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SOME ASPECTS OF THE PRODUCTION AND QUALITY OF SET YOGHURT USING FAT-SUBSTITUTES AND VEGETABLE OILS

by

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ABSTRACT

Yoghurt is a popular fermented milk product where it is consumed in appreciable quantities in most countries in the world. In the present study the quality of natural set yoghurt was evaluated taking into consideration the following aspects: first, the production of low-calorie products where the milk fat was substituted by modified starches (*e.g.* Litesse™, Lycadex® 100 and 200, N-Oil® II, Paselli® SA2 and P-Fibre 150 C and 285 F) and microparticulated whey protein (*e.g.* Simplesse® 100 dry and wet) because these products minic the fat mouth-feel and contain low energy values when compared with milk fat. Second, the manufacture of dietetic yoghurt by replacing the milk fat with different types of vegetable oils (*e.g.* olive, corn, sunflower and groundnut) that are high in mono- and poly- unsaturated fatty acids.

Yoghurt was made from reconstituted skim milk powder ($\sim$14% total solids) fortified with fat-substitutes, vegetable oils or anhydrous milk fat (AMF) at 1.5%., warmed to 60°C, homogenised at 17 MPa, heated to 95°C for 5 min, cooled to 45°C, inoculated with commercial starter culture type MY 087 (*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), dispensed into cups, incubated for $\sim$6 h or until pH drops to 4.6, removed to the cold store ($\sim$5°C) and evaluated when fresh and after storage for 20 days.

No processing difficulties were experienced for the production of fat-substitute or vegetable oil yoghurts with the exception of P-Fibre 285 F where some precipitation occurred during the storage period.

The chemical composition of these experimental yoghurts could be summarised as follows:- (a) the low-calorie yoghurts contained minute quantities of fat which was a carry over from the skim milk powder, (b) yoghurts made with modified starches had higher carbohydrate or fibre contents while the Simplesse® 100 yoghurts increased the protein level in the product and as a consequence the calorific value of these yoghurts were lower when compared with the product made with AMF, (c) the composition and

energy values of AMF and vegetable oil yoghurts were similar, but the latter products contained higher amounts of mono- and poly- unsaturated fatty acids, and (d) the organic acids content (orotic, citric, pyruvic, lactic, uric, formic, acetic and hippuric) of all the yoghurts were similar, but slightly lower values of lactic acid contents was observed in stored yoghurts when compared with fresh products; however, quantitiatively the HPLC method needs to be evaluated further in order to overcome the analytical difficulties experienced in the yoghurts.

All the different types of yoghurts (fresh and stored) were produced under excellent sanitary conditions. The non-lactic acid bacteria, coliforms, yeasts and moulds were <100, <10 and <10 colony forming unit (CFU) g^{-1}. The yoghurt starter organisms were recovered in high numbers (streptococci x 10^8 CFU g^{-1} and lactobacilli x 10^5 CFU g^{-1}) thus ensuring the safety of the product. The substitution of AMF with modified starches, microparticulated whey proteins or vegetable oils did not affect the starter culture activity during the fermentation stage.

The rheological properties of all different types of yoghurts were different from the product made with AMF. Both syneresis and firmness showed higher and lower figures respectively when compared with the control. For all the yoghurts, whey separation decreased with storage time, while firmness increased with time.

All the experimental yoghurts were acceptable by the taste panellists; however, few sensory attributes (based on flavour and aroma) were identified by the judges to be significantly higher in yoghurt made with AMF for example, the 'pea' taste of P-Fibre fat-substitutes and the 'typical' flavours associated with some vegetable oils. This could be attributed to 'carry over' flavour effect rather than a flavour fault, and overall acceptability of the yoghurts seemed to be most influenced by flavour and aroma.

Consumers at SAC - Auchincruive, (students and personnel) have evaluated strawberry flavoured low-calorie and vegetable oils yoghurts. Yoghurts made with fat-substitutes appeared to be highly acceptable among large numbers of consumers, but products containing vegetable oils, especially groundnut, were less liked due to the 'oily' flavour. This could be attributed to the fact that UK consumers are not accustomed to such flavours coming from the vegetable oils used.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

AOAC:	Association of Official Analytical (formerly Agricultural) Chemists
BSI:	British Standards Institution
C:	Coarse
CFU:	Colony forming units
CVA:	Canonical Variates Analysis
DM:	Dry matter
DVI:	Direct to vat inoculation
F:	Fine
FA:	Fatty acids
GLC:	Gas-liquid chromatography
HPLC:	High performance liquid chromatography
IDF:	International Dairy Federation
MPa:	Mega pascals
N:	Newtons
PCA:	Principal Components Analysis
r	Correlation coefficient
RSMP:	Reconstituted skim milk powder
SAC:	Scottish Agricultural College
SED:	Standard Error of Difference
SEM:	Scanning electron microscopy
SNF:	Solids non-fat
SMP:	Skim milk powder
TA:	Titratable acidity
TEM:	Transmission electron microscopy
TS:	Total solids
UV:	Ultraviolet
WHO:	World Health Organisation

CHAPTER ONE:

INTRODUCTION

CHAPTER ONE: INTRODUCTION

One of the oldest methods of preserving milk and imparting to it special favourable organoleptic qualities is the acidification of milk by fermentation. Normally, lactic acid bacteria are used as starter cultures to acidify the milk. Different methods of processing are used in various parts of the world to give rise to a wide range of fermented milk products excluding cheeses (Kurmann *et al.*, 1992).

The nutritional aspects of fermented milk products have been recognised by mankind for many years. However, the association of yoghurt, for example, with 'good health' was first reported by the turn of this century by Metchnikoff (1908). He advocated that the longevity of life is dependent on the appropriate intestinal flora that minimises the auto-intoxication condition which is produced by the wrong type of bacteria. Metchnikoff's theory has been a matter of controversy over the years; nevertheless, the presence of lactic acid bacteria in the intestinal tract is considered beneficial for a healthy life.

In the 1950's, yoghurt became a popular fermented milk product in Europe and North America and since then the possible health benefits has been thoroughly researched in terms of methods of processing and selection of starter cultures which have therapeutic properties and beneficial effects to the consumer.

Since the 1970's many consumers in North America and Europe have been modifying their eating habits for health reasons. This has largely involved reducing the amount of fat, sugar, salt, cholesterol and certain additives in the diet. Food technologists and manufacturers have responded to such demands and over the past few years there has been rapid market growth in perceived healthy foods and drinks, including dairy products (Hendley and Seymour, 1988; Singer, 1990). Such products are marketed under different names such as: 'low-fat', 'light', 'sugar free' or 'low-calorie', and 'healthy'. These innovative product developments can be achieved by using one or combinations of the following methods: (a) replacement of the carbohydrate with intense sweeteners, (b) reducing the carbohydrate content using fibre or bulking ingredients (*i.e.* hydrocolloids such as carrageenan or guar gum), (c) lowering the fat

content in traditional food products, (d) using fat-substitutes to replace fat in food whilst keeping the same functional and organoleptic properties as fats without the calories, and (e) using vegetable oil to replace the animal fat.

Fat-substitutes are newly developed food ingredients that 'mimic' the mouth feel of fat in foods without adversely affecting the rheological and organoleptic properties of 'low-calorie' products (Anderson, 1990; Iyengar and Gross, 1991). Technically developed fat-substitutes, are basically divided into two types: first, modified starches which have good emulsifying or gel properties along with low energy values, second, modified whey and/or egg protein known as microparticulated proteins, and third, compounds modified by esterification (*e.g.* glycerol ethers, pseudofats and carbohydrate fatty acids esters) and which provide fat-like properties of natural fats and no calories (Casella, 1989). In food formulations, fat-substitutes have been used to replace up to 50% of the fat and their application in the food industry including dairy products which has significantly increased during the past few years (Bruhn *et al.*, 1992; Dexheimer, 1992; Morrison, 1992; Blenford, 1992, 1992-93).

'Filled' milk products are mainly manufactured in Third World countries from recombined skimmed milk powder, but the milk fat (*i.e.* cream, un-salted butter or anhydrous milk fat) is replaced with vegetable fats or oils. The primary objective of using indigenous fats and oils in these countries for the production of a wide range of dairy products is to reduce the importation expenditure of milk fat. Some information regarding the influence of vegetable fats and oils on the quality of 'filled' milk products have been recently reported by Sjollema (1990). Although, the term 'filled' is vague regarding the nature of these dairy products and is not supported by the International Dairy Federation, 'filled' milk products have been produced for more than 30 years. These products are capable of benefiting the consumer in the Third World countries and to satisfy the nutritional requirements of 'filled' milk products, the addition of vitamins A and D is recommended (Newstead *et al.*, 1979). The possibility to manufacture yoghurt containing corn oil was patented in the USA a few decades ago (Metzger, 1962) and a dietetic acidophilus milk made in the former USSR from skimmed milk fortified with 2% corn oil was reported by Kurmann *et al.* (1992).

The cause of death in humans due to coronary heart disease is more common in the industrialised countries and is rarer in most Third World countries and relatively low in Japan (Truswell, 1992). This disease is attributed to a range of factors, but the human diet is one of the most probable fundamental controllable factor. Since the 1980s health

authorities including the medical profession in most countries have been advising the consumer to reduce their energy intake derived from fat and to replace animal fat with vegetable types including oils (Anon., 1984, 1987, 1988 a; IDF, 1989). However, the current debate on health among doctors and scientists in Europe and North America suggests that there is no need for low-fat or reduced-cholesterol milk products in the diet which has little or slight effect on the cholesterol level in the blood serum (Shaper *et al.*, 1991; Renner, 1990; McNamara, 1992; Wardlaw and Snook, 1990; Pietinen *et al.* 1988). Conversely, similar effect in the diet was also observed when comparing the use of hydrogenated vegetable oils and butter (Mensink and Katan, 1990; Connor, 1991; Clevidence *et al.*, 1992; Hunninghake *et al.*, 1993; Hølund, 1993; Kris-Elherton *et al.*, 1993).

At present, the "Mediterranean diet" of a balanced intake of carbohydrates (starches, cereals), fat (mainly olive oil), protein (vegetable origin, fish) and crude fibre (fruits, vegetables) is considered a less health risk approach to control cholesterol levels in the blood (Sirtori *et al.*, 1986; Aravanis *et al.*, 1988; Mensink and Katan, 1989; Schulpi and Scarpalezou, 1989; Klinger and Zucconi, 1989; Nicaud and Ducimetière 1990; Gonzalez *et al.*, 1990; Buzina *et al.*, 1991; Katsouyanni *et al.*, 1991; Bosaeus *et al.*, 1992).

The awareness of the British consumer on dietary aspects of food in relation to cardiovascular disease has changed over the past decade. The trend in food consumption in Britain, which is monitored by the National Food Survey by the Ministry of Agriculture, Fisheries and Food (MAFF) on a regular basis since 1940 (Buss, 1990; Anon., 1992 a) suggests the following aspects of dairy products consumption: (a) full-fat milk consumption declined and it has been offset by increased consumption of skimmed or semi-skimmed milk (b) butter consumption has been replaced first by soft margarine high in polyunsaturated fatty acids and recently by low-fat yellow spreads and vegetable oils and (c) yoghurt consumption has increased by 6-fold since the 1970s because this product is perceived by the consumer as healthy food and good for slimming purposes. Despite the fact that low- and reduced-calorie foods have been buoyant and have grown steadily over the past few years, the average daily fat intake by the British consumer has fallen by 10% since 1980. However, fats and oils still provide ~42% of energy in the diet (Buttris *et al.*, 1991), compared with the recommended target level of ~30%.

Since the 1960s the *per capita* consumption of yoghurt has increased in most countries in the world including the UK (IDF, 1982 a, 1992 a), and it has been illustrated that the pattern of consumer habits has changed towards eating healthy diets. It could be argued, however, that such current changes in consumer attitude towards energy intake might negatively affect the dairy industry. Thus, the industry in general should not undermine the fact that the current trend of consumer perception is towards eating low-calorie foods and the preference of poly-unsaturated fatty acids that originate from vegetable sources. Consequently, by adopting such approach these non-dairy components could be utilised with different dairy ingredients for new product formulations (IDF., 1989; Glesson, 1991; Gurr, 1988). No data is available on the manufacture and quality of 'filled' and 'low-calorie' yoghurts using vegetable oil or fat-substitutes, and for these reasons some aspects of the parameters evaluated in this thesis could be summarised as follows:

(a)   the use of different types of fat-substitutes and vegetable oils for the production of set type yoghurt, and the effect of these ingredients on the quality of yoghurt;

(b)   the effect of such additives on the activity of a commercial yoghurt starter culture during the acidification of milk and storage of the product;

(c)   the assessment of the rheological properties and sensory attributes of the experimental yoghurts;

(d)   the determination or identification of some of the flavour characteristics of these yoghurts;

(e)   the acceptability of fruit flavoured yoghurts made with fat-substitutes and vegetable oils using large number of consumers;

(f)   the effect of these additives or replacement of the milk fat on the microstructure of yoghurt.

CHAPTER TWO:

LITERATURE REVIEW

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2.1 Introduction

2.1.1 Historical Background of Yoghurt

The recognition of milk as the cornerstone of the human diet is well documented, and fermented milks and cheeses have been recognised to be the major part. Their origin is not well established, but it is believed that fermented milks have existed many civilisations ago (Rasic and Kurmann, 1978; Kosikowski, 1982; Tamime and Robinson, 1985; Oberman, 1985; Tamime and Robinson, 1988; Robinson and Tamime, 1990, 1993). The same authors have provided an ample review about the origins and developments of fermented dairy products. It is safe to assume that yoghurt and other fermented milks were first produced in the eastern parts of the Mediterranean, India, eastern Europe and the Balkans. It is also possible to suggest that the spread of fermented milks within these geographical areas could have been favoured with the migration of herdsmen or nomad in search of pastures, water and new land for settlement (Vedamathu, 1991). A summary of some fermented milks with the date first noted is shown in Table 2.1.

Table 2.1   Reference to fermented milks and their names with the date first recorded

Year	Type of fermented milk	Country
2000 BC	Koumiss	Russia
1300 BC	Cultured cream	Mesopotamia
800-300 BC	Butter-milk and Dahi	India
AD 633	Laban	Arab countries
AD 900	Yoghurt	Turkey
AD 1253-1255	Airan	Central Asia
AD 1336-1405	Kheran	Russia
AD 1500	Tarho	Hungary

Data compiled from Kurmann (1984).

Helferich and Westhoff (1980) reported the classical story about the beginning of yoghurt as follows: a nomad who wandered out into the desert with fresh milk stored in a goat's skin bag beside the camel's body. The temperature from the animal gave optimum conditions for growth of the indegineous bacteria, possibly the lactic acid type. Several hours later, the nomad found the milk turned to a semi-solid mass or coagulum, *i.e.* the birth of yoghurt, and when he consumed the clotted milk, the taste was delightful and refreshing. In the beginning, fermented milks were made mainly from sheep's and buffalo's milks and partly from goat's and cow's milks. It is safe to assume that fermented milks were originally obtained by allowing the milk to sour naturally and later by using a small quantity of previously fermented milk to inoculate fresh warm milk. The next development in the production of fermented milk was to use boiled milk which was partly concentrated to produce a thick coagulum. However, the use of starter cultures for the manufacture of fermented milk was only realised by the turn of this century (Rasic and Kurmann, 1978; Tamime and Robinson, 1985).

The therapeutic and nutritional properties of fermented milks have been well recognised in past civilisations. For example, according to a Persian legend that Abraham's longevity and fertility was due to the consumption of yoghurt (Tamime and Robinson, 1985). Ancient physicians in the Middle East prescribed yoghurt or fermented milks for curing disorders of the stomach, intestines and liver or for stimulating the appetite (Rasic and Kurmann, 1978). The same authors reported that fermented milks and in particular yoghurt have been used for the preservation of meat against spoilage during the summer and for cosmetic applications by Persian women.

The earliest scientific knowledge about yoghurt took place at the beginning of this century by a Russian bacteriologist (Metchnikoff, 1908). He advocated that the longevity of the Balkan people was attributed to large consumption of yoghurt, and as a consequence the lactic acid bacteria colonise the intestinal tract which minimises the growth of toxic anaerobic sporeforming bacteria. Metchnikoff's theory of longevity has been a matter of controversy and as a result it has stimulated voluminous research work over the years which have influenced the popularity and spread of yoghurt in most countries of the world.

2.1.2 Definition and Classification

According to the International Dairy Federation (IDF, 1992 b) fermented milks are prepared from milk and/or milk products by the action of specific micro-organisms,

which result in reduction of pH and coagulation. These micro-organisms shall be viable, active and abundant in the finished product at the time of sale for consumption. The milk and milk products may be homogenized or not and must be at least pasteurised. Yoghurt is obtained by the acidification of milk which is achieved by using a mixed starter culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. The addition of *Lactobacillus acidophilus* and *Bifidobacterium* sp. enhances the therapeutic quality of the product. Yoghurt is characterised as being a smooth and viscous gel with delicate walnutty flavour (Kosikowski, 1982).

In some countries statutory instruments provides detailed description of the yoghurt regulations, and these regulations may differ slightly. In the UK, there are no regulations but recommendations proposed by the Dairy Trade Federation and Ministry of Agriculture, Fisheries and Food. At present the regulatory bodies within the European Economic Community (EEC) are in the process of standardising these regulations in all member states. A comparative study of yoghurt laws and regulations within the EEC regarding the compositional specifications and type of starter culture used has been reviewed by Pappas (1988).

According to Robinson and Tamime (1990) yoghurt can be categorised by the following features:- (a) existing or proposed chemical legal standards (full, medium or low fat), (b) method of production, (set, stirred or fluid), (c) flavours (natural, fruit or flavoured), and (d) post-incubation processing (heat treatment, freezing, drying or concentration). However, a wide range of fermented milk products are produced in different countries and an appropriate scheme of classification of these products have been reported by Kurmann (1984) and Robinson and Tamime (1990). In both schemes yoghurt is classified as a pure lactic fermented product where thermophilic starter cultures are employed to acidify the milk.

2.1.3 Patterns of Production and Consumption

Yoghurt and other fermented milk products are manufactured and consumed in the majority of the countries of the world, but the consumption habits are different (see Table 2.2). For example, the highest *per capita* consumption of fermented milks including yoghurt in the world is in Finland (37 kg head^{-1}) and the lowest is in Poland (~0.7 kg head^{-1}).

Table 2.2   *Per capita* annual consumption (kg head^{-1}) of yoghurt and other fermented milks in different countries

Country	1970 Yoghurt	1970 Others	1980 Yoghurt	1980 Others	1990 Yoghurt	1990 Others
Australia	-	-	1.8	-	3.5	-
Austria	1.8	2.6	5.8	2.0	7.5	-
Belgium	3.5	-	4.9	-	6.5	1.2
Canada	0.3	-	1.7	-	3.2[a]	-
Chile	-	-	1.4	-	3.9	-
Czechoslovakia	-	-	1.7	2.5	-	-
Denmark	1.7	5.7	9.1	7.8	7.8	6.9
Finland	2.7	31.2	8.4	28.5	11.7	25.4
France	6.1	-	9.3	-	16.4[b]	-
Germany	3.8	0.7	6.7	1.2	10.6	0.8
Iceland	-	0.1	5.7	-	9.9	14.7
India	-	-	3.7	-	4.7	-
Ireland	6.9	-	2.0	-	3.1[a]	-
Italy	-	-	1.3	-	2.6	1.4
Japan	0.3	8.9	1.0	1.4	3.9	3.9
Luxembourg	2.3	1.5	5.1	-	6.1	-
Netherlands	13.7	-	17.8	-	21.8	-
Norway	0.2	6.9	2.2	7.9	4.3	10.6
Poland	-	2.0	0.1	0.6	-	-
Sweden	0.7	13.6	4.2	19.7	7.4	21.7
Switzerland	7.5	-	13.8	-	17.3	-
UK	0.7	-	2.8	-	4.3	0.1
USA	0.1	-	1.2	-	-	-
USSR	6.2[b]	-	6.2[b]	-	-	-

- Data not available.

[a] Consumption data for 1988.

[b] Data of yoghurt represents other fermented milk products.
After IDF (1982 a, 1992 a).

Tamime and Robinson (1985) reported that factors, which might be contributing to the trend of increasing consumption of yoghurt and other fermented milks, are: (a) level of income, (b) availability of an organised distribution system, (c) diversification in the type of products sold to the consumer and (d) advertisement. These factors will be of paramount importance for the development of yoghurt markets in Latin American countries. Improvements in the formulation of yoghurt (*i.e.* rich in protein and calcium, low-calorie and healthy food) and good advertisement have contributed in the expansion of the market such as Japan (Vedamuthu, 1991).

The UK market of low- or reduced-calories and 'light' dairy products (*i.e.* skimmed or low fat milk, cheese including Cottage, yoghurt and low fat yellow spreads) have been buoyant over the past decade and is growing steadily. The retail economic value of skimmed milk, yoghurt and Cottage cheese has increased from £565 m in 1985 to £1335 m in 1990 reflecting a growth by 2.4 fold (Tamime *et al.*, 1993). In the USA the 'light' dairy products market was estimated at $23.7 billion in 1991 and cultured dairy products, including yoghurt, amounted to 7.5 per cent of the total sales (Anon, 1992 b).

British yoghurts are inherently low in fat, ranging between 0.5 to 1.5% (excluding Greek type yoghurts) (Tamime *et al.*, 1987). Low-calorie yoghurts can be produced by one or more combinations of the following methods:- (a) reducing the fat and milk solids-not-fat contents in the milk base, (b) the use of stabilisers or bulking agents to replace milk solids, (c) replacing the high energy sucrose by low-calorie sweeteners and (d) replacing the milk fat with low-calorie products known as fat-substitutes. One such example is low-calorie fruit flavoured diet yoghurt containing fat-substitute (41 k cal 100 g^{-1}) when compared with low fat fruit flavoured yoghurt (90 k cal 100 g^{-1}) (Holland *et al.*, 1991). In the UK, the total volume of yoghurt production (thousand tonnes) and its economic value (£ million) between 1984 to 1990 is shown in Figure 2.1, and the market share of 'diet' yoghurt (*i.e.* by volume of production) has increased from 2 to 17% during the same period (Anon., 1985, 1988 b, 1991).

2.2 · Technology of Yoghurt Manufacture

The traditional method for the production of yoghurt would have included the following stage: (a) boiling of milk to reduce the volume to cause partial concentration, and (b) to use previous day yoghurt to inoculate a subsequent batch of milk. Such process has laid
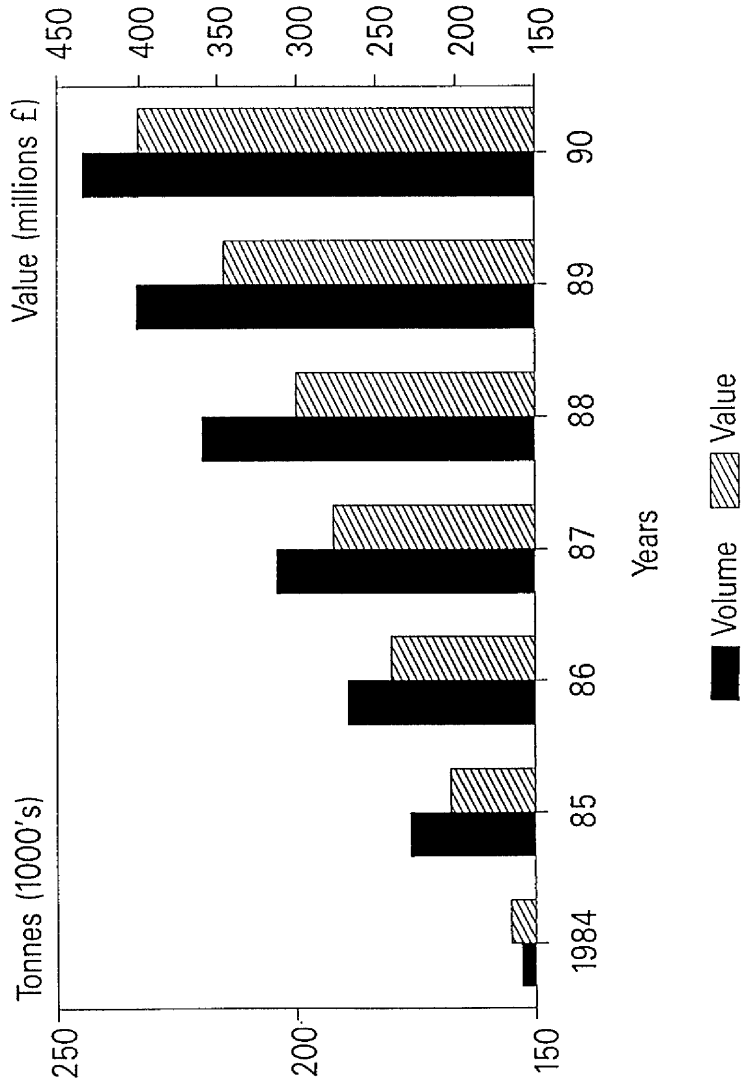
Figure 2.1   Production figures and economic value of UK yoghurt market in the 1980s.

Data compiled from Anon. (1985, 1988 b, 1991).

the basic foundation of present day technology; however, the use of milk from different species of mammals and the inherent variation in the chemical composition of milk during lactation, the traditional method may introduce an unpredictability of the quality of the finished product.

The industrial procedure for the manufacture of set- and stirred-type yoghurt is shown in Figure 2.2. The technology of yoghurt making has been extensively reviewed (Humphreys and Plunkett, 1969; Robinson and Tamime, 1975; Tamime and Deeth, 1980; Tamime and Robinson, 1988) and textbooks (Rasic and Kurmann, 1978; Kosikowski, 1982; Tamime and Robinson, 1985; Robinson and Tamime, 1993; Chandan and Shahani, 1993) have been published, and the present review is aimed summarising the important aspects that influence the characteristics of set-type yoghurt.

2.2.1 Milk as Raw Material

Milks from different species of mammals have been utilised for the manufacture of yoghurt in different parts of the world. Table 2.3 shows the major differences in the chemical composition of these milks. Since cow's milk is widely produced in most countries of the world (Tamime, 1993), the main emphasis in this review will be on the use of this type of milk for the manufacture of yoghurt. However, even when considering cow's milk (Table 2.3) there are large differences in the chemical composition within the various breeds. Also the composition of fresh milk varies from day to day depending on factors such as: stage of lactation, age of cow, milking interval and efficiency, season of the year, breed of cows and breeding programme, nutrition, hormones, mastitis, and environmental temperatures.

In order to overcome these inherent variations in the composition, fresh liquid milk is standardised and/or fortified. Therefore, in the present study it was decided to use recombined skimmed milk powder where the butterfat could be replace with fat-substitutes or vegetable oils (see sections 4.1.1 and 5.1.1). Also the practical application of recombination is widely used for the manufacture of different dairy products in developing countries including Costa Rica.

2.2.2 Preliminary Treatment of Milk

In developing and industrialised countries, milk collection from farms is carried out in bulk, and the facilities available for milk reception and storage in creameries may

Preliminary treatment   {Cooling and Storage
       of milk   {Filtration
                               {Clarification
          ↓
Preparation of the   {Standardisation of fat
   yoghurt base   {Fortification of milk solids
                                   (addition of powder, evaporation,
          ↓   ultrafiltration or reverse osmosis)
                               {De-aeration

Homogenization
(15-20 MPa)

          ↓

Heat treatment

          ↓

Cooling
(35-45°)

          ↓

Inoculation with starter culture

          ↓
        ←→

Set   **Stirred**

Package[a]   Incubate in bulk

   ↓   ↓
Incubate in retail carton   1st stage cooling

   ↓   ↓

Cool   Addition of fruit

   ↓   ↓

Dispatch   Packaging

                                                                ↓

                                                         2nd stage cooling

                                                                ↓

                                                           Dispatch

Figure 2.2   An outline of the major stages for the production of yoghurt.

[a] Sucrose and/or fruit or flavourings can be added at this stage.

