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Controlling the texture of fermented dairy products: the case of yoghurt

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8.1 Introduction

Starting with comments on the historical background and the economic importance of yoghurt, the first part of this chapter deals mainly with some basic biochemical and microbiological aspects of yoghurt production. An outline of different manufacturing methods resulting in various types of yoghurt is followed by a description of the main technological factors which are known to influence rheology and texture properties. These factors comprise the preparation of the base milk, including dry matter enrichment, homogenisation and pre-heat treatment, as well as incubation conditions and post-incubation treatments, which vary largely depending on whether set-style or stirred yoghurt is produced. Interrelations between technological factors and physical parameters are given and, where possible, are explained on the basis of micro-structure. The following section gives an overview of rheological methods commonly used for the testing of set-style and stirred yoghurt, and presents some tools for the numerical treatment of response data. Some empirical methods, which are the basis for simple product testing in routine laboratories or manufacturing plants, are then presented. Prior to an outline of presumable future trends and sources of additional information, a few comments deal with sensory procedures used for the evaluation of physical properties of yoghurt.

8.2 The manufacture of yoghurt

Fermentation is one of the oldest procedures for transferring raw materials of plant or animal origin into products with extended shelf-life, and it is assumed

that the fermentation of milk dates back approximately 10 000 years (Stanley, 1998). The term 'fermented milk' or 'cultured milk' refers to products such as yoghurt, sour milk, cultured buttermilk and sour cream, which are usually made from cows' milk by pure lactic acid fermentation. Additionally, some products are made from milk from other species such as ewes, goats or mares, and combined fermentation (by, e.g., lactic acid bacteria and yeasts) results in products known as kefir or koumiss.

Yoghurt represents the most popular fermented milk product worldwide and originates from countries around the Balkan and the Eastern Mediterranean Sea (Staff, 1998; Walstra *et al.*, 1999). Generally, yoghurt is manufactured from pre-heated milk, with fat and dry matter content varying with respect to region and legislation, either in the plain form or with added material such as fruits or fruit premixes, sugar, cereals, or additives such as gelling agents, flavourings or colourants. Legislation and codex regulations differ widely around the world; in the one or other country, the use of additives is prohibited, or the presence of a certain number of viable starter bacteria in yoghurt is required (e.g., 10^7 bacteria per gram in the USA; Mistry, 2001). Consumption statistics for fermented milks show highest *per capita* consumptions throughout Europe and a continuous growth in nearly all major markets. Exceptions are countries with an already existing high consumption level, such as the Netherlands and Iceland (Table 8.1).

Generally speaking, cultured or fermented milk products are made by inoculation of milk with a specific combination of microorganisms, which are able to convert lactose into lactic acid. Milk is a complex fluid containing

Table 8.1 Consumption of fermented dairy products including yoghurt

Country	Consumption (kg per capita)				
	1980	1990	1993	1998[a]	2000[a]
Australia	1.8	3.5	4.8	5.6	5.7
Austria	9.8	10.4	13.0	16.4	21.4
Belgium	7.7	9.6	11.9	20.5	21.1
Canada	2.3	3.7	3.5	3.7	4.9
Czech Republic	—	—	—	10.6	13.8
Denmark	26.7	21.6	20.7	25.9	26.2
Finland	41.0	38.3	38.1	38.8	40.7
France	9.3	16.4	17.3	27.8	28.5
Germany	—	—	—	24.7	26.5
Netherlands	27.3	32.5	29.7	45.0	44.8
Norway	10.1	14.9	—	19.3	16.6
Slovakia	—	—	—	5.4	6.7
Spain	6.0	8.0	9.8	14.7	15.7
Sweden	24.0	29.1	28.6	37.9	38.0
USA	6.2	—	3.5	2.4	2.7

Compiled from various sources (Anon., 1982, 1992, 1995, 2001).
[a] Data include milk drinks and fermented milk products.

Table 8.2 Proximate composition (g/kg) of bovine, goat and ewe milks

Component	Bovine	Goat	Ewe
Protein	34	29	55
Casein	28	25	46
Fat	37	45	74
Lactose	46	41	48
Ash	7	8	10

Source: Jensen, 1995.

relatively high amounts of proteins and minerals which, as it is intended to nourish young mammals, varies in composition according to the species' needs (Table 8.2). Especially the major part of the milk proteins, the casein, which occurs in conjunction with calcium phosphate in the form of colloidal particles 100–500 nm in diameter and of MW approximately 10^8 Da (Buchheim and Welsch, 1973), is of great importance for the functional behaviour of the final acidified product. The colloidal calcium phosphate (CCP) plays an important role in maintaining the integrity of the casein micelles, which are in dynamic equilibrium with their surroundings. Therefore, a lot of structural research has been undertaken to explain the mechanisms of the stability of casein micelles and their sub-units, irrespective of whether or not these are present in the form of sub-micelles (Holt, 1993; Holt and Horne, 1996; Rollema, 1992; Schmidt and Both, 1982; Visser, 1992; Walstra, 1990; Walstra *et al.*, 1999).

During fermentation of yoghurt, the milk sugar in the base milk is partially converted into lactic acid by the action of various enzymes, originating from the growth of thermophilic lactic acid bacteria. This causes a sufficient decrease in the pH, resulting in a dissociation of the CCP, a destabilisation of the casein micelles and even some liberation of individual casein molecules, accompanied by reaching a maximum in voluminosity (Dalgleish and Law, 1988; Lucey and Singh, 1998). Below a pH of 5.5 the casein micelles begin to swell and, as almost all CCP is dissociated, start to precipitate. This precipitation leads to a sufficient decrease in the voluminosity of casein micelles (van Hooydonk *et al.*, 1986) and to the formation of clusters and chains that link together to form a gel, composed of a continuous three-dimensional network with the milk serum containing whey proteins, lactose and salts entrapped as liquid phase (the amount of whey proteins depends on heat treatment; see below). Electron microscopy shows the particulate character of acidified milk gels with empty spaces or pores in the network where the serum was entrapped (Kalab, 1979, 1993; McManus *et al.*, 1993).

The classical yoghurt starter culture is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, with a cocci–rods ratio of usually 1:1 (Hassan and Frank, 2001; Hutkins, 2001). These organisms grow in a protocooperative relationship, resulting in rapid acidification by stimulating each other. Depending on type and activity of the starter cultures,

other metabolites such as carbon dioxide, acetic acid, diacetyl, acetaldehyde, large molecular weight exopolysaccharides or several other compounds are produced besides lactic acid, resulting in the characteristic properties of the products regarding flavour, texture and aroma. Since *Streptococcus thermophilus* is weakly proteolytic its growth is stimulated by the rods, which liberate free amino acids and small peptides from casein. The cocci in turn encourage the growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* by producing formic acid and carbon dioxide (Matalon and Sandine, 1986; Rajagopal and Sandine, 1990). Nowadays, microorganisms such as *Bifidobacterium* spp. and *Lactobacillus acidophilus* are often added for therapeutic purposes (Mistry, 2001; Yucuchi *et al.*, 1992). Generally, and based on the accumulating knowledge from well-defined, randomised and placebo-controlled studies (Fondén *et al.*, 2000), health-promoting effects of some strains used for yoghurt fermentation become more and more evident. Because of their slow acid production, these bacteria are usually used in combination with classical yoghurt starters, resulting in so-called 'yoghurt-like products'; depending on local legislation, this distinction might be of great importance (Hassan and Frank, 2001; Marshall and Tamime, 1997). Lactic acid bacteria that produce high molecular weight extracellular polysaccharides (EPS) are now commonly used in the yoghurt industry to improve product texture, partly replacing the addition of stabilisers and gelling agents, by enhancing yoghurt viscosity, independent of the fat content.

