flavouring the product with a fruit purée made yoghurt attractive to anyone who liked the flavour of fruits such as strawberries, raspberries or cherries, while the addition of sugar tended to mask the acidity disliked by many potential consumers. As a consequence, the dairy industry had found a massive new outlet for liquid milk and skim-milk powder, and cartons of stirred fruit yoghurt could be found in the chill cabinets of every supermarket.

Inspired by this success, manufacturers of dairy products began to seek additional options for marketing yoghurt. The first obvious target for improvement was the shelf-life because, compared to other dairy foods such as Cheddar cheese or butter, an in-store holding time of 14–21 days at < 10°C posed problems for some small retailers. Pasteurisation of fruit yoghurt does enable shops to hold cartons over a 2- to 3-month period at ambient temperature, but the sensory properties could be affected. Frozen yoghurt is sold at – 18°C, and such products offer an alternative route to a longer shelf-life yoghurt.

If the ideal formula for a yoghurt to be sold from the deep-freeze cabinet was proving elusive, the production of a frozen yoghurt or yoghurt ice cream that could be eaten in the manner of normal dairy ice cream has achieved more success (see section 9.4).

However, the share of the total yoghurt market taken by stirred fruit yoghurts during the 1980s and 1990s, and later by fruit-flavoured 'bio-yoghurts', suggested that consumers liked both the mouth-feel of stirred yoghurt and the fact that an individual carton of 125–150 mL provided a thoroughly satisfying conclusion to a 'snack lunch'. The obvious extension to this pattern of consumption was the development of yoghurt-based desserts, and a number of manufacturers offer a variety of products in this category. Apart from providing an opportunity for the introduction of some interesting new and complex flavours and textures, e.g. a rhubarb crumble and custard yoghurt, these products have attracted a new group of consumers for whom even fruit yoghurts tasted too acidic.

Products such as quark, cultured cream and fromage frais that are fermented with low acid-producing, mesophilic cultures also appeal to those buyers who

like the smooth, viscous consistency of stirred yoghurt, but also want a mild taste. Fruit-flavoured mesophilic milks have already become a major component of the fermented milk sector, and savoury dips based on natural stirred yoghurt, cultured cream or crème fraiche are extremely popular for serving with drinks at a party. What is also attractive to consumers is the fact that the consistency of these types of product can vary from 'spoonable' to 'spreadable'. In other words, some products can be served in bowls with crisps or pieces of celery to act as 'spoons', while others can be spread on crackers and, equally important, each type can be purchased in a whole range of flavours.

While these various yoghurt-based or related products have not had the market impact of the original stirred fruit yoghurt, they have extended consumer interest in fermented milks and, in that context, their production and properties merit detailed consideration.

9.2 Cultured/sour/fermented cream

9.2.1 *Background*

Cultured cream is an extremely viscous product, and has been used for years in many countries. The flavour and aroma is similar to cultured buttermilk, but with a fat content ranging between 10–12 and 20–30 g 100 g^{-1}. The product is utilised warm or cold in many dishes, such as sauces, soups and dressings. Cultured cream is normally produced without any additives, but some products may contain stabilisers and spices. In addition, some manufacturers may, optionally, add citric acid or sodium citrate to enhance the metabolic activity of mesophilic lactic acid bacteria, i.e. cit^+ *Lactococcus* and *Leuconostoc* species (Robinson *et al*., 2002), and some suggested processes for making cultured cream are detailed in Table 9.1.

Cultured cream is marketed under different names such as smetana (in Russia and Eastern European countries) and crème fraiche (in France). The product has a bright appearance and uniform structure. The taste is rather mild and slightly acid, with a pH of ~ 4.5.

The starter culture used contains a blend of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*. The latter two organisms are the main aroma-forming bacteria in the product.

Cultured cream has a limited shelf-life at < 10°C, and strict hygiene is required during production to ensure good quality and safety of the product. However, yeasts and moulds can grow on the surface of the product in packages that are not airtight. In the event of extended storage, the enzymes of the lactic acid bacteria can hydrolyse the β-lactoglobulin and cause bitterness in the product. Cultured cream may also lose its flavour because of the diffusion of aromatic substances through the packaging material.

Table 9.1 Some suggested processes employed during the manufacture of cultured cream.

Process	References
Entrapment of the starter culture in Ca-alginate gel beads was successfully used for the production of cultured cream with greatly reduced counts of the starter organisms (e.g. 150–1800 times less); also the fermentation time was reduced	Prevost & Divies (1992)
The use of nisin-producing starter cultures in cultured cream production helped to control the growth of spoilage micro-organisms and some pathogenic bacteria	Khattab *et al*. (1992)
The use of single strength rennet (0.066 mL L^{-1}) decreased the sensory scores of the body, texture and appearance of cultured cream, but increased the viscosity, proteolysis and gel firmness of the product	Lee & White (1993)
The addition of *Lactobacillus casei* or *Bifidobacterium bifidum* to the starter culture produced a good-quality cultured buffalo's cream	El-Kenany (1996)
Exopolysaccharide-producing sour cream starter cultures increased the adhesiveness and gumminess of the product	Adapa & Schmidt (1998)
The sensory properties of sour cream (11 g fat 100 g^{-1}) were influenced by the starter culture blend	Folkenberg & Skriver (2001)

Long shelf-life cultured cream for ambient distribution can also be produced by post-fermentation heat treatment of the product prior to aseptic packaging. Stabilisers are added to ensure product stability in a similar manner to fermented milk drinking products.

9.2.2 *Production methods*

Cultured cream can be produced either as a stirred- or set-type, and the manufacturing stages are similar to yoghurt or other fermented milk products (Fig. 9.1 – Alt. 1 and Alt. 2, respectively; see also Anon., 2003). For the former type, the cream is fermented in tanks, cooled and packaged. However, cultured cream containing a high fat content is very viscous and is difficult to cool in a plate heat exchanger without damaging the structure of the product. To overcome this negative effect, the fermented cream is packaged and is cooled in the container. In addition, the cream coagulum is highly sensitive to mechanical treatment and, as a consequence, the transportation of the product from the incubation tanks to the filling machine must be done with care.

For the manufacture of set cultured cream, the processed cream is cooled, mixed with the starter culture, packaged, and fermented and cooled in the container. The product has a firm and unbroken coagulum.

Stirred-type (cold distribution)

The fat content in the cream is standardised to the desired level, and deaerated before it is homogenised. Deaeration will improve the viscosity and stability of the

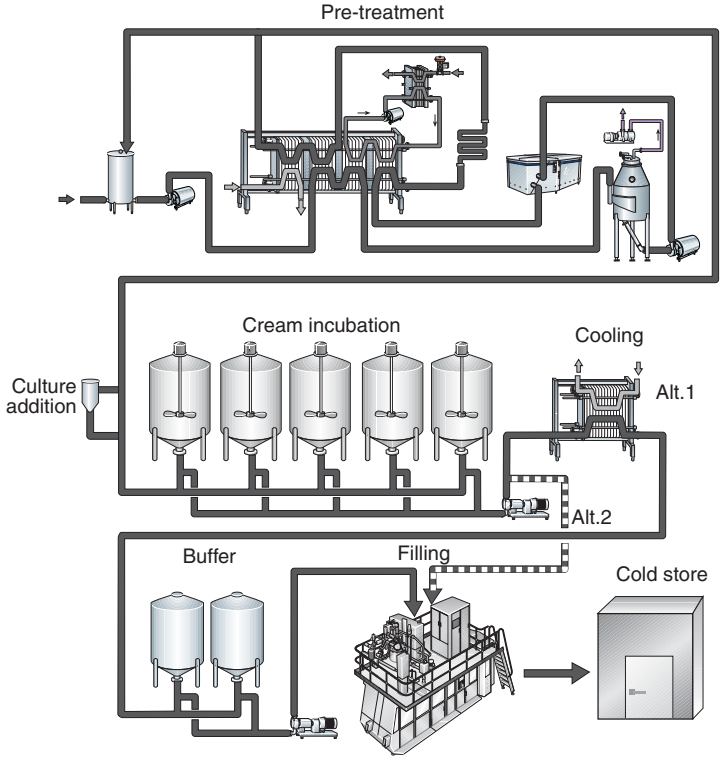


Fig. 9.1 Production lines for stirred- and set-types of cultured cream for cold distribution. By permission of Tetra Pak AB, Lund, Sweden.

cultured cream as well as the running conditions of the homogeniser. Moreover, deaeration will also minimise the risk of fouling of the equipment during the heat treatment stage.

The homogenisation pressure applied is adapted to the fat content of the product. In general, a lower pressure is used for high-fat cultured cream, and some guidelines of pressures used in the industry are:

- 10 g fat 100 g^{-1} 15–20 MPa
- 18 g fat 100 g^{-1} 12–17 MPa
- 38 g fat 100 g^{-1} 3–5 MPa

Homogenisation of the cream is often done at 60–70°C, but a temperature below 60°C is recommended if starch is added. Afterwards, the cream is heated to 90–95°C for ~ 5 min, cooled to the incubation temperature (e.g. 22–24°C), inoculated with the starter culture (i.e. 0.01 g 100 g^{-1} using direct-to-vat inoculation (DVI) or 1–2 g 100 g^{-1} using bulk starter culture) and incubated for 18–20 h or to pH 4.5.

Low-fat cultured cream, which is not very viscous, is cooled to ~ 15°C in a plate heat exchanger before packaging. The design of the cooler should ensure gentle

mechanical treatment of the product, and positive pumps are used to transfer the product from the incubation tank to the plate heat exchanger, buffer tank(s) and filling machines. Incidentally, the incubation tank is normally emptied within 1 h in order to maintain the uniformity of the cultured cream. After filling, the cultured cream is transported to the cold store, where it is further cooled. If the production line has been optimally designed, the consistence and viscosity of the cultured cream will improve during storage.

Alternatively, high-fat cultured cream is packaged directly from the incubation tank without cooling. The cooling of the product will then take place in the cold store, in a specially designed cooling room or cooling tunnel. It is important that the palletised packages are designed for quick cooling (i.e. large area of the packages must be exposed to the cold air and there must be enough distance between the packages to allow the circulation of the cold air around the packages).

Set-type (cold distribution)

The pre-treatment of the cream base for the production of set-type cultured cream is as described above for the stirred-type. After cooling the cream to 22–24°C, the starter culture is inoculated in-line (i.e. using a positive metering pump), packaged, fermented in incubation cabinets and cooled at pH 4.5.