Table 2.3   Chemical composition (% w/w) of milk

I. Different species of mammals

Species	Fat	Protein	Lactose	Ash
Ass	2.5	2.0	6.0	0.5
Buffalo	8.0	4.2	4.9	0.8
Camel	4.2	3.7	4.1	0.9
Cow	3.8	3.3	4.7	0.6
Goat	4.5	3.3	4.6	0.6
Mare	1.5	2.6	6.2	0.7
Reindeer	22.5	10.3	2.5	1.4
Sheep	7.5	5.6	4.4	0.9

II. Different cows' breed

Ayrshire	3.9	3.4	5.0	0.7
Friesian	3.4	3.2	4.6	0.7
Guernsey	4.9	3.9	5.0	0.8
Jersey	5.1	3.8	5.0	0.8
Shorthorn	3.7	3.3	4.8	0.7

Data compiled from Tamime and Robinson (1985).

included:- (a) metering or weighing in-coming milk, (b) filtering the milk to remove cellular material and any other contaminants and (c) cooling the milk to $<5°C$ using a plate cooler before storing in a silo (Tamime and Kirkegaard, 1991).

2.2.3 Preparation of the Yoghurt Base

In order to comply with existing and/or proposed legal standards, the fat content has to be standardised to a set level, normally between 0.1% to 10.0% (Robinson and Tamime, 1990). The fat content in milk could be adjusted to the desired level by using one of the following methods: (a) removal of part of the fat from milk, (b) mixing whole milk with skimmed milk and (c) addition of cream or anhydrous milk fat to skimmed milk (fresh or recombined). In the UK, the major share of the market is taken by low-fat yoghurt ~1.5% (Tamime *et al.*, 1987).

The next stage of processing is to fortify the level of solids-not-fat (SNF) because raising the level of protein in milk increases the firmness of the coagulum and minimises syneresis/whey separation after the fermentation stage. The two main methods, which are widely used in the industry to enhance primarily the level of SNF in the milk are: (a) the addition of powders (full cream, skimmed, buttermilk, whey, caseinates and/or ultrafiltered retentate) and (b) concentration of the liquid mix by evaporation, ultrafiltration or reverse osmosis. Thus, the method(s) adopted for the elevation of milk solids in the yoghurt base can ultimately affect the level of casein, whey proteins, lactose and fat. It is the casein level that is critical in relation to the strength of the coagulum and detailed discussion regarding the principle effect of the fortification method used on milk constituents have been reported by Robinson and Tamime (1990, 1993). However, a target ratio of casein to non-casein (~3.4) in the yoghurt base has been recommended by Tamime *et al.*, (1984).

Other ingredients such as stabilisers, sweeteners, preservatives, colouring matter and/or flavouring material can be added to the yoghurt base. In the present study only natural yoghurt without any of the above mentioned additives was undertaken and these compounds will not be reviewed. For further details see Rasic and Kurmann (1978) and Tamime and Robinson (1985).

2.2.4 Homogenization

Homogenization of the yoghurt base is usually carried out prior to the heat treatment stage where the product flows through a series of restrictions. The homogenization process is carried out at 15-20 MPa and between 50 and 70°C. According to Tamime and Robinson (1985, 1988) and Robinson and Tamime (1993) the effect of homogenization of the yoghurt base offers certain advantages, namely:

(a)   The fat globule size in the milk is reduced and this prevents the fat globules from coalescing and rising to the surface.

(b)   Whey separation/syneresis is minimised due to the protein - protein interaction and as a result the water holding capacity is improved.

(c)   The small fat globules are adsorbed onto the casein micellar structure of the coagulum and this may increase the viscosity of the product.

(d)   Un-dissolved particles of added ingredients such as powder will be broken down.

(e)   The end product becomes whiter in colour because the increase in the number of very small fat globules, improves light scattering .

2.2.5 Heat Treatment

The temperature and time used for the heat treatment of the yoghurt base ranges between 85-115°C for 3 s up to 30 min (Rasic and Kurmann, 1978; Tamime and Robinson, 1985). The heat induced changes in the milk constituents have been reported by many authors (Tamime and Deeth, 1980; Puhan, 1988; Dannenberg and Kessler, 1988 a, 1988 b; Haque and Kinsella, 1988; Mottar *et al.*, 1989), and the aims of this stage of process can be summarised as follows:

(a)   Destruction of pathogens and other undesirable micro-organisms which could be present in the milk and thus, avoiding the competition with the starter culture.

(b)   Inactivation of certain enzymes that may cause rancid or bitter off flavour in the yoghurt.

(c)   Reduction in the amount of oxygen present in the milk, so providing micro-aerophilic conditions required by the starter organisms.

(d)   Production of certain compounds which might stimulate the starter culture.

(e)   Production of volatile compounds which may contribute towards the flavour of yoghurt.

(f)   Re-distribution of minerals (*i.e.* between the soluble and colloidal states) which may lead to a decrease time of coagulum formation.

(g)   Denaturation of whey proteins (>90%) where both β-lactoglobulin and α-lactalbumin aggregate and later interact with κ-casein; such physio-chemical

interaction increases the viscosity of the coagulum and enhances the water holding capacity of the yoghurt gel.

Heating of milk at high temperature reduces slightly the nutritional property of the yoghurt due to destruction of certain water soluble vitamins, but the metabolic activity of the starter culture can synthesis folic acid and niacin acid during the fermentation stage.

2.2.6 Fermentation Process

The fermentation of the yoghurt base is achieved as the result of the biological activity of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus*. The biological activity that can occur in the yoghurt base by these micro-organisms could be summarised as follows:-

(a)   Lactose is utilised for respiration and/or cell division, and such metabolic activity yields the production of lactic, acetic and other organic acids.

(b)   Production of antibacterial substances such as bulgarican which inhibit the growth of pathogenic micro-organisms in the product.

(c)   Production of carbonyl compounds (acetaldehyde, acetone, acetoin and/or diacetyl) which are essential for the flavour of natural yoghurt.

(d)   The acid formation helps to transform liquid milk into a gel possibly through the following physio-chemical changes of the casein micelle: demineralisation, aggregation, contraction and charge neutralisation at the iso-electric point (Heertje *et al.*, 1985).

(e)   Slight production of proteolytic enzymes may contribute towards the flavour of the product.

(f)   Certain strains of the yoghurt organisms produce polysaccharide material which helps to increase the viscosity of the gel and prevent syneresis.

For the manufacture of set-yoghurt, the fermentation of the yoghurt base takes place in the retail container as compared with stirred-yoghurt where the milk is fermented in bulk in large tanks. Thus, the fermentation stage takes place in incubating cabinets or chambers and tunnels. The latter system is used by large-scale manufacturers.

2.2.7 Cooling and Storage

The aim of cooling is to reduce the metabolic activity of the starter culture and their enzymes. For set-yoghurt, *e.g.* ~ pH 4.6, the containers are transferred to the cold store to be cooled to ~5°C.

2.3 Microbiology of Starter Cultures

2.3.1 Introduction

The yoghurt starter cultures are mixed strain starters which are classified in the genus *Streptococcus* and *Lactobacillus* (Tamime, 1990). *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* are termed as 'thermophilic' because their optimum growth temperature lies between 37 and 45°C, and they are normally propagated together at 42°C. The differentiating characteristics of the yoghurt starter cultures are shown in Table 2.4.

2.3.2 Symbiotic Relationship

It has well been established that when *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* are grown together in milk, the rate of acid development is faster. Such synergistic relationship between these micro-organisms is known as symbiosis because the stimulation of the *Streptococcus* sp. is attributed to the proteolytic activity of *Lb. delbrueckii* subsp. *bulgaricus*, whilst the stimulation of the *Lactobacillus* sp. is due to a factor which originates from the metabolic activity of *Str. salivarius* subsp. *thermophilus*.

For the past four decades the symbiotic relationship that exists between the yoghurt starter cultures have been studied extensively by many scientists in different laboratories. According to the review by Robinson and Tamime (1990) the stimulatory factors that have been identified are:

(a)   *Str. salivarius* subsp. *thermophilus*
- wide range of amino acids
- casein hydrolysate

(b)   *Lb. delbrueckii* subsp. *bulgaricus*
- Formic acid
- Sodium formate
- Carbon dioxide

Table 2.4   Selected differentiating characteristics of the yoghurt starter cultures

	Str. salivarius subsp. *thermophilus*	*Lb. delbrueckii* subsp. *bulgaricus*
G + C[a] (mean %)	40	49-51
Lactic acid configuration	L (+)	D (-)
Growth at 10°C	-	-
45°C	+	+
Requirements for		
Thiamine		-
Riboflavin		+
Pyridoxal		-
Folic acid		+
Thymidine		+
Vit. B_{12}		+
Carbohydrate utilisation		
Aesculin	-[b]	-
Amygladin		-
Arabinose	(-)[c]	-
Cellobiose		-
Fructose	+[d]	+
Galactose		-
Lactose	+	+
Maltose	-	-
Mannitol	-	-
Mannose		-
Melezitose		-
Melibiose		-
Raffinose	(-)	-
Ribose		-
Salicin	-	-
Sorbitol		-
Sucrose	+	-
Trehalose	-	-
Xylose	(-)	-

[a] Mean % of guanine and cytosine in DNA.
[b] Negative reaction by 90% or more strains.
[c] Positive reaction by 11-89% of strains.
[d] Positive reaction by 90% or more strains.

Data compiled from Tamime (1990).

2.3.3   Biochemistry of Fermentation

Although some of the main components such as fat and proteins in the yoghurt base including some minor constituents are slightly affected during the manufacture of yoghurt, the hydrolysis and metabolism of lactose is considered to be the major contributor towards flavour development and gel formation of the product.

The biochemistry of fermentation of fermented milks including yoghurt was reviewed by Marshall (1987), and Table 2.5 illustrates the main activity of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* in the yoghurt base.

2.4   Nutritional and Therapeutic Aspects of Yoghurt

Yoghurts have maintained a reputation of being highly nutritious and even therapeutic due in part to such anecdotal claim that *Lb. delbrueckii* subsp. *bulgaricus* can be implanted in the intestinal tract and inhibits the growth of undesirable micro-organisms. Wide range investigations have shown that *Lb. delbrueckii* subsp. *bulgaricus* does not implant the intestine. Nevertheless, this brief review will summarise the recent nutritional and perhaps the therapeutic value of yoghurt.

2.4.1   Nutritional Composition

Milk is well recognised a highly nutritional food which is suitable for babies, adults and the elderly. Also, milk has a high nutrient density *per se* in relation to the calorific content especially for people who can not eat bulky foods (Scott *et al.*, 1984; Cheeseman, 1991; Gurr, 1992 a).

The overall composition of yoghurt is similar to milk, but differences may occur due to: (a) level of fortification of the yoghurt base and (b) the microbial activity during the fermentation stage (Deeth and Tamime, 1981; Tamime and Robinson, 1985).

2.4.2   Digestibility

The factors, which have been identified that yoghurt is more digestible than milk, are: (a) softer curd due to high heat treatment of the yoghurt base, (b) the gel is easily digested and (c) the microbial activity increases the peptide and free amino acids content

Table 2.5   A summary of the biochemistry of yoghurt

Milk component	Comments
Lactose	- Hydrolysis to lactic and other organic acid via the Embden Meyerhoff Pathway (EMP) and some of the enzymes involved are β-D-galactosidase and lactate dehydrogenase.
	- Alcohol dehydrogenase is involved in the production of acetaldehyde.
	- D(-) and L(+) lactic acid isomers are produced and the amount of each in yoghurt is dependent on the ratio of the starter organisms.
	- Formation of long chain polysaccharide or polymer production.
Protein	- Partial liberation of amino acids due to the activity of lactobacilli.
	- Conversion of amino acid threonine to glycine by threonine aldolase results in the release of acetaldehyde, and the aldolase activity is increased at 42°C.
	- Conversion of methionine and valine to acetaldehyde.
Nucleic acid	- Thymidine is converted to acetaldehyde by the deoxyriboaldolase activity.
Fat	- Partial hydrolysis to volatile fatty acids may contribute towards the flavour of yoghurt.

Data compiled from Tamime and Robinson (1985) and Marshall (1987).

in the product. These effects help to increase bioavailability of yoghurt, for example the biological value of milk is 84 when compared with yoghurt 87-90 (Shahani and Chandan, 1978; Deeth and Tamime, 1981; Doyle *et al.*, 1981; Rasic, 1987). The digestibility of protein in yoghurt *vis-a-vis* with milk requires half the time (Salji, 1989; Odet, 1990).

2.4.3 Lactose Intolerance

The benefits of yoghurt to 'lactose maldigestion' individuals are attributed to the following aspects: (a) up to 50% of the lactose content is utilised by the starter cultures during the fermentation of yoghurt base, and (b) 5-fold increase in β-D-galactosidase activity in the gut due to the cell lysis of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* (Rasic and Kurmann, 1978; Deeth and Tamime, 1981; Saviano, 1989; Alm, 1991; Gilliland, 1991). However, fortification of the yoghurt base increases the lactose content to around 7% and the level drops to ~4% after the fermentation stage. Such level is similar to the lactose content in milk, but in the presence of β-D-galactosidase in the gut, the remaining lactose is hydrolysed.

2.4.4 Lactic Acid Isomers

D(-) and L(+) lactic acid are produced by the starter cultures (see Table 2.4). The D(-) lactate isomer is not actively metabolised (hence, it could lead to acidosis) and as a consequence the daily intake should be restricted especially to infants (Doyle *et al.*, 1981).

Fresh yoghurt may contain 45-60% L(+) lactic acid and the D(-) lactate may increase during storage due to the metabolic activity of *Lb. delbrueckii* subsp. *bulgaricus*. A higher proportion of L(+) lactate isomers could be achieved in yoghurt by adopting one or both of the following approaches: (a) increase the ratio of streptococci to lactobacilli and (b) replace the lactobacilli by other lactic acid bacteria capable of producing L(+) lactic acid only.

2.4.5 Mineral Absorption

At low pHs the calcium ions become more solubilised and this increases the bioavailability of mineral absorption during the consumption of yoghurt (Drüeke, 1989; Sellars, 1989). The improved protein digestibility of yoghurt greatly enhances calcium

and magnesium absorption. In addition yoghurt is a good source of calcium (415 mg g^{-1}) when compared with milk, bread and potato (291, 190 and 227 mg g^{-1} respectively) (Rasic, 1987).

2.4.6 Vitamins Content

Low-fat yoghurts contain less fat soluble vitamins when compared to milk. It is generally recognised that folic acid and niacin contents of yoghurt has shown marked increase (Deeth and Tamime, 1981; Alm, 1991). Some factors, which may affect the vitamins contents in yoghurt, are: (a) original vitamins contents of raw milk,. (b) type of SMP used, *i.e.* low, medium or high heat powder, (c) presence of oxygen and degree of heat treatment applied to the yoghurt base, (d) strains of starter culture used and (e) the addition of stabilisers and fruit flavours. Factors that can reduce the vitamins contents of yoghurt may include (a) post-fermentation heat treatment of the coagulum and (b) storage period.

2.4.7 Miscellaneous Health Attributes

Yoghurt and other fermented milk products have been associated with a wide range of health attributes. Some examples include: (a) production of antimicrobial agents, (b) antitumor activity, (c) effect on blood serum cholesterol, (d) inhibition of potential carcinogens formation and (e) stimulation of the immunological host system. The relevance of these finds to the treatment of human diseases is unknown because most of these studies were carried out *in vitro* on laboratory animals, and for this reason these aspects will not be reviewed.

2.5   Health, Development and Future Trend of Yoghurt

2.5.1 Introduction

As mention elsewhere (see Chapter One) consumers in North America and Europe have modified their eating habits for health reasons such as for slimming purpose and combating coronary heart disease. Thus, consumers attitude has largely involved reducing the amount of fat, sugar, cholesterol and other additives in the diet. Despite the fact that the majority of the therapeutic attributes of yoghurt are not substantiated scientifically, yoghurt is inherently low in fat and is perceived by the consumer as healthy food (Gurr, 1992 b). Furthermore, in the U.K. the market share of 'diet' yoghurt (*i.e.* by volume of production) has increased significantly from 2 to 17% over

the past decade. Also during the same period the British consumers have decreased their butter consumption which has been first replaced by soft margarine high in poly-unsaturated fatty acids and recently by low-fat yellow spreads and vegetable oils. Since the mid 1980s consumption of butter and margarine has declined and consumption of vegetable oils has increased (Buss, 1990) possibly due to the health claim associated with the Mediterranean diet.

The use of fat-substitutes, *i.e.* products that 'mimic' the mouth feel of fat without increasing the calorific value of the product, for the manufacture of low-calorie yoghurt can also help to improve the health image of the product.

Therefore, if the current consumer attitude is maintained, it is possible to suggest that the future market development of yoghurt should offer low-fat, fat-free, high in fibre and/or mono- and poly-unsaturated fatty acids products. These aspects can be achieved by replacing the animal fat with fat-substitutes and vegetable oils.

2.5.2 Fat-Substitutes

Low-calorie yoghurt can be achieved by using one or combinations of the following methods: (a) replacement of the sucrose with intense sweetness, (b) bulking the yoghurt base with fibre and hydrocolloids rather than milk solids, (c) lowering the fat content, for example from 1.5% to <0.5% and (d) using fat-substitutes to replace the fat. The last method has been widely used in innovative product development including dairy products for the manufacture of low-calorie foods. In general, fat-substitutes 'mimics' the functional and organoleptic properties as fats in food without the calories.

The main approach to classify these products is based on the chemical nature and origin of fat-substitutes which are available on the market. These products have been recently reviewed by Tamime *et al.* (1993 - in press); however, a variety of natural gums and thickening agents have been used to reduce the fat and solids-not-fat contents in yoghurt. Technically, developed fat-substitutes, are basically devided into the following categories: first, modified starches such as dextrins and maltodextrins, second, modified egg and/or dairy proteins known as microparticulated proteins and third, ester bonds that have been modified, for example glycerol ethers, pseudofats and carbohydrate fatty acids esters. All these products provide fat-like properties of natural fat and no calories (Casella, 1989).

Fat-substitute products have been extensively reviewed in text books (Murray, 1988; Hendley and Seymour, 1988; Lee, 1989; Singer, 1990; Keuning, 1990; Anderson, 1990; Iyengar and Gross, 1991). A summary of the classification of fat-substitutes including some technical information and their application in dairy products is shown in Table 2.6. However, an alternative approach for the classification of fat-substitutes is to consider their functional characteristics as shown in Figure 2.3. It is possible to suggest that both approaches provide food and dairy technologists with the appropriate specifications of the different fat-substitutes available on the market.

2.5.3 Vegetable Oils

Oils have similar chemical structure like fat, but differ in their physical state. Fats are solid and oils are liquid, for example, at room temperature conditions. Their main physiological roles in animals and humans are as follows: (a) a convenient way to store energy, (b) provide flexibility and/or rigidity of the tissues, (c) act as carriers for certain vitamins, colours and flavouring components, and (d) improve the acceptability of food (*i.e.* mouth feel) product.

Chemically, oils are complex mixture of triacylglycerols which are triple esters formed from three carboxylic acids and the trihydric alcohol glycerol (propane -1, 2, 3 -triol). The pattern of the constitutent carboxylic acids is characteristic of the source of the oil which could be influenced by many factors such as: climate, season, geographical area, soil condition, fertiliser application and botanical species (Clark, 1992).

Most natural fatty acids possess unbranched chains with an even number of carbon atoms and the length ranges from C4 to C22, but the most abundant containing C16 and C18. Chains may be formed entirely through single bonds (saturated) or may contain one or more double bonds (mono- or poly-unsaturated). The arrangement of different substituents on two carbon atoms joined by a double bond introduces another form of stereoisomerism known as *cis* or *trans* isomer. These isomers have different structures and properties which can have an important influence on the physical and nutritional properties of triacylglycerols and oils (Gurr, 1983; Clark, 1992).

Numerous textbooks, reviews and scientific articles have been published on vegetable oils and some comprehensive data in this field regarding the production, refining and technical aspects have been published by de Man (1980), Min and Smouse (1985),

Table 2.6   Classification, differentiation and technical information of fat-substitutes

Trade name/ manufacturer	Type/ calories	Technical information	Application[a]
I. Hydrocolloids and modified starches			
Many sources	Guar gum Carrageenan Xanthan		IC, FD
Maltrin® 040 (Grain Processing Corporation)	Corn 4 k cal g⁻¹	Enzymatic hydrolysis of corn starch; has a low dextrose equivalent (DE) (4-7) maltodextrin containing 98% penta- and higher oligosacchrides; it has good film forming characteristic, low hydroscopicity and no sweetness; it is fully digestible.	FD, IC, SC
N-Oil® II (National Starch	Tapioca 3.75 k cal g⁻¹	It is a dextrin that is prepared by acid-catalysed hydrolysis of the starch; it is also produced as a pre-gelatinised form.	FD, SC, Y, BM
Paselli® SA2 (Avebe b.a.)	Potato 4 k cal g⁻¹	Enzyme (α-amylase) modified amylopectin and amylose of the starch which are partially hydrolysed and DE value is 3.	IC, FD
Litesse™ (Pfizer Chemicals Incorporation)	Glucose 1 k cal g⁻¹	Polydextrose prepared by thermal polymerisation of glucose in the presence of citric acid and sorbitol; around 60% is excreted, i.e. not digestible.	Y, FD, IC
Lycadex® (Roquette Frèses)	Potato Waxy maize k cal (NR)[b]	Maltodextrins obtained by partial hydrolysis of high amylose starch (Lyc.-100) and amylopectin starch (Lyc.-200); DE value is ~5.	B, C, IC, PC
Crestar SF (Euro Centre Food)	Potato 3.8 k cal g⁻¹	Enzymatic conversion of modified waxy starch to yield maltodextrin; DE value is 2-5.	IC

Table 2.6 (continued)

Trade name/ manufacturer	Type/ calories	Technical information	Application[a]
Nutrio P-Fibre (Danish Sugar Factories)	Pea 1.8 k cal g^{-1}	The fibre is prepared by water extraction of de-hulled yellow pea.	Y, DD
Avicel® (FMC - Food & Pharmaceutical Division)	Cellulose 0 k cal g^{-1}	Isolated microcrystalline cellulose from fruits and vegetables	FD
Slendid® (Copenhagen Pectin)	Pectin 0 k cal g^{-1}	Citrus peel hydrocolloid consisting mainly of methyl esters of polygalacturonic acid; the acid groups are partly neutralised with Ca, K and Na ions.	SC, C
Quaker oatrim (Rhône-Poulenc Food Ingredients)	Oats k cal (NR)	Heat stable maltodextrin obtained from oats and is available in a soluble formulation and as an insoluble fibre.	IC, PC, CD, DD, SM
II Microparticulated protein			
Simplesse® (Nutrasweet Company)	Protein 1.5-3.6 k cal g^{-1}	Milk proteins subjected to heat and high shear results in controlled denaturation of of the protein and the formation of tiny spheroidal particles ($<$ 2µm in size); the process is known as microparticulation.	BS, Y, SC, DD, PC
Trailblazer® (Kraft General Foods)	Gum and protein k cal (NR)	Modified xanthan gum/soy, egg and/or dairy protein to produce fibre formation; protein/xanthan fibres are <10 µm in length and ratio ranges 2:1 to 4:1.	FD

Table 2.6 (continued)

Trade name/ manufacturer	Type/ calories	Technical information	Application[a]
Lita® (Opta Food Ingredients)	Corn protein k cal (NR)	Corn protein (zein), which is highly hydrophobic, is microparticulated (<3 μm in size) by using alcoholic precipitation process under controlled conditions (*i.e.* pH, additives, agitation and temperature); the colloidal suspension is ultrafiltered and alcohol is removed by diafiltration.	IC, BS
Nutrifat (Reach Associates)	Carbohydrates and proteins 1 k cal g^{-1}	It contains lightly hydrolysed dextrins and proteins from animal and vegetable sources.	IC
Miprodan (MD Foods)	Milk protein k cal (NR)	Modified milk casein which becomes more soluble.	Y, BS, IC, FY
III. Modified ester bonds			
Olestra (Procter & Gamble Company)	Sucrose and FA^c 0 k cal g^{-1}	Sucrose polyester (SPE) is a process based on solvent free interesterinfication of sucrose and long–chain fatty acid methyl ester followed by refining and extraction.	IC, C
EPG (Arco Chemical Company)	Glycerol and propylene epoxide k cal (NR)	Esterified propoxylated glycerol (EPG) is an acetylated epoxide extended polyols and is prepared by the base-catalysed reaction of glycerol with propylene epoxide.	BS, IC
DDM (Frito-Lay Inc.)	Malonic acid k cal (NR)	Dialkyl dihexadecylmalonate is a combination of fatty alcohol esters of malonic and alkylmalonic acids.	
CCE (East Hanover)	FA and alcohols k cal (NR)	Carboxy/carboxylate esters in a complex polyol esters with at least two distinct acid residues (*i.e.* fatty acids and ester/ether) which have an acidic function.	FD, PC

Table 2.6 (continued)

Trade name/ manufacturer	Type/ calories	Technical information	Application[a]
TATCA (Best Foods)	FA and alcohols k cal (NR)	Trialkoxytricarballylate is a thermally stable polycarboxylic acids having 2 to 4 carboxylic acid groups esterified with saturated and unsaturated alcohol with straight or branched chains composed of 8 to 30 carbon atoms.	BS
MCT (Stepan Karlshamus Company)	Medium chain tri-glycerides k cal (NR)	Such tri-glycerides are prepared by: (a) splitting of fat to obtain fatty acids and (b) followed by distillation of fatty acids and re-esterification with glycerol.	MP
DKS (Dai-Ichi Kogyo Seyaku Company)	Sucrose ester k cal (NR)	Sucrose esters are relatively tasteless.	
DUR-LO (Durkee Ind. Foods)	Diglyceride 9 k cal g^{-1}	Although DUR-LO provides 9 k cal g^{-1}, its efficiency allows it to be used at lower level as an emulsifier.	
Jojoba oil (Lever Brothers Company)	Oil seed k cal (NR)	Oil is extracted from *Simmondsia chinensis* seeds which contains mixture of linear esters of mono-saturated long-chain fatty acids and fatty alcohol.	
Polysiloxanes (Dow Corning)	Silica k cal (NR)	These are synthetic organic compounds which are derivatives of silica and have a linear polymeric structure.	

a   Dairy products are identified as follows: IC: ice-cream, FD: frozen dessert, SC: sour cream, Y: yoghurt, BM: buttermilk, C: cheese, PC: processed cheese, CD: cream dip, DD: dairy desserts, SM: skimmed milk powder, BS: butter spread, FY: frozen yoghurt, MP: milk powder.

b   NR: not reported.

c   FA: Fatty acids.

Data compiled from LaBarge (1988), Lee (1989), Casella (1989), Iyenger and Gross (1991) and Tamime *et al.* (1993 - in press).

FAT-SUBSTITUTES
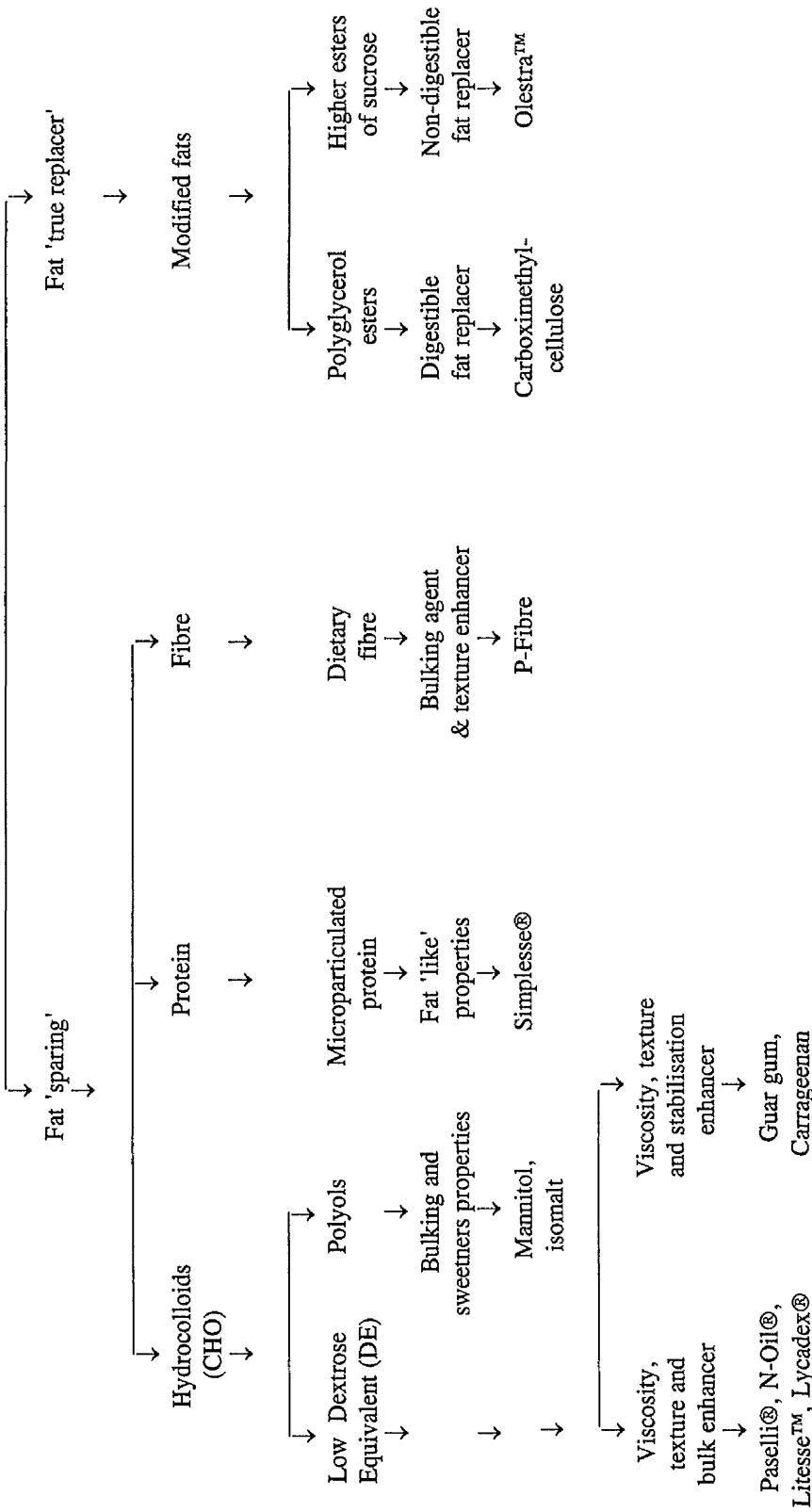
Figure 2.3   **A schematic illustration for the classification of fat-substitutes.**

Adapted from Anderson (1990) and O'Sullivan and Jones (1991).