Manufacturing methods vary considerably and, for example, depend on the country, the type of product manufactured, the raw materials used and the product formulation. However, a number of common principles are generally applied (Staff, 1998):

- The total solids content of the base milk is increased to enhance the water-holding capacity of the product.
- A heat treatment of the base milk, usually >80ºC for some time, is applied to achieve a proper denaturation of the whey proteins, also increasing the water-binding capacity.
- Inoculation with a specific starter culture and subsequent incubation with a time-temperature profile depending on the properties of the starter, and on technical requirements.
- Cooling and addition of appropriate ingredients (fruit premixes, flavours).
- Packaging and chilled storage.

Yoghurt types are usually distinguished according to their physical state in the retail container, which results from differences in the manufacturing process. Apart from set yoghurt and stirred yoghurt (Fig. 8.1), with production figures varying from country to country, there is a generally increasing demand for yoghurt drinks consisting of yoghurt mixed with skimmed milk, whey or water, and of yoghurts with increased shelf-life such as frozen or thermised yoghurt.

Incubation of set yoghurt takes place in retail containers (plastic cups or glasses of different sizes) until the required pH (around 4.4–4.7) is reached,

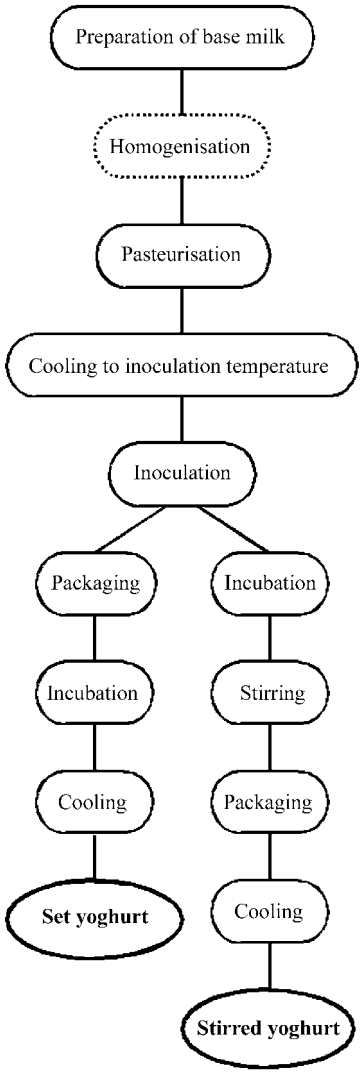

Fig. 8.1 Basic steps in the production of set and stirred yoghurt.

leading to an undisturbed gel. The viscoelastic gel network consists of aggregated spherical casein particles forming a continuous structure and enclosing fat globules and serum. From a structural point of view, yoghurt belongs to particulate gels with disordered structures (Horne, 1999; Walstra *et al.*, 1999). Stirred yoghurt is inoculated and incubated in large fermentation vessels; the formed gel is then gently stirred to obtain a smooth and viscous, but still pourable, product, and finally packed. By breaking up the gel, a highly viscous, non-Newtonian liquid is formed, which shows a strongly shear-rate and time-dependent flow behaviour.

Drinking yoghurt is produced from low solid milk on the basis of the stirred manufacture process, or regular stirred yoghurt is diluted to some extent. Increased shelf-life of yoghurt may be achieved either by freezing or by thermisation of the fermented product. Whereas the thermisation process is designed to reduce the number of potential spoilage microorganisms and, therefore, results in a partial inactivation of the starter culture, the freezing procedure, provided that appropriate methods are applied, leaves the culture bacteria viable. In frozen yoghurt, higher amounts of sugar and stabilisers are required to maintain the air bubble structure during the freezing process (Tamime and Deeth, 1980).

8.3 Factors affecting yoghurt texture

It is generally accepted that three main factors determine the physical properties of yoghurt. These factors are the preparation of the base milk, comprising several possible treatments, the fermentation process and the post-fermentation treatment.

8.3.1 Preparation of the milk

Dry matter enrichment

The amount of total solids in the base milk, to a large extent, determines the physical properties of the final yoghurt product. The dry matter content of the base milk typically ranges from 9% in the case of skim milk yoghurt without fortification up to more than 20% for certain types of 'concentrated yoghurt', with the most usual values of commercial products ranging between 13% and 17%. There are several possibilities for the fortification of the base milk (Kulkarni *et al.*, 1990), either procedures which increase milk solids proportionally, e.g., evaporation of the base milk to the desired dry matter level, particular membrane processes (i.e., reverse osmosis), the addition of skim milk powder or the addition of evaporated milk, or procedures which increase milk solids disproportionally. These methods comprise the addition of certain types of protein powders, either whey protein or casein based, the addition of whey protein concentrates, or the application of several types of membrane processes such as ultrafiltration or nanofiltration. The selection of a particular method is largely determined by the availability of raw materials (e.g., whey for concentration) and the equipment of the production plant.

Independent of the type of fortification, the protein content of the base milk is the most important factor which influences rheological and physical properties of yoghurt. An increase of the protein content increases the amount of bound water and, consequently, the firmness of the resulting gel. According to Snoeren *et al.* (1982), milk casein is able to immobilise as much as 2.82 g H_2O per g protein and shows a voluminosity of 3.57 ml/g. Corresponding hydration values for undenatured and denatured whey protein are 0.32 g H_2O per g protein and

2.34 g H_2O per g protein, respectively. Data on both quantitative and qualitative effects of dry matter enrichment on physical parameters such as apparent viscosity, gel firmness or susceptibility to syneresis are available from various authors (Table 8.3).

The use of concentrated whey proteins in yoghurt manufacture is of increasing interest, both for utilisation purposes but also from a nutritional point of view; increasing the whey protein/casein ratio improves the biological value of the mixture (Meisel, 1989). Results on firmness and syneresis of yoghurt with a modified whey protein/casein ratio are somewhat contradictory. There is, however, a tendency towards less viscous, softer gels for products with an increased whey protein content, compared to products showing a similar protein content on casein basis (Buchheim *et al.*, 1986; Jelen *et al.*, 1987; Modler *et al.*, 1983; Morris *et al.*, 1995). In any case, it was suggested to limit the increase in the whey protein/casein ratio due to potential off-flavour effects (de Boer, 1996).