Alternatively, the processed cream is cooled, pumped to a tank, mixed with starter culture, warmed to 22–24°C in a plate heat exchanger and packaged. The stacked pallets are fermented in incubation cabinets for 18–20 h (to pH 4.5), cooled and handled as described above for the stirred-type.

Long shelf-life (ambient distribution) cultured cream

Long shelf-life cultured cream for ambient distribution is manufactured in many countries, and a typical production line is shown in Fig. 9.2. The preliminary treatment of the cream is very similar to the stirred-type product that is retailed under refrigerated conditions, but differs with regard to the addition of stabiliser and the post-fermentation heat treatment. However, the product may be low in viscosity (i.e. thin and fluid-like coffee cream) or thick like a paste, and the factors that can affect the rheological properties of long shelf-life cream are:

- The level of fat in the cream (e.g. 15–25 g 100 g^{-1});
- The use of stabiliser (e.g. starch is added to the cream before the heating stage, while pectin is added to the fermented product before the final heat treatment); and
- The processing parameters.

As mentioned elsewhere, the stabiliser tends to minimise whey separation or sedimentation of the proteins, influence viscosity (i.e. thin or thick) and affect the mouth-feel of the product. In addition, the use of starch will produce a viscous product, while pectin ensures the fluidity of the cultured cream. The former type of

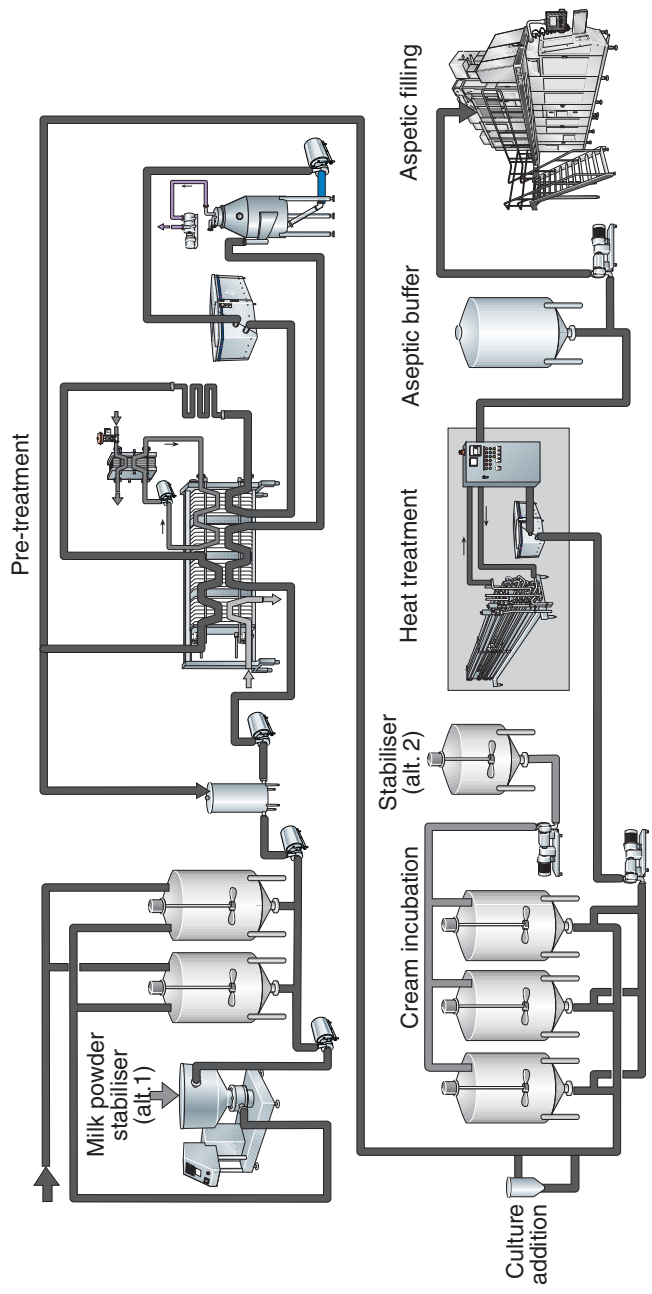


Fig. 9.2 Production line for long shelf-life cultured cream for ambient distribution. By permission of Tetra Pak AB, Lund, Sweden.

stabiliser is normally added to the cream before the homogenisation stage, and at a temperature to ensure that the starch granules remain unswollen after leaving the homogeniser. Incidentally, the stabilising effect of pectin in low-viscosity cultured cream is greatly improved if it is added to the product after the fermentation stage (see also Chapter 5).

The pre-treated cream is incubated in tanks at 22–24°C for 18–20 h. If the product is stabilised with starch, the fermentate will be too firm for pumping; hence, the fermentation tank(s) should be fitted with an adapted agitator to break the coagulum and facilitate easier flow of the product to the pump when emptying the tank. Alternatively, pectin solution (3–5 g 100 g^{-1} is prepared in water at > 70°C) is added to the fermented cream in the fermentation tank and no special agitator is required to ensure proper mixing of the stabiliser because the product is not very viscous.

With the addition of pectin solution, the fat content of the cultured cream will be slightly diluted and, to compensate for the reduction in the fat content, full-fat milk powder can be added to the cream before the heating and fermentation stages. The stabiliser solution is added to the cultured cream at pH 4.5 in the fermentation tank (i.e. batch mixing) or in-line before the final heat treatment.

No post-fermentation homogenisation of the product is required when starch is used as a stabiliser; however, when pectin is used, it is important to homogenise the cultured cream before the final heating stage in order to obtain the optimal stabilising effect. The homogenisation pressure applied is dependent on the fat content in the cream and the type of stabiliser used. For example, with a product containing 20 g fat 100 g^{-1} cream, a homogenisation pressure of at least 5 MPa should be used in order to ensure the stabilisation effect of the pectin, but more often a pressure of 15 MPa is used for optimal effect. Nevertheless, it is difficult to recommend an exact homogenisation pressure because the final characteristics of cultured cream may vary from one country to another, and on-site trials should be performed to optimise the processing conditions for any manufacturer. Incidentally, the homogenisation stage will also have a great influence on the consistency of the end-product.

The cultured cream is heated in a plate heat exchanger or tube heat exchanger at 85–90°C for a few seconds before it is cooled to 25–30°C, aseptically packaged and stored at ambient temperature. The product is 'commercially' sterile and no further growth of micro-organisms takes place. However, not only is the shelf-life dependent on the micro-organisms in the product, but the duration of storage at ambient temperature will affect the appearance of the cultured cream. In addition, the aroma and colour of the product may change with time, and the effect of the added stabiliser may be reduced. Storing the cultured cream at elevated storage temperatures will enhance these changes.

9.3 Pasteurised stirred and fruit flavoured yoghurt

A post-fermentation heat treatment of stirred and fruit flavoured yoghurt helps to prolong the shelf-life of the product, since the application of heat inactivates the starter culture bacteria (e.g. *Streptococcus thermophilus* and *Lactobacillus*

delbrueckii subsp. *bulgaricus*) and their enzymes, as well as other contaminants, such as yeasts and moulds. The destruction of the starter organisms may, in some countries, cause a controversy with the existing definition of yoghurt because most standards stipulate the product should contain an abundant and viable count of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*. It is possible to suggest, however, that heat-treated product should be designated as pasteurised, ultra high temperature (UHT) or long shelf-life yoghurt.

Different processing conditions have been reported by many authors (see the review by Tamime & Robinson, 1999), and the shelf-life of the product may be extended up to one year.

For the manufacture of long shelf-life yoghurt, the base is produced as detailed in Chapter 3, and a starch-containing stabiliser (e.g. Grindsted® SB 550A, which contains modified starch and pectin) is normally added to the milk base before homogenisation and heat treatment. However, a different stabiliser blend (e.g. Grindsted® NP, which contains guar gum and pectin) is added with the fruit before post-fermentation heat treatment. A typical example is shown in Table 9.2.

9.4 Frozen yoghurt or yoghurt ice cream

9.4.1 *Background*

Frozen yoghurt was introduced to the market in the 1970s as a frozen dairy product similar to ice cream, but it has been marketed as a healthy alternative to standard ice cream. This has been achieved with some success, especially in the 1980s in the USA, and the current worldwide production figure is around 200m litres, despite the fact that the market has declined slightly. Yoghurt has a good image among consumers due to its unique properties in relation to digestion, lactose intolerance, calcium absorption and even improved human immune response. Furthermore, yoghurt is associated with a fresh taste, which to some extent can be transferred to the frozen product, depending on the process (Fig. 9.3).

Table 9.2 Formulation for the production of long shelf-life stirred and fruit flavoured yoghurt.

Ingredients (g 100 g^{-1})	Full-fat (3.2 g 100 g^{-1})	Low-fat (1.0 g 100 g^{-1})
Whole milk (3.5 g fat 100 g^{-1})	91.9	30.0
Skimmed milk	—	60.7
Skimmed milk powder	2.8	3.8
Sucrose	4.0	4.0
Stabiliser (Grindsted® SB 550A)	1.3	1.5

Note: The base milk including the stabiliser (Grindsted® SB 550A) is homogenised at 60–65ºC and 20 MPa pressure, heated to 90–95ºC, cooled to 42ºC, inoculated with a yoghurt starter culture (e.g. YO-MIX™) and fermented to pH 4.5. The fermentate is stirred, pH adjusted to 4.0 by adding food grade lactic acid, mixed with fruit preparation (15 g 100 g^{-1}) and stabiliser (Grindsted® NP; 1.5–2.0 g 100 g^{-1}), heated to 80–85ºC for 15–20 s, homogenised, cooled to 25ºC, packaged aseptically and stored at 5ºC.
Grindsted® SB 550A contains modified starch and pectin, while Grindsted® NP contains guar gum and pectin.

Fig. 9.3 Yoghurt ice cream or frozen yoghurt – a healthy and delicious alternative to conventional ice cream. By permission of Danisco A/S, Brabrand, Denmark.

In addition, the healthy image of frozen yoghurt or yoghurt ice cream is often associated with low-fat or fat-free, low-cholesterol, low-calorie, low-sugar or, lately, low-carbohydrate varieties. In fact, low-fat recipes (e.g. 3.5 g fat 100 g^{-1}) have helped to produce a fresher product that goes well with the other properties of frozen yoghurt.

In practice, frozen yoghurt and/or yoghurt ice cream can be produced in several different ways, but must meet fairly limited restrictions in terms of statutory standards. In some countries, there are requirements for minimum cell counts of the yoghurt organisms, while in others the product does not have to contain live cultures, but has to be made from yoghurt.