Gunstone *et al.* (1986), Pomeranz (1991), Clark (1992), Gurr (1992 b). The fatty acid composition of a wide range of vegetable oils including butter fat is shown in Table 2.7. The health aspect of vegetable oils is associated with the presence of mono- and poly-unsaturated fatty acids and in particular the long chain type. However, most vegetable oils, with the exception of coconut and palm oils, do not contain C4 to C14 saturated fatty acids. Mustard and rape seed oils may contain 30-50% erucic acid (Clark, 1992), and its presence in the diet can seriously affect the normal function of the heart. This was a drawback, but with improved plant breeding, new varieties of rape seed now yield oil with negligible erucic acid content (Lanning, 1991).

2.6   Conclusion

Yoghurt has always been recognised by consumers and the medical profession for its excellent nutritional value mainly based of the well-known properties of the milk components and the metabolites (organic acids, partial hydrolysis of proteins, synthesis of viatmins and enzymes, *e.g.* β-D-galactosidase). Taking into account the current consumers attitude towards eating 'healthy' foods, *i.e.*, low in fat and high in unsaturated fatty acids, the formulation of the yoghurt base could be developed to meet such demand by adopting the following approaches:- firstly, reducing the calorific value by replacing the butterfat with fat-substitutes that provides the functional properties of fat without the calories and secondly, substitution of the fat with vegetable oils.

In order to achieve these beneficial effects in set-type, natural/plain flavoured yoghurt, it was decided for the present investigation to evaluate a wide range of fat-substitutes and vegetable oils since very limited data is available for the manufacture a quality product. Not all the fat-substitute products shown in Table 2.6 are available for scientific investigions either because they are not fully developed or because they are awaiting establishment of their safety status. As a consequence, the availability of fat-substitute products is somewhat restricted. Nevertheless, in response to a circular to many companies, samples of fat-substitutes have been received (*e.g.* Litesse™ - improved Polydextrose, Paselli® SA2, N-Oil® II, Lycadex® 100 and 200 - Maltodextrin, P-Fibre 150 C and 285 F and Simplesse® 100 (wet and dry) - microparticulated protein). However, the choice of vegetable oils was governed mainly by the following aspects: (a) the availability of such oils in both the U.K. and Costa Rican markets, (b) the increase popularity and awareness of the consumer regarding the Mediterranean diet which includes high consumption of olive oil, (c) consumers

Table 2.7   Fatty acids composition (%w/w) of some fats and oils

Fatty acids		Butter	Coconut	Palm	Corn	Sunflower	Groundnut	Olive	Cotton	Soya
Saturated										
Butyric	C4:0	3	-	-	-	-	-	-	-	-
Caprioc	C5:0	1	0.5	-	-	-	-	-	-	-
Caprylic	C8:0	1.5	7	3.5	-	-	-	-	-	-
Capric	C10:0	3	6	3.5	-	-	-	-	-	-
Lauric	C12:0	4	46	46	-	-	-	-	-	-
Myristic	C14:0	12	18.5	16.5	-	-	-	-	1	-
Palmitic	C16:0	25	9.5	9	12	6	11	10	24	11
Stearic	C18:0	9	3	2.5	2	4	3	2	2	4
Unsaturated										
Myristoleic	C14:1	1	-	-	-	-	-	-	-	-
Palmitoleic	C16:1	4	-	-	-	-	-	1	-	-
Oleic	C18:1	-	7.5	16.5	27	22	46	76	18	25
Linoleic	C18:2	2	2	2.5	57	66	31	10	54	50
Linolenic	C18:3	0.5	-	-	1	-	1	1	-	8

Data compiled from Sjollema (1990), Pomeranz (1991) and Clark (1992).

choice/preference for the utilisation of a variety of vegetable oils, and (d) the use of vegetable oils that does not contain saturated fatty acids.

These ingredients were used for evaluation during the production and storage of set-yoghurt using a commercially available direct-to-vat-innoculation starter culture.

CHAPTER THREE:

EXPERIMENTAL MATERIALS AND METHODS

CHAPTER THREE: EXPERIMENTAL MATERIALS AND METHODS

3.1   Raw Materials

3.1.1 Skimmed Milk Powder

Throughout this investigation, antibiotic free skimmed milk powder (SMP) was used for the production of yoghurt. The powder was obtained from Express Foods Ingredients Ltd., Middlesex, U.K. in 25 kg bags and stored in a cold place.

3.1.2 Anhydrous Milk Fat (AMF)

AMF was obtained from Aberdeen and District Milk Marketing Board, Scotland; it was packaged in 800 plastic containers and stored at -40°C until required.

3.1.3 Fat-Substitutes

Ten different types of fat-substitutes were evaluated in the present study, and they were classified into the following categories: (A) Modified starches: Litesse™ -improved Polydextrose (Pfizer Food Science Group, Kent, U.K.), N-Oil® II (National Starch and Chemicals, Manchester, U.K.), Lycadex® 100 and 200 -Maltodextrin (Roquette 'UK' Ltd., Kent, U.K.), Paselli® SA2 (Avebe 'UK' Ltd., South Humberside, U.K.) and P-Fibre 150 C and 285 F (Grinsted Products Ltd., Suffolk, U.K.), and (B) Modified milk proteins: Simplesse® 100 (dry and wet) is a microparticulated whey protein which was obtained from NutraSweet® Europe, Burospace, France.

All the modified starch fat-substitutes and Simplesse® 100 (dry) were received as powder and they were stored in a cool and dry place until required. Simplesse® (wet) was dispatched by air a few days before use from Holland and stored at 5°C; three batches were used during this investigation.

3.1.4 Vegetable Oils

Four different types of pure vegetable oils were studied: corn (Mazola™, CPC 'UK' Ltd., Surrey, U.K.), sunflower (Flora®, Flora Food Co., Burgess Hill, U.K.), groundnut (KTC, Wednesbury, U.K.) and olive (Filippo Berio & Co., di Lucca, Italy). The oils were purchased from a local supermarket and have a shelf-life for ~ 15 months. The oils were stored in a dark place at room temperature.

3.1.5 Starter Culture

Pure commercial freeze-dried yoghurt starter culture MY 087 (Textel/Rhône Poulenc 'UK' Ltd., Cheshire, U.K.) was used as direct-to-vat inoculation (DVI) at a rate of 16 g 100 l^{-1}. The freeze-dried starter culture organisms were *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*.

3.1.6 Strawberry Fruit Flavour

This fruit flavour was obtained from G.R. Spinks & Co. Ltd., Devon, U.K. in sealed metal cans. The containers were stored at 5°C until required.

3.2 Processing Equipment and Utensils

3.2.1 Batch Pasteuriser

A cylindrical batch pasteuriser (T. Giusti & Son Ltd., London, U.K.) was used for recombination of the SMP at 40°C.

3.2.2 Homogenizer

A homogenizer model Lab 4746/72 (Rannie Machine Works Ltd., DK-2620 Albertslund, Denmark) was used for the homogenization of yoghurt bases.

3.2.3 Thermometer

A portable digital thermometer Testo 900 (Testoterm Ltd., Hampshire, U.K.) was used for temperature checking and recording.

3.2.4 Hydrogen Ion Meters

A portable pH stick meter model PHK-120-B (Gallenkamp Express, Leicestershire, U.K.) and a bench type PYE 290 MK2 (PYE Unicam Ltd., Cambridge, U.K.), which was fitted with a standard combined glass electrode, were used to measure the pH value in the yoghurt base and product.

The equipment were adjusted with buffer solutions of pH 7 and 4 (BDH Chemicals Ltd., Poole, U.K.) taking into consideration the temperature of the buffer solutions and the sample before starting the measurement.

3.2.5 Incubation Cabinet

An electric and thermostatically controlled incubator type P-33 A-18 (LEEC Electrical Engineering Ltd., Nottingham, U.K.) was used to ferment the yoghurt base.

3.2.6 Plastic Container

The plastic cups used (150 ml white and 50 ml clear) were supplied by MONO Containers Ltd., Middlesex, U.K., and they were suitable for 'press-on' plastic lids.

3.3 Production of Set-Yoghurt

Set-yoghurt was produced as shown in Figure 2.2. SMP was reconstituted to ~14% (w/w) total solids at 40°C, warmed to 60°C and divided into 10 kg equal portions. The AMF was melted at 65°C and added at a rate of 1.5% (w/w) to the first batch of milk (*i.e.* control). The experimental ingredients were also added at the same rate as follows:-

Experiment	Ingredient	No of replicas
1st	Modified starch (7)	2
2nd	Vegetable oil (4)	3
3rd	Microparticulate protein (3)[a]	3

[[a] *Simplesse® 100 (wet) was also used to yield an increase in dry matter content equivalent to 1.5%; figures in parenthesis represent number of ingredients studied*].

The AMF and the experimental ingredients were mixed with the milk using a hand whisker to form a coarse emulsion. Each batch of yoghurt base was then homogenized at 17.3 MPa, heated to 90°C for 5 min and cooled to 45°C in a water bath where steam was injected into the water during the heat treatment stage and mains water was circulated during the cooling stage. The processed milks were inoculated with starter culture at a rate of 16 g 100 l^{-1} dispensed into plastic cups (40 and 150 ml) fitted with press-on lids and incubated for 6-7 h at 42°C or to pH 4.6. The yoghurts were then transferred to a cold store at ⁻5°C.

3.4   Analysis of Skimmed Milk Powder (SMP)

3.4.1   Determination of Fat

Fat content of SMP was determined according to the method of the International Dairy Federation (IDF) Standard No. 22B (IDF, 1987 a) which is based on the Rose-Gottlieb analytical method.

3.4.2   Determination of Total Nitrogen

Total nitrogen content of SMP (expressed as percentage of protein) was determined according to the improved micro-Kejeldahl method of the Association of Official Agricultural Chemists (AOAC, 1990). Kejeldahl copper catalyst tables (BDH Chemicals Ltd.) were used instead of mercuric oxide. A standard solution of 0.02 N hydrochloric acid (HCl) was used as a receiver during distillation. The excess of acid was titrated with a standard solution of 0.02 N sodium hydroxide (NaOH) using Kjeltec Auto 1030 Analyser (Tecator AB, Hoganas, Sweden).

3.4.3   Determination of Total Solids

The method of the IDF Standard No. 21A (IDF, 1982 b) was used for the determination of total solids in SMP. Around 3 g of sample was weighed on a AE 166 balance (Mettler Instruments Ltd., Buckinghamshire, U.K.) and dried at 102°C for 2 hours in a hot air oven to a constant weight (Gallenkamp Express).

3.4.4 Determination of Ash

Ash content of SMP was determined according to the method of BS: 1741 Part 9 (BSI, 1988) by drying 6 g of sample, charred and ashed at 550°C using a muffle furnace (Baird & Tatlock, London, U.K.).

3.4.5 Determination of Titratable Acidity

Titratable acidity of SMP was determined by the method specified by the American Dry Milk Institute (ADMI, 1983).

3.4.6 Determination of the Heat Number

The heat number of SMP was determined according to the method of the IDF Standard No 114 (IDF, 1982 c).

3.4.7 Solubility Index

The solubility index of SMP was determined according to the method described by the American Dry Milk Institute (ADMI, 1983) in order to measure the ability of SMP to dissolve in water.

3.4.8 Scorched Particles

The method of the American Dry Milk Institute (ADMI, 1983) was used to determine the scorched particles content of SMP.

3.4.9 Antibiotic Residue

The disc assay method of Galesloot and Hassing (1962) as modified by Crawford and Galloway (1964) was used for the detection of antibiotics in skim milk powder. This test detects the present of antibiotics or other inhibitory substances in milk at a level of less than 0.02 international units (IU) of penicillin ml^{-1}. Test organism was *Bacillus stearothermophilus* var. *calidolactis*, and the plate was then incubated at 55°C for 2½ hours. Normal growth of the test bacteria indicates that the sample is free of antibiotics. Antibiotics or other inhibitory substances, when present in the milk sample, diffuse into

the agar medium round the disc, thus preventing the growth of the organism and therefore resulting in the formation of a circular clear zone.

3.4.10 Total Viable Count

The American Dry Milk Institute method (ADMI, 1983) was used to determine the colony count. Plate Count agar CM 325 (Oxoid Ltd., Basingstoke, U.K.) was used and the plates were incubated at 30°C for 3 days.

3.4.11 Thermoduric Count

Plate Count agar CM 325 (Oxoid Ltd.) was used to determine the count according to the method reported by Harrigan and McCance (1976). The plates were incubated at 55°C for 2 days.

3.4.12 Enterobacteria Count

The method of the IDF Standard No. 73A (IDF, 1985) was used to enumerate the enterobacteria. The test medium used was Violet Red Bile Lactose CM7 (Oxoid Ltd.). The plates were incubated at 30°C for 48 hours; the presence of acid and gas in a tube of broth meant a positive reaction.

3.4.13 Yeasts and Moulds Count

The method of the IDF Standard No. 94B (IDF, 1990) was used to enumerate yeasts and moulds. An agar medium, which consisted of 5 g yeast extract L21 (Oxoid Ltd.), 20 g dextrose analar 10117 (BDH Chemicals Ltd.), 15 ml agar 0140-01 (Difco Lab. Ltd., Surrey, U.K.) and chloramphenicol (Sigma Chemical Co. Ltd., Poole, U.K.), were used. The added antibiotic inhibits the growth of organisms other than yeasts and moulds.

3.5   Analysis of Anhydrous Milk Fat (AMF)

3.5.1 Determination of Fat and Moisture

Fat content was determined according to the method described in section 3.3.1. The moisture content was determined by the Karl Fisher method as described by IDF Standard No. 23A (IDF, 1988 a).

3.5.2 Determination of the Peroxide Value

The reference method of the IDF Standard No. 74A (IDF, 1991) was used to determine the peroxide value of the AMF.

3.5.3 Determination of Fatty Acids

The fatty acids content in AMF was determined using gas liquid chromatography (GLC) according to the method of BS: 684 - section 2.34 (BSI, 1980). The column used for the GLC was 2 m long, 2 mm internal diameter and packed with cyansilicone derivative (SP 2330) on 100-120 mesh chromasorb (W/AW).

The chromatograph used was Model 93 equipped with flame ionisation detector S 100183 (Ai Scientific Cambridge Ltd., Cambridge, U.K.) and an integrator Model SP 4290 (Spectra-Physics) which was obtained from San Jose, California, U.S.A. Samples were injected by an automatic liquid auto sampler Phillips Model PU 4700 supplied by PYE Unicam Ltd., fitted with a $1\mu l$ syringe. The temperature programme was 50°C for 2 min (iso-thermal temperature) then increasing to 200°C at a rate 20°C min^{-1} and held at 200°C for 10 min. Nitrogen gas was used as a carrier with a flow rate of about 20 ml min^{-1}, and hydrogen flow rate was about 20 ml min^{-1}.

A set of fatty acid standards prepared in di-isopropyl ether containing 4 per cent formic acid supplied by BDH Chemicals Ltd. was used for calibration of the chromatograph. Response factors were automatically determined by data processor using the n-Nonanoic acid (C9) as internal standard. The chromatograms were quantified on the processor by relating the corrected peak areas to the peak area of the C9.

Around 50 mg of AMF was weighed into a Q/Q stoppered test-tube and mixed with 2 ml hexane until dissolved. Later 0.2 ml of methanolic potassium hydroxide (*i.e.* 2%

(w/v) $K(OH)_2$ in methanol) was added and shaken until the solution becomes clear. The glycerol fraction settles to the bottom of the test-tube after standing for ~10 min.

3.5.4 Microbiological Analysis

Total viable count, thermoduric, enterobacteria, yeasts and moulds in the AMF were determined according to the methods described by Harrigan and McCance (1976).

3.5.5 Lipolytic Count

Harrigan and McCance (1976) method was used to determine lipolytic bacteria in AMF. Tributyrin agar PM4 (Oxoid Ltd.) was used and the plates were incubated at 30°C for 36 hours. Colonies surrounded by well defined clear zones were counted as lipolytic bacteria.

3.6   Analysis of Vegetable Oils

3.6.1 Determination of Moisture

The moisture content in vegetable oils was determined according to the method described in section 3.5.1.

3.6.2 Determination of Peroxide Value

Peroxide value was determined according to the method described in section 3.5.2.

3.6.3 Determination of Fatty Acids

Fatty acids content was determined according to the method described in section 3.5.3.

3.6.4 Microbiological Analysis

Total viable count, thermoduric, enterobacteria, yeasts and moulds and lipolytic count in the vegetable oils were determined according to the methods described in section 3.5.4.

3.7   Analysis of Fat-Substitutes

3.7.1   Determination of Total Nitrogen and Total Solids

Total nitrogen and total solids contents of fat-substitute samples were determined according to the methods described in section 3.4.2 and 3.4.3 respectively.

3.7.2   Determination of Organic Acids

The organic acids content in the fat-substitutes was determined according to the method of Marsili et al. (1981), but the concentration of sulphuric acid in the eluent was reduced from 0.009 N to 0.0045N. A known mixture or organic acids in solution was chromatographed and by using 0.0045 N sulphuric acid it was possible to achieve a separation of orotic and citric acids, which co-eluted when the higher concentration was used, without a great effect on the retention times of the other acids which were being determined (see Mahdi et al., 1990).

A Spectra-Physics HPLC system (San Jose) was used which consisted of an auto sampler (Model SP 8780 XR), a detector (model LC 871 UV-VIA - PYE Unicam Ltd.) and a pump (model SP 8770 isocratic - Santa Carla, California, USA). The column effluent was monitored at a wavelength of 220 nm. Calculation of organic acids was carried out by measuring the peak area using an integrator model SP 4270 (San Jose). Analysis was performed isocratically at flow rate of 0.7 ml min^{-1} at 65°C.

The HPLC organic acid analysis column (Bio-Rad Lab., Richmond, California, USA) was used which was 300 mm long and 7.8 mm internal diameter. The column was packed with Aminex HPX - 87H i.e. a strong cation exchange resin (8% crosslinked and 9 μm diameter) which separates organic acids by ion exclusion and partition chromatography.

The chromatograph was calibrated with an aqueous organic acid calibration standard mixture covering a range of acids prepared from a stock solution. This standard mixture contained orotic, citric, pyruvic, lactic, uric, acetic, propionic, butyric and hippuric acids at the following concentrations: 20.4, 1000, 50, 1680, 6.39, 880, 925, 1230 and 6.7 μg g^{-1} respectively.

The sample (5 g) was mixed with 5 ml distilled water and 20 ml HPLC grade acetonitrile (BDH Chemical Ltd.) in a 50 ml beaker. The mixture was filtered through a filter paper Whatman No. 1 (Whatman Ltd.) 20 µl aliquot of the filtrate was injected into the HPLC for analysis.

3.7.3 Microscopic Analysis

2.7.3.1 Scanning Electron Microscopy (SEM)

Each fat-substitute powder was spread in a thin layer on the sticky surface of a dry-mount film disc attached to an SEM aluminium stub using a silver-based cement (Ladd Industries, Burlington Vermont, U.S.A.) as described by Kalab *et al.* (1989). Additional spreading was done with a fine sable brush. The powders were sputter-coated in a Hummer II Technics sputter coater to form a gold layer approximately 20 mm thick and were examined in an ISI DS-130 scanning electron microscope operated at 30 kV.

3.7.3.2 Light Microscopy

Light microscopy Olympus Vanox (Olympus Optical Co., London, U.K.) of fat-substitutes was carried out on samples in liquid paraffin and Gram's iodine solution (1 g iodine, 2 g potassium iodide and 300 ml distilled water). Nomarski and brightfield illuminations, respectively were used.

3.8   Analysis of Strawberry Fruit Flavour

The energy value of the fruit was 187.6 k cal 100 g^{-1} and it was calculated using Adiabatic Bomb Calorimeter - System 3.

3.9   Enumeration of the Starter Cultures

The freeze-dried starter cultures viable cell count was expressed as colony forming units (CFU) g^{-1} by using the method of the IDF Standard 117A (IDF, 1988 b). *Str. salivarius* subsp. *thermophilus* was enumerated using M17 medium CM 785 (Oxoid Ltd.) with added 5% lactose solution (BDH Chemicals Ltd.) and MRS medium CM 361 (Oxoid Ltd.) was used for *Lb. delbrueckii* subsp. *bulgaricus*. The poured plates were incubated at 37°C for 72 h and the anaerobic condition for the lactobacilli was achieved by placing the plates in a   Gaspack ANF-402-R   (Gallenkamp Express)   jar.   The

anaerobic condition within the jar was created using a microaerophilic sachet type 71034 BBL® Campy Pack™ (Becton Dickinson Microbiology Systems, Oxford, U.K.) dissolved in water.

3.10   Analysis of Yoghurt Base

3.10.1 Chemical Analysis

Fat, total nitrogen, total solids and ash contents were determined according to the methods described in sections 3.4.1, 3.4.2, 3.4.3 and 3.4.4 respectively.

3.10.2 Determination of Titratable Acidity

Titratable acidity in the yoghurt base was determined according to the method of BS: 1741 (BSI, 1963).

3.10.3 Determination of Carbohydrate

Galactose, glucose and lactose in the yoghurt base were determined by using different methods which could be described as follows:

(a)   Enzymatically by an ultraviolet (UV) method developed by Boehringer (Anon., 1989 b). UV spectrophotometer model SP 1800 (PYE Unicam Ltd.) was used at a wavelength of 340 nm.

(b)   The polarimeter method described by Biggs and Szijreto (1963) was used for the determination of lactose using a digital polarimeter model AA-100 (Digital Activity Ltd., Cornwall, U.K.). A pump (*i.e.* serial No. 9138), which was obtained from Watson-Marlow Ltd., Cambridge, U.K., was attached to the polarimeter. Sodium light was used as a source of light and the tube length was 17 cm. The reagent solution was prepared by mixing given proportion of zinc acetate ($C_2H_3O_2$) 12.5% (w/v), dudeca-phosphotungstic acid (H_3PO_4 $12WO_3$ $24H_2O$) 6.25% (w/v) and glacial acetic acid (CH_3 O_3H) 10% (w/v). All reagents were analar grade obtained from BDH Chemicals Ltd. Filter paper No. 42 (Whatman Ltd., Maidstone, U.K.) was filtered before use to clarify the test solution. A standard sucrose solution (BDH Chemicals Ltd.) was prepared to given an optical rotation of 3.460.

Ten ml of reagent was added to 40 g of the yoghurt base and the filtrate was analysed at 20°C.

3.10.4 Determination of Organic Acids

The organic acids content in the yoghurt base was determined according to the method described in section 3.7.2.

3.10.5 Viscosity Assessment

The viscosity of the yoghurt base, *i.e.* after the heat treatment stage and before the addition of the starter culture, was determined according to the methods of BS: 734 - Part 2, 734 - Part 2, and 188 (BSI, 1959, 1973, 1977). A 'U' shape capillary tube type D-BSI was used.

3.10.6 Determination of Fatty Acids

The fatty acids content in the yoghurt base was determined according to the method described in section 3.5.3. However, 50 mg of extracted lipids from the yoghurt base by the Rose-Gottlieb method was used.

3.10.7 Determination of pH

The hydrogen ion concentration in the yoghurt base was measured using the pH meter described in section 3.2.4.

3.10.8 Determination of the Non-Casein

The casein content in the un-heated yoghurt base was determined according to the method of the IDF Standard No. 29 (IDF, 1964), and the non-casein content was calculated by difference (*i.e.* non-casein = protein - casein). Acetic acid (10% w/v) and sodium acetate (1N) solutions were used to precipitate the casein and were obtained from BDH Chemicals Ltd.

3.10.9 Enumeration of the Starter Cultures

The enumeration of the yoghurt organisms (*i.e. Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) were determined according to the method described in section 3.9. The counts were expressed as CFU ml $^{-1}$.

3.10.10 Microscopic Analysis

The microstructure of the yoghurt base was examined using scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The sample was warmed to 40°C and a few drops were mixed with molten water agar (3% w/v). After solidification, the sample was cut into thin strips 5 mm in width, fixed in glutaraldehyde solution (2.8% of v/v) according to the method of M. Kalab (Personal Communication) and mailed to Centre of Food and Animal Research, Agriculture Canada, Ontario, Canada for electron microscopy. The preparation of sample for microscopic analysis is illustrated in section 3.11.7.

3.10.11 Calorific Value

Total energy in yoghurt base was expressed in k cal 100 g^{-1}, and was calculated using energy conversion factors (*i.e.* protein 4, carbohydrates 4, fat 9 and organic acids 3) as described by Holland *et al.* (1991).

3.11 Analysis of Yoghurt (Fresh and Stored)

3.11.1 Chemical Analysis

Fat, total nitrogen, total solids, ash, titratable acidity, carbohydrate, organic acids, fatty acids and pH were determined according to the methods described in sections 3.4.1, 3.4.2, 3.4.3, 3.4.4, 3.10.2, 3.10.3, 3.7.2, 2.5.3 and 3.10.7 respectively. However, titratable acidity of yoghurt was determined by titrating 10 g rather than 10 ml, also the drying time of the sample in the oven was 4h rather than 2h.

3.11.2 Microbiological Analysis

Total viable count of non-lactic acid bacteria in the yoghurts was determined according to the method of the IDF Standard No. 100A (IDF, 1987 b); the growth medium used was plate count agar CM 325 (Oxoid Ltd.). Thermoduric, enterobacteria, yeasts and moulds, and lipolytic counts in the yoghurt (fresh and stored) were determined according to the methods described in sections 3.4.11, 3.4.12, 3.4.13 and 3.5.5 respectively.

3.11.3 Enumeration of the Starter Culture

Str. salivarius subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* in fresh and stored yoghurts were enumerated according to the method described ins section 3.9 and the counts were expressed as CFU g^{-1}.

3.11.4 Syneresis/Serum Separation Assessment

Serum separation of yoghurt (fresh and stored) was estimated using a drainage test (ml) according to Dannenberg and Kessler (1988 a). The weight of the hemisphere of yoghurt was ~21 g and the test was carried out for 2h period at 5°C. A mesh size of 0.4 mm was used. Figure 3.1 (A) illustrates the utensils of such analysis.

3.11.5 Firmness/Compression Response Assessment

The firmness of yoghurt (fresh and stored) was expressed in newtons (N) and was measured using the Stevens Texture Analyser (C. Stevens & Son Ltd., Hertfordshire, U.K.) as described by Tamime *et al.* (1991). The analyser was equipped with a cylindrical probe (type TA3-TFE-105-504), 25 mm in diameter and 35 mm long. The probe penetrated the sample to a depth of 15 mm at a speed of 0.5 mm s^{-1} and the force exerted on the probe was recorded. Firmness was evaluated on samples immediately after removal from the cold store at ~5°C. An illustration of the analyser is shown in Figure 3.1 (B).