Homogenisation

Whole milk is homogenised at pressures of 10–20 MPa in a temperature range of 55–65ºC, usually prior to heat treatment, to prevent creaming during fermentation. The process results in the disruption of the milk fat globules, which are stabilised by a specific fat globule membrane consisting mainly of proteins, phospholipids and neutral glycerides, from native size (approximate range 1–5 μm) into much smaller ones. Commonly used homogenisation techniques and the mechanism of the fat globule size reduction are described in detail by Kessler (1996) and Walstra (1995). The covering of the homogenisation-induced, enlarged fat globule surface area with fragments of milk proteins leads to the development of a secondary fat globule membrane, which is of great importance for the characteristics of fermented dairy products (Schkoda, 1999). Electron microscopic investigations (Buchheim and Dejmek, 1990) confirmed the model of the fat globule membrane of homogenised milk as proposed by Walstra and Jenness (1984), showing casein micelles and whey proteins as part of the layer: depending on the homogenisation temperature either casein micelles (at 40ºC), or micelle fractions (or submicelles) and whey proteins (>60ºC) represent the main part of the newly built membrane.

Large fat globules as present in unhomogenised milk may decrease firmness of fermented products by interrupting the gel network (Aguilera and Kessler, 1988). It is generally accepted that the partial replacement of the native fat globule membrane with other milk proteins allows the fat globules to be incorporated into the gel by crosslinking them to the matrix (van Vliet and Dentener-Kikkert, 1982). However, the effects of homogenisation depend on the layout of the production process. Schkoda (1999) stated that aseptic homogenisation, with the pre-heat treatment preceding the mechanical treatment, causes a higher load to the membrane due to the aggregation of denatured whey proteins to each other and to casein; on the other hand, heating after homogenisation (septic homogenisation) leads to a partial aggregation of membrane proteins to each other, resulting in the formation of aggregates of

Table 8.3 Effects of various fortification methods on physical properties of yoghurt

Reference	Fortification method[a]	Results
Becker and Puhan, 1988	SMP, UF, EV	Increase in gel firmness, less syneresis
Guirguis *et al.*, 1987	RO (compared to milk powder addition)	Increase in gel firmness, less syneresis
Jaros *et al.*, 2002a, 2002b	Reconstituted SMP to 10, 12 and 14% dry matter with two different starters (non-ropy, ropy)	Increase in gel firmness, viscosity and serum-holding capacity; oscillatory measurements of EPS yoghurt gels showed almost no effect of the protein content
Kulkarni *et al.*, 1990	Whey protein concentrate	Decrease of viscosity and firmness with increasing whey protein
Lankes *et al.*, 1998	Comparison of SMP, VEV and UF techniques	Higher firmness and viscosity for UF (higher protein content) due to membrane characteristics
Modler *et al.*, 1983	NaC, UF-MPC, SMP, UF-WPC	Increase of firmness, decrease of syneresis; WPC less firm than casein-based products
Rohm, 1993a; Rohm and Schmid, 1993	SMP, NaC, UF	Increase in viscosity, most for NaC fortification; decrease in syneresis
Savello and Dargan, 1995, 1997	SMP, UF	UF yoghurts have higher firmness and viscosity than SMP
Schkoda *et al.*, 2001	Increase of protein content from 3.5 to 7.0% by nanofiltration of skim milk	Increase in gel firmness, viscosity and serum-holding capacity
Tamime *et al.*, 1984	RO (compared to milk powder addition)	Increase in firmness, less syneresis

[a] RO, reverse osmosis; SMP, skim milk powder; UF, milk protein concentrate from ultrafiltration; (V)EV, (vacuum) evaporation; NaC, sodium caseinate; UF-WPC, whey protein concentrate from ultrafiltration.

linked fat globules. Studies of physical properties of high-fat yoghurt made from whey protein-enriched base milk homogenised either aseptically or septically revealed large differences, with higher viscosity and firmness values for products where homogenisation was performed after heat treatment (Kulkarni *et al.*, 1990). The authors dedicated the different effects of heating to different mechanisms of membrane loading, enhanced by the relatively high amount of whey proteins, and concluded that casein micelles incorporated in the secondary fat globule membrane built during homogenisation will be covered with whey proteins. Their denaturation during subsequent heating might prevent the active contribution of the fat phase to the gel properties (Sharma and Dalgleish, 1994; Tamime and Marshall, 1997).

The effects of incorporating the fat globules into the gel network also depend on the globule size and, consequently, the homogenisation procedure. After homogenising whole milk at different conditions, Plock *et al.* (1992) found a linear increase in gel firmness with decreasing fat globule diameter, and achieved a higher efficiency by multiple stage homogenisation at low homogenisation pressures.

Pre-heat treatment

Heating of the base milk is essential in yoghurt manufacture, and temperature–time conditions may be varied to adjust physical properties of yoghurt products. Generally, heating conditions are much more intense than necessary for preservation purposes, causing a sufficient denaturation of whey proteins, which are then able to associate with casein micelles (Law, 1996; Pearce, 1995). Heating increases voluminosity and water-binding capacity of whey proteins (Snoeren *et al.*, 1982) and decreases their solubility. Reactive side groups of globular whey proteins, especially thiol groups, are exposed due to unfolding, resulting in an oxidation to disulphide linkages and associations between whey proteins and casein. Additionally, denatured whey proteins may associate with casein micelles via hydrophobic interactions with κ-caseins (Smits and van Brouwershaven, 1980). All possible interactions result in a significant contribution of the denatured whey proteins to the properties of the yoghurt gels.

In commercial yoghurt production temperature–time profiles usually ranging from 80–85ºC for 30 min to 90–95ºC for 5 min are applied (Lucey and Singh, 1998). Dannenberg and Kessler (1988a, 1988b) demonstrated the close relationship between the degree of β-lactoglobulin (β-lg) denaturation, which was linked to heating conditions by a kinetic approach (Dannenberg and Kessler, 1988c), and selected physical properties of yoghurt. Within a β-lg denaturation range of 60–99%, susceptibility to syneresis decreased linearly, whereas yoghurt gel firmness showed an optimum at a residual β-lg level of 10%. Lucey *et al.* (1997) compared acid gels made of reconstituted skim milk from powder subjected to different heat treatments during manufacture, by further subjecting reconstituted milk to several heating conditions. Increasing heating temperature and time led to higher denaturation of β-lactoglobulin and α-lactalbumin (α-la), with β-lg being more heat sensitive (i.e., 95% denaturation after heating at 90ºC

for 15 min). Dynamic rheological measurements showed a marked increase in the storage modulus (G') for heating conditions higher than 80ºC for 15 min, indicating higher gel firmness. The authors suggested that denatured whey proteins in heated milk become susceptible to aggregation during acidification, as the isoelectric points of whey proteins are approached.