Developments in frozen yoghurt dessert technology have been reviewed by Tamime and Robinson (1999) and Mitchell *et al.* (1999), and may include the following.

- Frozen yoghurt or yoghurt ice cream prepared by replacement of skimmed milk powder with whey solids had an overrun of 77%, melting time 5.4 min and a starter culture count of 7.2×10^8 cfu g^{-1}; however, the sensory properties of the product were further enhanced by blending the yoghurt with an ice-cream base mix at a ratio of 40 : 60; the viable cell count marginally decreased after storage at –20ºC for 10 days (Venkateshaiah *et al*., 1997). Jayaprakasha *et al.* (2000) recommended the use of whey protein concentrate (WPC) to replace 50% of the skimmed milk solids in the yoghurt base to produce a quality product, which improved the overrun to 87.7% as compared to 56.7% in the control product.
- Storage of frozen yoghurt or yoghurt ice cream for longer than 2 months affected the sensory properties of the product; an orange-juice flavour was preferred by the taste panel (Özdemir *et al.*, 1999).

- Milk, liquid whole egg and sugar were used to make the yoghurt, and the fermented product, which was also known as 'sour curdling egg-milk', was mixed with an ice-cream base, homogenised, aged and frozen; the product was stable for > 3 months storage at –18°C (HuiYan *et al.*, 2000).
- The use of capsule-forming strains of lactic acid bacteria in making frozen yoghurt increased the apparent viscosity and overrun, reduced the rate of melting, made the culture more resistant to heat shock, and the product was more acceptable than a parallel product made with non-capsule-forming starter culture (El-Rahman *et al.*, 2000).
- Cultured buttermilk, fermented skimmed milk (or a mixture of both) and replacing the fat with reduced cholesterol butteroil were used successfully during the manufacture of yoghurt ice cream or frozen yoghurt; the product that contained cultured buttermilk had more volatile fatty acids and acetaldehyde, increased overrun and had good flavour, body and texture when compared with the control frozen yoghurt (Mostafa *et al.*, 2001).
- Miscellaneous factors that have been reported to affect the quality of yoghurt ice cream or frozen yoghurt are: the level of sugar and fruit concentration (Güven & Karaca, 2002); and replacing sucrose with stevia (*Stevia rebaudina*) sweetener by 75% (Salem & Massoud, 2003).

9.4.2 *Yoghurt ice cream or frozen yoghurt with live bacteria*

The product with live bacteria can be manufactured by adding high (40–70 g 100 g^{-1}) or low (< 40 g 100 g^{-1}) amounts of yoghurt to the ice-cream base. Alternatively, dried yoghurt powder with live bacteria can be used instead of the fresh liquid product. A concentrated yoghurt starter culture can also be added to the mix to ferment the ice-cream base, or just to incorporate live bacteria in the formulation in order to meet the standard of identity for frozen yoghurt.

Frozen yoghurt with high yoghurt content

Frozen yoghurt made with 40–70 g 100 g^{-1} yoghurt has a low pH and a distinctive acid taste, unlike a frozen product made with < 40 g yoghurt 100 g^{-1}. As a consequence, the product is manufactured from three separate mixes, i.e. an ice-cream base, a sugar base and a plain/natural yoghurt base with live bacteria. The total solids content of most commercial natural yoghurt ranges between 12 g and 15 g 100 g^{-1}, which is rather low when compared with a standard ice-cream mix. Hence, the yoghurt used must be sufficiently concentrated in order to achieve the desired level of solids in the finished product. In order to avoid excessive viscosity in the base mix, part of the sugar is added in a special sugar mix in combination with a stabiliser, e.g. locust bean gum, and a guar blend, such as Cremodan® DC 100. The emulsifier is added separately to the base mix. A typical formulation is shown in Table 9.3.

It is necessary to increase the solids content in the yoghurt when using more than 50 g 100 g^{-1} in the product formulation (Table 9.3). An upper limit of 70 g 100 g^{-1} of

Table 9.3 Formulation for the production of yoghurt ice cream or frozen yoghurt made with high yoghurt content (50 g 100 g^{-1}).

Components	Base mix (g 100 g^{-1})	Sugar mix (g 100 g^{-1})	Yoghurt mix (g 100 g^{-1})	Finished mix (g 100 g^{-1})
Fat	5.0	—	3.5	3.5
Milk solids-not-fat (SNF)	19.4	—	9.0	11.3
Sucrose	15.4	44.0	—	12.0
Dextrose	—	13.0	—	2.0
Glucose syrup	—	13.0	—	2.0
Emulsifier (Cremodan® Super)	0.85	—	—	0.3
Stabiliser (Cremodan® DC 100)	—	1.3	—	0.2
Total solids (TS)	40.65	71.3	12.5	31.3
Mixing ratio	35.0	15.0	50.0	100.0

Preparation of the different mixes: the base mix is pre-heated to 80ºC, homogenised at 22.5 MPa of pressure, heated to 80–85ºC and cooled to 5ºC; the sugar mix is heated to 80–85ºC and cooled to 5ºC; the yoghurt mix (i.e. milk) is pre-heated to 75ºC, homogenised at 20 MPa of pressure, heated to 80–85ºC, cooled to 42ºC, inoculated with starter culture and fermented to pH 4.2, and cooled to 5ºC.
Production of frozen yoghurt: blend the different mixes (i.e. base, sugar and yoghurt in a ratio of 35, 15 and 50, respectively), age at 5ºC, freeze at –5ºC (e.g. 70–90% overrun), harden at –30ºC and store at –25ºC.

yoghurt mix can be used, but highly concentrated yoghurt should be prepared, and it is not recommended to use a higher level as it will lead to an inferior quality of finished product. Frozen yoghurt made with a high yoghurt content is very suitable for blending with fruit preparations. The acidity level and the acetaldehyde contents of yoghurt are extremely compatible with most fruit flavours, and are also believed to enhance such flavours – as in the case of conventional fruit flavoured yoghurt. Fruit juice or fruit concentrate can be added to the mix immediately before freezing, but the addition of fruit before the heating of the mixes may lead to precipitation of the milk protein due to the low pH level. After freezing the blended mixes, it is then possible to add pieces of fruit to the semi-frozen product.

In some instances, the pH level is adjusted with lactic or citric acid, which must be done immediately before freezing. Commercial flavourings (natural extracts, natural equivalent or synthetic) are often added to the mixes with the fruit juices and/or fruit concentrate before freezing in order to achieve a well-balanced fruit taste in the frozen yoghurt.

Sucrose is the main factor controlling the freezing point depression (FPD) in frozen desserts. In order to improve the taste and texture of the frozen product, the total sugar content should be higher than that of ice cream. Insufficient sugar levels will reduce the FPD, and will lead to a brittle and coarse structure. To avoid excessive sweetness, it is possible to substitute part of the sucrose with sugar that has a higher FPD, e.g. dextrose (Table 9.3).

Yoghurt ice cream with low yoghurt content

Yoghurt ice cream containing < 40 g yoghurt 100 g^{-1} is produced by using a two-mix system (Table 9.4). An ice-cream base is mixed with 20–40 g yoghurt 100 g^{-1}. This

Table 9.4 Formulation for the production of yoghurt ice cream or frozen yoghurt made with low yoghurt content (20 g 100 g^{-1}).

Components	Ice-cream mix (g 100 g^{-1})	Yoghurt mix (g 100 g^{-1})	Finished mix (g 100 g^{-1})
Fat	2.0	3.5	2.3
Milk solids-not-fat (SNF)	11.3	9.0	10.8
Sucrose	15.0	—	12.0
Glucose syrup solids	8.0	—	6.4
Flavourings	+	—	+
Emulsifier/stabiliser blend (Cremodan® 816 Creamline)[1]	1.0	—	0.8
Total solids	37.3	12.5	32.3
Mixing ratio	80.0	20.0	100.0

[1] The stabiliser blend contains mono- and diglycerides of fatty acids, locust bean gum, and guar gum; it has a high emulsifier action to produce a 'creamy' taste in the frozen product.
Preparation of the different mixes: the ice-cream mix is pre-heated to 80ºC, homogenised at 22.5 MPa of pressure, heated to 80–85ºC and cooled to 5ºC; the yoghurt mix (i.e. milk) is pre-heated to 75ºC, homogenised at 20 MPa of pressure, heated to 80–85ºC, cooled to 42ºC, inoculated with starter culture and fermented to pH 4.2, and cooled to 5ºC.
Production of frozen yoghurt: blend the ice-cream and yoghurt mixes (i.e. at a ratio of 80 to 20, respectively), age at 5ºC, freeze at –5ºC (e.g. 70–90% overrun), harden at -–0ºC, and store at –25ºC.

formulation will eventually increase the pH value, and reduce the yoghurt flavour compared with parallel product made with a higher level of yoghurt. Incidentally, the yoghurt can be bought from another dairy factory if facilities are not available to ferment the milk base.

Direct fermentation of the ice-cream mix

Instead of adding 'live' yoghurt to the ice-cream mix, it is possible to ferment the mix by using a DVI starter culture; this method of production has a marketing advantage where the finished product is made totally from yoghurt. In addition, direct fermentation of the ice-cream mix is a much simpler procedure for producing frozen yoghurt compared with the methods described above, as it requires less processing equipment, and the ageing tanks (i.e. fitted with temperature control) can be used to incubate the mix at 42°C.

Due to the high sugar and SNF (total solids 30–40 g 100 g^{-1}) content and low water activity of the ice-cream mix, only very robust starter cultures (e.g. YO-MIX™ 621) are able to ferment the mix, and the inoculation rate is rather high, i.e. 3 to 5 times greater than conventional yoghurt. Despite the high inoculation rate, the fermentation time is still very long, and the acidity and flavour (acetaldehyde) will be less pronounced compared to the addition of commercial yoghurt to an ice-cream mix. Furthermore, most stabiliser systems for ice cream will contain emulsifiers, which are known to inhibit the growth of starter culture. It is therefore necessary to use a stabiliser system with low or specially selected emulsifiers and, in order to increase the protein protection, the use of a pectin-based stabiliser system is recommended.

Unfermented mixes

In some countries, one approach that can be used during the manufacture of yoghurt ice cream is simply to add starter cultures to the ice cream to meet the standard of identity of the product, and then freeze the mix. This method of production simplifies the process compared to fermenting the ice-cream mix or adding live yoghurt. However, the product lacks the true taste of yoghurt; a yoghurt-like taste can be achieved by adding yoghurt flavours combined with adjusting the pH level using citric or lactic acid. Alternatively, yoghurt powder can be added to the ice-cream mix to improve the flavour profile of the frozen product.