3.11.6 Calorific Value

Total energy in yoghurt was calculated using the conversion factors described in section 3.10.11.

3.11.7 Organoleptic Assessment

In the present study different organoleptic schemes were used to evaluate set-yoghurt (10-12 trained judges and large number for consumers acceptability of strawberry flavoured yoghurt) which could be described as follows:

(a)   Pearce and Heap (1974): The yoghurt samples (fresh and stored) were evaluated by 10 panellists from the Food Science and Technology Department. The scores were awarded on a five-point Hedonic scale ranging from 'Excellent' (5 points)
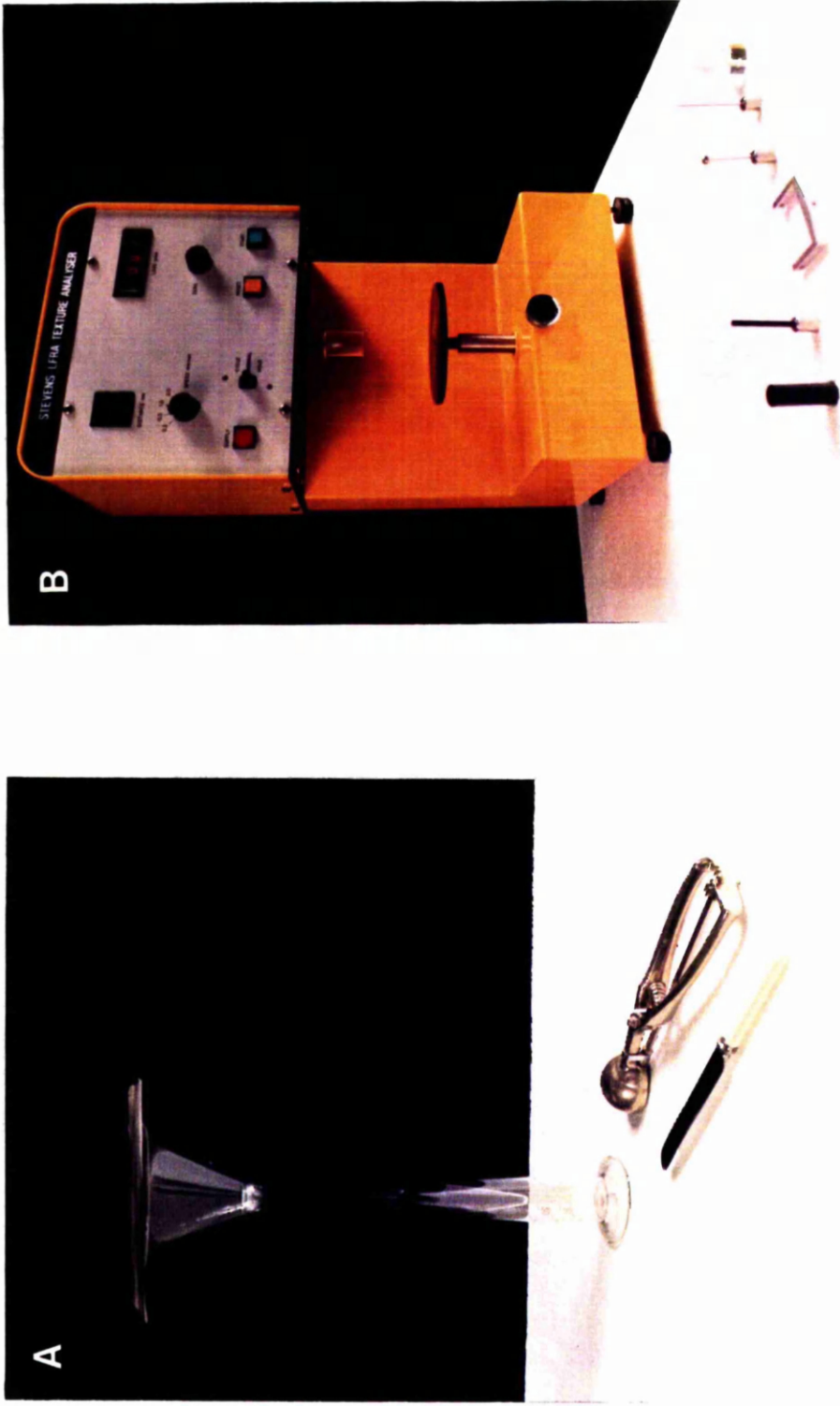
Figure 3.1   Illustrations for the equipment used for measuring syneresis (A) and firmness (B) of yoghurt.

to 'Poor' (1 point) for four attributes: appearance and colour, body and texture, flavour and aroma, and overall acceptability. The flavour and aroma score is multiplied by 2 and the overall score is obtained by addition. Maximum score awarded is 20.

(b) Land and Shepherd (1988): Strawberry flavoured stirred-yoghurts (using fat-substitutes or vegetable oils) were assessed by consumers at SAC-Auchincruive. The acceptability score was awarded on a nine-point Hedonic scale ranging from 'Like-Extremely' (9 points) to 'Dislike - Extremely' (1 point).

(c) Powers (1988), Land and Shepherd (1988) and Muir and Hunter (1992): Unstructured or graphic scale (*i.e.* 125 mm in length) usually has anchors only at the ends was also used for the organoleptic evaluation of yoghurt. Eleven sensory attributes were chosen taking into account odour, flavour, after-taste and texture. The evaluation of the yoghurt was carried out using panellists from Food Science and Technology Department and the Hannah Research Institute.

All yoghurts were presented to the judges in 50 g plastic cups fitted with press-on lids at -8°C. The order presentation of the yoghurt samples were arranged according to William's square (Mcfie *et al.*, 1989) This ensures that the presentation order was fixed and designed to create balance in order of testing and carry over effects. Furthermore, the judges were instructed before tasting the first sample and, between samples, to eat a piece of plain biscuit and rinse their palate with water.

The overall illustration of the organoleptic schemes used in the present study are shown in Appendix I.

3.11.8 Microscopic Analysis

Yoghurt columns or cubes (0.5 cm in width) were fixed in 2.8% (v/v) glutaraldehyde (Sigma Chemical Co. Ltd.) solution and mailed to Centre of Food and Animal Research, Agriculture Canada, Ontario, Canada for electron microscopy (Allan-Wojtas, 1984). After arrival, the yoghurt columns were prepared for scanning microscopy (SEM) and transmission electron microscopy (TEM) according to the methods described by Tamime *et al.* (1991).

For SEM, the fixed yoghurt columns were cut into prisms, 1 x 1 x 10 mm, and dehydrated in a graded ethanol (20, 40, 60, 80, 95 and 100%) series, defatted in chloroform, and returned into ethanol. Then the samples were frozen in Freon 12 at

-150°C and freeze-fractured under liquid nitrogen. The frozen fragments were thawed in absolute ethanol and critical-point dried from carbon dioxide,. The fragments, sputter-coated with gold, were examined in an ISI DS-130 scanning electron microscope operated at 30 kV.

For TEM, the yoghurt samples were cut into 0.5 mm cubes, postfixed in a 2% osmium tetroxide solution in a 0.05 M veronal-acetate buffer, pH 6.75, and embedded in medium hard Spurr's low-viscosity medium (J.B. Em Service Inc., Pointe Claire-Dorval, Quebec, Canada). Sections, approx. 90 nm thick, were stained with uranyl acetate and lead citrate solutions and examined in a Phillips EM-300 transmission electron microscope operated at 60kV.

3.12   Statistical Analysis

The data were analysed by univariate (analysis of variance, regression) and multivariate [Canonical Variates Analysis (CVA), Principal Components Analysis (PCA)] by the Genstat computer programme (copyright Lawes 1990) Agricultural Trust, Rothamsted Experimental Station, and Minitab Release 8 (Minitab Inc., Pennsylvania State College, PA 16801, U.S.A.).

CHAPTER FOUR:

PRODUCTION OF SET-TYPE YOGHURT USING DIFFERENT TYPES OF CARBOHYDRATE-BASED FAT-SUBSTITUTES

CHAPTER FOUR: PRODUCTION OF SET-TYPE YOGHURT USING DIFFERENT TYPES OF CARBOHYDRATE-BASED FAT-SUBSTITUTES

4.1   Preliminary Studies

4.1.1   Quality of the Skim Milk Powder (SMP)

As mentioned in section 2.2.1, SMP was used as the basic raw material for the manufacture of set-yoghurt in the present study in order to minimise the inherent seasonal variation in the chemical composition of fresh milk.

The specifications (chemical, physical and microbiological) of SMP for recombination are important and can affect the quality of the product. Different selected criteria were chosen, and the analysis of SMP were carried out according to the methods described in section 3.4. Table 4.1 shows the compositional, physical and microbiological, qualities of SMP when compared with specifications reported by Sjollema (1988). These results indicate that the SMP used was suitable for yoghurt making, and similar specifications were recommended by Wilcek (1990). The heat number of the SMP was 81, and the powder used is classified as 'medium heat' suitable for the production of fermented milk products (Wilcek, 1990). SMP of 'high heat' number is not suitable for the production of yoghurt because the excessive denaturation of whey proteins can affect the mechanism(s) of the gel formation.

4.1.2   Quality of Anhydrous Milk Fat (AMF)

The AMF was used in the recombination process to manufacture yoghurt which was known as a 'control'. The compositional and microbiological qualities of the AMF is shown in Table 4.1 (see also section 3.5). Similar specifications were reported by Sjollema (1988) and Tamime and Kirkegaard (1992) for the AMF which was

Table 4.1   Some selected analysis and specifications for skim milk powder and anhydrous milk fat for recombination

Test	Skim milk powder		Anhydrous milk fat	
	SAC[a]	Sjollema (1988)	SAC	Sjollema (1988)
Protein (%)	36.35	-	-	-
Fat (%)	0.72	max 1.0	99.85	min 99.9
Moisture (%)	3.30	max 4.0	0.15	max 0.10
Ash (%)	8.20	-	-	-
Lactose (%)[b]	51.43	-	-	-
Titratable acidity (%)	0.15	max 0.15	-	-
Peroxide value [c]	-	-	0.16	max 0.20
Scorched particle	Grade A	min B	-	-
Insolubility index (ml)	<0.1	max 0.5	-	-
Heat number	81.0	-	-	-
Antibiotics	-ve	-	-	-
Total viable count (CFU g^{-1})	18.5 x 10^1	max 50 x 10^3	130	max 100
Plate count 55°C (CFU g^{-1})	<10[d]	max 10 x 10^3	<10	-
Enterobacteriaceae (CFU g^{-1})	<10	max 10	<10	max 10
Yeasts and moulds (CFU g^{-1})	<10	max 100	<10	max 10
Lipolytic bacteria (CFU g^{-1})	-	-	<10	-

[a]   Scottish Agricultural College.
[b]   Lactose was calculated by difference.
[c]   meq O_2 kg^{-1}.
[d]   No growth at 10^{-1} dilution.
(-)   Not reported or not specified.

recommended for recombination. In addition, no lipolytic bacteria was present in the AMF at 10^{-1} dilution; thus, the product is free from off-flavours. The total fatty acids content is shown in Table 5.2

4.1.3 Enumeration of the Starter Culture

One type of thermophilic lactic starter culture (MY 087 Texel/Rhône Poulenc) was chosen for the acidification of milk for the manufacture of set-yoghurt. As mentioned in section 3.1.5 the freeze-dried culture was suitable for DVI application and was stored at -40°C in a freezer. *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were recovered on plating out as 2.9 x 10^{11} cfu g^{-1} and 6.8 x 10^8 cfu g^{-1} respectively. The high proportion of streptococci in the yoghurt culture was probably designed for the production of low-acid mild yoghurt.

4.1.4 Compositional Quality of Fat-Substitutes

Low-calorie yoghurt was produced by replacing the milk fat with seven different types of carbohydrate-based fat-substitutes (Litesse™, N-Oil® II, Lycadex® 100 and 200, Paselli® SA2 and P-Fibre 150 C and 285 F). However, studies on microparticulated whey protein fat-substitutes were evaluated separately (see Chapter 6) because there was a delay in receiving the samples. The moisture and protein contents in seven types of fat-substitutes were determined as described in sections 3.4.2 and 3.4.3, and the results are shown in Table 4.2. Components such as fat, ash, carbohydrates (*e.g.* oligosaccharides) and fibre contents were not determined for the following reasons: firstly, lack of analytical facilities to qualitatively determine the contents of dietary fibres and oligosaccharides, secondly, since the fat-substitutes were used in the yoghurt base at a rate of 1.5% (w/w), the technical data provided by the supplier was sufficient in order to estimate their presence in the product by calculation (see Table 4.2).

As mentioned elsewhere (see Table 2.5 and Figure 2.3) these fat-substitutes are carbohydrate-based and only the P-Fibre (150 C and 285 F) contains substantial amount of dietary fibre, *e.g.* 35% and 80% respectively (Table 4.2).

The spectrum of organic acid contents (µg g^{-1}) in the fat-substitutes were determined as described in section 3.7.2. Only butyric acid was detected (Table 4.2) and the level ranged between 90µg g^{-1} in Paselli® SA2 to 1040 µg g^{-1} in Lycadex® 200. Although

Table 4.2   Approximate composition (%w/w)[a] of seven different types of fat-substitutes

Component	Litesse™	Lycadex® 100	Lycadex® 200	N-Oil® II	Paselli® SA2	P-150 C	P-285 F
Moisture	4	6	6	5	9	7	8
Protein	–[b]	–	–	–	–	9.5	5
Ash[c]	0.3	0.1	0.1	–	1	2	3
Carbohydrates[c]	96	99	99	97	99	48	3
Fat[c]	–	–	–	0.2	–	0.2	–
Dietary fibre[c]	–	–	–	–	–	35	80
Soluble	–	–	–	–	–	66	88
Insoluble	–	–	–	–	–	34	12
Butyric acid (μg g^{-1})	722	561	1040	631	90	–	240

[a]   Based on dry matter content.
[b]   Not present or not reported.
[c]   Data compiled from technical specifications provided by the suppliers.

butyric acid is not found in yoghurt, the levels shown in Table 4.2 are not considered important because the fat-substitutes were used at a low rate (1.5% w/w).

4.2   Production of Set-Yoghurt

A total of two trials, *i.e.* eighteen batches of set-yoghurt using different types of fat-substitutes were produced as described in section 3.3 and as illustrated in Figure 2.2.

4.2.1 Viscosity of the Yoghurt Base

The viscosity measurement of nine different types of yoghurt bases is shown in Figure 4.1 and Appendix II. The pattern of viscosities were similar for both trials, but the measurements were slightly lower in some yoghurt bases in the second trial. This reduction in viscosities could be due to the level of solids in the yoghurt base (see Table 4.3). The protein and lactose contents were marginally lower in the second trial.

The highest viscosities in both trials ranged between 5.6 to 6.7 and 4.7 to 5.2 centipoise were observed in yoghurt bases containing Lycadex® 200 and P-Fibre 150 C respectively. This could be attributed to the composition and water binding properties of these fat-substitutes. For example, Lycadex® 200 is made from a high amylopectin containing starch and P-Fibre 150 C contains around 35% of dietary fibre of which 66% is in the soluble form. Although P-Fibre 285 F contains higher amounts of fibre (80%), the viscosity of the yoghurt base was lower and contrary to what was expected. This was attributed to some precipitation of P-Fibre 285 F which was observed in the yoghurt base and the product after fermentation. As a consequence the functional property of the fibre was not realised. However, the precipitation of the fibre could have been avoided if the homogenization of the yoghurt base was carried out at 70°C rather than 60°C (K. Buchbjerg- personal communication).

The viscosity measurements of the yoghurt bases was analysed using analysis of variance, and the results indicated that there was a significant difference between the trials (variance ratio = 10.87; P< 0.05) and between the yoghurts (variance ratio = 58.59; P< 0.001).
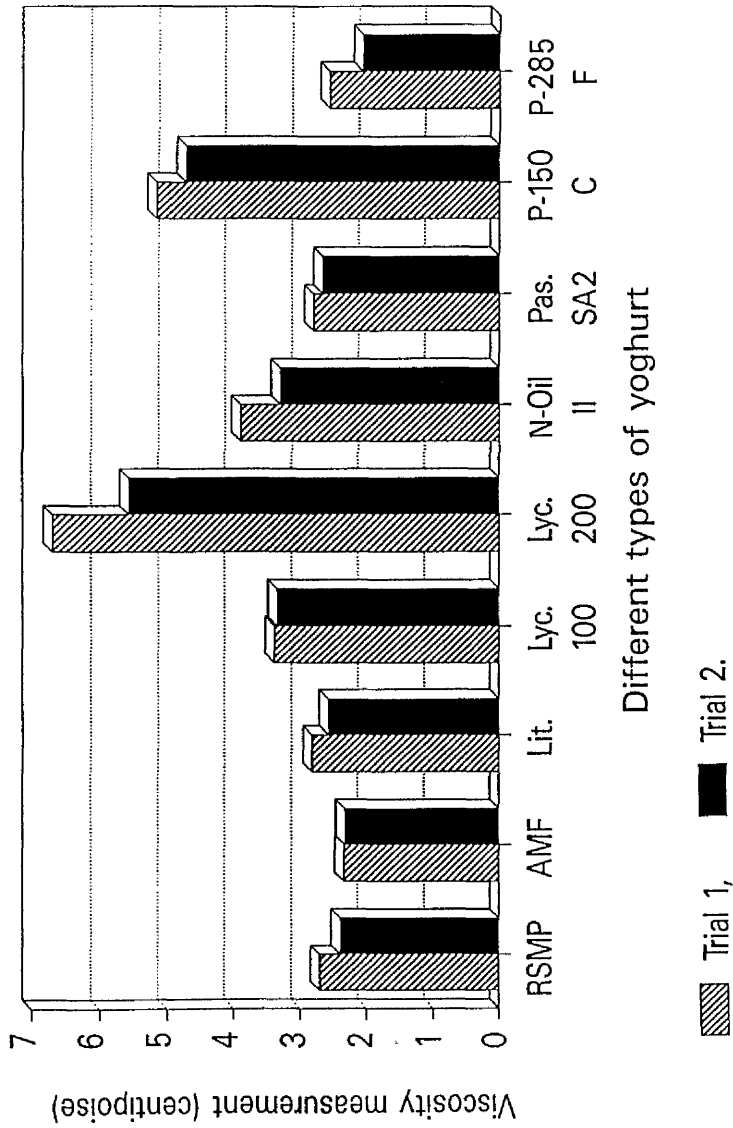
Figure 4.1   Influence of fat-substitutes on the viscosity of yoghurt bases[a] (20°C)

a   Results are average of two determinations performed on the same sample.

Standard Error of Difference (SED) = 0.24.

Table 4.3   Chemical composition (%w/w) of yoghurt bases using different types of fat-substitutes[a]

Product	Protein	Fat	Ash	Carbohydrates			Total solids	pH	Acidity	
				Lactose[b]	Others[c]	Fibre[c]			T.A.[d]	HPLC[e]
1st Trial										
RSMP	5.37	0.10	1.17	7.30	-	-	14.17	6.20	0.22	0.24
AMF	5.34	1.53	1.10	7.22	-	-	15.36	6.21	0.21	0.19
Litesse™	5.31	0.10	1.12	7.50	1.40	-	15.59	6.25	0.21	0.14
Lycadex® 100	5.32	0.10	1.10	7.18	1.40	-	15.27	6.31	0.21	0.17
200	5.36	0.20	1.05	7.40	1.40	-	15.43	6.34	0.21	0.15
N-Oil® II	5.34	0.10	1.11	7.57	1.40	-	15.67	6.32	0.21	0.15
Paselli® SA2	5.33	0.10	1.10	7.18	1.40	-	15.26	6.35	0.21	0.15
P-150 C	5.55	0.10	1.14	7.40	0.70	0.50	15.56	6.32	0.21	0.18
285 F	5.48	0.10	1.16	7.35	0.10	1.10	15.44	6.41	0.22	0.15
2nd Trial										
RSMP	5.32	0.10	1.15	7.22	-	-	13.96	6.20	0.21	0.17
AMF	5.28	1.50	1.11	7.10	-	-	15.14	6.21	0.23	0.17
Litesse™	5.28	0.10	1.10	7.24	1.40	-	15.27	6.23	0.20	0.15
Lycadex® 100	5.27	0.10	1.13	7.15	1.40	-	15.20	6.31	0.20	0.14
200	5.29	0.10	1.11	7.10	1.40	-	15.13	6.35	0.19	0.14
N-Oil® II	5.26	0.10	1.12	7.38	1.40	-	15.41	6.35	0.21	0.15
Paselli® SA2	5.28	0.10	1.15	7.15	1.40	-	15.23	6.32	0.21	0.15
P-150 C	5.49	0.10	1.15	7.32	0.70	0.50	15.36	6.35	0.22	0.10
285 F	5.40	0.10	1.14	7.04	0.10	1.10	15.04	6.32	0.19	0.16

a   Results are average of two determinations performed on the same sample.
b   Lactose was calculated by difference.
c   Calculated on the basis of the technical data shown in Table 4.2.
d   Titratable acidity.
e   Data compiled from Appendix IV.
(-)   Not reported.

4.2.2 Chemical Composition of Yoghurt Base

The chemical composition and acidity measurements of eighteen batches of yoghurt bases are shown in Table 4.3. In general the milk solids-not-fat contents were ~14% and the total solids and protein contents ranged between 13.96 to 15.67% (SED = 0.10) and 5.26 to 5.55% (SED = 0.01) respectively. Such variations were mainly attributed to the ingredients used. The fat contents were ~0.1% with the exception of the batch which contained AMF (1.5%, see Table 4.3). Thus, the fat contents met the proposed specifications for yoghurt in the U.K. and Costa Rica (Anon., 1975; Anon., 1989 c) as either skimmed (< 0.3 or < 0.5) or partly skimmed (1.0 - 2.0 or 0.5 - 3.0%) respectively.

The acidity measurements (*i.e.* pH, titrable acidity and total organic acid contents) were similar in all batches and in both trials, and averaged 6.4, 0.21% and 0.16% respectively.

Variations in the lactose content of two determinations on the same sample were observed by using the enzymatic method for analysis (Anon., 1989 b). The supplier was consulted and parallel samples were analysed by Boehringer in their laboratory in the U.K. Both results are shown in Table 4.4, and from these limited trials and the results from both laboratories indicate the following aspects: (a) the lactose content in the yoghurt bases were different, (b) the results were significantly lower than expected (*i.e.* ~7.25% by difference), and (c) the margins of error between the duplicates were high (> 2% - see Table 4.4) than the recommended acceptable level by the supplier and IDF (1991). The main factor, which could affect quantitatively the level of lactose when using the enzymatic technique, is that the lactose may be bound to the protein due to heating (*e.g.* during the production of SMP or preparation of the yoghurt base). In such cases the bound lactose can not be determined enzymatically (IDF, 1991). It is unlikely that the fat-substitutes may have interfered with the analytical methodology because lactose content in the RSMP and AMF yoghurt bases were similar to the experimental batches (Table 4.4). From these results it was decided that the lactose content in the yoghurt bases would be calculated by difference.

Table 4.4   **Results of anhydrous lactose content (%w/w) of yoghurt bases**
conducted at different laboratories

Product	SAC - Auchincruive			Boehringer		
	$\chi_1{}^a$	$\chi_2{}^b$	% Diff.[c]	χ_1	χ_2	%Diff.
1st Trial						
RSMP	6.34	6.41	1.10	6.83	6.66	**2.50**
AMF	6.74	6.87	1.91	6.67	6.61	1.00
Litesse™	6.48	6.36	1.87	6.53	6.48	0.80
Lycadex® 100	6.39	6.69	**4.59**	6.31	6.36	0.80
200	6.94	6.87	1.01	6.48	6.51	0.50
N-Oil® II	6.39	6.53	**2.17**	6.52	6.23	**4.60**
Paselli® SA2	6.64	6.61	0.45	6.32	6.32	0.00
P-150 C	6.29	6.59	**4.66**	6.28	6.21	1.10
285 F	6.48	6.57	1.38	6.24	6.38	**2.10**
2nd Trial						
RSMP	6.05	6.35	**4.84**	6.70	6.70	0.00
AMF	6.38	6.36	0.31	6.64	6.33	**4.47**
Litesse™	6.66	6.41	**3.82**	6.35	6.25	1.50
Lycadex® 100	6.53	6.48	0.77	6.31	6.24	1.00
200	6.68	6.51	**2.57**	6.42	6.45	0.50
N-Oil® II	6.50	6.36	**2.18**	6.37	6.35	0.20
Paselli® SA2	6.28	6.26	0.32	6.36	6.26	0.00
P-150 C	6.50	6.41	1.39	6.46	6.44	0.20
285 F	6.64	6.54	1.41	6.36	6.34	0.30

[a]   1st reading.
[b]   2nd reading.
[c]   % differences ($\chi 1 - \chi 2$).

Bold type script for which sample results of duplicates are not acceptable according to Boehringer.

Table 4.5 Compositional quality (%w/w) of fresh and stored yoghurt made with different types of fat-substitutes[a]

Product	Protein	Fat	Ash	Carbohydrates				Total solids
				Lactose	Galactose	Other[b]	Fibre[b]	
Fresh Yoghurt								
RSMP	5.35	0.10	1.13	4.66	0.98	-	–	14.00
AMF	5.30	1.50	1.12	4.59	0.99	-	–	15.04
Litesse™	5.27	0.10	1.14	4.74	0.93	1.40	–	15.19
Lycadex® 100	5.28	0.10	1.12	4.58	1.02	1.40	–	15.17
200	5.30	0.10	1.15	4.36	1.02	1.40	–	15.24
N-Oil® II	5.26	0.10	1.15	4.77	0.98	1.40	–	15.42
Paselli® SA2	5.31	0.10	1.15	4.68	0.91	1.40	-	15.12
P-150 C	5.27	0.10	1.11	4.66	0.99	0.70	0.50	15.12
285 F	5.48	0.10	1.16	4.82	1.03	0.10	1.10	14.40
SED[c]	0.05	0.00	0.02	0.16	0.04	-	-	0.17
Stored Yoghurt								
RSMP	5.34	0.10	1.16	4.36	0.94	-	–	14.03
AMF	5.32	1.50	1.16	4.35	0.87	-	–	15.03
Litesse™	5.23	0.10	1.16	4.40	0.91	1.40	–	15.20
Lycadex® 100	5.33	0.10	1.15	4.34	1.17	1.40	–	15.14
200	5.28	0.10	1.18	4.39	0.70	1.40	–	15.25
N-Oil® II	5.28	0.10	1.18	4.46	0.77	1.40	–	15.36
Paselli® SA2	5.31	0.10	1.15	4.50	0.80	1.40	-	15.20
P-150 C	5.28	0.10	1.18	4.41	0.74	0.70	0.50	15.10
285 F	5.47	0.10	1.14	4.45	0.82	0.10	1.10	14.34
SED	0.05	0.00	0.04	0.13	0.14	-	-	0.16

a   Above results are average of single sample analysed in duplicate in each of the two trials.
b   Calculated on the basis of the technical data shown in Table 4.2.
c   Standard Error of Differences
(-) Not reported.

4.2.3 Compositional Quality of Yoghurt (Fresh and Stored)

The average composition of eighteen yoghurts (fresh and stored) is shown in Table 4.5 while Appendix III illustrates the results of each trial. The protein, fat, ash and total solids contents in fresh and stored yoghurts were similar to the yoghurt bases (see Table 4.3) with the exception of total solids in fat-substitute yoghurt containing P-Fibre 285 F where slight precipitation occurred (see section 4.2.1). Nevertheless, analysis of variance showed no significant change for these constituents when compared with the yoghurt bases (variance ratio = 1.9; P> 0.23).

The only change, which was evident, was a reduction of the lactose content and partial accumulation of galactose in yoghurt (fresh and stored) due to the metabolic activity of the starter culture. Despite the fact that some problems were encountered during the enzymatic determination of lactose in the yoghurt bases, the duplicate results of the fermented products were very similar. Also the amount of lactose utilisation by the starter culture MY 087 amounted to ~35-40% (see Table 4.6) similar to what have been reported in the literature. Thus, the results of determination of lactose using the enzymatic method were used for comparative purposes and also to facilitate the approximate calculations of the calorific value of these yoghurts.