However, details on the mechanisms of the heat-induced interactions of α-lactalbumin and β-lactoglobulin with casein micelles are not yet clear. Since native whey proteins, added to the milk after heat treatment, do not contribute to the gel matrix (Lucey *et al.*, 1998a, 1999; Mahaut and Korolczuk, 1992), it appears that denatured whey proteins building associations with casein micelles during heat treatment then act as bridging material by interacting with other denatured whey proteins. Corredig and Dalgleish (1999) performed heating procedures between 70 and 90ºC on skim milk after addition of different amounts of purified α-la and β-lg and reported on two main interaction mechanisms: a direct interaction of β-lactoglobulin with casein micelles, via κ-casein binding, and the formation of soluble aggregates of both types of whey proteins as an intermediate before reacting with the casein. Furthermore, the presence of β-lg was necessary for the occurrence of any association with the casein micelle, and binding sites seem to be limited.

8.3.2 Incubation conditions

Cultures

The selection of the starter culture also determines the physical properties of stirred yoghurt to a large extent. As described above, some extracellular polysaccarides (EPS) produced by lactic acid bacteria are known to cause an increase in apparent viscosity, thus leading to improved physical stability of the fermented product.

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (e.g., Faber *et al.*, 2002; Grobben *et al.*, 2000; Petry *et al.*, 2000; van Calsteren *et al.*, 2002), but the functionality of the EPS in fermented milk is still not completely clear. It seems that not the amount of polysaccharide, but rather the type, charge and molecular mass of EPS, are important for rheological properties (Bouzar *et al.*, 1997; Laws and Marshall, 2001; Marshall and Rawson, 1999; Pleijsier *et al.*, 2000; Ruas-Madiedo *et al.*, 2002). The amount of EPS is, however, correlated to viscosity properties when a particular type of EPS is considered (Sebastiani and Zelger, 1998). Some authors suggested that the effects of EPS on yoghurt texture derive from a possible attachment to the casein matrix (e.g., Domínguez-Soberanes *et al.*, 2001; Skriver *et al.*, 1995), which has been shown in micrographs obtained by conventional SEM. From these figures, it was concluded that the junctions between polysaccharides and casein strands are responsible for increased viscosity values of yoghurt made with EPS-producing starters. However, Kalab (1993) referred to some previous work done by himself (Tamime *et al.*, 1984) and stated ‘In micrographs of samples which had been

dehydrated prior to electron microscopic examination, the mucus appears in the form of filaments. This appearance is an artifact; since the polysaccharides cannot be fixed chemically, they shrink on drying and form filaments.' It is more likely that EPS are either excreted outside the cell walls and remain there, thus forming a type of capsule (this was shown by confocal laser scanning microscopy by Hassan *et al.*, 1995), or are loosely attached to the cells or even excreted into the serum phase (Cerning, 1995).

Oscillatory measurements on intact yoghurt gels revealed no effects of non-charged EPS on the storage modulus of the gel network at a dry matter level of approximately 12%, whereas improved stiffness of products with lower dry matter contents was found (Jaros *et al.*, 2002a; Pleijsier *et al.*, 2000). It may be assumed that non-charged EPS are dissolved in the serum phase in the pores of the network and, therefore, do not contribute to the strength of the protein network. This might be the reason for the significant effect they show on the viscosity of stirred yoghurt, as measured either in thixotropic loops in large deformation measurements or by empirical funnel flow measurements (Jaros *et al.*, 2002b). Permeability measurements showed lower values for yoghurt gels fermented with EPS-producing cultures compared to non-EPS gels, indicating a larger resistance to capillary flow (Jaros *et al.*, 2002b; van Marle and Zoon, 1995). Van Marle *et al.* (1999) also distinguished between two different types of EPS on the basis of steady-shear viscosity measurements, with viscosities of yoghurt serum being five times higher for starter cultures producing EPS which are released into the aqueous phase.

Temperature–time regimes of fermentation

The conditions of incubation may additionally influence the properties of the final product. Generally, thermophilic lactic acid bacteria show an optimum temperature ranging around 40–43ºC. In the dairy industry two different fermentation procedures are usually applied: the short incubation method and the long incubation method. By providing the microorganisms their optimum temperature range, incubation times of approximately 2.5–4 h can be achieved. When incubation should take place overnight due to technical requirements, temperature has to be reduced to 30–32ºC, leading to a fermentation time of 10–12 h to reach the desired end-pH. This retarded activity of the starter organisms results in a slower rate of acidification and leads to a difference in the kinetics of protein network formation. It was generally accepted that the lower the fermentation temperature, the longer it takes to reach a certain pH and therefore firmness, but the final product is much firmer (Walstra *et al.*, 1999). However, Lankes *et al.* (1998) compared yoghurts manufactured at either 30ºC or 42ºC and found higher gel firmness and higher viscosity for products fermented at 42ºC. Haque *et al.* (2001) found a systematic increase in gel strength for set-type yoghurt and viscosity for stirred yoghurt with increase in the temperature of fermentation from 37ºC to 46ºC. A possible explanation for these contradictory results was suggested by Horne (1998), who recently introduced a model of the casein micelle as a complex balance of hydrophobic attraction and electrostatic repulsion.

8.3.3 Post-incubation treatments

Set-style yoghurt

Since set-style yoghurt is fermented directly within the retail container, no treatment further than cooling is necessary after fermentation. Usually, incubation is stopped at a pH slightly above the desired pH of the product, as a sufficient period of time is necessary for cooling the cups in ventilated chambers. When using separated incubation and cooling chambers, it is essential to avoid any vibrations of the packages during transportation as the gel is still weak and susceptible to local fracture with subsequent syneresis. Generally, rapid cooling is important to diminish the continued growth of the lactobacilli, otherwise leading to excessive acid production. Furthermore, enhanced acidification to pH values below 4 may lead to body and texture defects such as gel shrinkage and syneresis.

Stirred yoghurt

After fermentation of yoghurt in large vats, the gel is broken by stirring, thus forming a viscous non-Newtonian liquid, which is strongly shear-rate thinning. Defining the stirring regime is a crucial process which induces considerable changes in the rheological properties of the final product. At a given shear rate, the apparent viscosity of stirred yoghurt depends on the firmness of the gel before stirring, giving higher viscosity with higher firmness. Additionally, higher gel firmness allows more vigorous stirring, consequently leading to smoother products which do not become too thin. Higher firmness of the gel in the vat also lowers the risk of syneresis, which might lead to less viscous and more lumpy products.

There are no generally accepted rules for the layout of the time–temperature profile during stirring and cooling, and the applied procedures vary from manufacturer to manufacturer. However, it is generally accepted that the stirred product needs some time after stirring to rebuild some structure. Typically, after reaching a particular pH, the product may be slowly stirred in the fermentation vat to achieve a homogeneous temperature distribution during cooling. Upon reaching 22–24ºC, the product may then be pumped to the filling and packaging unit, where relatively high shearing forces are applied. During the subsequent cooling process of the packed product, a desired increase in viscosity will be achieved.