In some standards of yoghurt ice cream or frozen yoghurt, a minimum cell count of the starter cultures is required but, as can be expected, some death of the strains over the shelf-life period cannot be avoided, especially if the storage temperature fluctuates. To ensure that the finished frozen product will contain a minimum viable count of 10^6 cfu g^{-1} of the starter organisms, it is anticipated that $2–3 \times 10^7$ cfu g^{-1} should be added to the ice-cream mix. It is recommended that the starter cultures should be added to the mix immediately before the freezing stage, or mixed into the semi-frozen product. In addition, probiotic organisms can also be added to the ice-cream mix with the starter culture.

9.4.3 *Yoghurt ice cream without live bacteria*

An alternative method of simplifying the manufacture of yoghurt ice cream is to heat the mix containing the live yoghurt organisms before ageing and freezing; the process is known as the direct method. Up to 80% of yoghurt can be used in the mix; however, the low pH of yoghurt and the application of heat may cause the milk protein to precipitate, especially if additional skimmed milk powder is added to the mix. Therefore, it is recommended to increase the SNF level and to use a more acid-tolerant dairy powder, such as whey powder. This latter powder will enhance the taste of the frozen product, but reduce the water-binding effect compared to casein and, as a consequence, the final product lacks the desired body characteristics and has a fluffy texture. Furthermore, slightly higher amounts of emulsifier and stabiliser have to be added to the mix compared to conventional ice cream, but heating the mix at lower temperature (e.g. 75°C for a few seconds) still ensures the bacteriological safety of the product.

9.5 Dried yoghurt

9.5.1 *Introduction*

The primary objective of manufacturing powdered yoghurt is to produce a product that is stable during prolonged storage and readily utilisable. Although a wide range of patents have been filed in many countries (see review, Tamime & Robinson, 1999), the processes were not successful in producing a gel-type yoghurt when the powder was rehydrated. Nevertheless, dried yoghurt is widely used in the food sector in

the manufacture of sauces, soups, baked goods and baby food; the manufacturing techniques of drying milk and yoghurt have been reviewed by Masters (1991), Carić (1994), Knipschildt and Andersen (1994), Pisecky (1997) and Tamime (2003).

Nevertheless, the addition of hydrocolloids during the manufacture of spray-dried yoghurt has improved the following characteristics of the product when compared with ordinary dried yoghurt: retention of volatile compounds such as acetaldehyde; and solubility and dispersion of the powder during reconstitution (Ramirez-Figueroa *et al.*, 2002; Crofskey *et al.*, 2004).

9.5.2 *Method of manufacture*

Powdered yoghurt, which is sometimes known as dried or instant yoghurt, is manufactured in small quantities and using normal driers, and some of the manufacturing techniques have been patented. Low-fat yoghurt presents no difficulties when spray dried, but some precautionary measures that should be considered include the following:

- Concentrate the product, before drying, at low temperature (~ 50–60°C) to minimise scorching on the surface of the evaporator and/or discolouration of the final product.
- The drying conditions must be moderate in order to ensure a high viable cell count of the starter culture in the dried product.
- Package the product with nitrogen or carbon dioxide to extend its shelf-life during storage at ambient temperature.

An example of a process to dry yoghurt has been reported by Tamime (2003), and the product is dried in a three-stage drying plant. The yoghurt is concentrated to 35 g 100 g^{-1} total solids, pre-heated and nozzle atomised into the drying chamber (first-stage drier). The inlet and outlet drying temperatures are 160 and 65°C, respectively, and the partially dried yoghurt falls down onto the first fluid bed drier, which is integrated into the bottom of the drying chamber. The semi-dried particles form a fluidised layer, which is further dried (second-stage drier). Finally, the powder is transferred to an external fluid bed drier for final drying and cooling (third-stage drier).

The exhausted drying air from both the drying chamber and the external fluid bed drier is drawn through different cyclones to recover the powder fines from the air. The fines are fed back to the external fluid bed drier where they are mixed with the bulk of the powdered yoghurt, thus increasing the yield. In general, the maximum temperature of the powder during drying is 55°C, and the temperature of the product outlet is 25°C. A typical powder contains 2 g moisture 100 g^{-1}, and has a tapped bulk density of 0.5 g cm^{-3}.

9.6 Yoghurt-based desserts

Some dairy desserts are made from a fermented base, and are aimed to increase indirectly the market segment for desserts. This allows manufacturers of yoghurt and other fermented milk products to enter the dessert market, and, at the same time, encourages the current dairy dessert manufacturers to produce yoghurt-based products with a healthier image than their current products.

9.6.1 *Yoghurt mousse*

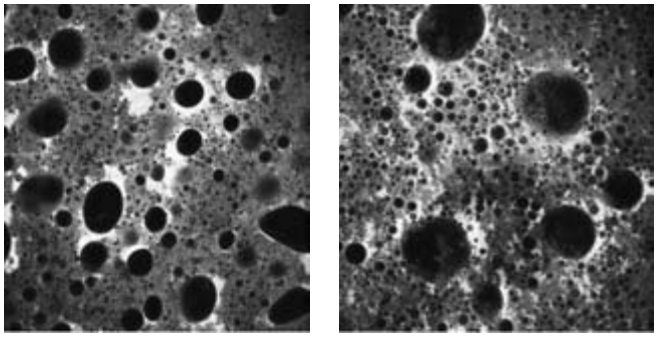
Aerated desserts are products that have a stabilised foam structure, and are mainly based on dairy or dairy analogue ingredients. Aerated desserts are characterised by a high and stable overrun, and can be stored at < 5°C or frozen. The acidity of the products varies from neutral to low pH; the latter category of products is based on using yoghurt or fresh cheese.

Stabilisation of aerated dairy desserts

Whipped desserts, including yoghurt mousse, are aerated oil-in-water emulsions, and surface-active ingredients, such as lipid-based emulsifiers, are often added to these products due to their ability to increase aeration and stabilisation of the foam by reducing the interfacial tension and thus facilitating the creation of bubble surfaces.

Emulsifiers are hydrophilic-lipophilic substances, which reduce the interfacial tension between oil and water. They cover part of the 'naked' fat globules, which are the result of homogenisation, increase the amount of desorbed protein from the fat globules and facilitate crystallisation of the fat phase. Furthermore, emulsifiers facilitate the incorporation of air due to reduced surface tension and controlled agglomeration of the fat globules, which stabilise the air cells. The most commonly used emulsifiers are mono- or diglycerides of fatty acids and lactic acid esters of monoglycerides. In general, mono- and diglycerides impart a uniform distribution of large air cells, typically described as an open structure, and enhance the desired level of creaminess. Lactic acid esters produce smaller air cells, typically described as a closed structure, and increase creaminess of the product (Figs 9.4a,b). In addition, lactic acid esters of monoglycerides generally produce a stronger destabilising effect compared to diglycerides. If problems occur with regard to achieving a constant high overrun during production, a lactic acid ester is the correct choice.

Stabilisers increase the viscosity of a mousse mix, which makes it easier to whip air into the product. After setting, certain stabilisers can give the mousse a gelled foam structure and ensure that the product does not collapse or lose its overrun during storage, distribution and retailing. Only a limited range of stabilisers is suitable for providing a gelled structure during the aeration of desserts. Until recently, gelatin was the predominantly used stabiliser but, in response to market demand, excellent gelatin-free stabilisers based on carrageenan, alginate and pectin are now available on the market.



(a) (b)

Fig. 9.4 Confocal laser scanning micrographs of mousses made with Grindsted® mono- and diglycerides (a) and Grindsted® Lactem (b) (lactic acid ester of mono- and diglycerides). The protein structure (i.e. the serum phase) is shown in green and the fat in red and yellow (see coloured plate), while the dark circular patches show the air bubbles. By permission of Danisco A/S, Brabrand, Denmark. Reproduced in colour as Plate 7, after page 110.

Method of production

Basically, there are two fundamental methods for the manufacture of a fermented mousse. The most common and popular method involves working with two mixes – a standard yoghurt or any other fermented milk base and a neutral pH cream base to which the stabiliser system is added (Table 9.5). This method avoids any limitations on the choice of stabiliser and emulsifier level. The viscosity of the cream base will be potentially high and, therefore, the temperature of the cream should be ~ 30°C before it is blended with the fermented base.

The other method is known as a single-mix fermentation (Table 9.6), which places some restrictions on the choice of the stabilising system because several hydrocol-

Table 9.5 The production of yoghurt mousse using two different mixes (cream base and plain yoghurt base).

Components	Cream base formulation (g 100 g^{-1})
Cream (38 g fat 100 g^{-1})	32.25
Skimmed milk	29.25
Skimmed milk powder	6.25
Sucrose	27.50
Emulsifier/stabiliser blend (Cremodan® Mousse 38)[1]	4.75
Flavouring	+

[1] The stabiliser and emulsifier blend contains lactic acid esters of mono- and diglycerides of fatty acids, E472b and gelatin.

Heat all liquid ingredients of the cream base to 40ºC, and add all the dried ingredients; the mix is pre-heated to 70–75ºC, homogenised at 15 MPa of pressure and heated in sequence at three different temperatures as follows: at 90ºC for 30 s, followed by ultra-high temperature (UHT) at 140ºC for 4 s and finally at 80–85ºC for 20–40 s; the processed cream base is cooled to 15–18ºC, blended with a plain yoghurt base (e.g. 3.5 g 100 g^{-1}) at a ratio of 40 to 60, respectively, aged for 30 min, whipped in an aerator to 60–80% overrun (optional with fruit addition), packaged and stored at 5ºC.

The overall total solids content of the product is ~30 g 100 g^{-1}).

Table 9.6 The production of yoghurt mousse using one base.

Components	Base formulation (g 100 g^{-1})
Cream (38 g fat 100 g^{-1})	18.20
Skimmed milk	65.80
Skimmed milk powder	3.50
Sucrose	11.00
Emulsifier/stabiliser blend (Cremodan® Mousse 38)[1]	1.50
Flavouring	+

[1] The stabiliser and emulsifier blend contains lactic acid esters of mono- and diglycerides of fatty acids, E472b and gelatin.

Add all dried ingredients to the cold milk and cream and mix vigorously; the mix is pre-heated to 65–75ºC, homogenised at 15 MPa of pressure, heated to 80ºC for 20 s, cooled to 42ºC, inoculated with DVI starter culture (e.g. YO-MIX™ 621) and fermented to pH < 4.5; the fermented base is cooled to 10ºC, whipped in an aerator to 60–80% overrun (optional with in-line fruit addition), packaged and stored at 5ºC.