4.2.4 Acidification of the Yoghurt Base and Post-Acidification of the Product

The incubation period of all the yoghurts in both trials was 6-7 h at 42°C. The increase in titratable acidity and total organic acid during the fermentation period and storage of yoghurts for 20 days at 5°C is shown in Appendix III while the decrease in pH measurements of the same yoghurts is illustrated in Figure 4.2.

For example, in the first trial the pHs of fresh and stored yoghurts ranged between 4.49 to 4.64 and 4.30 to 4.45 respectively. A similar pattern was also observed in the second trial. The mean pH decrease during 20 days storage of all yoghurts was ~0.18 units. The tendency to post-acidification in yoghurt after storage was evidently due to the continued metabolic activity of the starter culture (Tamime *et al.*, 1987; Becker and Puhan, 1989). and the use of fat-substitutes did not affect the activity of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Barrantes and Tamime, 1992).

Table 4.6   **Lactose content (%w/w) of yoghurt bases and products**

Product	Yoghurt				
	Base	Fresh		Stored	
1st Trial					
RSMP	7.32	4.56	(39)	4.36	(42)
AMF	7.22	4.48	(40)	4.43	(40)
Litesse™	7.50	4.86	(36)	4.36	(43)
Lycadex® 100	7.18	4.74	(36)	4.39	(40)
200	7.40	4.23	(44)	4.18	(45)
N-Oil® II	7.57	4.88	(32)	4.49	(38)
Paselli® SA2	7.18	4.61	(36)	4.41	(40)
P-150 C	7.40	4.86	(36)	4.58	(40)
285 F	7.35	4.78	(37)	4.48	(39)
2nd Trial					
RSMP	7.22	4.77	(35)	4.36	(41)
AMF	7.10	4.70	(35)	4.27	(41)
Litesse™	7.24	4.62	(37)	4.43	(40)
Lycadex®100	7.15	4.42	(39)	4.29	(41)
200	7.10	4.78	(34)	4.60	(37)
N-Oil® II	7.38	4.65	(38)	4.42	(41)
Paselli® SA2	7.15	4.70	(36)	4.41	(42)
P-150 C	7.32	4.78	(36)	4.32	(43)
285 F	7.04	4.13	(36)	4.52	(40)

Above results are average of single sample analysed in duplicate.

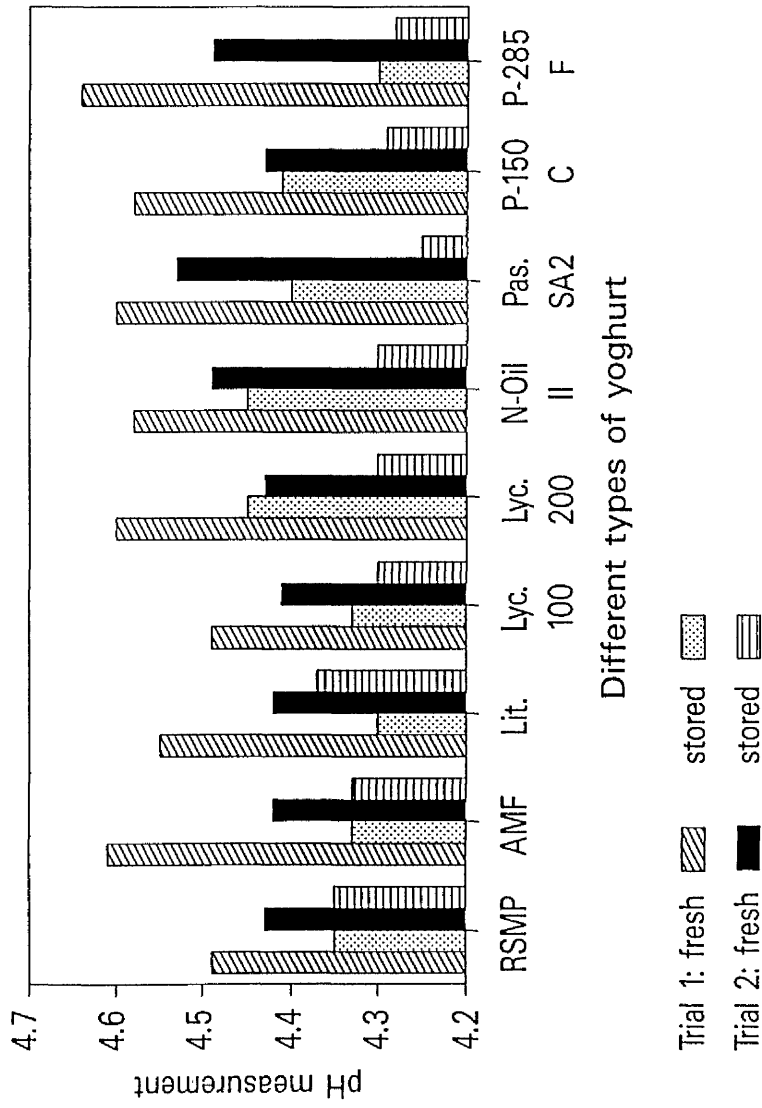Figures in parenthesis represent % of lactose utilisation.

Figure 4.2   **Influence of storage on acidification of yoghurts[a] after 20 days at 5°C.**

a   Results are average of two determinations performed on the same sample.

Statistical evaluation of the pH and titratable acidity change of all the yoghurts showed significant difference (P <0.001) between trials and between fresh and stored yoghurts. However, no significant difference was observed between the yoghurts made with different fat-substitutes in each trial.

Starter cultures including *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* utilise the lactose in yoghurt base as their source of energy for respiration and/or cell division. In both trials the average consumption of lactose in fresh and stored yoghurts was 37% and 40% respectively (SED = 2.11). Lactose is metabolised only after hydrolysis to glucose and galactose. Glucose is metabolised to a proportionately greater extent than galactose (Marshall, 1987), which is also excreted by the starter organisms and thus accumulates in the yoghurt (see Table 4.5).

4.2.5 Analysis of Organic Acids

Organic acids in fermented milk products are important because these components contribute towards the taste and flavour of yoghurt. The type of organic acids content in the product also provides relevant data about the metabolic activity of the starter culture (Rasic and Kurmann, 1978; Tamime and Robinson, 1985).

The spectrum of organic acid contents (μg g^{-1}) in yoghurt bases, fresh and stored products is shown in Figure 4.3 and Appendix IV illustrates the results of each trial. The pattern of organic acid contents in fresh and stored yoghurts (*i.e.* increase or decrease) was mainly influenced by the metabolic activity of the starter culture. The main feature is an increase in lactic and acetic acids in the yoghurts compared with yoghurt bases.

Some data on the organic acid contents of yoghurt has been reported, but the age of the product was not specified (Marsili *et al.*, 1981; Ashoor and Wetty, 1984; Bevilacqua and Califano, 1989). No data is available on fat-substitute yoghurts. In the present study the following observations could be made:

(a)   Orotic and citric acids had been utilised by the starter culture;

(b)   Pyruvic acid was slightly increased;

(c)   Lactic and acetic acids had increased by 30 and 108 fold respectively;

(d)   Uric/Formic acid had increased after the fermentation period, then decreased slightly after storage;
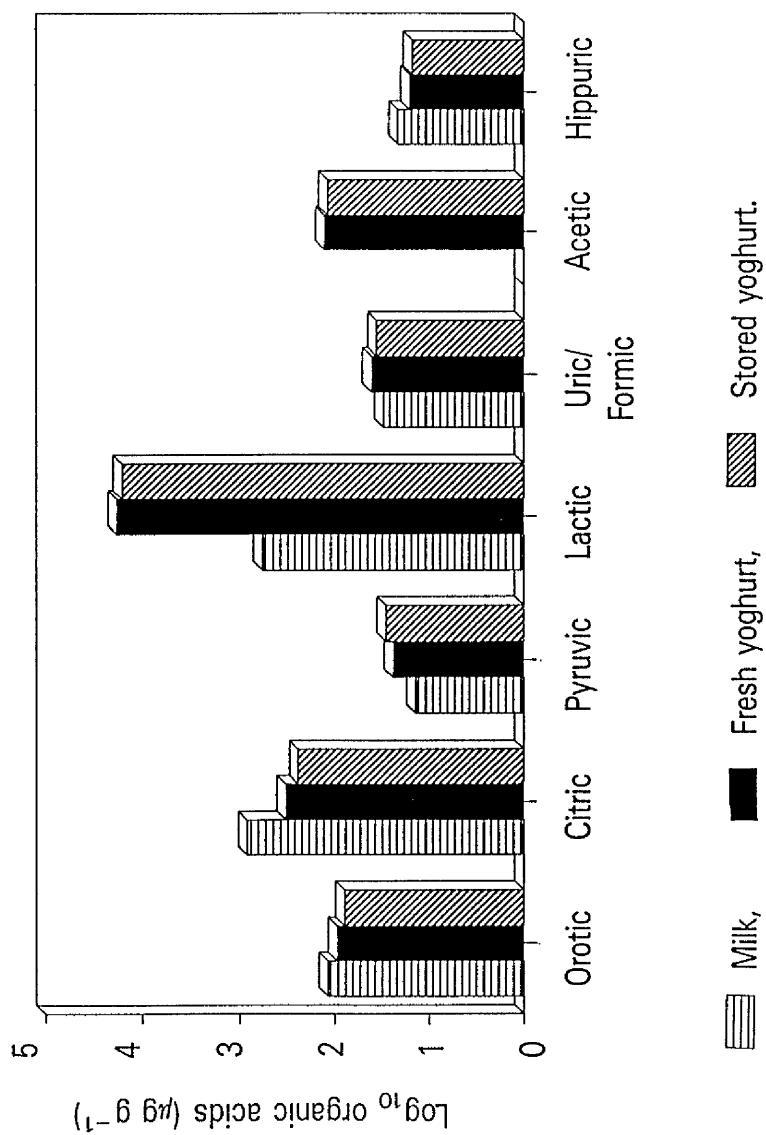
Figure 4.3   Organic acid content (μg g^{-1}) of different yoghurt bases and product[a].

[a]   Results are average of two trials and of two determinations performed on the same sample.

(e)   Hippuric acid had been metabolised after the fermentation stage and the content
        remained constant after 20 days storage;

(f)   Propionic and butyric acids were not found in the yoghurt bases or any of the
        products in these trials.

The organic acid contents in all the yoghurts (Appendix IV) were similar to those
reported in the literature (Okonkwo and Kinsella, 1969; Rasic and Kurmann, 1978;
Marsilli et al., 1981; Mahdi, 1990) with the exception of hippuric acid. Such organic
acid was partially utilised by the yoghurt starter culture bacteria while other workers
reported complete disappearance of hippuric acid probably as a result of hydrolytic
process (Rasic and Kurmann, 1978). However, the different pattern found in the
present study could be attributed to: (a) the amount present in the yoghurt base, i.e.
~ 22 µg g^{-1} as compared to ~ 8 µg g^{-1} reported by Mahdi (1990) or (b) the variation
that can exist among strains of Str. salivarius subsp. thermophilus and Lb. delbrueckii
subsp. bulgaricus.

Analysis of variance showed no significant differences in the organic acid contents
(increase or decrease) among all the different types of yoghurts and between the two
trials.

As mentioned elsewhere, no data were reported in the literature comparing the pattern
of organic acid contents in fresh and stored yoghurts. A unique phenomenon was
observed regarding the major organic acid present in yoghurt where the lactic acid
content dropped after 20 days storage. Such observation merited further investigation in
order to find out whether such effect was due to microbial activity or chemical
interaction(s). The work of Condon (1983), Murphy and Condon (1984 a, b), Murphy
et al. (1985) and Lucey and Condon (1986) suggested that under certain conditions
some starter cultures (e.g. Lactobacillus plantarum and Leuconostoc spp.) were
capable of converting lactate to acetate in the growth medium. Reconstituted skim milk
powder and a yoghurt base containing AMF were prepared and processed as shown in
Figure 4.4. The objectives were to compare quantitatively the lactic acid contents in
milk fermented with the yoghurt starter culture vis-a-vis with direct acidification.
Parallel samples of these products were subjected to heat treatment to inactivate the
starter culture and analyses were carried out on both fresh and after storage for 20 days
at 5°C. Furthermore, the lactic acid content was determined using the HPLC and
enzymatic technique (Anon., 1989 b) for comparative purposes.

Reconstitute SMP to 14% total solids

Process I - Fermentation

Process II - Direct Acidification

Process milk as for yoghurt making (see Figure 2.2)

Autoclave at 115°C for 15 mins

Thermised
Heat the fermented milk to 85°C

Cool

Cool

Live

Cool

Add food grade concentrated
lactic acid to obtain ˜ 1% acidity

Analyse for lactic acid when
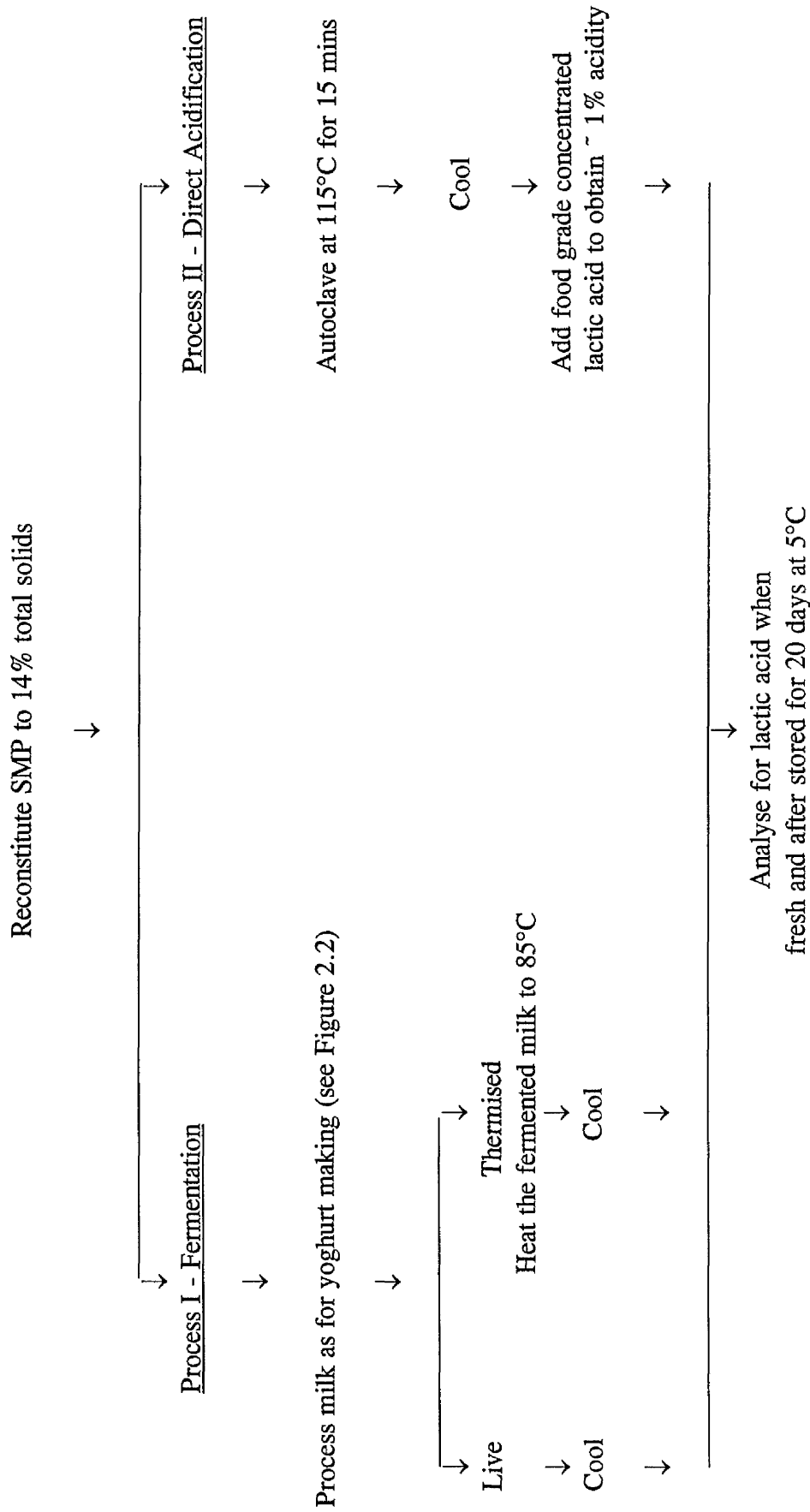fresh and after stored for 20 days at 5°C

Figure 4.4   A schematic illustration showing different processes of acidified milk.

Table 4.7   Lactic acid content (%w/w) in recombined milks subjected to different treatments

Product/ process	HPLC	Enzymatic		
		L(-)	D(-)	Total
Autoclaved (110°C)				
RSMP	0.00	0.00	0.00	0.00
RSMP + l.a.[a] (fresh)	1.28	0.34	0.44	0.78
(stored)	1.12	0.32	0.34	0.66
RSMP + AMF	0.00	0.00	0.00	0.00
RSMP + AMF + l.a. (fresh)	1.03	0.41	0.40	0.81
(stored)	0.96	0.32	0.32	0.64
Live Yoghurt				
RSMP (fresh)	1.87	0.86	0.06	0.92
(stored)	1.56	0.93	0.01	0.94
RSMP + AMF (fresh)	1.44	0.81	0.00	0.81
(stored)	1.61	0.90	0.00	0.90
Thermised Yoghurt				
RSMP (fresh)	2.01	0.98	0.00	0.98
(stored)	1.82	0.92	0.00	0.92
RSMP + AMF (fresh)	1.83	0.93	0.03	0.96
(stored)	1.71	0.80	0.02	0.82

[a]   Lactic acid.

Above results are average of two determinations performed on the same sample.

The lactic acid contents of the fermented milks and direct-acidification including the thermised products are shown in Table 4.7. It is evident that: (a) the lactic acid contents in all the stored products were lower than the fresh samples except the 'live' yoghurt containing AMF and (b) quantitatively, the HPLC method gave higher readings when compared with the enzymatic technique. From these limited results it is possible to conclude that freshly made fermented or direct-acidified products may contain "lactate" compounds which are measured when determining the lactic acid (*i.e.* by HPLC and enzymatic methods). However, after storage for 20 days at 5°C these "lactate" compounds may have dissociated as a result of chemical hydrolytic process or react with other component in milk causing no further interference in the test method used. The yoghurt starter culture could not have been involved in the mechanism(s) of lactate conversion, because in 'thermised' yoghurt samples (see Table 4.7) the lactic acid content in the stored samples was lower than the fresh product. Such hypothesis was confirmed when the lactic acid peak of the HPLC chromatogram was viewed using higher attenuation where a 'shoulder' peak became evident in all the fresh yoghurt samples containing AMF or fat-substitutes. Figure 4.5 B (see arrow) shows such a peak, and when the peak area was edited the difference in peak area between the fresh and stored samples was negative (Figure 4.5 D) indicating that the stored product contained more lactic acid. Editing these peaks for all the yoghurts was carried out and still some of the fresh samples contained more lactic acid. This was thought to be due to the difficulty in accurately determining the effect of the 'shoulder' adjacent to the lactic acid peak.

4.2.6 Approximate Calculation of the Calorific Value

Total calorific value in yoghurts is expressed in k cal 100g^{-1} and was calculated using energy conversion factors as described by Holland *et al.* (1991) (*i.e.* protein 4, carbohydrates 4, fat 9 and organic acids 3 - see section 3.10.11). The data shown in Tables 4.1, 4.3 and 4.5 and Appendix IV was used to calculate the calorific value in different types of yoghurt bases and products (fresh and stored). The calculated energy value in different yoghurt bases ranged between 52 and 65 k cal 100g^{-1} (Table 4.8). The highest energy value was observed in yoghurt base containing 1.5% AMF and the lower values were for yoghurt bases made with RSMP and fat-substitutes.

The calorific contents of the different types of yoghurts (fresh and stored) was likely to similar to, or slightly lower than the calculated energy values for the different yoghurt
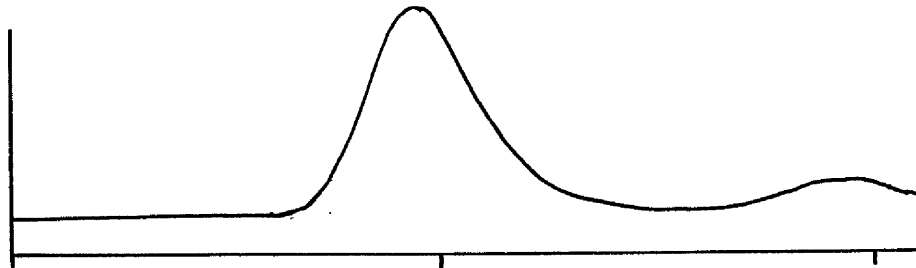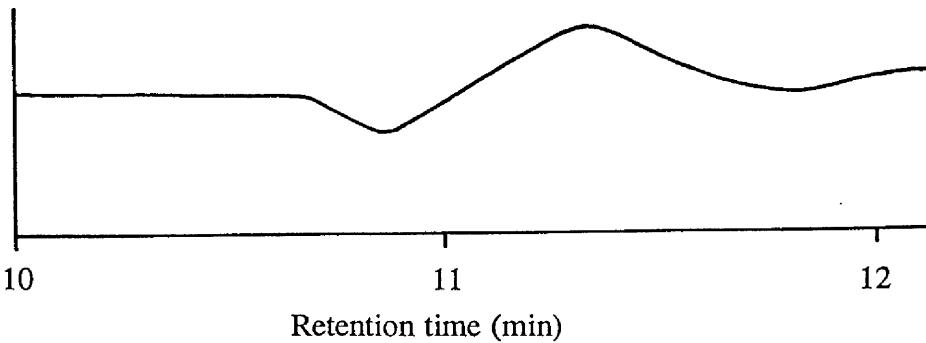
(A)   Standard
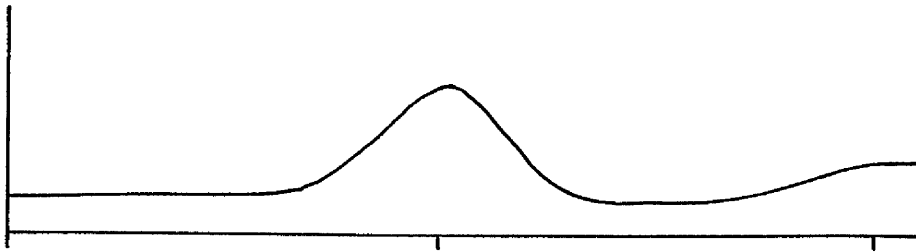
(B)   Fresh yoghurt
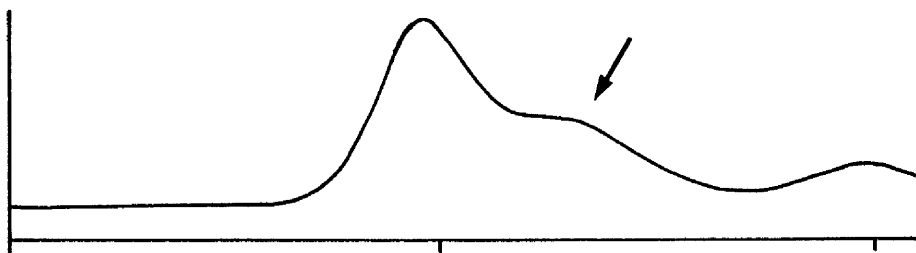
(C)   Stored yoghurt

(D)   Difference between fresh and stored yoghurts

10   11   12

Retention time (min)

Figure 4.5   HPLC chromatogram of the lactic acid peak of yoghurt containing AMF.

Table 4.8   Calculated calorific content[a] (k cal 100 g^{-1}) in yoghurt bases and
yoghurts (fresh and stored)

Product	Yoghurt		
	Milk	Fresh	Stored
1st Trial			
RSMP	53.0	50.6	48.5
AMF	65.3	62.7	60.8
Litesse™	54.6	51.9	49.2
Lycadex® 100	57.8	56.9	54.6
200	58.5	54.6	51.6
N-Oil® II	59.2	56.8	53.4
Paselli® SA2	57.2	55.9	52.7
P-150 C	56.7	54.5	51.2
285 F	53.8	50.9	48.2
2nd Trial			
RSMP	52.3	50.0	49.6
AMF	64.2	61.7	61.0
Litesse™	53.4	51.2	49.2
Lycadex® 100	57.2	53.9	53.9
200	57.0	55.5	54.7
N-Oil® II	58.1	54.3	53.8
Paselli® SA2	57.1	55.2	54.3
P-150 C	55.8	55.1	52.8
285 F	52.1	50.2	47.9

bases. The overall pattern in the changes in the calorific content of the different types of yoghurt was mainly due to:

(a)   The chemical composition of the yoghurt bases or the ingredients used where the highest energy values was for yoghurt containing AMF, and lowest for Litesse™ and P-Fibre 285 F yoghurts. These fat-substitutes contain only 1 k cal g⁻¹ and high amounts of non digestible fibre respectively.

(b)   The metabolic activity of the starter culture had utilised 37 to 40% of the lactose present in the yoghurt base and such apparent loss was compensated with production of organic acids (see Figure 4.3 and Appendix IV) and the partial accumulation of galactose in the different yoghurts (see Table 4.5).

(c)   When calculating the calorific content of yoghurts, different energy conversion factors were used. For example 4 for carbohydrates as compared with 3 for organic acids (Holland *et al.*, 1991).

Analysis of variance on the calculated calories, which were found to be statistically significant were between the yoghurts (variance ratio = 55.11; P <0.001) and after storage for 20 days at 5°C (variance ratio =33.19; P <0.001). No significant difference was observed between the first and second trials.

Analysis of variance of yoghurt containing AMF x the other types of yoghurts was significant at P<0.01. When RSMP was analysed against the other yoghurts, the degree of significance could be summarised as follows:

-       P< 0.01 for AMF, Lycadex® 100 and 200, N-Oil® II, Paselli® SA2 and P-Fibre 150 C yoghurts;

-       Not significant for Litesse™ and P-Fibre 285 F.

4.3   Microbiological Quality of Yoghurt

4.3.1   Microbiological Analysis of Yoghurt

The coliform, yeasts and mould counts of all yoghurts (fresh and stored samples in both trials) tested were <10 CFU g⁻¹ *i.e.* absence of such micro-organism in 10⁻¹ dilution (see Table 4.9). The virtual absence of coliform, yeasts and moulds from all the yoghurts analysed in this study, indicate that the yoghurts were produced under good hygienic standards and sanitary conditions (Barnes *et al.*, 1979; Tamime *et al.*, 1987).

Table 4.9 Microbiological quality (CFU g^{-1}) of different yoghurts

	Fresh yoghurt			Stored yoghurt		
	Total count	Coliform	Yeasts & moulds	Total count	Coliform	Yeasts & moulds
1st Trial						
RSMP	<100[a]	<10[b]	<10	<100	<10	<10
AMF	<100	<10	<10	<100	<10	<10
Litesse™	<100	<10	<10	<100	<10	<10
Lycadex® 100	<100	<10	<10	<100	<10	<10
200	<100	<10	<10	<100	<10	<10
N-Oil® II	<100	<10	<10	<100	<10	<10
Paselli® SA2	<100	<10	<10	<100	<10	<10
P-150 C	<100	<10	<10	<100	<10	<10
285 F	<100	<10	<10	<100	<10	<10
2nd Trial						
RSMP	<100	<10	<10	<100	<10	<10
AMF	<100	<10	<10	<100	<10	<10
Litesse™	<100	<10	<10	<100	<10	<10
Lycadex® 100	<100	<10	<10	<100	<10	<10
200	<100	<10	<10	<100	<10	<10
N-Oil® II	<100	<10	<10	<100	<10	<10
Paselli® SA2	<100	<10	<10	<100	<10	<10
P-150 C	<100	<10	<10	<100	<10	<10
285 F	<100	<10	<10	<100	<10	<10

[a] No growth at 10^{-2} dilution.
[b] No growth at 10^{-1} dilution.
Results are the average of two determinations performed on the same sample

The total count of non-lactic acid bacteria in all the yoghurt samples tested was low, *i.e.* < 100 CFU g^{-1} (see Table 4.9), which is an acceptable target count (Tamime, *et al.*, 1987)

4.3.2 Enumeration of Starter Organisms

The viability of starter culture organisms in fermented milks such as yoghurt has a substantial bearing on the safety of the product. For this reason, in both trials the effects of fat-substitutes and storage on the counts of streptococci and lactobacilli were considered in detail (Appendix V) and are shown in Figure 4.7.