8.4 Measuring the rheological and textural properties of yoghurt

8.4.1 Rheological and other physical methods

Set yoghurt

It is obvious that rheological properties of intact yoghurt gels can only be accessed when the fermentation process is performed *in situ*, i.e., within a specific geometry of any rheometer. Measurements may be performed either

after the gel forming process is finalised, or even during fermentation provided that some basic requirements are fulfilled. These include, e.g., the choice of an appropriate geometry, sufficient protection against evaporation during fermentation (this can be achieved by avoiding a milk–air interface using a low viscosity oil), and an appropriate instrument setup. It is absolutely essential that the strain applied to the gelling system is kept as small as possible to minimise any disturbance of the gelation process and to achieve reliable results. This can easily be achieved by using strain-controlled rheometers but might be difficult in the case of stress-controlled instruments where the smallest applicable stress, particularly in the early stage of fermentation when almost liquid milk is within the system, will result in a sufficient angular deformation (Hemar *et al.*, 2000). By comparing gelation profiles Haque *et al.* (2001) recently concluded that slippage along supporting surfaces occurs easily when using horizontal geometries (i.e., cone-and-plate and plate–plate), whereas cup-and-bob systems are much more robust towards artifacts.

Rheological studies on intact yoghurt gels fermented within a rheometer system were performed by, e.g., Fiszman *et al.* (1999), Haque *et al.* (2001), Rohm (1993b), Rohm and Kovac (1994), Rönnegard and Dejmek (1993), van Marle and Zoon (1995) and Vlahopoulou and Bell (1995). Generally, mechanical spectra obtained within the linear viscoelastic region, i.e., a strain lower than approximately 1–3%, reveal a response typical for biopolymer gels (Ross-Murphy, 1994), with the storage modulus G' (Pa) exceeding the loss modulus G'' (Pa) by a factor of about 5–7, and little dependency of either G' or G'' on angular frequency ω (rad s^{-1}), with power law slope exponents in the range 0.1–0.2 and, consequently, another power law relation between complex viscosity η^* (Pa.s) and ω (here, slope exponents are $1 - n$). As experimental conditions (base milk, heat treatment, starter, analytical conditions, etc.) vary to a large extent, a G' of 10^3 Pa can only be taken as a rough estimate of the gel firmness of set yoghurt.

Stirred yoghurt

From the rheological point of view stirred yoghurt is a complex viscoelastic fluid which exhibits shear-thinning and time-dependent properties. A complete characterisation of flow properties of yoghurt therefore requires a large set of experiments, considering both the dependency on shear rate and time effects. It is somewhat complicating that, under practical conditions and especially when using stress-controlled instruments, a stress region is observed below which no flow takes place. Recently, the existence of a yield stress has been demonstrated on the basis of several different rheological methods (Dimonte *et al.*, 1998). However, Barnes and Walters (1985) and Barnes (1999) insisted on finite viscosity values for almost all materials, with the response at small stresses to be treated as a Newtonian plateau, and considered the yield stress as a mathematical constant for modelling purposes.

Applying constant shear rate for a specific period of time results in typical decay curves for viscosity versus time, and viscosity usually decreases at any

time when the experiment is repeated with increased shear rate. Although an equilibrium viscosity was not achieved within one hour (Butler and McNulty, 1995; O'Donnell and Butler, 2002; Ramaswamy and Basak, 1991; Schellhaass and Morris, 1985), the decrease of viscosity diminishes with increased time of shear, and quasi-equilibrium values were assumed for shearing times ranging between 10 and 20 min (Benezech and Maingonnat, 1993; van Marle *et al.*, 1999). Although these differences might not be large when expressed in figures, it is important for modelling purposes to decide whether steady-shear conditions at a specific time are met or not.

For the latter condition comprising stress decay *ad infinitum*, analytical data may be fitted to the logarithmic time model originally described by Weltman (1943):

$$\tau = A + B \ln\left(\frac{t}{t_m}\right) \qquad 8.1$$

where τ (Pa) corresponds to shear stress at time t (s), t_m (s) is the time at which maximum stress is measured, and A and B (Pa) refer to intercept and slope in the Weltman model, respectively (consequently, A corresponds to τ for $t = t_m$).

Whereas Weltman coefficients have been related linearly to the applied shear rate $\dot{\gamma}$ (s^{-1}) ranging from $100\,s^{-1}$ to $500\,s^{-1}$ by Ramaswamy and Basak (1991), O'Donnell and Butler (2002) used a power law fit and a logarithmic equation to describe the shear-rate dependency of A and B, respectively.

Assuming an approach to equilibrium viscosity η_e (Pa.s) at finite time, the model of Tiu and Boger (1974), based on the theory of Cheng and Evans (1965), may be used:

$$\tau = \lambda \tau_m \qquad 8.2$$

Here, shear stress is related to the maximum shear stress τ_m (Pa) times a dimensionless structural parameter λ ($0 < \lambda \leq 1$). Depending on the model to be used for relating shear stress to shear rate, one will find that

$$\tau_m = \lambda K \dot{\gamma}^n \qquad 8.3$$

or

$$\tau_m = \lambda(\tau_0 + K\dot{\gamma}^n) \qquad 8.4$$

for the power law equation (O'Donnell and Butler, 2002) and the Hershel Bulkley model (Butler and McNulty, 1995), respectively. In equations 8.3 and 8.4, τ_0 (Pa) corresponds to a yield stress, and K (Pa.s^n) and n (–) are fitting coefficients, with K referring to the apparent viscosity at $\dot{\gamma} = 1\,s^{-1}$, and $0 \leq n \leq 1$ as yoghurt is shear-thinning.

The time dependency of λ at constant shear rate is given by

$$\frac{1}{\lambda - \lambda_e} = \frac{1}{\lambda_m - \lambda_e} + k_1 t \qquad 8.5$$

with λ_m corresponding to λ at t_m and λ_e being the structural parameter at equilibrium conditions. Substituting equation 8.2 into $\eta = \tau/\dot{\gamma}$ yields

$$\lambda = \frac{\eta\dot{\gamma}}{\tau_m} \qquad 8.6$$

which also holds for maximum and equilibrium conditions and can be further substituted into equation 8.5 giving

$$\frac{1}{\eta - \eta_e} = \frac{1}{\eta_m - \eta_e} + \left(\frac{k_1\dot{\gamma}}{\tau_m}\right)t \qquad 8.7$$

A plot of $1/(\eta - \eta_e)$ versus time results in a straight line with a slope equal to $k_1\dot{\gamma}/\tau_m$ and, if repeated for a number of shear rates, the relation between k_1 and $\dot{\gamma}$ can be established. A similar treatment can be applied for the Hershel Bulkley model by using equation 8.4 instead of equation 8.3 to express the denominator in equation 8.6, τ_m. With the above-mentioned tools it will be possible to model initial maximum stress and equilibrium stress as well as λ_e by a power law (or whatever is intended) function of shear rate, thus completely describing shear-thinning and thixotropic behaviour.