The overall total solids content of the product is ~30 g 100 g^{-1}.

loids have an inhibiting effect on the starter culture. Thus, only free starch, milk proteins and pectin blends can be used for stabilisation of the yoghurt base, while the level of emulsifier used and total dry matter of the mix are other limiting factors if a stable fermentation is required.

9.6.2 *Demoulded-type or gelled yoghurt*

Any yoghurt or sour cream base can be easily transformed into a dairy dessert that has a gelled texture. The yoghurt dessert can have either a live starter culture or no yoghurt organisms (i.e. the base is heated post-fermentation to obtain a long shelf-life product).

Long shelf-life product

Yoghurt or sour cream base is mixed with a highly jellifying stabiliser system after fermentation, heated and packaged hot (Table 9.7). This gives a gelled structure to the yoghurt and enables it to be removed from the cup without breaking the product. The demouldable product is a nice indulgent alternative to conventional yoghurt or sour cream.

Table 9.7 Production of long shelf-life demoulded or gelled yoghurt.

Preparation of the yoghurt base	Production of demoulded yoghurt
Mix full-fat milk with cream, pre-warm to 65ºC, homogenise at 17.5 MPa of pressure, heat to 95ºC for 6 min, cool to 42ºC, inoculate with starter culture and ferment to pH 4.5, stir and cool to 10ºC.	Mix the sugar with the stabiliser and add slowly to the yoghurt (optional – add other ingredients, such as fruit juice concentrate, water, flavouring material, anti-microbial agent and/or colouring matter), mix for 5–10 min, adjust the pH to 4.2, pre-warm to 55ºC, heat to 95ºC for 15 s, cool to 70ºC, package and cool in the refrigerated store.

Due to the potential health problem associated with bovine spongiform encephalopathy (BSE) ('mad cow disease', a chronic and degenerative disease affecting the central nervous system of cattle and which can be transmitted to humans), in certain countries gelatin is no longer used in desserts. Despite its unique jellifying properties, it has a bad marketing image. The hot-fill process shown in Table 9.7 illustrates that it is possible to manufacture a good gelled fermented product without the use of gelatin. Instead, a stabiliser system containing carrageenan, starch and pectin is used with good results. Since the yoghurt base has been heated after the fermentation stage, the local legislation has to be checked to see whether the term 'yoghurt' can still be used.

Short shelf-life product

Unlike the process for long shelf-life demouldable yoghurt, the stabiliser system is added to the yoghurt base before the heat treatment, and the manufacturing stages are very similar to the production of standard yoghurt or sour cream. The dried ingredients (stabiliser, sugar and milk powder) are mixed and blended with full-fat milk or water at ~ 45°C. The milk base is pre-heated to 65°C, homogenised at 20 MPa pressure, heated to 95°C for 6 min, cooled to 42°C, mixed with a starter culture and fermented to pH 4.5, cooled in a plate heat exchanger to 25°C, packaged and stored at 5°C.

To obtain the desired gel structure, it is essential to use a good quantity of jellifying hydrocolloids. As already mentioned, gelatin has superior jellifying properties, but its use is governed by market forces. However, to obtain an excellent gelled structure in a cold-filled and low pH product, it is necessary to use gelatin as the primary jellifying hydrocolloid, possibly combined with modified starch. Pectin can be added as well to increase the viscosity and mouth-feel at ambient temperatures due to its higher gel strength at such temperatures.

9.7 Conclusion

It is evident from this brief review that a wide range of products can be manufactured from cream and yoghurt and, in part, the primary objective is to extend the shelf-life by the use of heating, freezing or drying. The ability to name some of these products as 'yoghurt' may not be applicable in some countries in view of existing legal standards but, ultimately, development of yoghurt-based products will benefit the consumer in the long term. However, innovations in yoghurt or cream-based products rely heavily on the use of stabilisers to provide the desired sensory characteristics, such as stability of structure and mouth-feel, and future developments of closely related products will be entirely orientated towards improvements or developments in the stabiliser blends.

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10 Mechanisation, Automation and Future Developments

H. Elaisson

10.1 Introduction

Today, a strong brand name and a good company image are of utmost importance in order to succeed as a food producer in the increasingly competitive global food market. One of the most important aspects when building and protecting a brand name is to produce and deliver a product that is consistent in quality. Consumers expect their favourite product, for example yoghurt, to have the same taste and texture every time it is consumed, regardless of when or where it was purchased and under what circumstances it was manufactured.

In just a few decades, the dairy industry has developed from small production units operated by a number of skilled dairy personnel, performing most of the operations manually, to large-scale production lines where product quality and production efficiency are equally essential for success. Mechanisation and the introduction of automation and production management systems have major roles in this development, and in the production of yoghurt several operations are very well suited to mechanisation and automated control (Anon., 2003).

The main advantage of having an automated production line is that each procedure in the operation is repeated in exactly the same controlled manner every time, which ensures high precision of repeatability and safety. At present, the rapid development of electronics and information technology also allows for much more than just the process control to be built into an automated production system, for example, production management systems with work execution, data collection and data analysis, and integration to local or global business systems.

The benefits from such developments are improved quality, safety and control, but also increased possibilities to map, visualise, analyse, develop and optimise the product and the process as well as the production management and the production facilities.

In recent years, a number of accidents and scandals have led to a call for increased food safety. Consumers around the world are raising their voices on this issue and local health authorities are introducing regulations that require all food producers to ensure that food is handled properly and safely.

All these demands from a global consolidating food industry drive the development of tools that are precise, efficient and effective, tools that can assist the human brain and body when it comes to managing a food processing enterprise in an optimal way.

10.2 Structure and different levels of automation

As the field of automation grows and solutions become more complex in the liquid milk/food processing industry, there is a need to structure and standardise the way automation is applied. Influenced by other industries such as the pharmaceutical industry, which has been more advanced in this field, standards such as S88 and S95 (ANSI/ISA, 1995, 2000) are being recognised and becoming a requirement for automation solutions in the liquid milk/food industry as well.

The different levels defined for an Enterprise System are shown in Fig. 10.1. The top section illustrates the business planning and logistic level (level 4) where the enterprise resource planning (ERP) systems are found. These systems are global for an enterprise, and each production facility is hosting a part of the global system. The next section (level 3) is the manufacturing operation and control level where the specific plant is being planned, operated and followed up; in this level management information systems (MIS) and manufacturing execution systems (MES) are found. The final section is production control (levels 2, 1 and 0) from where the process equipment in the plant is actually controlled and, in this section, the basic process logic control (PLC) and user interface functionalities are found.

The standards S88 and S95 (ANSI/ISA, 1995, 2000) set out the definitions, models and structures of an Enterprise System from the enterprise level all the way down to the control module, the smallest individual item to be controlled (Fig. 10.2). The S88 standard primarily focuses on the lower parts of the system, which are related to production control. It defines the rules and structures for the logic that is used to programme these parts of the system using phase logic. The S95 is used to define structures and flow of information between different parts of the system, and is primarily focused on levels 3 and 4 (Fig. 10.2).

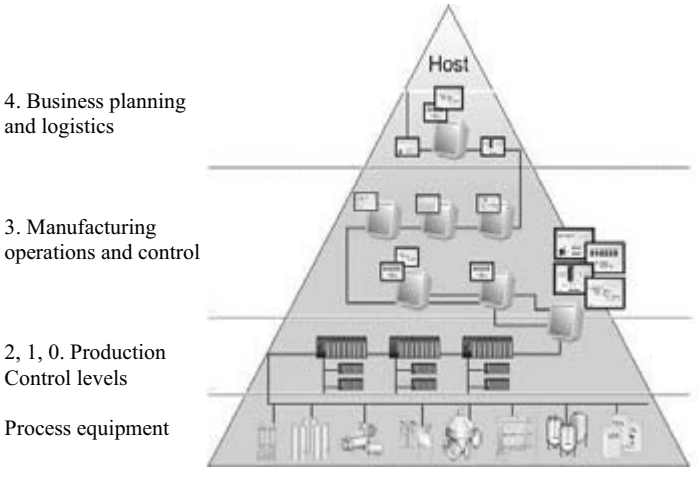

Fig. 10.1 Overview of the different levels of automation in a yoghurt/food production line. By permission of Tetra Pak processing system AB, Lund, Sweden.

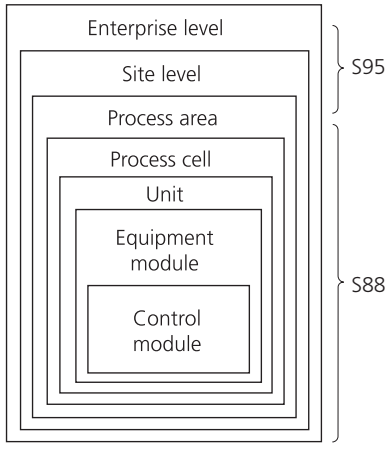

Fig. 10.2 Illustration of the structure used to model an enterprise according to specific standards (ANSI/ISA-S88 and ANSI/ISA-S95). By permission of Tetra Pak processing system AB, Lund, Sweden.

The fact that the S88 standard has been developed for batchwise production, as is the predominant case in the pharmaceutical industry, drives some new thinking in how to apply these standards to meet processing conditions and requirements in the more continuous or semi-continuous production lines in the dairy industry.

The vision for the future is to have a system that is connected all the way from the ERP system down to the control equipment. An incoming order is processed in the ERP system, broken down and planned in detailed work orders that are despatched down to the execution level and, after execution, all the information and history related to that production is collected, stored for future analysis and relevant parts fed back to the ERP level where the business transactions are finalised (Fig. 10.3).

In addition to the production control and business systems, a number of systems closely related to the production must be integrated, such as material resource planning (MRP) systems, laboratory information and management systems (LIMS), warehouse management systems (WMS), maintenance management systems (MMS) and building management systems (BMS). Each system is developed to fulfil its specific task.

The development of such a wide range of different systems covering different aspects of an enterprise must, for obvious reasons, take place in a cautious manner. It is important to have the whole life cycle in mind and to allow for maintenance activities and expansion, in terms of both capacity and new functionality. It must include open, flexible systems that are able to communicate in an understandable and structured way. This is not only a matter of a successful hardware-to-hardware communication protocol, which today is a minor problem even between hardware of different brands. The major issues are to find and use common standards for defining the structure of the data, and the communication and the interpretation of its content. Collecting data and turning data into useful information requires a

Fig. 10.3 Illustration of the flow of data and information between enterprise resource planning (ERP) and production control (PC) levels. By permission of Tetra Pak processing system AB, Lund, Sweden.

major effort. The S95 standard arises from this need, and its development is actively proceeding, covering new ground and measurements (ANSI/ISA, 2000).