The type of fat-substitute, trial and storage for 20 days at 5°C had no statistical significant effect on numbers of streptococci and lactobacilli in yoghurts (fresh and stored). After the fermentation period the viable counts of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* in fresh yoghurts had increased by 2 and 3 $\log_{10}$ cycles respectively which reflects their metabolic activity during the fermentation period (Barrantes and Tamime, 1992). It is evident that the streptococci had dominated the culture organisms of the yoghurt, reflecting the initial ratio of the inoculum (*Str. salivarius* subsp. *thermophilus* x 10^{11} CFU g^{-1} and *Lb. delbrueckii* subsp. *bulgaricus* x 10^8 CFU g^{-1} - see also section 4.1.3). From these results (Figure 4.7), it is likely that the high ratio of streptococci : lactobacilli ensures low post acidification during the storage period of the product.

### 4.4 Rheological Properties of Yoghurt

The rheological properties of nine different types of yoghurt were assessed by monitoring the rate of serum separation/syneresis and firmness of the product as described in sections 3.11.4 and 3.11.5.

4.4.1 Measurement of Serum Separation/Syneresis

The syneresis measurements of yoghurts containing AMF and seven different types of fat-substitutes is shown in Figure 4.8 and Appendix VI illustrates the results in each trial.

After two days storage at 5°C, the yoghurts' syneresis values ranged between 3.0 and 3.8 ml 2 h^{-1} with the exception of P-Fibre 150 C which had a mean syneresis measurement of 1.75 ml 2 h^{-1} (see Figure 4.8). Although this variation is sizeable there was no statistically significant difference (variance ratio test; $P > 0.25$) detected between
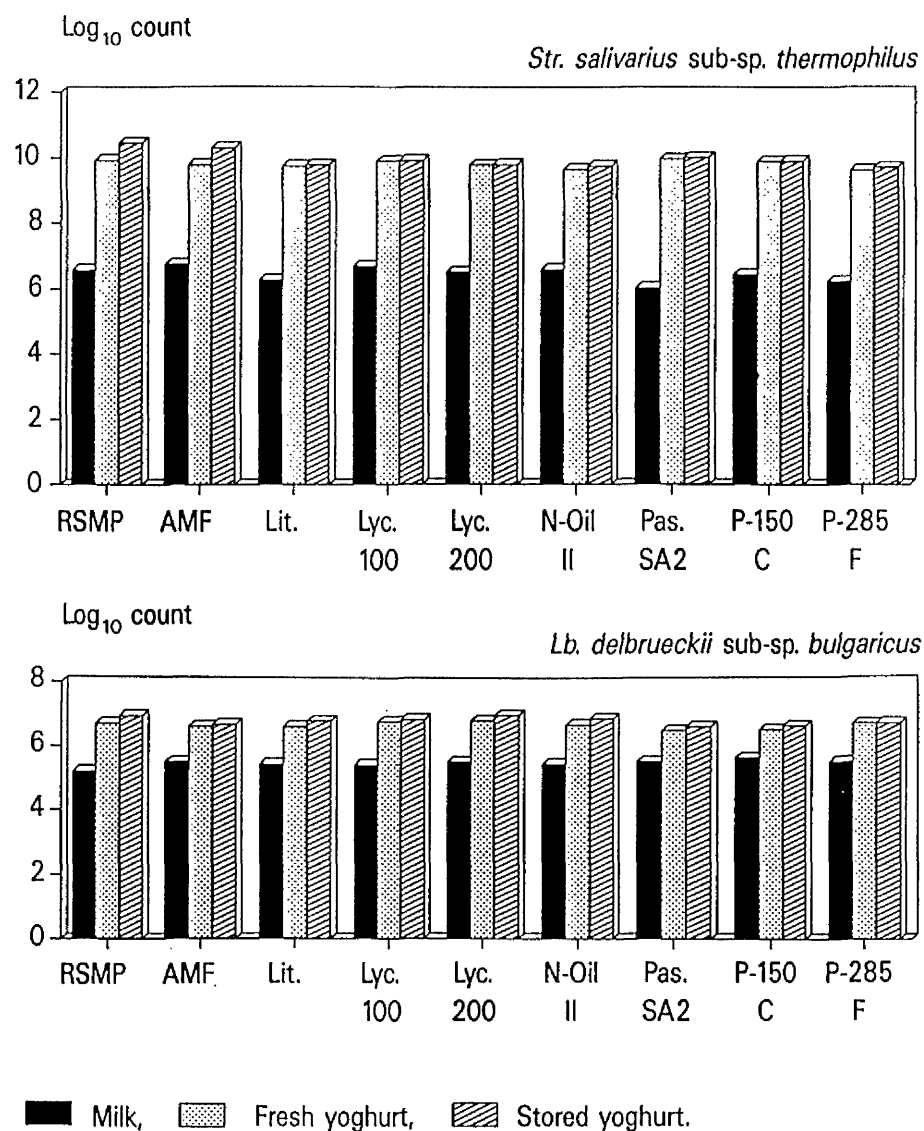
Figure 4.7   The enumeration of starter cultures during the production and storage of different types of yoghurts[a].

[a]   Results are average of two trials

the nine yoghurts at this first observation date. This was due to the large variation between samples of the same yoghurt (SED = 0.69). During storage the syneresis scores decreased to values between 1.3 and 3.0 ml 2 h^{-1} with P-Fibre 150 C assuming the minimum value. The unexplained variation remained high (SED = 0.55) and the differences between the yoghurt means was not statistically significant (P> 0.10).

An analysis of the data over the entire storage period revealed that it was likely that the differences observed between the yoghurts was greater than we would expect by chance (0.10>P> 0.05). This was mainly due to the P-Fibre 150 C yoghurt having lower values than the rest.

Using orthogonal polynomials the decrease in syneresis with time was modelled. It was found that the reduction was linear but that the linear component was marginally different for each yoghurt (0.10 >P> 0.05). This was mainly due to the rate of decrease for the P-Fibre 285 F and N-Oil ® II being greater than the rest. Inspection of the raw data suggested that the rate of decrease might be greater at the beginning of the storage period than towards the end of the 20 days. This effect was added to the model by including the quadratic polynomial component of time. This improved to fit significantly (P< 0.05). The curvature was not significantly difference for these yoghurts (P> 0.25). Fitted values from this model have been plotted in Figure 4.8.

It is evident that the presence of fibre in the yoghurt improves the extent of water hydration of the product. However, P-Fibre 285 F yoghurt did not have the same effect on syneresis as P-Fibre 150 C possibly due to its chemical composition and functional properties (Barrantes et al., 1993 - in press - see also section 4.1.4). When the serum separation data for the P-Fibre yoghurts were omitted from the analysis, no significant differences were observed between RSMP, AMF, Litesse™, Lycadex® 100 and 200, N-Oil® II and Paselli® SA2 yoghurts.

4.4.2 Measurement of Firmness

The firmness of measurements of different yoghurts is shown in Figure 4.8 and Appendix VII illustrates the results in each trial. At the beginning of the storage period, the firmness measurements ranged between 1.7 and 2.2 N with no evidence to suggest that there were any statistically significant differences between them (variance ratio test; p> 0.80). The firmness of each yoghurt increased with time to values ranging between 2.0 and 2.7 N at the final sampling date (Figure 4.8). Analysis of variance performed
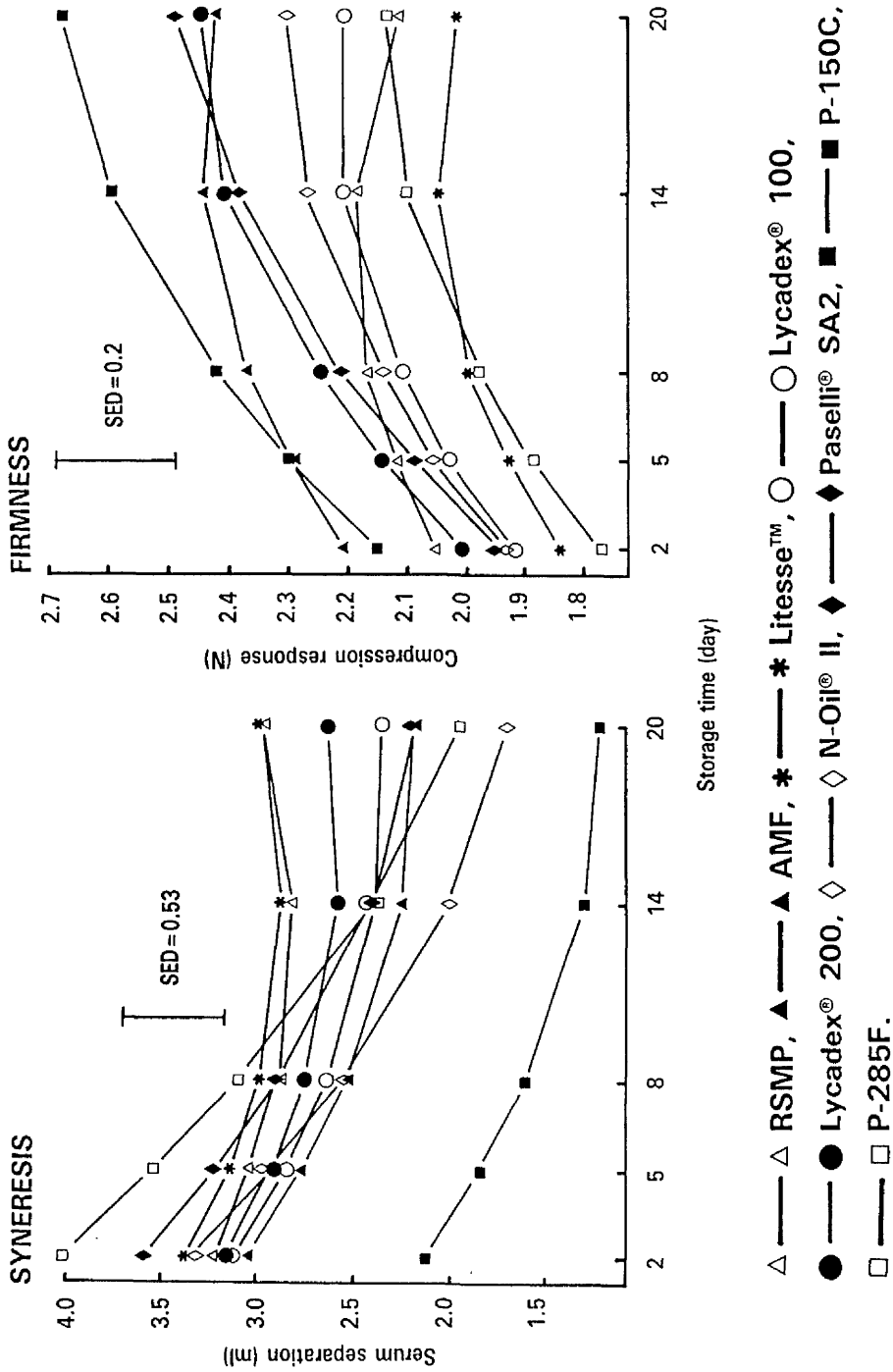
Figure 4.8   Changes in syneresis and firmness of yoghurts[a] during 20 days storage at 5°C.

[a]   Results are average of two trials.

on the stored samples data suggested that the observed differences were statistically significant (P< 0.05) and that P-Fibre 150 C was the firmest.

The data were then analysed as a whole with the increase in firmness being modelled by orthogonal polynomials of time. It was found that the yoghurts' means were not significantly different (P> 0.10). However, the way in which the firmness of each yoghurt changed with time varied significantly. In particular, the linear component of the increase was significantly different for the yoghurts (P<0.01) and in particular RSMP and Litesse™ yoghurts (i.e. decreased in firmness after 8 and 14 days storage respectively), but single quadratic term modelled the change in the rate of increase sufficiently well. Fitted values from this model have been plotted in Figure 4.8.

4.4.3 Combined Analysis of Syneresis and Firmness

The syneresis and firmness measurements provided complementary information concerning the rheological properties of the nine yoghurts (correlation coefficient = 0.543). In particular, both indicated significant changes with length of storage time and both identified the P-Fibre 150 C as being marginally or significantly distinct from the others. This alliance was confirmed by performing a Principal Component Analysis (PCA) , which examine the inter-relationships between several variables. The technique achieves this by participating the total variation of all the variables into components for each of a set of new variables. The first variable in this new set explains as much of the total variation as possible. The second new variable must be independent of the first and explain as much as possible of what is left of the total variations. Similarly for the third and fourth. These new variables are formed as linear combinations of the old variables, for example, the coefficients used indicate the weight given to each of the old variables in forming the new one. When all variables are positively correlated, all of the coefficients (loadings) for the first Principal Component (PC) will have the same sign.

The PCA performed on the two variables (i.e. firmness and syneresis) produced a single principal component (PC) which accounted for 77% of the total variation (i.e. essentially a weighted average of the two variables) (see Figure 4.9). Analysis of the obtained PC suggested that the difference between P-Fibre 150 C and the rest of the yoghurts was significant (P< 0.01).

As a measure of the degree of association between the rheological measurements (firmness and syneresis) and the chemical and 'physical' properties (protein and
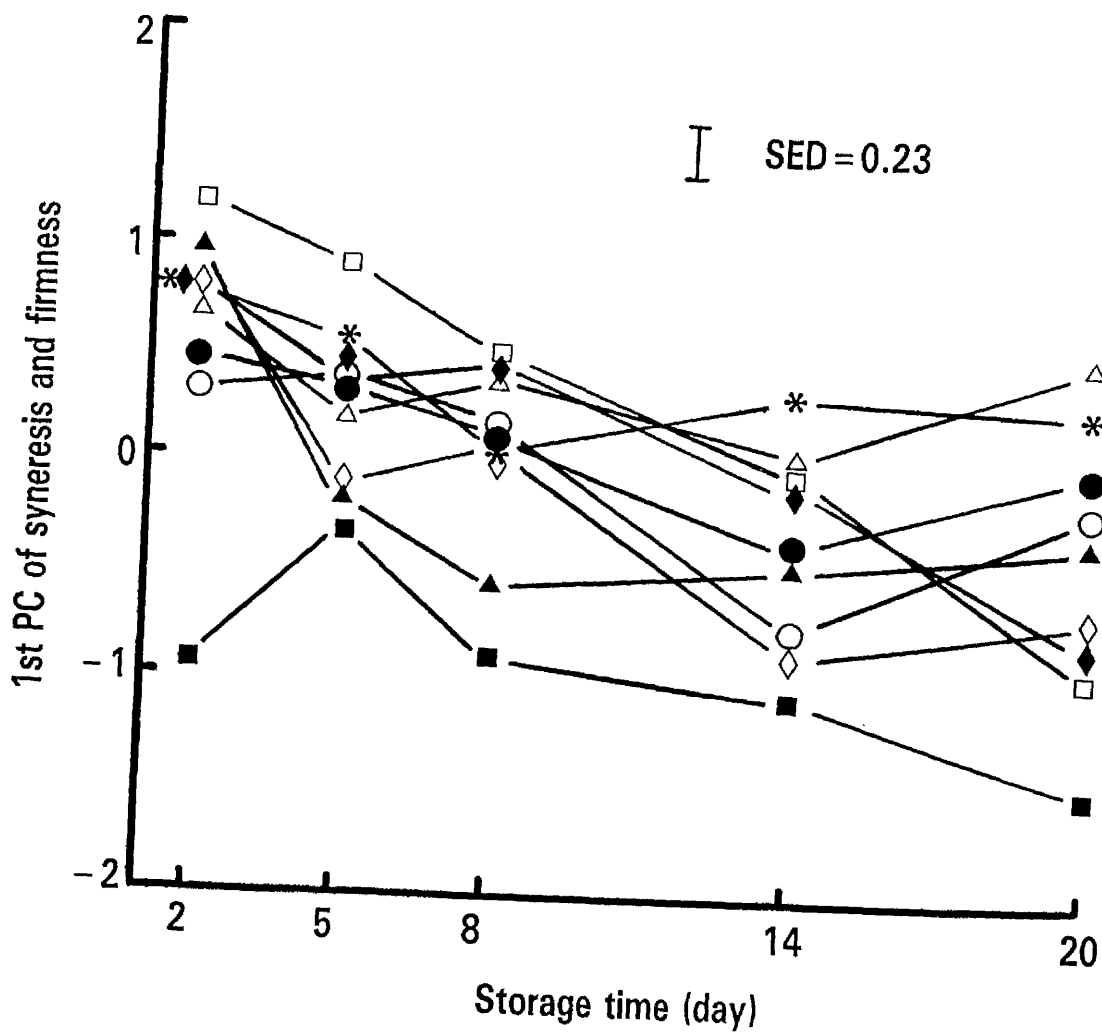
Figure 4.9   First Principal Component of syneresis and firmness measurements using correlation matrix.

viscosity) of the milks, correlation coefficients were calculated and displayed in Table 4.10. Yoghurts with a high milk viscosity tended to have a high firmness measurement after storage. Conversely, yoghurts with a high milk protein content tended to have a lower amount of serum separation. This applied both to fresh and stored yoghurts. Similarly yoghurt with a high milk viscosity tended to have a low syneresis value when fresh. Firmness was negatively related to syneresis for both fresh and stored yoghurts. However, the correlation was only statistically significant after storage.

4.5   Organoleptic Evaluation

The nine different yoghurts were assessed when fresh and after storage for 20 days at 5°C by ten trained panellist according to the scheme described in Appendix I (A). The average results of 2 trials for the organoleptic assessment of different types of yoghurts (fresh and stored) in terms of appearance and colour, body and texture, flavour and aroma, and overall acceptability is shown in Figure 4.10. From this data the following observations can be made:

<u>Appearance and colour</u>: all yoghurt samples (fresh and stored) had scores ∼ 3.

<u>Body and texture</u>: scores greater than 3 were awarded to most yoghurts with the exception of the fresh product made with P-Fibre 150 C in the first trial.

<u>Flavour and aroma</u>: In both trials the lowest scores (<5) were awarded to the yoghurts made with P-Fibre 150 C and 285 F. The panellists had a preference for the second yoghurt when compared with fresh parallel samples.

<u>Overall acceptability</u>: the pattern of awarded scores was similar to those observed for flavour and aroma.

When the attributes scores were analysed using the univariate analyses of variance (*i.e.* trial, type of yoghurt and fresh/stored product) the pattern emerging suggested the following: (a) Neither the effect of trial nor its interaction with the effect of storage were statistically significant. This merely suggests that the panellists were awarding similar overall mean scores for both trials and were consistently giving higher scores to the stored yoghurts than the fresh. Similarly, the interaction between yoghurt and the effect of storage was not statistically significant, which implies that all yoghurts in both trials tasted 'better' after storage; (b) The effect of storage was statistically significant for both appearance and colour (P< 0.05) and for overall acceptability; (c) The effect of type (or blend) of yoghurt and its interaction with trial were statistically significant (P< 0.05) for all four attributes mentioned above. The yoghurt effect was mainly due to the two P-Fibre yoghurts receiving relatively low scores in both trials. The

Table 4.10   Correlation coefficient of rheological properties of yoghurts with viscosity and protein content in the milk base

	Yoghurt				
	Fresh			Stored	
	r	Sig. [a]		r	Sig. [a]
Firmness x protein	0.048	NS		0.383	NS
Firmness x viscosity	0.104	NS		0.484	P<0.05
Syneresis x protein	-0.530	P<0.05		-0.495	P<0.05
Syneresis x viscosity	-0.478	P<0.05		-0.162	NS
Firmness x syneresis	-0.148	NS		-0.519	P<0.05

r: Correlation coefficient.

[a]:   Significant.

(NS)   Not significant.
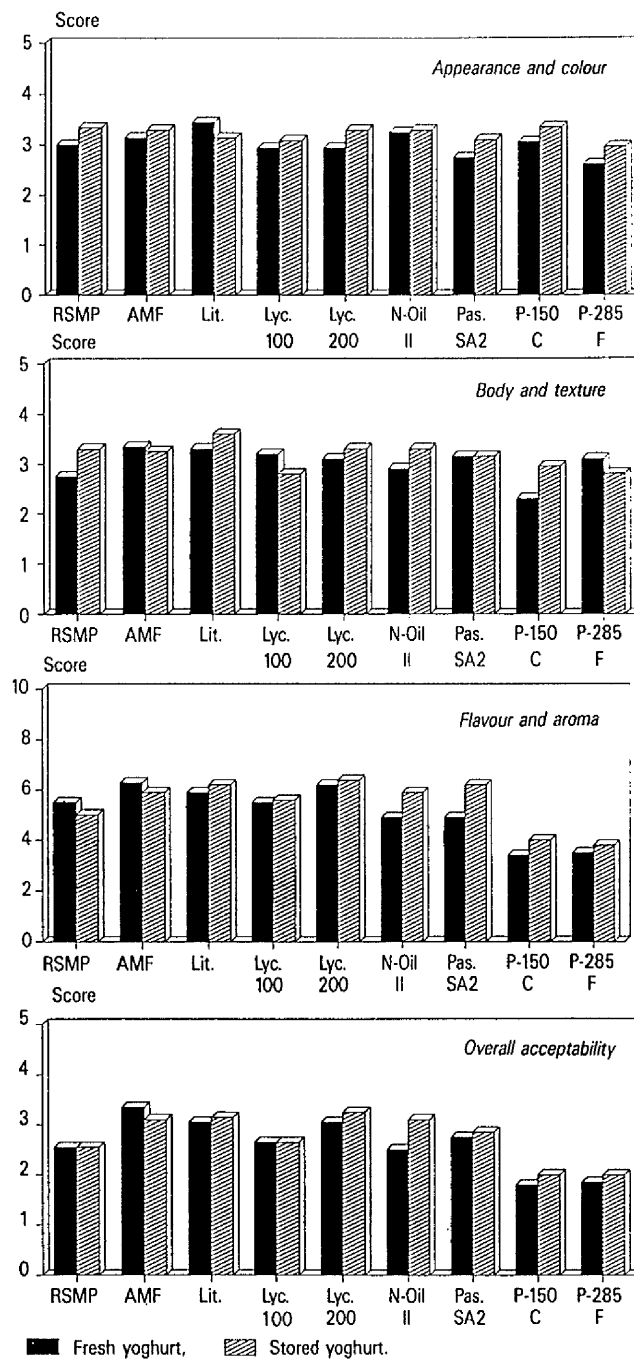
Figure 4.10 Average results[a] of 10 panellists for organoleptic assessment of fat-substitute yoghurts (fresh and after 20 days storage at 5°C).

[a]   Results are average of 2 trials.

interaction is due to the large and inexplicable discrepancy of scores awarded to N-Oil® II in the two trials (see Figure 4.11) and to the swapping of rank of the two P-Fibre yoghurts between trials 1 and 2. Although these two results are difficult to explain, the fact that both the flavour and aroma and the overall acceptability attributes show this so clearly we are inclined to believe it.

It is evident from the statistical analysis that the yoghurts, which appear to have the lowest scores, were the products made with P-Fibre 150 C and 285 F. This was mainly due to the detection of pea flavour in the product. Also the results for the overall acceptability scores by the panellists were very similar to the results of the flavour and aroma attribute (correlation coefficient = 0.835), and this suggests that the panellists were basing their acceptability scores almost entirely on flavour and aroma (see Figure 4.11).

In order to visualise the relationships between the 9 yoghurts and their inter-relationships with the 4 attributes, principal components analysis (PCA) was used and a PCA Biplot was produced for each trial (see Figure 4.12). In both trials a large proportion (65%) of the total variation is explained by the first principal component (PC) where the loadings for each of the 4 attributes are positive and similar in magnitude. This PC, therefore, gives some indication of overall preference and therefore separates the P-Fibre yoghurts from the rest in the first trial and this highlights the apparent reduction in popularity of N-Oil® II between trial 1 and trial 2.

The second PC accounts for a further 20% and 18% (in the first and second trial respectively) of the total variation where the loadings indicate that this PC is contrasting samples that score highly for the appearance and colour attribute with samples that score highly for the other three attributes. Although this PC accounts for a fairly large percentage of the total variation, very little of this variation can be attributed to differences between yoghurts (6% and 11% for the first and second trial respectively). This is demonstrated in the way the mean yoghurt scores are not well separated on the second PC.

AMF, Litesse™, Lycadex® 200 and N-Oil® II yoghurts scored highly on this PC, followed by RSMP, Lycadex® 100 and Paselli® SA2 yoghurts. As mentioned elsewhere the P-Fibre 150 C and 285 F yoghurts scored least on this PC (Figure 4.12).
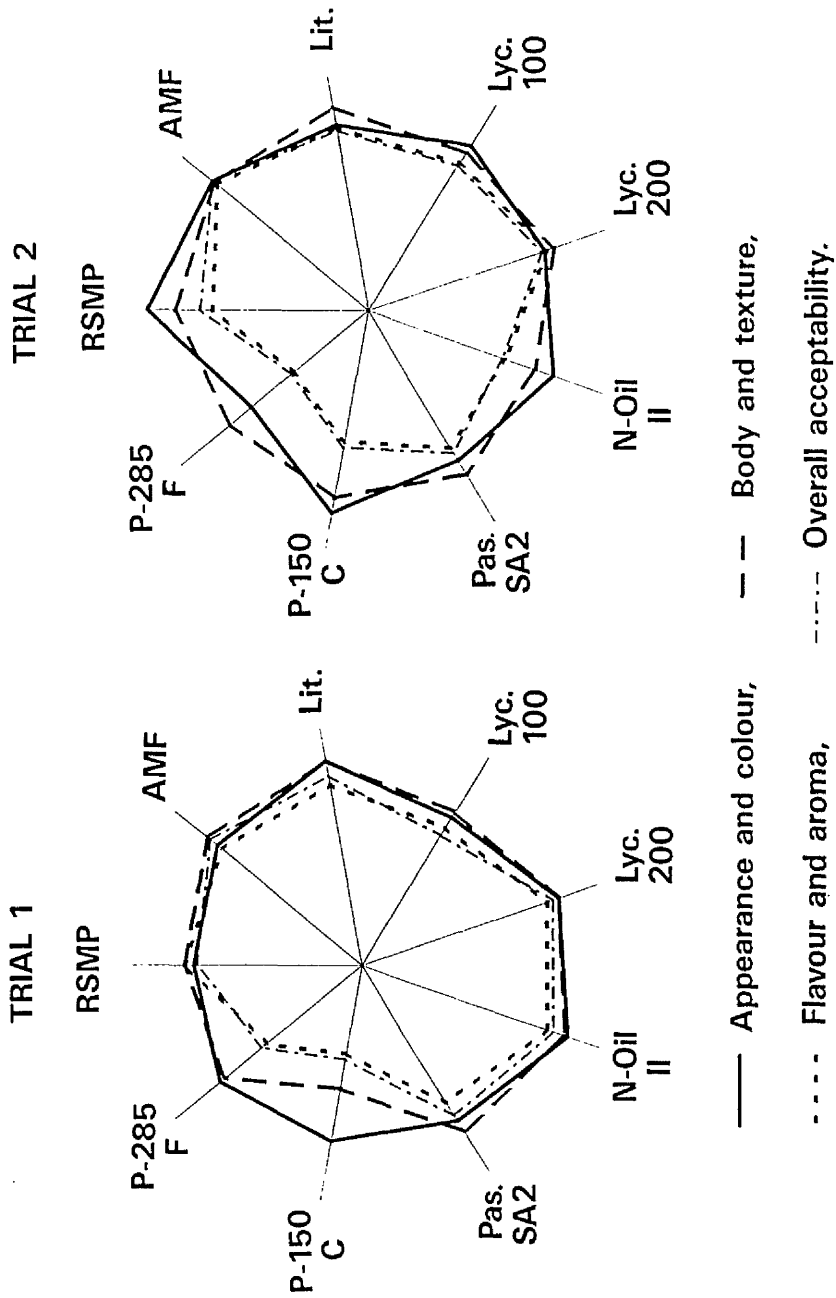
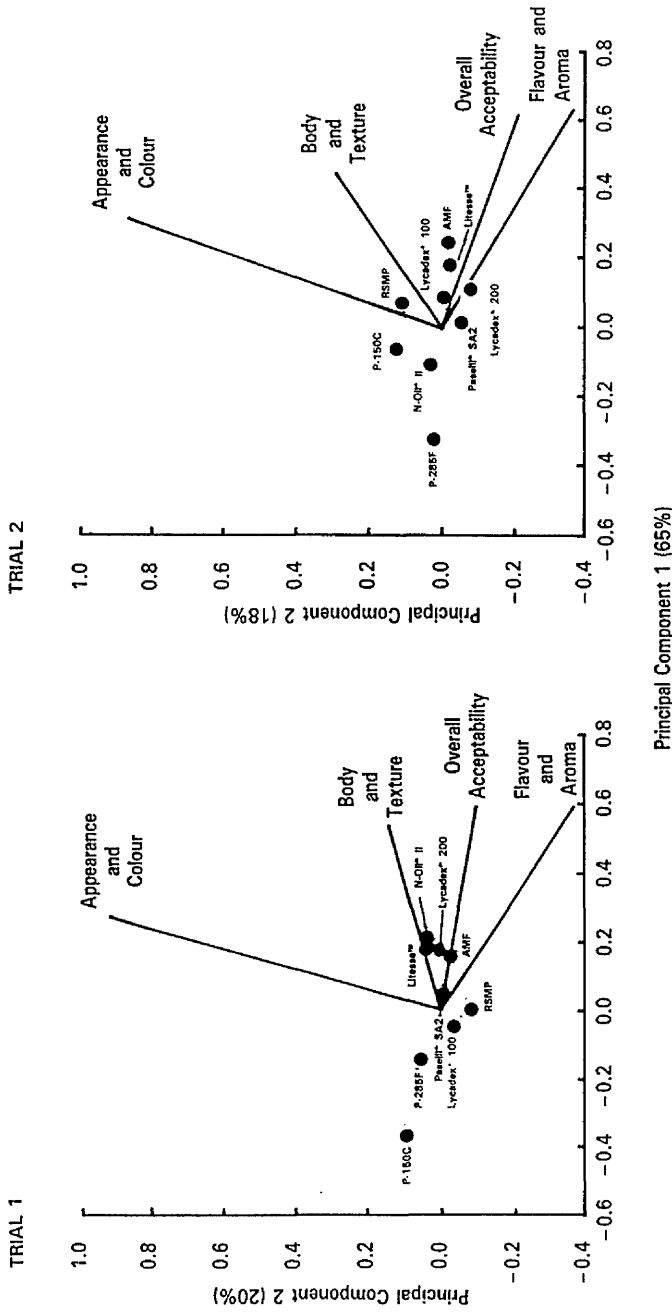Figure 4.11   Web diagram illustrating the organoleptic properties of yoghurts.