Several authors used a more qualitative approach to study stirred yoghurt, either by increasing shear rate stepwise or by increasing shear rate linearly with time, followed by a decrease until $\dot{\gamma} = 0\,s^{-1}$. Flow curves were fitted by means of the power law (Abu-Jdayil *et al.*, 2000; Geraghty and Butler, 1999; Keogh and O'Kennedy, 1998; Parnell-Clunies *et al.*, 1986; Schellhaass and Morris, 1985), the Casson equation (Parnell-Clunies *et al.*, 1986; Skriver *et al.*, 1993) or the Hershel Bulkley equation (Hassan *et al.*, 1996; Ramaswamy and Basak, 1991, 1992; Rohm, 1993a; Rohm and Schmid, 1993). However, it has to be noted that any equation coefficients obtained by regression analysis will depend heavily on the setup of the test, i.e., the acceleration of shear rate, due to the time-dependent viscosity decay of yoghurt (Rohm, 1992). Some additional information can be drawn from the area included between the upward and downward curves when applying the 'thixotropic loop technique'. The area is given in terms of ($N/m^2 \times s^{-1}$) or, if related to the volume of the sheared sample, can be treated in terms of the power necessary for structure degradation.

A number of studies deal with oscillatory methods applied to stirred yoghurt (Jaros *et al.*, 2002b; Ozer *et al.*, 1998, 1999; Rohm and Kovac, 1995; Skriver *et al.*, 1999, Steventon *et al.*, 1990). Qualitatively, mechanical spectra resemble those of set yoghurt, with the moduli being 8–10 times lower. Afonso and Maia (1999) and Skriver (1995) compared the results of dynamic measurements of stirred yoghurt with apparent steady shear viscosity and observed that the empirical Cox-Merz rule, which should result in identity of η^* versus $\emptyset$, ω and η_{app} versus γ-curves (Cox and Merz, 1958), was only obeyed after introducing a horizontal shift factor as has been suggested by Bistany and Kokini (1983) and Rao and Cooley (1992). Most recently, Haque *et al.* (2001) demonstrated the importance of the sample loading procedure by showing a sufficient (approximately 25%) increase of the modulus after the first few minutes after loading, attributable to some structure recovery.

Figure 8.2 provides some interesting detail of a comparison of two samples of stirred yoghurt produced at laboratory scale under identical conditions (i.e., base

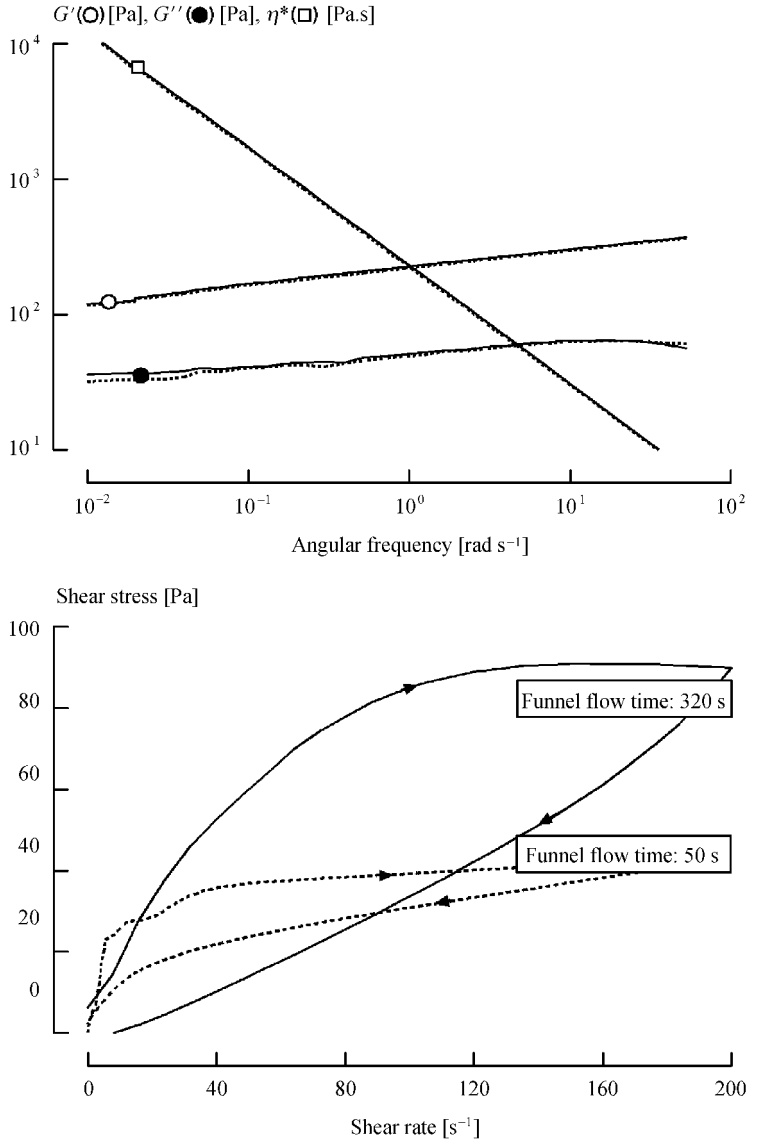

Fig. 8.2 Mechanical spectra (upper graph) and flow curves of stirred yoghurt (lower graph). Full lines, yoghurt A inoculated with a highly viscous starter; dotted lines, yoghurt B inoculated with a standard starter. For further explanations see text.

skim milk 14% total solids, pre-heat treatment 90ºC for 30 min; incubation temperature 43ºC, final pH 4.5). Whereas the mechanical spectra showed no noticeable differences, yoghurt A inoculated with a highly viscous starter revealed a completely different response to an up-and-down shearing cycle (shear rate acceleration $1\,s^{-2}$) than did sample B fermented with a standard

starter, with a much smaller area enclosed between the upward and the downward curves. This means that, in both cases, the breakdown of structure exactly compensates for the strain rate increase that would result in a higher shear stress response if the sample remained unchanged.

Permeability

A special method, originally described for rennet milk gels but later adopted to acidified milk gels (Lucey *et al.*, 1997; van Marle and Zoon, 1995), has been introduced by van Dijk and Walstra (1986) and Roefs *et al.* (1990). Small glass tubes containing *in situ* fermented gels are placed in whey, with the gel surface below the whey level thus causing a pressure difference. The whey flux through the gel network is usually observed as a function of time and may serve as an indicator for the occurrence of microsyneresis, i.e., some rearrangement and condensation in the casein network, leading to an increase in the size of micropores, or it might be helpful in the detection of interactions between milk constituents and polysaccharides produced by starter cultures.