10.3 Hardware development

The rapid development of electronics, microprocessors and computers has made it possible and economically feasible to mechanise and automatically control the different processes in yoghurt production. The development of the hardware has reached a stage where hardware no longer represents a bottleneck or limitation to what can be done. As a consequence, the current task is to combine the design of the process and automation into an integrated production solution, and to make sure that this solution integrates efficiently with all other systems applied in the various parts of the factory. At present, there are still too many isolated 'islands' of systems and information that, if integrated, would be extremely powerful for any enterprise.

10.3.1 *Programmable logic controllers*

In the past, the first major step in the automation of any dairy production line was the introduction of relatively cheap programmable logic controllers (PLCs), which controlled the opening and closing of valves and the start and stop of motors and

pumps. Currently, a wide range of PLC brands are available on the market, and the relation between performance and price has developed rapidly, as in the rest of the computer industry.

10.3.2 *Communication and valve tops*

In a large modern automated dairy, some thousands of valves are operated by PLCs and, to do this in an effective way, a network for signal exchange is built. Each individual valve needs to communicate with the PLC, and this can be done either in the traditional way by a direct connection to each valve or via a serial communication on a field bus. Field bus communication to a control top on each valve is the predominant technology in modern plants, and more and more intelligence is being built into the tops. Sending the signal is not just to operate and give feedback on the valve's position, but also to convey other information regarding overall status, maintenance and remote settings. The same development is seen in instrumentation, which also works on field buses; in this case, information such as actual values, mean values, accumulated values, status and maintenance information is communicated over the field bus. Benefits from this development are not only getting all this information into the system, but also reducing costs of installation, commissioning and troubleshooting.

10.3.3 *Programme controller and servers*

In large complex systems, there is a need to structure and handle the communication between the PLC level and the programme controller (PC) level where all the servers for the MIS and MES related software are situated. This is performed by an input/output (I/O) system, normally built up around an open protocol communication (OPC) server, which basically can be seen as a server for signal traffic management and directing communication to the right parts of the system. For all central PC-based software applications such as user interface, MIS and MES applications, industrial grade PC computers are used. These are powerful, robust models, normally incorporating hardware redundancy (e.g. double processors and hard drives that prevent unnecessary shutdowns of the computer).

10.4 Building an integrated plant and automated system

The evolution from a manual production plant to a fully integrated plant control and management system can be divided into steps. These can be taken one by one if an old production unit is to be modernised, or the process can be carried out as a single major revamp. A new plant for value-added products such as yoghurt would normally be designed for the fully integrated solution. However, there is still a market for simplified solutions designed for quality assurance on another level.

10.4.1 *Local process control and user interface*

Starting from the base, introducing a PLC with a user interface makes it possible to operate and supervise a process unit from a local panel somewhere in the process. In such a system a certain amount of security can be obtained by making sure, for example, that the same sequence of openings of valves is repeated each time regardless of which operator is doing it and other circumstances. Control and supervision of critical control parameters such as temperature are also obtained in such a solution. Altogether, the introduction of a process control/user interface system is a step that gives an increased potential to secure a consistent quality of the product that is being produced and, in a modern production facility, this is the basis for the production management system. The production may still contain a number of operations that are performed manually, such as adding a small ingredient into a tank or connecting a pipe to cleaning-in-place (CIP) position. Such actions from operators can be taken into account in the programming of the logic, and different control measures can be put in place. A proximity switch can be used to control the position of the pipe for cleaning-in-place; a lamp and a push button can be used to remind the operator of the need to add an ingredient and for confirmation of the performance of such activity. In this scenario, the operators are out and about, working in the process on the local units.

10.4.2 *Process line control*

The next step is to connect different process units having their own internal control to interact and communicate with each other in a line control. This can be done in a simple way by signal exchange between the PLCs or by introducing one PLC to coordinate others. In such a solution, the production can be trimmed and optimised in order to reduce product losses, waste of CIP chemicals, energy and other utilities. The user interface in a line control is normally placed centrally, and one operator can control many units from one position. The user interface in a line control is working as the extended eyes and ears of the operator, and the information in it serves as an important decision base. It provides real-time information that enables the operator to make the correct decision on what to do at each and every second. As such, a user interface is the working environment of the operators and must be designed in an ergonomically sound way. There are a number of standards and guidelines on how to use colours and how much information to display on one screen, and other matters (ISO, 2004a; IEC, 2000).

10.4.3 *Plant control and management*

Over the last decade, developments in industrial hardware and software have opened up a wide range of opportunities for using information technology (IT) to manage and develop a production plant. This is done by integrating the process control in a network where an I/O system handles the communication of signals from the con-

trol level up to a PC-based level, i.e. software for data collection, data storing and data analysis are used. This opens up possibilities for keeping production records in order to document critical control points, traceability and any other important information. Having access to such data is essential when it comes to improving product quality, production efficiency and the process. This data collection represents the information flow from the control level to the higher levels. There is also a possibility of introducing a flow of data in the other direction, from the level of ERP systems down to the production and production control levels. A batch control system operates the production by recipe control. One recipe is used to control one process cell for making one batch of a product. The recipe is programmed to include all the information needed concerning the equipment, materials, procedures and parameters. The batch manager sends signals down to the required process control modules, and the operator interaction can be limited to just starting the batch and providing strict control over the production. A batch control system stores all recipes in a database, which provides a flexible and convenient environment for modifications and development of new products.

Between the MES and ERP levels a connection can be made in the form of systems for production planning and work despatch. Such software handles the breakdown of an order coming into the ERP system to a plan of the production and despatch of the work that needs to be done in the batch system, i.e. executing the work through the process control level. The above-mentioned MIS systems report the actual outcome and conclude the flow of data described by the S95 standard shown in Fig. 10.3 (ANSI/ISA, 2000).

Although the technological developments of computers and communication hardware and software certainly allow for this flow of data signals to occur automatically, cycle after cycle, currently this is not fully implemented in the industry for various reasons. In most cases, the ERP systems and the manufacturing systems are separate and are bridged by human interaction. Some items of software are still not fully developed; in many cases a value is perceived in having a human evaluation before execution starts; it also gives a flexibility that has a premium if being programmed into a system.

10.5 The yoghurt production process

Considering the different manufacturing stages of a yoghurt production line, a number of operations are particularly well suited to benefit from the values of a modern automation solution. Areas of specific interest are quality control, recipe management, traceability, and product and production analysis. As the process normally starts with continuous processing of raw milk, evolving into parts that are performed batchwise, there are many benefits from introducing a batch management system with recipes controlling the different production stages. In such a solution, a database contains master recipes for the different process cells, such as mixing of starter cultures, incubation and addition of fruit or flavour.

10.5.1 *Quality control and interlocking*

The quality of the raw materials used is of utmost importance, and quality control of raw materials, intermediate products and the final product is needed at several points in the production of yoghurt.

The recording of all incoming raw material is a legal requirement in many countries (i.e. traceability). Getting all the relevant information into the system can be done either by manual typing or by a barcode reader. The latter approach requires that all materials coming into the factory be marked with a barcode, and it is both time-saving and quality-enhancing since the risk of mistyping is eliminated.

A laboratory information management system (LIMS) is a system that handles samples, evaluations and results of quality checks. The sampling can be done manually from tanks, or when in-line instrumentation is available it can be monitored from such a device. In an integrated system, the result from a quality check can be connected to the specific batch of material it was taken from. The LIMS is normally a separate system connected to the MIS database in the production management system, and the information from the LIMS is transferred to the production reports. The results from the LIMS can also be used to release materials or intermediate products for further production. Taking a raw milk tank, for example, the quality is checked by taking a sample and, until that sample is confirmed as suitable by the LIMS or by laboratory personnel, it is not possible to start any transport of milk from that tank for processing. This interlocking feature is used in all parts of the plant where a certain condition needs to be fulfilled before an operation is allowed to start. It can also be related to CIP, for example, to check that the tank is cleaned before it is allowed to be used for a certain operation; in addition, it can check the time-lag since the last CIP cycle; if it is too long, risking possible reinfection, the interlock will require a new CIP treatment before use.

10.5.2 *Strict batch control by recipe management*

The batch method of yoghurt production certainly benefits from being controlled by a batch control system, where each batch is strictly controlled by a recipe. The recipes are stored in a database, and can be changed and fine-tuned without any reprogramming needed.

The S88 model for recipes is detailed in Fig. 10.4. For a global producer with several production sites, this provides an efficient way of handling recipes with a central–local transparency. A general recipe, developed after scaling up at a central research and development (R&D) department, is maintained at corporate level. This general recipe defines materials and process dependencies. The general recipe is then transferred to a site where local adjustments are made; this could be related to language, local units or any other factors (ANSI/ISA, 1995).

The master recipe is the processing stages or cell-specific recipe that defines exactly how a product is to be made in a specific process cell. The master recipe is used as the template for a control recipe that is the actual recipe to be used for

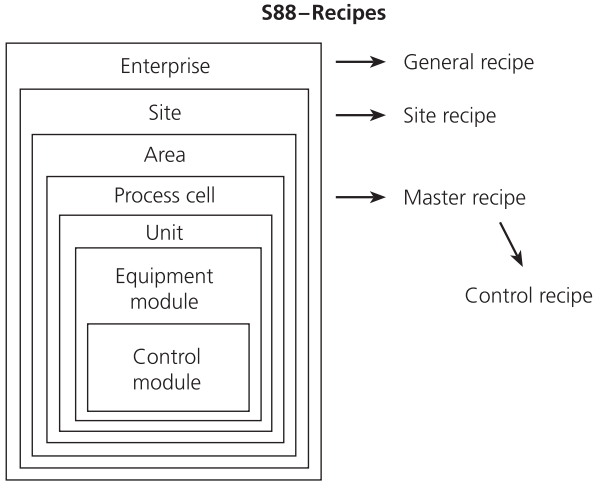

Fig. 10.4 Illustration of a structure for recipes on enterprise and control levels according to ANSI/ISA-S88 standard. By permission of Tetra Pak processing system AB, Lund, Sweden.

the execution of a specific batch. One control recipe controls the production of one batch of material in one process cell and consists of four parts:

(1) Equipment required for processing (source of raw milk or pre-treated milk, starter culture(s) tank, incubation tank);
(2) Raw materials used for blending (pre-treated milk, starter cultures);
(3) Manufacturing procedures to be adopted (transport, agitation);
(4) Parameters to be used (e.g. revolutions per minute (rpm), flow rate per hour).