Figure 4.12 Principal Component Biplot.

According to the supplier of P-Fibre (Nutrio in Denmark) for low-calorie stirred yoghurt P-Fibre 150 F is recommended rather than P-Fibre 150 C. The former type is extracted from the cell wall fibre of the pea and is finer in particle size (K. Buckbjerg - personal communication). N-Oil® II yoghurt scored highly in the first trial when compared with the second trial. It is likely that the pH level could have caused this effect since it is recommended that for this type of fat-substitute the pH level should not be below 4.4.

No statistically significant correlation was observed between the rheological measurements of yoghurts (*i.e.* firmness and serum separation) with the organoleptic property of the body and texture scores of the panellists.

4.6   Microscopic Analysis

Microscopy is a valuable procedure for understanding raw materials and finished products which are important to the industry. These techniques are used to show the spatial distribution of components and the overall structure of the ingredients in the finished products. Microstructure studies have been used to investigate dairy and food products for many years and many papers have been published. However, the recent review by Kalab (1993) highlights the current advances of microscopy in dairy research.

In general, different methods of microscopy could be employed which include light, electron, X-ray microanalysis and image analysis. Optical microscopy is normally used for microbiological analysis and its application for studying dairy foods is limited because of its limited resolution ˜ 0.2 μm and the depth of focus is very shallow. However, light microscopy allows optical properties to be deduced and allows staining to reveal the location of starch, protein and fat. In contrast, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) extends the resolution to several nanometers where minute particles of dairy products (*e.g.* casein micelles, and fat globules membrane) can be seen (Kalab, 1993).

Thus, the structural material of the different fat-substitutes have been examined by light or optical and SEM while the microstructure of the yoghurt bases and the products were examined by SEM and TEM as described in section 3.11.8.

### 4.6.1 Analysis of SMP and Fat-Substitutes

The microstructure and characteristics of milk powder and other dried food ingredients are dependent on many factors such as processing methods, drying temperature or conditions and the chemical composition of the concentrates prior to drying (Mistry *et al.*, 1989). Figure 4.13 illustrates the SEM microstructure of skim milk powder and 7 different types of fat-substitute. The dried skim milk powder particles (Figure 4.13 A) are spherical in shape and widely ranging in diameter; however, the exterior of the particle is either smooth or wrinkled and many particles are hollow and porous. Frequently, some smaller dried skim milk powder particles are inside the large vacuole in the large particles. Some of the fractured particles of dried skim milk powder consisted of compact interior structure with few vacuoles (Figure 4.13 A). The structure of good quality skim milk powder, which was used for the manufacture of yoghurt, was similar to what was reported by other authors (Kalab, 1979; Saito, 1985; Caric and Kalab, 1987; Kalab *et al.*, 1989; Mottar *et al.*, 1989). The structure of the fat-substitutes used for the production of yoghurt is shown in Figure 4.13 B-H and a description based on light microscopy is as follows:

Litesse™: The particle shape is irregular with fairly rough outer surface which has some angular edges (Figure 4.13 B). The surfaces of the Litesse™ appeared to have fracture marks consistent with them having broken down. On mounting in iodine solution the particles dissolved. The possible size of such fat-substitute particle is up to 150 μm.

Lycadex® 100 and 200: The particle shape (Figure 4.13 C and D) resembles a typical spray dried milk powder (Figure 4.13 A) but the outer surface has a more angular appearance due to collapse during drying. Lycadex® 200 particle consists of larger centre vacuole rather than the small one shown in a skim milk powder particle. The particle size of Lycadex® 100 and 200 ranged between 10 to 75 μm and up to 120 μm respectively. On mounting in iodine solution the particles dispersed leaving air bubbles and a purple coloration.

N-Oil® II: The overall appearance is similar to Lycadex® 200, but the large particle surface contains more smaller particles (Figure 4.13 E). On mounting in iodine solution the particles dispersed leaving blue stained 'ghosts'.

Paselli® SA2: The particle structure is also similar to Lycadex® 200 and the size is up to 200 μm (Figure 4.13 F). On mounting in iodine solution the particles dispersed leaving coloured flakes and strands.
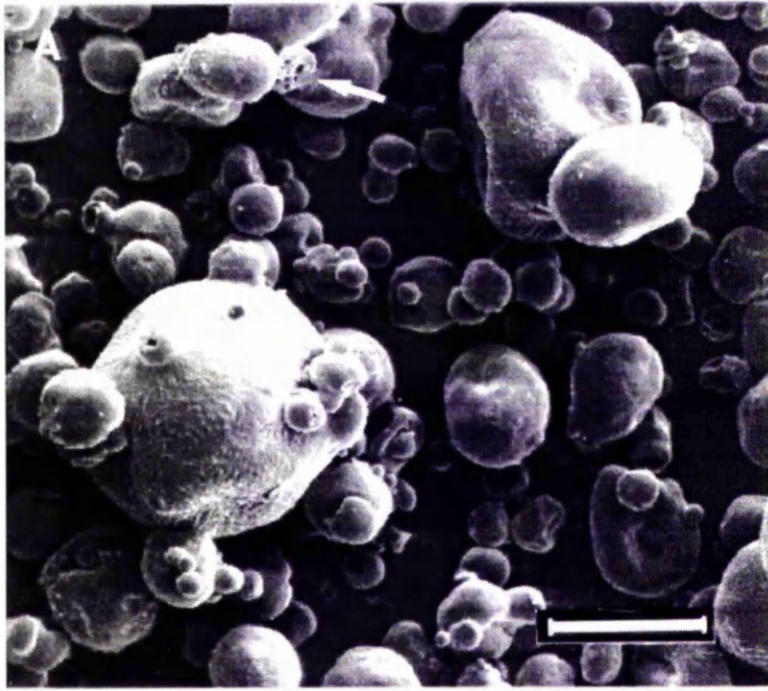
A)   Skim milk powder - the arrow illustrates a fractured particle and notice the compact interior structure.
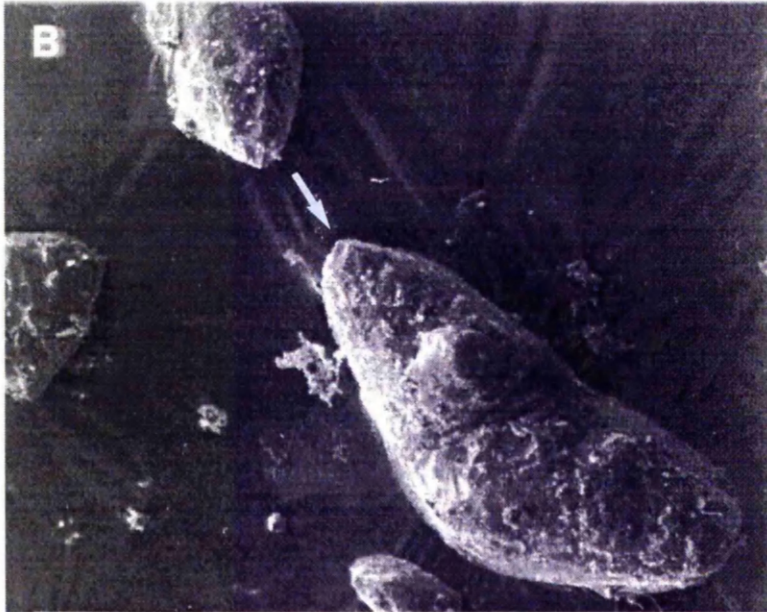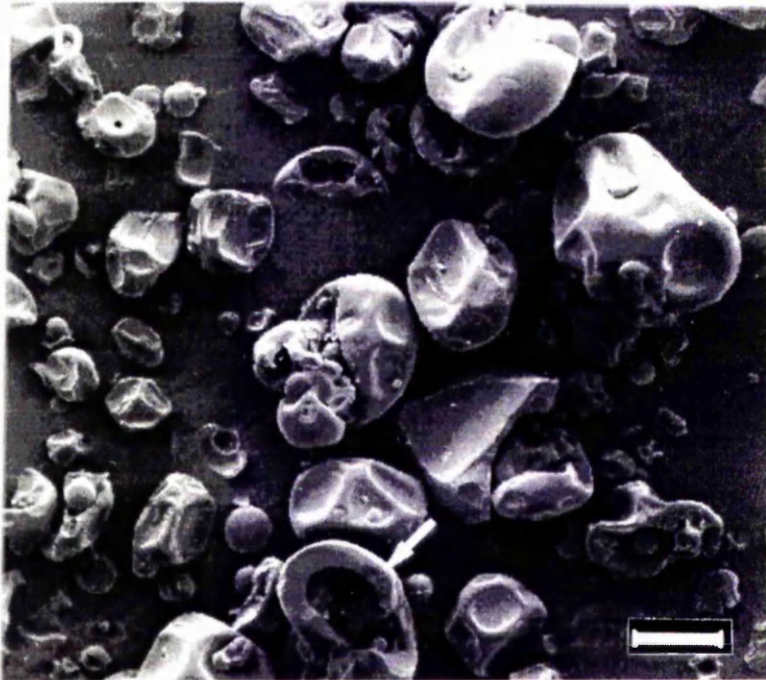

B)   Litesse™ - notice the angular edges (arrow).

Figure 4.13   Microstructure (SEM) of fat-substitutes used for the production of yoghurt (bar size = 50µm).
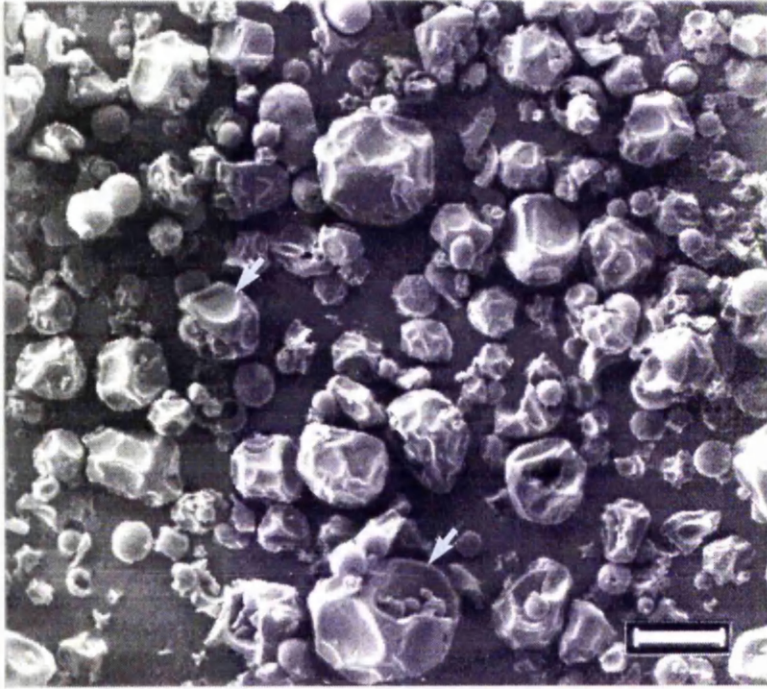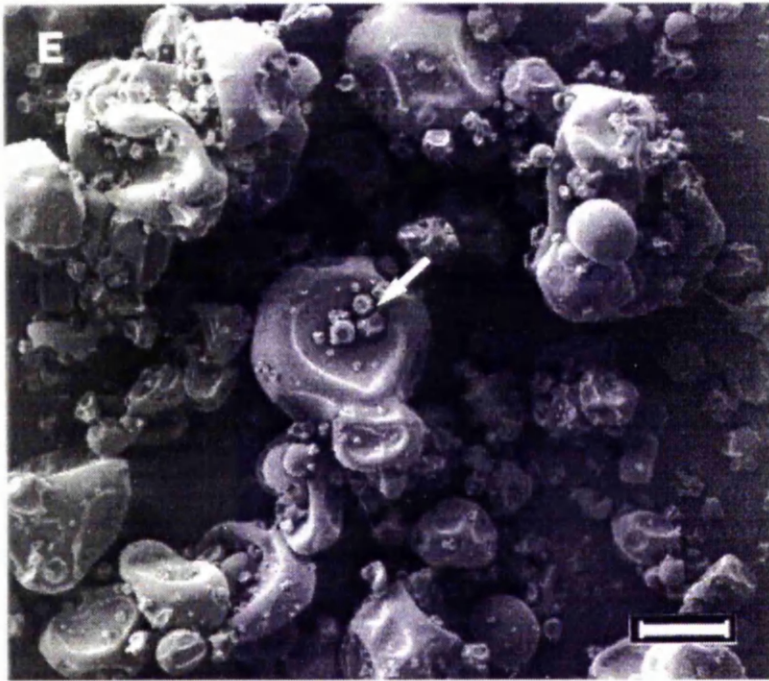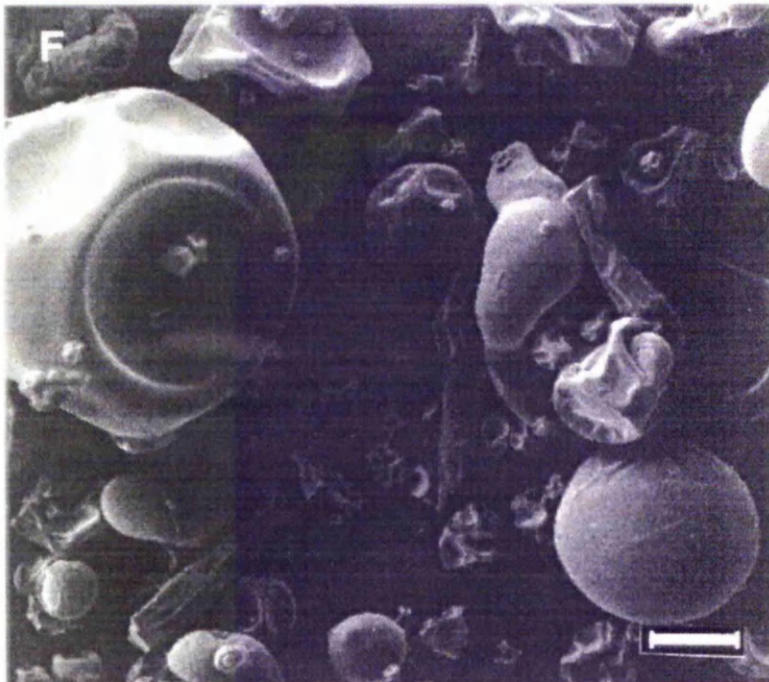
C)   Lycadex® 100 - arrows indicate that the outer surface angular appearance.

D)   Lycadex® 200 - arrow shows the large centre vacuole.
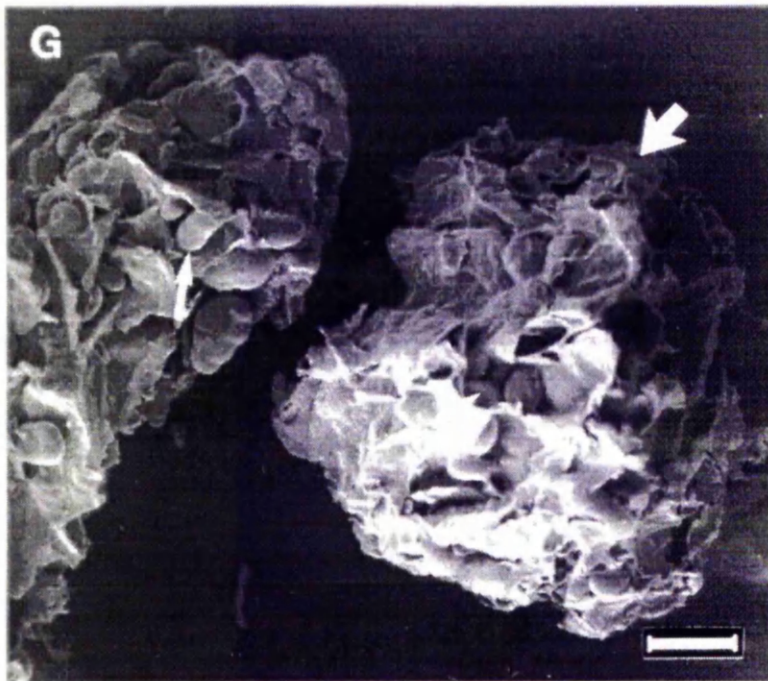
Figure 4.13   (continued)

E)   N-Oil® II - the angular outer surface appearance contains more smaller particles (arrows).

F)   Paselli® SA2 - notice the large particle size as compared with Lycadex® 200.

Figure 4.13   (continued)

G)   P-150 C - arrows (small) showing legume starch granules and (large) plant cell debris.
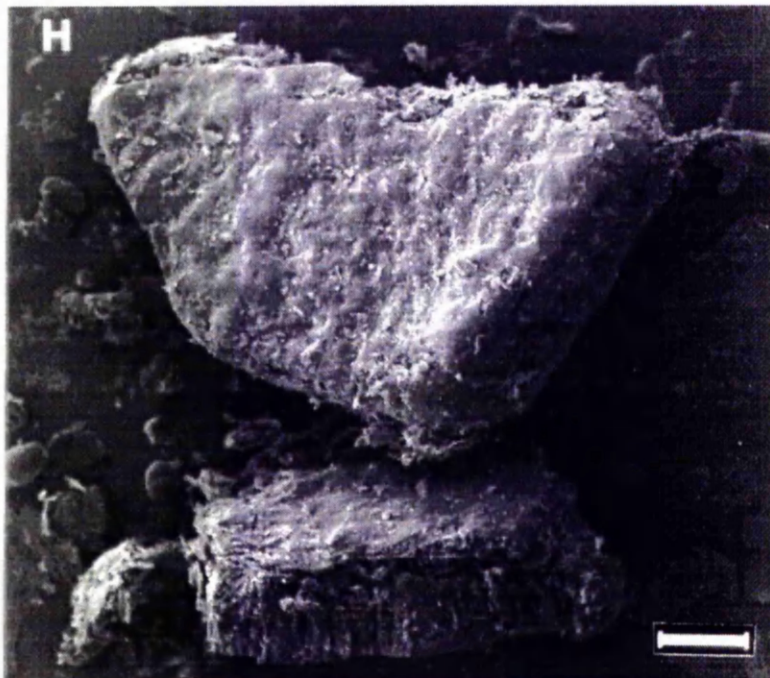
H)   P-285 F - notice the flake like appearance of the fibre particle.

Figure 4.13   (continued)

P-Fibre 150 C: The particle structure of Pea-Fibre 150 C consists of plant cell debris
containing typical legume starch granules. The particles are large in size ranging
between 400 to 500 μm (Figure 4.13 G), and the surface is more rough when compared
with P-Fibre 285 F. Some of the particles could be identified as from the palisade layer
of the pea (Winton and Winton, 1935). Other particles comprised more general
parenchyma cells with a fair amount of air included in the fragments. Staining with
iodine revealed starch grains within the parenchyma fragments. The starch grains were
mostly oval or kidney shaped and were around 40 μm in length. A few starch grains
were seen to be separate from the particles.

P-Fibre 285 F: The particle appearance is more flake like in nature and has smoother
surface when compared with P-Fibre 150 C (Figure 4.13 H). There were more smaller
particles in P-Fibre 285 F than P-Fibre 150 C, with a large number of particles being
less than 100 μm. Fewer starch grains were seen after staining with iodine and these
tended to be separate from the larger particles. As with P-Fibre 150 C palisade tissue
could be recognised as well as more general plant tissue.

4.6.2 Microstructure of the Yoghurt Base

The images of casein micelles in 9 different yoghurt bases were similar and resemble
those reported by other authors (Mottar *et al.*, 1989). However, the main differences in
the microstructure of these milks could be summarised as follows:-

(a)   The yoghurt base, which was made from reconstituted skim milk powder, had
'spikes' or 'hair' line structures on the casein micelles (Figure 4.14 A). This was due to
interaction between denatured whey proteins and κ-casein because the yoghurt bases
were heated to 90-95°C (Mottar *et al.*, 1989).

(b)   Milk containing anhydrous milk fat (see Figure 4.14 B) was different from the
rest of the samples. Cluster formation of the casein micelles is more evident
surrounding the milk fat globule. Also, the fat globule membrane becomes more
apparent surrounding the globule. The lighter areas in the fat particle may consist of
larger quantity of saturated fatty acids.

(c)   The search for the presence of fat-substitutes in the yoghurt bases was difficult to
observe possibly because these ingredients were used at low concentrations and they are
highly soluble. However, in some samples the fat-substitutes material appear in clusters
(see Figure 4.14 C and D) either heavily or lightly stained.
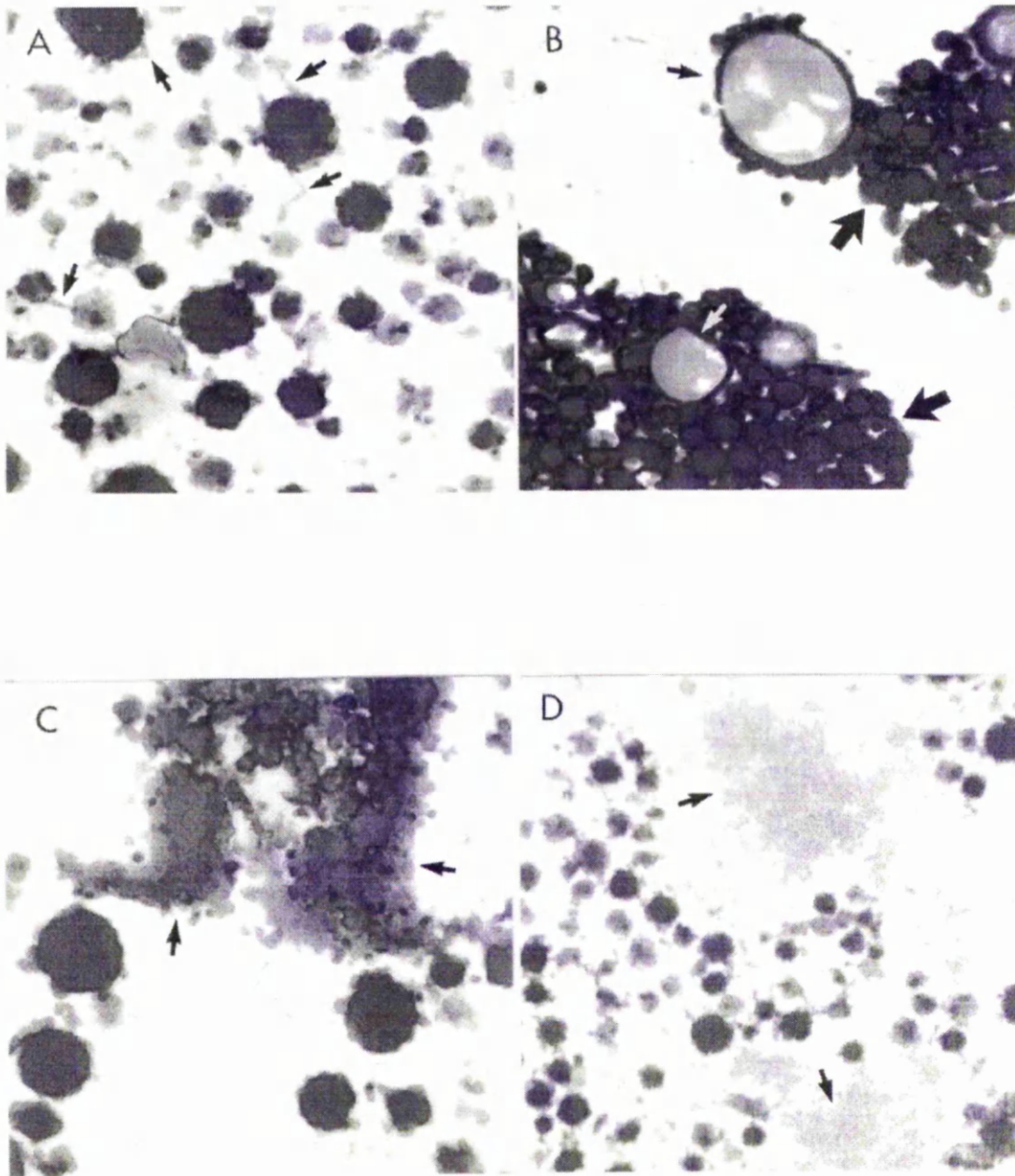
A) Reconstituted skim milk powder - 'hair' line
    structure (small arrows) on the casein micelles
    (11650 x).

B) AMF - notice the casein aggregates (large arrows) and
    fat globules (small arrows); the irregular lines throughout
    the sample are agar fibre artefacts (7190 x).

C) Lycadex® 200 - large aggregates of fat-substitute
    appear in clusters (arrows) (11650 x).

D) P-150 C - clusters of fat-substitute which lightly stained
    (arrows) (7190 x).

Figure 4.14   Illustrations of protein structure (TEM) in yoghurt bases.

(d)   'Spikes' and 'hair' line aggregates surrounding the casein micelles are evident in some samples (see Figure 4.15 A) which are lightly stained when compared with the milk proteins. Also, the milk containing P-285 F fat-substitute can be seen surrounding the bacterial cell (see Figure 4.15 B). The fine fibres, most probably of polysaccharide material can be see to radicate from the bacterium.

4.6.3   Microstructure of Yoghurt

No major differences in the microstructure of all the yoghurts (fresh and stored) when examined by SEM. The protein matrix of all the product was uniform and resembled the structure of the product made from skim milk powder only (see Figure 4.16 A). Such structure consisted of casein particle chains and clusters. The only noticeable difference was observed in yoghurt made with Lycadex® 100 where the protein matrix was less compact and consisted of slightly larger pore size in the fresh product (see Figure 4.16 B). However, greater aggregation of casein micelles was evident and the structure of yoghurt became more compact after the storage period (see Figure 4.16 C). This could be due to the greater interaction between the milk proteins and Lycadex® 100 as the pH drops slightly during the storage period (see Figure 4.17).

The subtle differences in the structure of the yoghurt bases (see Figure 4.14) could not be observed in the resulting yoghurts. The casein clusters in the yoghurt base became unfolded and the casein micelles have sharper outline, and an example is shown in Figure 4.17. Occasionally, some fat globules were evident in the structure fat-substitute yoghurts because the skim milk powder contain very small quantities of fat. Only the yoghurts made with P-Fibre 150 C and 285 F where the fat-substitute could be observed in the structure of the product (see Figure 4.18 A and B) when examined by TEM.