8.4.2 Empirical methods

Gel firmness

Gel firmness measurements of set yoghurt are usually performed by means of constant speed penetration on universal testing machines or similar instruments, using cylindrical plungers (15 mm < d < 40 mm) and crosshead speed values ranging between 10 and 100 mm/min, most likely below room temperature. With up-to-date equipment the force–response, which is affected by plunger size and penetration speed, is monitored as a function of penetration depth. Several authors (e.g., Barrantes *et al.*, 1996; Ferragut *et al.*, 2000; Hassan *et al.*, 1996; Lorenzen *et al.*, 1999; Tamime *et al.*, 1991, 1996) used force values (or force values related to plunger diameter) at a predefined penetration depth to express gel firmness.

Another way to evaluate firmness from penetration data is based on the work of Schmidt and Ahmed (1972), who proposed to express firmness as the elastic response from the undisturbed sample by calculating the initial slope of the force–penetration curve. The advantage of applying regression analysis to the response curve (Fiszman and Salvador, 1999; Fiszman *et al.*, 1999; Jaros *et al.*, 2002a; Rohm, 1995; Rohm and Kovac, 1994) is that the parameter, denoted as 'apparent firmness modulus (N/mm)', resembles mainly the elastic response of the yoghurt gel and is not influenced by upward shear forces occurring after sufficient penetration of the plunger. These shear forces along the circumference of the plunger obviously cause some fluctuations in the force–penetration curve and, finally, may result in poor repeatability (Fig. 8.3).

The use of cones with varying dimensions on constant mass penetrometers, which read penetration depth after a predefined period of time, has been described by, e.g., Davi and Shah (1998), Mistry and Hassan (1992) and Ozer and Atamer (1999).

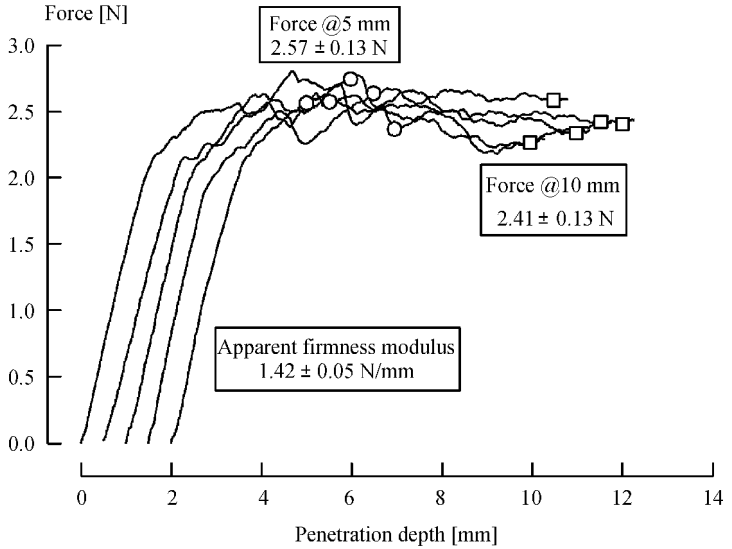

Fig. 8.3 Evaluation of different parameters from force–penetration curves applied to set yoghurt.

One-point measurements of viscosity

Although apparent viscosity of stirred yoghurt depends heavily on shear rate, shearing time (at constant rate) and shear history, single-point measurements have been performed in a number of studies to achieve appropriate indicator values. Most commonly, shear rate in the case of rotational viscometers (Abu-Jdayil *et al.*, 2000; Daubert *et al.*, 1998; Moreira *et al.*, 2000; Schkoda *et al.*, 2001) or rotations per minute in case of Brookfield-type instruments (Fernandez-Garcia and McGregor, 1997; Fernandez-Garcia *et al.*, 1998; Skriver *et al.*, 1999; Trachoo and Mistry, 1998) of the shearing device were set to a predefined value, varying to a large extent from study to study, and torque, shear stress or apparent viscosity were recorded after a predefined period of time. In some other cases, flow curves comprising a particular shear rate–time regime were recorded, and apparent viscosity was calculated from the shear stress at a predefined shear rate (Hassan *et al.*, 1996; van Marle and Zoon, 1995).

Another indicator for apparent viscosity can be obtained from the flow time necessary for a predefined amount of stirred yoghurt to pass through devices such as the Posthumus funnel, which represents a cup with a narrow efflux tube. Funnel flow can be considered as an easy-to-perform and inexpensive method for viscosity evaluation, provided that the entire sample preparation procedure (e.g., prestirring of yoghurt, transfer into the funnel) is thoroughly defined (Beal *et al.*, 1999; Martin *et al.*, 1999). Although Hellinga *et al.* (1986) showed in a theoretical approach that shear rates occurring during the flow of the product through the orifice are much higher than shear rates expected during sensory

evaluation of yoghurt, Skriver *et al.* (1999) reported on a sufficient interrelation between funnel flow time and oral examination of viscosity, thus allowing one to predict sensory properties with the Posthumus device.

Syneresis

The stability of set yoghurt towards syneresis or whey drainage can be evaluated either under the influence of regular gravity or by applying additional gravitational forces by centrifugation. The afore-mentioned principle can easily be applied to both pilot plant and laboratory-scale products as well as to commercial yoghurts, whereas the procedure based on centrifugation requires the fermentation of yoghurt in special containers in the case of set yoghurt. A third method, though rarely used, which can only be applied to set yoghurt, is based on gel shrinkage after wetting.

Measurements at normal gravity

Generally, a certain amount of yoghurt supported by a container or moulded with a specific device is placed on a sieve, and the amount of separated whey is measured after a predefined period of time. Prior to measuring whey drainage, Modler *et al.* (1983) used a Cherry-Burrell knife to determine firmness of yoghurt gels. The containers were then inverted and placed on a stainless steel sieve supported by a funnel. The amount of whey was collected after 2 h of draining at 3ºC and taken as a measure of syneresis. Although originally intended for enzymatic coagulation, a special device based on this principle was proposed by Pompei *et al.* (1994). Guirguis *et al.* (1987) used a container to apply this method to stirred yoghurt. Several authors have formed a hemisphere of yoghurt gel by means of an ice-cream scoop and collected the drained whey (Dannenberg and Kessler, 1988a; Hassan *et al.*, 1996; Hoffmann *et al.*, 1997; Lorenzen *et al.*, 1999).

Several authors have evaluated spontaneous whey separation in yoghurt containers after a predefined storage period (Augustin *et al.*, 1999; Fiszman and Salvador, 1999; Fiszman *et al.*, 1999; Moreira *et al.*, 2000). However, this method shows some disadvantages, especially in firm yoghurt, as the amount of spontaneously released whey is usually very small.