The influence of the operator on the different parts of the recipe is variable in the design of the system. The system can prompt the operator with alternatives to choose from when a specific batch is being prepared or it can be pre-programmed in the recipe with certain rules that determine how it shall be done, i.e. use the first tank empty or the tank first cleaned.

Cooling temperatures, time of incubation period, speed of agitators, speed of pumps in in-line mixing operations, timing for hygiene demands and limit for time since last CIP cycle are typical parameters introduced into a recipe. Different products having different demands are effectively dealt with by having different recipes stored. It may also be the case that variations in the specification of the raw materials require that several different recipes are available for that product.

Manual interactions in the process can still be allowed, and are dealt with in the design by setting a level of operator interactions that is desired. When the recipe sequence comes to a step where a manual interaction is needed, the operator is alerted and asked for an interaction. The verification of the performance of the interaction can either be done automatically by a signal from a sensor or a transmitter, for

example, a measurement of a weight or flow rate, or by having the operator confirm that the action has been taken.

The batch control system continuously records what is happening, and all changes in the system made either by the system or by an operator are recorded; in the latter case, the operator should use password identification. The major benefit from introducing a batch control system is the strict control and easy handling of large quantities of recipes but, in the end, the full traceability of all activities controlled by the batch execution system is of major value as well.

10.5.3 *Logging production data*

Having knowledge of what is actually happening during production, and making it visible, is the first important step in all improvement work. Collecting and storing historical data in a database provides a number of possibilities of management; for example, keeping track of the production and CIP, critical control points [i.e. in hazard analysis critical control point (HACCP)], key performance parameters and traceability. In parts of the production where batch recipe control is not considered necessary, e.g. the continuous pasteurisation of raw milk, there is still a need to log important processing parameters to a database for documentation and analysis.

Having access to real-time process data provides information for early warnings and diagnosis of process deviations, as well as extended possibilities for trouble-shooting and identifying bottlenecks in production. At present, these types of data can be accessed over web-based applications, and viewed in illustrative and user-friendly graphical formats. It is possible to sit in corporate headquarters and follow the production process as it occurs, far away and in real time. However, for security reasons real-time access must be handled cautiously, and the systems designed in such a way that remote viewers do not interact with or disturb traffic in sensitive parts of the system where signals must be transferred in milliseconds (for more on security, see section 10.5.7).

10.5.4 *Traceability*

Legal demands

In the European Union, Regulation 178 was adopted by the Council of Ministers on 28 January 2002 (EU, 2002). This regulation describes the general principles and requirements of food safety, which establishes the European Food Safety Authority and lays down procedures in matters of food safety (Fig. 10.5). Articles 18 and 19 describe traceability and the responsibilities of a food business operator, ensuring that each part in the production chain keeps track of raw materials used and products sent further along the food retailing chain. It also states that this must be documented in a structured way and there must be a system in place for product recall and consumer notification if a suspect product is delivered to the marketplace (Fig. 10.6). The articles regarding traceability are effective from 1 January 2005, and cover all food being produced in or imported to the EU. Similar regulations are proposed or already implemented in the USA, Japan, Australia and other parts of the world.

Fig. 10.5 Coverage of EU regulation 178/2002.

Fig. 10.6 Summary of Articles 18 and 19 on traceability, EU regulation 178/2002.

Commercial and market demands

In addition to the legal perspective, consumers are becoming more and more concerned about food safety and food ethics, and in many ways the demands of the commercial market are greater than the legal requirements. Such aspects could range from requiring information on the source of raw materials to identifying products with ecological or other specific treatments to requiring assurance that no trace of allergenic substances are present. Hence, a food producer today should be able to show what happens to food ingredients through the production chain (i.e. from farm to consumer or from farm to fork) in order to protect the brand value of the product and ensure consumer loyalty.

Standards

A number of international organisations are focusing on setting standards to meet the legal requirements and enhance food safety, and a number of standards and guidelines are available and recognised by the industry. The ISO standard 9000 (ISO, 2000) defines the requirements for food traceabililty in each step of the production chain (Fig. 10.7), which can be summarised as keeping track of:

- the raw materials used;
- the products delivered;
- the processing history.

The ISO standard 22000 (ISO, 2004b) was released in September 2004, and covers not only the traceability issue but also describes how to implement a Food Safety Management System in all parts of the food production chain. This new standard is for guidance and audit certification.

Traceability resolution

In practice, traceability is more than regulations, standards and having an automation system. It is actually how the production is designed and operated that sets the level of traceability to be achieved. There are two major aspects of traceability: separation and resolution. Separation is related to how two batches of material are separated from each other, by CIP, by water flush, by tank emptying, etc. This and other rules of operation, such as whether it is permissible to fill and empty a tank at the same time, set the resolution of traceability in a certain production line, which gives the quantity of a specific material that needs to be identified and recalled if something goes wrong.

For example, if the daily production is running continuously, the received raw milk in silos is routed to the pasteuriser and collected in pasteurised milk tanks

ISO 9000:2000

'Ability to trace the history, application or location of that which is under consideration'

When considering product, traceability relates to
- the origin of materials and parts,
- the processing history, and
- the distribution and location of the product after delivery'

Fig. 10.7 Summary of ISO 9000: 2000 on traceability.

without keeping track of which tanker delivered the milk to which silo, and then from which silo to which tank the pasteurised milk was fed, the resolution of the raw milk will be *one day's production*. If, on the other hand, a resolution of *one truck* is desired, the volume of that truck must be kept separate all the way through the process and be given a unique identification after filling. As long as everything is being recorded properly, both cases, according to the laws and standards, are fulfilling the requirements of traceability, but there is a major difference in resolution. Figure 10.8 illustrates two different cases where the resolution of traceability

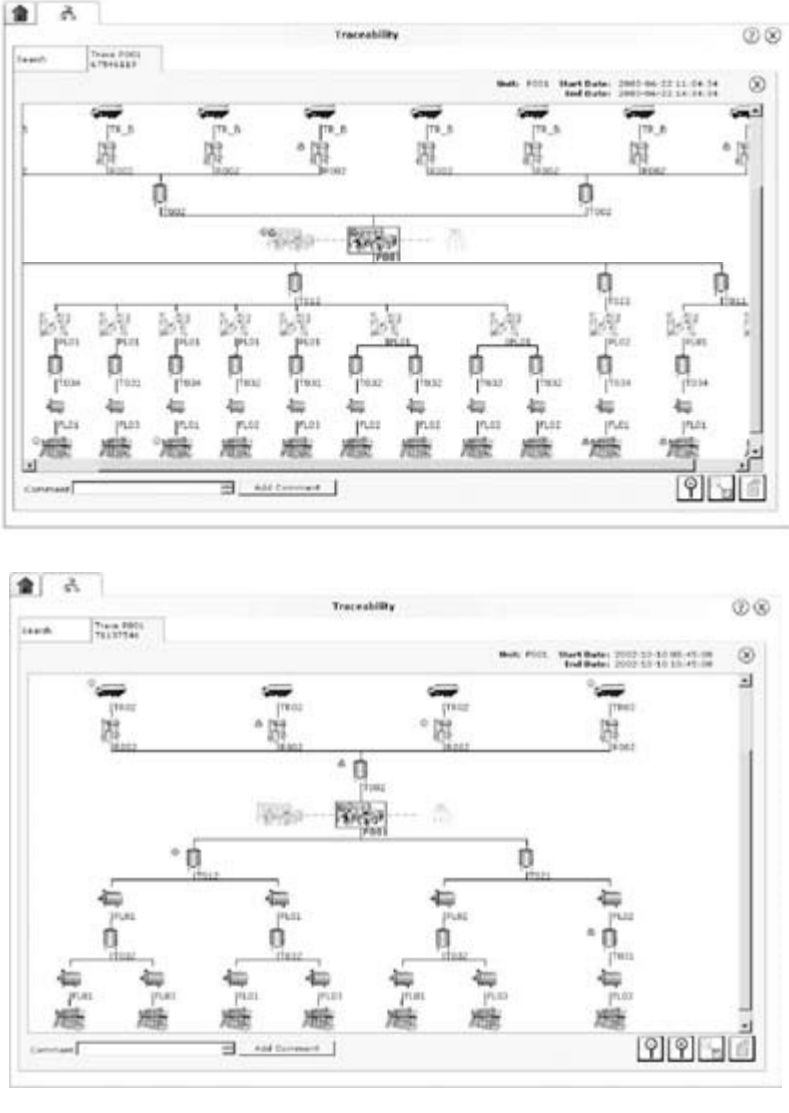

Fig. 10.8 Two different levels of resolution in traceability, focusing on the pasteuriser. By permission of Tetra Pak processing system AB, Lund, Sweden.

is different. Since there is a cost in keeping small volumes separated, i.e. number of tanks required, product losses related to CIP, utility and energy consumption, each producer must assess the risk and value of the assurance premium. For example, in a case of a product recall, what will be the cost of raw materials, production, the recall costs and the damage to the brand and consumer confidence? Obviously, for more valuable products and a higher brand image, it is important to implement higher assurances. Thus, the automated traceability system is a tool that makes a difference in being precise, efficient and effective in delivering the correct information fast in a situation of crisis. It does not create traceability, but it can report the traceability that the operation and design of the production system allows for.

In a traditional production plant with many manual operations, the information needed can be collected and handled manually, but now it is possible and economically feasible to do this in an automated system, which also opens up other possibilities. Introducing such a system for data collection and documentation is not only fulfilling the legal, standard or market demands, but also it provides a possibility that the same data can be used for analysis of the product, the process and the production. This gives a great opportunity to improve and optimise the whole operation, and the overall value of an automated system is related to:

- The labour cost of keeping manual records;
- The increased quality and consistency of automated records;
- The decreased damages in case of accident – thanks to fast access to correct information (e.g. early warnings and diagnosis, precise and accurate information to suppliers, customers and consumers); and
- Improvements in production from analysing the data collected.

10.5.5 *Analysing the production*

By using the collected data from an equipment point of view, it is possible to analyse how well the equipment is utilised; also it gives indications of where any bottlenecks are in a production line. For example, by recording the status of a stoppage (e.g. waiting for CIP or material from a previous operation), the cause can be identified and addressed. Figure 10.9 is an example of a report analysing production, giving key performance indicators (KPIs). Such indicators are often used as a benchmark of different operations; it is possible to ‘drill down’ into these figures and find out the cause of deviation from the benchmark, which is the key to constant improvement.