Centrifugation methods

Harwalkar and Kalab (1983) subjected yoghurt gels fermented in centrifuge tubes to centrifugal forces ranging between 30*g* and 2000*g* (6ºC, 10 min), then observed a sigmoid relationship between separated whey and *g*-value, and proposed the *g*-value at the inflection point of the curve as a measure of susceptibility to syneresis. However, this method is laborious as it requires a number of centrifugations per sample, and several authors have used the work of Harwalkar and Kalab (1983) as a basis for a simplified procedure, covering only one centrifugation step and taking into account the relative amount of separated whey (Ferragut *et al.*, 2000; Haque *et al.*, 2001; Lucey, 2001; Rohm and Kovac, 1994; Schkoda *et al.*, 2001). Both the magnitude of the *g*-values as well as

centrifugation time and temperature vary considerably; e.g., Lucey *et al.* (1998b) used 100*g* whereas Parnell-Clunies *et al.* (1986) applied as much as 13 500*g*.

The centrifugation method can be applied to set yoghurt (as long as the gel is fermented within the centrifugation tubes) as well as to stirred yoghurt. Generally, the amount of whey separated from the capillary space decreases with increasing dry matter content of yoghurt and is influenced by technological conditions such as pre-heat treatment of the base milk, milk homogenisation or the type of starter culture used.

Gel shrinkage measurement

Van Dijk and Walstra (1986) used the fact that milk gels in containers only start to shrink either after cutting or after wetting of the surface, and established a method for measuring the height of a defined slab of the gel after spraying whey or water onto the surface. This method was adopted for acid milk gels by Lucey *et al.* (1997).

8.4.3 Sensory assessments

Apart from simple difference testing (de Ancos *et al.*, 2000), issues related to quality perception and acceptance (Grunert *et al.*, 2000; Laye *et al.*, 1993; Muir and Hunter, 1992) as well as consumer studies using Free-Choice Profiling and Procrustes analysis (Gacula, 1997), most of the studies (e.g., Barrantes *et al.*, 1996; Biliaderis *et al.*, 1992; Faergemand *et al.*, 1999; Kneifel *et al.*, 1992; Martin *et al.*, 1999; Rohm *et al.*, 1994; Skriver *et al.*, 1999) dealing with sensory assessments of physical properties of yoghurt were performed by using the Quantitative Descriptive Analysis (QDA) originally described by Stone *et al.* (1974). Simply speaking, QDA is based on a vocabulary and sensory procedures selected by means of test products and established in a group process by subjects. In repeated measurements, data are collected by interval scales, and after applying analysis of variance, multivariate procedures (e.g., PCA, FA) may be performed to eliminate redundancy.

Descriptors for texture properties of yoghurt extracted from the literature differ to a large extent; additionally, some studies lack definitions and procedures for the assessed sensory parameters. A collection of descriptors and definitions is summarised in Table 8.4.

8.5 Future trends

Continuously increasing consumer health consciousness is responsible for the expanding worldwide interest in functional foods. Fermented dairy products such as yoghurt have long been known for their value in managing intestinal disorders such as lactose intolerance or acute gastroenteritis. Three different approaches in the dairy industry are applied to modify the intestinal microflora and thereby beneficially influence the health of the host. These include the

Table 8.4 Descriptors applied in sensory analysis of yoghurt

Reference	Texture descriptor	Sensory definition and/or procedure
Barrantes *et al.* (1996)	Perceived whey separation	Not specified
	Firmness	Not specified
	Lumpy/coarse	Not specified
	Gummy	Not specified
	Body and texture	Not specified
	Creamy	Not specified
Biliaderis *et al.* (1992)	Thickness	Not specified
	Graininess	Not specified
Faergemand *et al.* (1999)	Whey drainage	'Visual determination of liquid on top of yoghurt'
	Firmness	'When cutting the yoghurt gel with a spoon'
	Flakiness	'By cutting yoghurt surface with a spoon'
	Grittiness	'Oral determination'
	Creaminess	'Oral determination'
Kneifel *et al.* (1992)	Whey drainage	'Visual observation of gel surface after inserting a spoon into the gel'
	Texture	'Visual observation after stirring the product with a spoon'
Martin *et al.* (1999)	Smoothness	'Quantity of particles in the gel quantified by visual inspection of the spoon's back'
	Sliminess	'Product's ability to flow in a continuous way from the spoon'
	Thickness (non-oral)	'Product's ability to flow from the spoon'
	Thickness (oral)	'Product's flowing resistance assessed by crushing one spoonful of the product between the tongue and palate'
	Mouthcoating	'Product's ability to form a film lining the mouth'
Rohm *et al.* (1994)	Texture	'Gel firmness perceived by penetrating the gel with a teaspoon and removing an appropriate amount of yoghurt without exerting any shearing force'
	Mouthfeel	'Degree of smoothness perceived by squeezing yoghurt between tongue and palate'
	Viscosity	'Perceived resistance against stirring with a teaspoon'
	Ropiness	'Perceived cohesiveness of the stirred product after pouring it from a teaspoon'
Skriver *et al.* (1999)	Non-oral viscosity	'Penetrating the yogurt gel with a teaspoon, placing approx. 5 ml on the surface of the yoghurt gel and observing how fast it disappeared'
	Oral viscosity	'Perceived degree of thickness when eating the yogurt'

fermentation of milk with probiotics, such as various strains of lactic acid bacteria and *Bifidobacterium* spp., which inhabit the human gut; the addition of prebiotics, which are non-digestible food ingredients, supposed to stimulate the growth of various health-promoting bacteria in the human colon; and the use of synbiotics, defined as a mixture of probiotics and prebiotics. The application of each treatment potentially influences rheology and texture properties, as different starter cultures are used, or conventional starter cultures show other modified fermentation patterns.

Although technology has been applied with almost complete success to produce low-fat, low-calorie yoghurt with sufficient rheological properties, there is still a need for product optimisation. The addition of processed dairy ingredients (e.g., microparticulated whey proteins) might be a promising way to mimic fat properties and to improve physical properties and sensory characteristics. Whereas membrane processes, providing selective cut-offs of particular ingredients, may be used to enhance the quality of traditional products, new techniqes such as high pressure treatment might result in microstructural modifications, presumably leading to the development of completely new products.

8.6 Sources of further information and advice

Many different research groups around Europe and the United States are involved in studies concerning dairy products in general or yoghurt in particular. Apart from many publications in the scientific literature, a good overview of current or recently completed international projects is available on the Internet pages of the Community Research and Development Information Service of the European Union (http://www.cordis.lu). Currently, research on fermented milks is focused mainly on lactic acid bacteria, dealing with topics ranging from molecular biology and genetics to genetically engineered food products, which are supposed to improve product quality, and to consumer attitudes towards these food systems. Additionally, extensive work is being done on the isolation of new starter cultures from fermented dairy products and on human probiotics for fermented milks. The mechanisms and the controlled production of exopolysaccharides from lactic acid bacteria as natural thickeners and the improvement of the texture properties of yoghurt are still under investigation. In particular, a project finalised recently was dealing with the relationship between composition, processing conditions and gel texture of particle gel systems by means of modern technologies such as Brownian dynamics simulation of real and simulated systems.

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