10.5.6 *Analysing the product and the process*

By viewing the data from a product and process aspect, quality can be optimised. If a certain batch has been particularly good (i.e. a ‘golden batch’), samples and data from the various operations can be analysed and compared to those that were

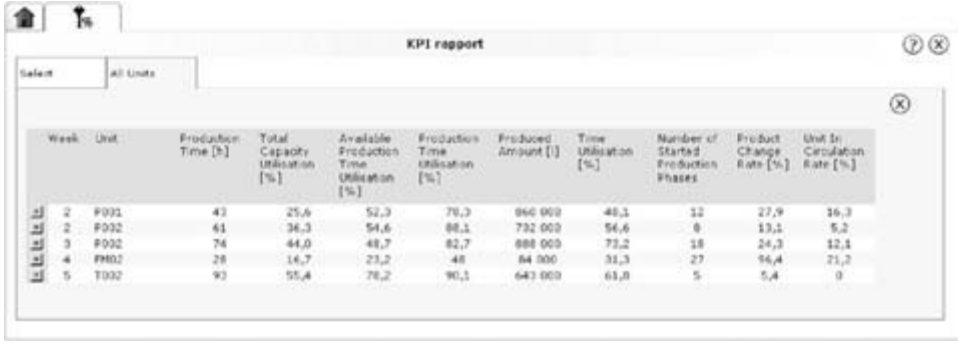

Fig. 10.9 Example of production analysis report. By permission of Tetra Pak processing system AB, Lund, Sweden.

not so successful; hence, gaps and possibilities for improvement can be identified. Comparing different batches or production runs is a valuable tool in the development of new products. Having this opportunity will be even more important in the future as the variety of products constantly increases and the life cycle of each product is getting shorter and shorter. Figure 10.10 is an example of a production detail report with quality information and a graph of a parameter of interest.

Fig. 10.10 Trend visualisation, from a typical production report. By permission of Tetra Pak processing system AB, Lund, Sweden.

10.5.7 *Security*

A totally integrated production control system is very powerful and thus needs to be safely protected from abuse (intentional or not) and accidents. This is basically achieved in two ways, by using software protection and hardware protection. Software is protected by passwords on different levels, which gives certain access and authorisation depending on what level is logged in, such as operator, maintenance personnel, production management or administration. Hardware is protected, first, by good design to ensure that the load is kept at a proper level, and that processors and memories are rightly sized; and second, if needed, backed up by redundancy and uninterruptible power supply (UPS) – a system, for example, that protects against variations and breakdown in the electricity supply.

Introducing the possibility of remote access and control via modem, intranet and internet requires extensive security assurance. Having separate networks for data traffic in the production environment and in administration is essential to ensure that the production network is not disturbed by non-production traffic. The exchange of data between systems must be designed according to such principles.

One major aspect to keep in mind is that the different parts of an integrated system are so very different in nature. The production control systems in the 'base' are working in real time (in milliseconds), and the business systems at the 'top' are huge and global with enormous numbers of data transactions. Integrating these aspects in meeting the needs of both environments but without jeopardising security is a challenge that is constantly dealt with in today's solutions.

10.5.8 *Remote connection*

Technology allows for remote access by various means, e.g. from laptop computers in the process to connection over intranet or internet for planning and control. For a service engineer, a handheld computer with the work orders and all manuals and drawings needed to perform the work and confirm that it is fulfilled makes the job more efficient, secures a certain level of quality and allows everything to be updated at all times – there are no bulky binders to move around, as used to happen a few decades ago.

The recording of raw material intake by wireless barcode readers is another example showing the gain in efficiency and quality of using modern technology. It is also possible to use a modem or the internet to control the whole production from a physical location that is remote. Obviously, doing this on a regular basis involves many risks, but there are major benefits in using a remote connection for troubleshooting, service and maintenance purposes in a controlled manner. Furthermore, experts remotely connected can guide local people on site in updating software and backing up databases. Another possibility is to have web cameras surveying certain parts of the process for display on the user interface of a centrally positioned operator.

10.6 Future developments

Developments in the automation field for the liquid food manufacturing industries are focusing very much on efficiency and transparency of operations. However, a production unit that is efficient and flexible at the same time is becoming more and more important for manufacturers of liquid food products. To manage this paradox, a clever design of the production solution is required by utilising the most suitable software in every part of the system. Yoghurt production is a typical example where the process changes from being continuous to batchwise in different parts of the plant. Today, this calls for different software tools in different parts in order to make the production process more efficient and flexible, as well as safe and user-friendly in all aspects. The software used in the liquid food industry today comes mainly from two directions, the batchwise production of the pharmaceutical industry and the intermittent production exemplified by the car industry. There are still some challenges in developing complete software solutions for the batchwise and continuous flexible production of liquid food.

Transparency can be seen from different aspects, i.e. internally between the different systems in an enterprise and externally linking the suppliers in the supplier chain closer to each other. From the internal perspective, there is a need to develop the link between the ERP system and the production control system, which contains the detailed planning of the production from the base of an overall ERP system. In many production plants, this is done manually using small stand-alone calculation tools. This is the final link between the business and the production systems that needs to be bridged to fulfil the vision of the total flow of data and information in a total enterprise system. From the external perspective, there is a drive for an increased transparency in the value chain, to give suppliers and customers updates on the requirements one step in each direction, i.e. aiming for decreased stock-keeping and improved precision in delivery time.

In the field of production analysis, many tools are aimed at further improvements in production efficiency. An interesting development would be to utilise the systems and data to monitor the actual cost of making a specific batch or work order of a product in terms of all raw materials, energy, utilities, losses and wastes, labour and everything else used to produce it.

To actually be able to monitor and document the cost of producing a specific batch of a certain product, it would be necessary to visualise the impact of production management on the running of smaller or larger batches, cleaning between batches and/or running certain products in certain sequences; it would provide a great opportunity to evaluate these cost aspects in the light of market demands.

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Note: page numbers in **bold** indicate tables or illustrations

Plate 1 Stabilised (right) and unstabilised (left) drinking-type yoghurt. With permission of Danisco A/S, Brabrand, Denmark.

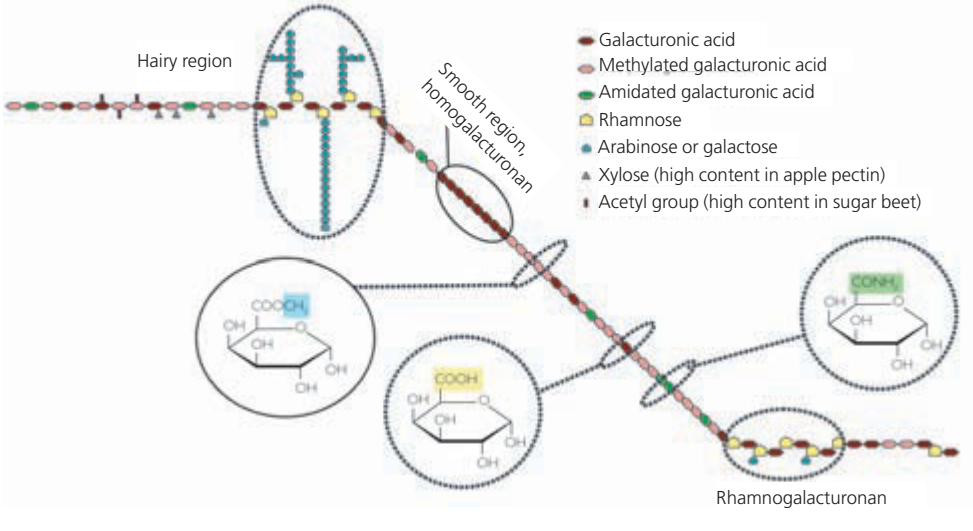


Plate 2 Pectin, a complex molecule built up of long chains of galacturonic acid (smooth regions) with interruptions of amidated or methylated galacturonic acid sequences and branched regions. With permission of Danisco A/S, Brabrand, Denmark.

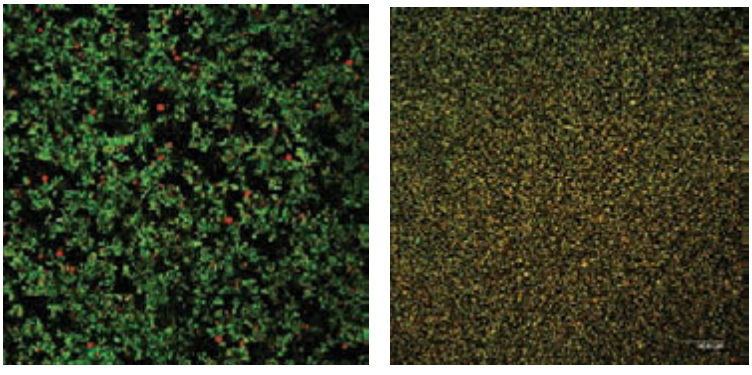


Plate 3 Confocal laser scanning microscopy (CSLM) showing the quality of drinking yoghurt with (a) or without (b) high ester pectins. The stabiliser used was Grindsted® AMD 780. With permission of Danisco A/S, Brabrand, Denmark.

Plate 4 The Malvern Mastersizer for measuring of particle size distribution. With permission of Danisco A/S, Brabrand, Denmark.

Plate 5 Stresstech CS rheometer for controlled stress viscosity measurement. With permission of Danisco A/S, Brabrand, Denmark, and Reologica, Sweden.

Plate 6 An illustration of the typical texture of viili.

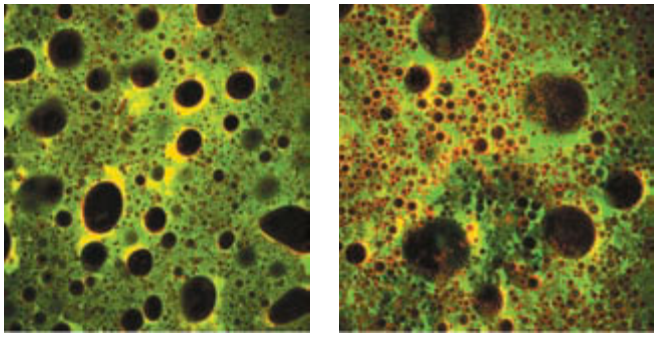
(a)  (b)

Plate 7 Confocal laser scanning micrographs of mousses made with Grindsted® mono- and diglycerides (a) and Grindsted® Lactem (b) (lactic acid ester of mono- and diglycerides). The protein structure (i.e. the serum phase) is shown in green and the fat in red and yellow, while the dark circular patches show the air bubbles. By permission of Danisco A/S, Brabrand, Denmark.