4.7   Consumer Acceptability of Strawberry Yoghurts Using Five Different Types of Fat-Substitutes

In the U.K., fruit flavoured yoghurts account to ˜90% of the total annual production (Anon., 1991) and strawberry is one of the most popular flavour by the consumer (Honer, 1993). As a consequence, strawberry yoghurt was made using five different types of fat-substitutes, and P-Fibres (150 C and 285 F) were excluded from this study for the following reasons: (a) due to difficulties experienced during the manufacture of natural set-yoghurt, and (b) these fat-substitutes were withdrawn from the market by the supplier.
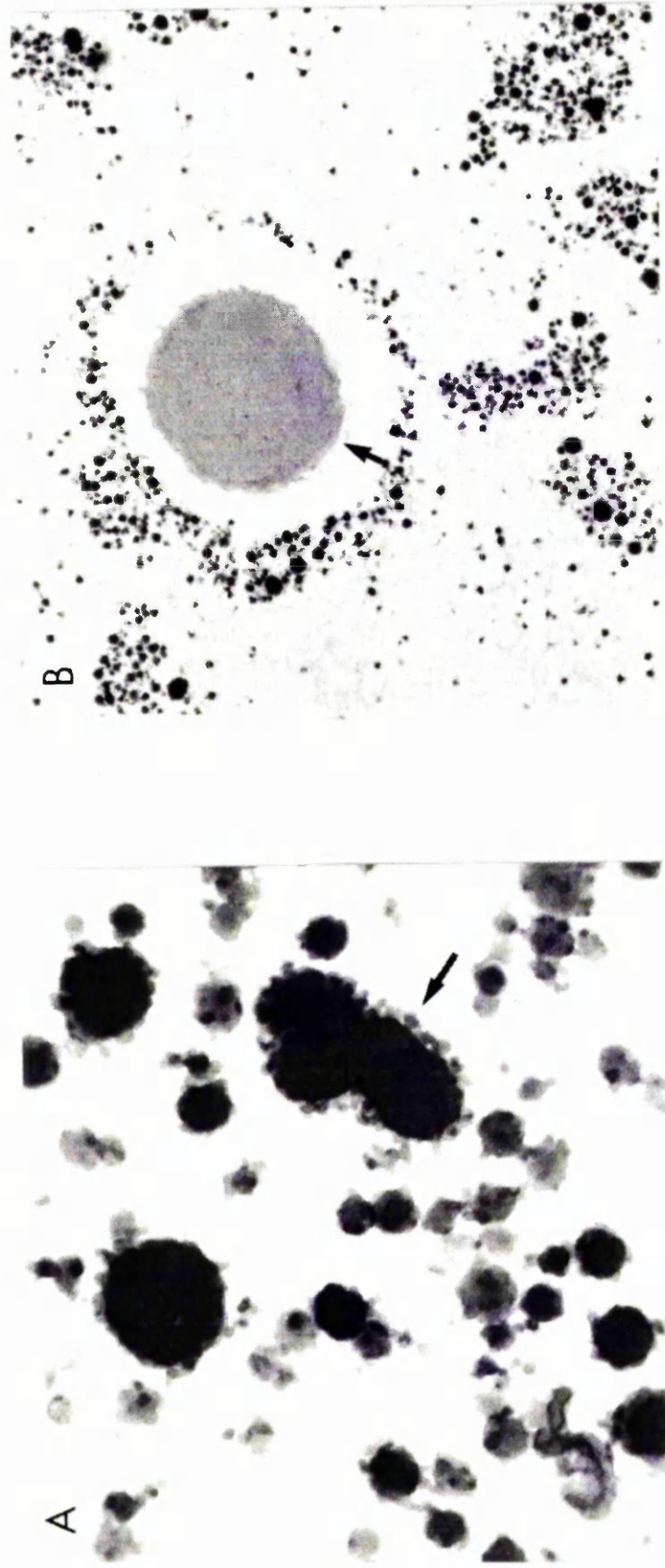
A)   Paselli® SA2 - arrows illustrate fat-substitute material (11650 x). B)   P-Fibre 285 F - arrows illustrate fat-substitute material (1940x).

Figure 4.15   Illustrations of some fat-substitute aggregrates surrounding the casein and bacterial cell .
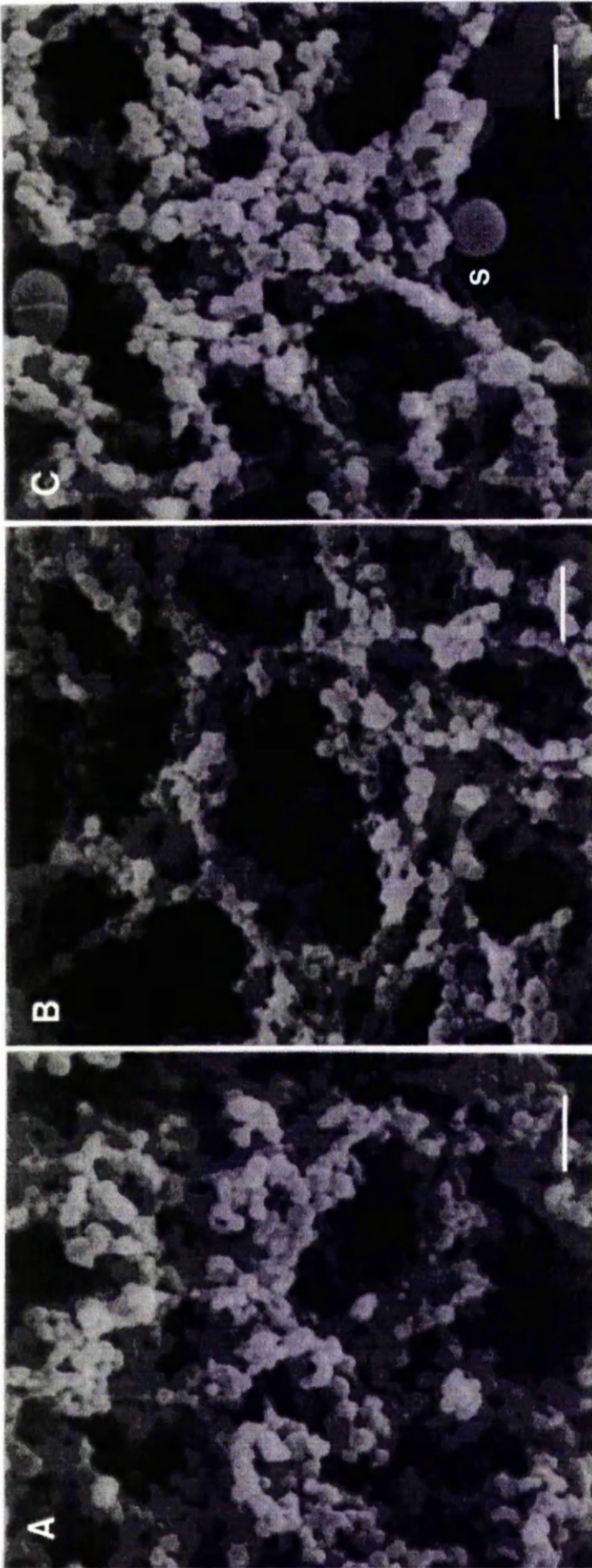
A) Stored skim milk powder yoghurt.   B) Fresh yoghurt containing Lycadex® 100.   C) Stored yoghurt containing Lycadex® 100

                                                                                                      s: streptococci.

Figure 4.16   Microstructure (SEM) of different yoghurts (Bar size = 1 μm).
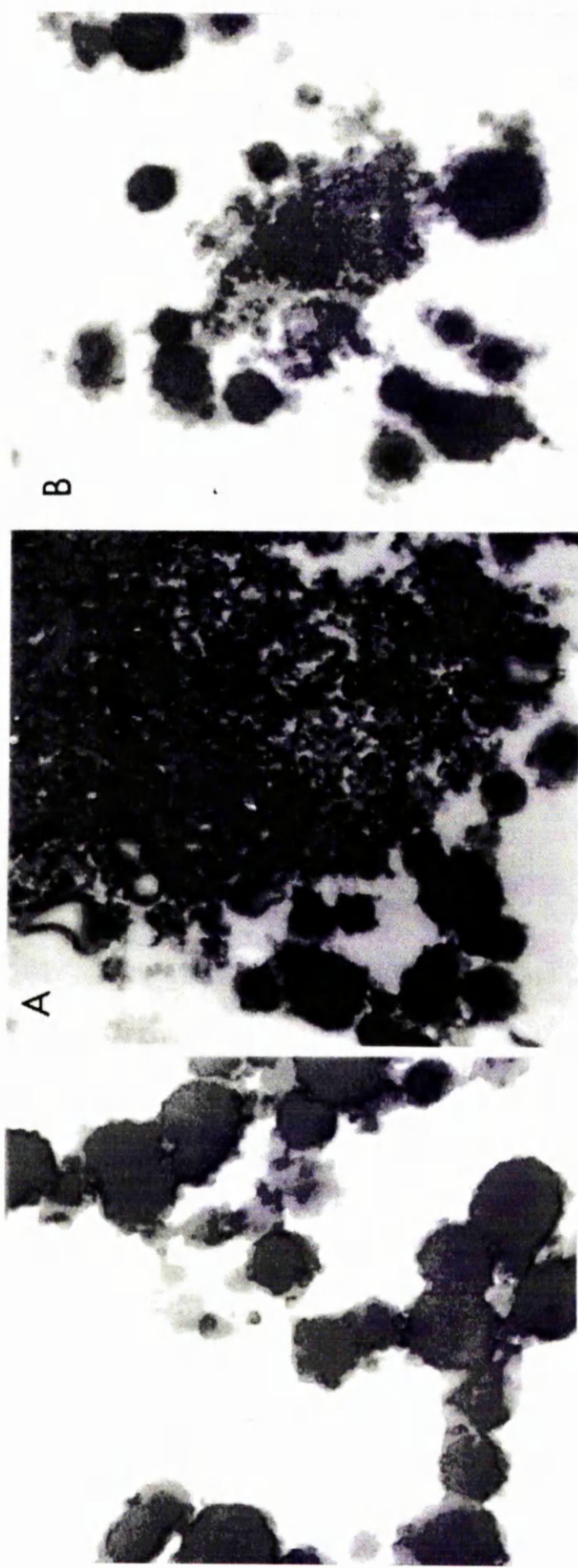
A) P-150C.

B) P-285 F - notice the large aggregrate structure.

Figure 4.17 Microstructure (TEM) of N-Oil® II yoghurt illustrating the sharper outline of casein micelles (11560 x).

Figure 4.18 Evidence in fat-substitute yoghurts (11650 x).

The yoghurt was produced from reconstituted SMP containing ~14% total solids and 1% sugar as described in Figure 2.2. A control batch, *i.e.* unfortified, was produced along with six batches fortified with anhydrous milk fat or fat-substitutes at a rate of 1.5% (w/w). The following fat-substitutes were studied: Litesse™, N-Oil® II, Lycadex® 100 and 200 and Paselli® SA2. The freeze dried starter culture MY-087 was used as direct-to-vat inoculation of the milk at a rate of 16 g 100 l^{-1}. The processed strawberry fruit was 187.6 k cal 100 g-1 (see section 3.10.11). The yoghurts were organoleptically evaluated by students and personnel at SAC- Auchincruive. The acceptability score, awarded on nine-point Hedonic scale (Appendix I -B).

4.7.1 Compositional Quality of Yoghurt

The compositional quality of the natural yoghurts were similar to those reported in Table 4.5 (*i.e.* ~14.0% solids-not-fat, 5.3% protein, 4.6% lactose and 1.7% total organic acids). Thus, the energy value of the strawberry yoghurts were calculated using the conversion factors reported by Holland *et al.* (1991). These values are shown in Table 4.11 and the calorific values were higher when compared with set-yoghurt because of the added sucrose (1% w/w) and strawberry flavour (15% w/w).

4.7.2 Microbiological Quality of Yoghurt

In this study, the coliforms, yeasts and moulds counts of the yoghurts were < 10 cfu g^{-1}. The level of total count of non-lactic acid bacteria in all the yoghurts tested was low (*i.e.* < 100 cfu g^{-1}). These results, for example, the virtual absence of coliform and the total count of non-lactic acid bacteria in 10^{-1} and 10^{-2} dilutions respectively from all the yoghurts analysed, indicate that the production of these samples were carried out under good hygienic standards and sanitary conditions (Tamime *et al.*, 1987; Barrantes and Tamime, 1992).

4.7.3 Organoleptic Evaluation

One hundred and eighty two consumers of yoghurt were (self) selected from staff and students at SAC - Auchincruive to sample seven types of yoghurts and score each for overall acceptability using the nine point Hedonic scale. The yoghurts were presented in a fixed sequence, determined by a design based on Williams' squares (Macfie *et al.*, 1989) to balance any effect of order of presentation and carry-over effect.

Table 4.11   Approximate calculation of the calorific content (k cal 100 g-1) of strawberry flavoured yoghurts containing fat-substitutes

Product	Yoghurt	
	Set/Natural[a]	Stirrred/Strawberry[b]
RSMP	50.3	76.3
AMF	62.2	84.4
Litesse™	51.6	77.5
Lycadex® 100	55.4	81.7
200	55.1	81.7
N-Oil® II	55.6	81.7
Paselli® SA2	55.6	81.7

[a] Data compiled from Table 4.8.

[b] The yoghurts contain sugar and strawberry flavour at a rate of 1% and 15% w/w respectively.

Four personal characteristics were recorded for each consumer: age, sex, weekly consumption of yoghurt and permanent address. For the purposes of the analysis the personal details were categorised in the following ways. Age was coded as: (a) less than 20 years, (b) between 20 and 30 years, and (c) greater than 30 years. Permanent address was reclassified into three regions: (a) living in Strathclyde, (b) not living in Strathclyde but within the U.K., and (c) living outwith the U.K. Weekly consumption was divided into: (a) less than or equal to 3 pots per week, (b) 4 or 5 pots per week, and (c) more than 5 pots per week. Sex was retained as male or female. The overall scores of the consumer tasters is shown in Figure 4.19. It can be observed that the majority of consumers scored the yoghurts between 5 and 9 points (*i.e.* 'liked').

The scores were analysed for order of presentation, effects of yoghurt, personal characteristics and the interaction of yoghurt with personal characteristics. Yoghurt mean scores ranged between 5.4 (Lycadex® 100) and 6.5 (AMF) (Figure 4.20). The variations between the yoghurt means was significantly greater than would be expected by chance (Wald statistic = 36.5; df = 6). Order or presentation of the samples was also found to be significant ($P < 0.05$) (Figure 4.21), in particular, yoghurts tasted first (6.3) and last (6.2) tended to score higher than the others (range 5.6 to 5.9; SED = 0.16), but this is overcome by the appropriate order of presentation of samples, based on Williams squares. There was no evidence to suggest that: (a) males preferred different yoghurts from females, (b) a consumer's preference depended on how much yoghurt he/she habitually consumed, or (c) yoghurt preference was dependant on age (Figure 4.22). Similarly, the interaction between geographic location and yoghurt was insignificant; however, consumers from outwith the U.K. had a tendency to have a higher preference for N-Oil® II and AMF and a greater dislike for Lycadex® 100 and 200 than consumers from within the U.K. The number of outside U.K. consumers was small (approximately 3%).

4.8   Conclusion

The use of different types of carbohydrate-based fat-substitutes offer new possibilities for the manufacture of diet yoghurt. It could be argued, however, that the energy value of the product could be further reduced by adopting one or combinations of the following methods: (a) lowering the level of milk solids-not-fat in the yoghurt base, (b) adding low-calorie bulking agent in order to maintain the rheological characteristics of the product, (c) using sweetener to replace the added sugar in the yoghurt base, and (d) adding fruit flavours high in fructose rather than sucrose.
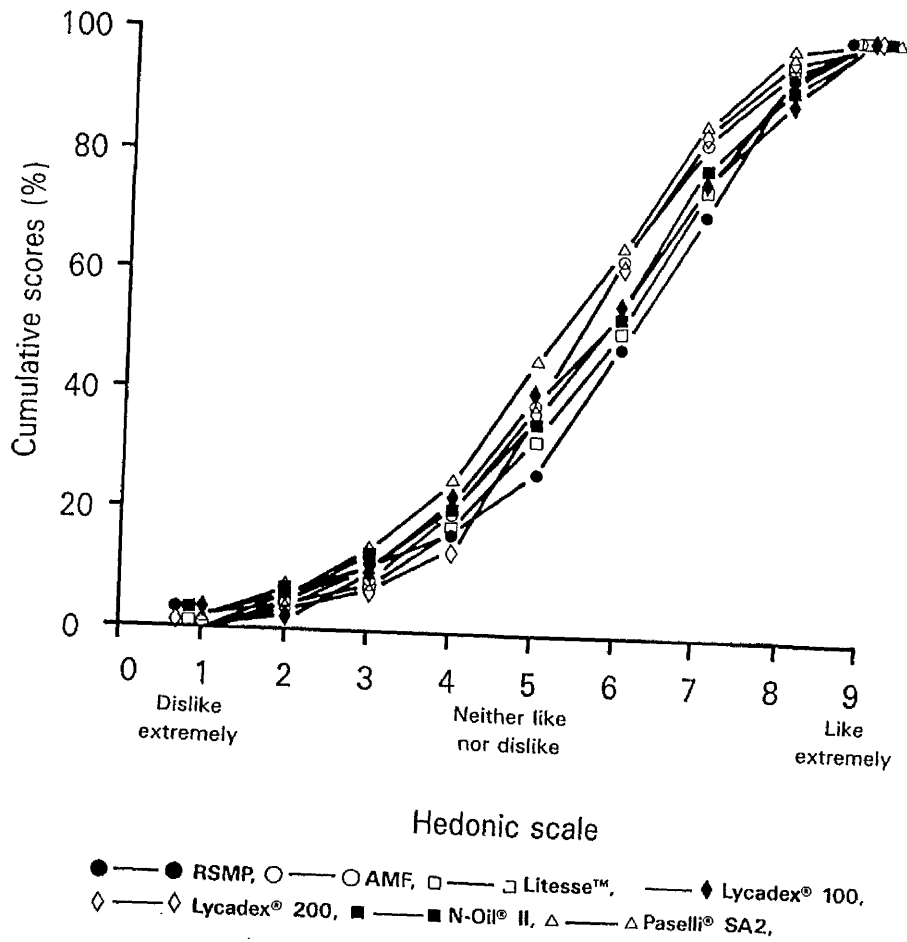
Figure 4.19 **Cumulative scores for organoleptic acceptability of different types of yoghurts.**
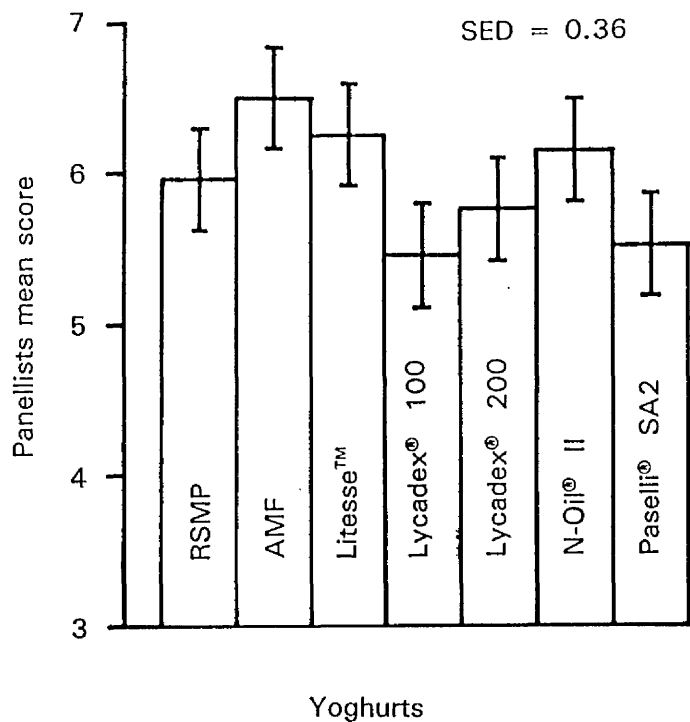
Figure 4.20   Yoghurt means scores of the consumers panel.

Sample means of presentation orderYoghurts
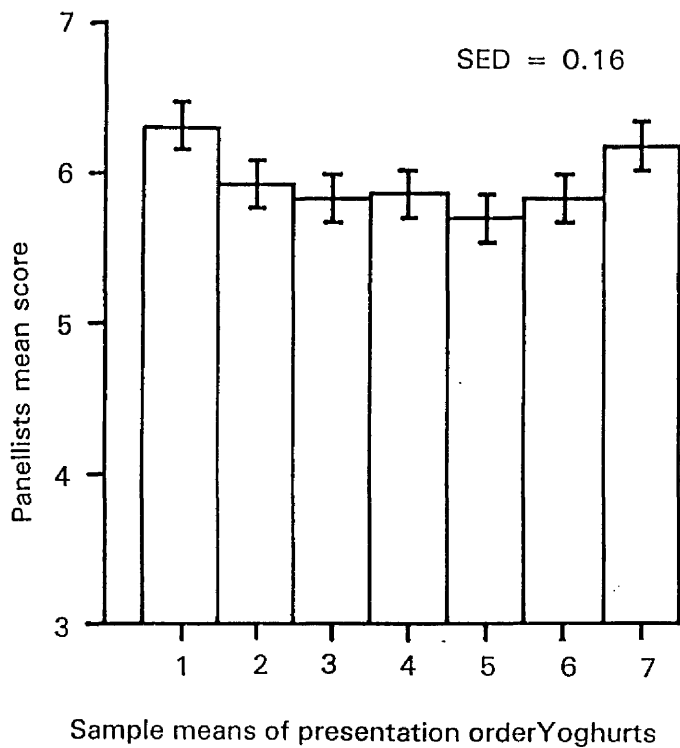
Figure 4.21   Effect of order of presentation of yoghurt samples on the consumer's scores.
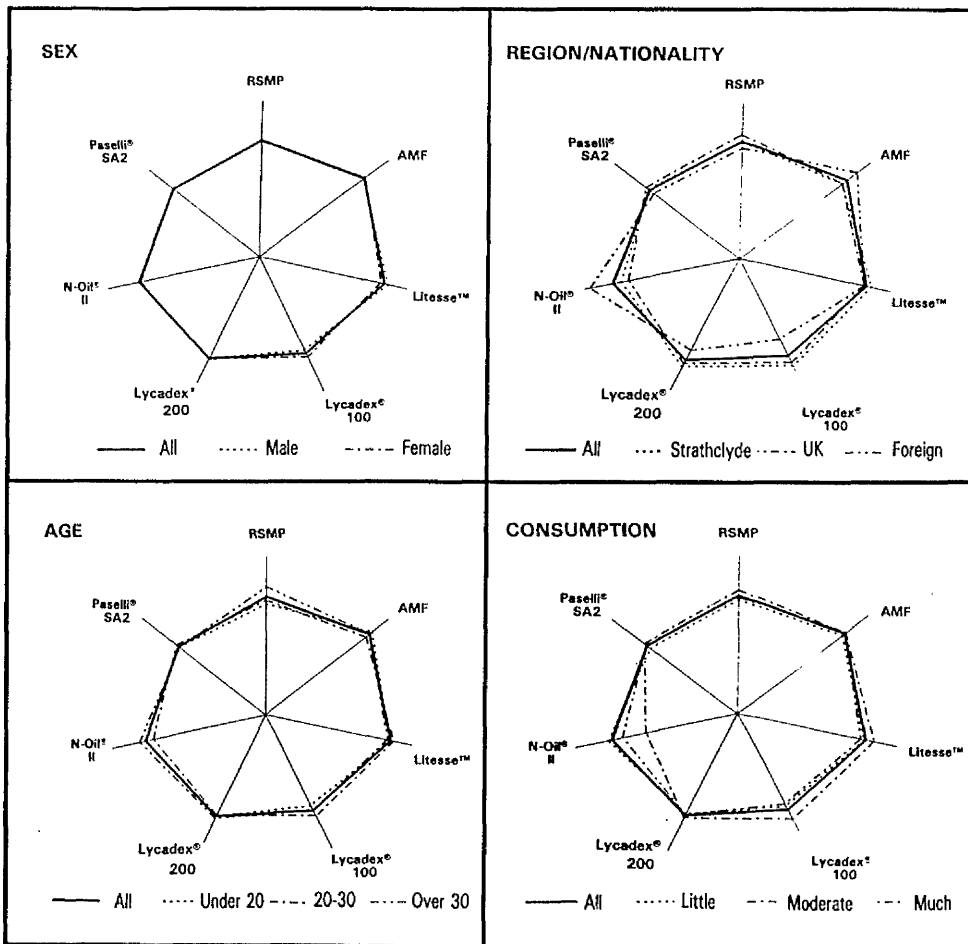
Figure 4.22   Web diagrams illustrating the organoleptic acceptability of fat-substitute yoghurts.

No processing difficulties were experienced for the production of fat-substitute yoghurts with exception of P-Fibre 285 F. No further investigations were conducted in order to overcome the precipitation of P-Fibre 285 F either in the yoghurt base or the product because this fat-substitute and P-Fibre150 C were withdrawn from the market by the suppliers.

All the majority of the fat-substitutes were suitable to produce good and acceptable natural set- and strawberry- yoghurts.

The use of fat-substitutes did not affect the activity of the starter culture during the fermentation period and the combined viable count of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* was high in all the yoghurt samples.

All the yoghurts made with different types of fat-substitutes (with the exception of P-150 C) were similar in their rheological properties to yoghurts made with RSMP and AMF. Serum separation/syneresis decreased in time while firmness increased with time. The rate of change was more rapid in the initial days of storage than at the end of 20 days.

Quantitatively the enzymatic and HPLC methods for the determination of lactose and lactic acid have to be evaluated further in order to overcome some of the analytical difficulties experienced in the yoghurt base or in the final products respectively.

Because the fat-substitutes were used at 1.5% (w/w) and highly soluble, their detection in milk and the parallel yoghurts were difficult to observe in SEM and TEM studies with the exception of P-150 C and 285 F. Subtle differences in the microstructure of the yoghurts could be observed and only the fresh product made with Lycadex® 100 had a slightly more open void structure when compared with the other yoghurts. It is possible to suggest that further studies are required to use these fat-substitutes at a higher rate to evaluate the quality of yoghurt and whether electron microscopy could detect these additives in the product.

CHAPTER FIVE:

PRODUCTION OF SET-TYPE YOGHURT USING DIFFERENT TYPES OF VEGETABLE OILS

CHAPTER FIVE: PRODUCTION OF SET-TYPE YOGHURT USING

DIFFERENT TYPES OF VEGETABLE OILS

5.1   Preliminary Studies

5.1.1   Quality of the Skim Milk Powder (SMP), Anhydrous Milk Fat (AMF) and Enumeration of Starter Culture

The same raw materials *i.e.* SMP, AMF and starter culture used for the production of yoghurt with fat-substitutes ingredients were utilised in this experiment. Hence, the specifications and analysis mentioned in sections 4.1.1; 4.1.2, 4.1.3 and Table 4.1 apply in this study.

5.1.2   Compositional Quality of Vegetable Oils

Four different types of vegetable oils were used to replace the milk fat in yoghurts (olive, groundnut, sunflower and corn). The moisture and peroxide values in these vegetable oils were determined as described in sections 3.6.1 and 3.6.2, and the results are shown in Table 5.1. The peroxide value measures the level of oxidation of fatty acids (peroxides), that can occur during the storage and handling of fatty products, especially poly-unsaturated oils. As shown in Table 5.1, the peroxide values ranged between 3.5 and 4.8 meq O_2 kg^{-1} and were well below those suggested by Kirk and Sawyer (1991), *i.e.* maximum 10 meq O_2 kg^{-1} for all the tested oils. However, Parodi (1979) and Sjollema (1990) reported that peroxide values above 1 meq O_2 kg^{-1} in vegetable oils used in milk products may enhance the detection of 'off-flavours', such as 'oxidised' or 'lipolytic'. The same authors also mentioned that once incorporated in 'filled' milk products the flavour of fat is quite stable and oxidation is prevented by natural antioxidants from the non-fat milk solids. Furthermore, the off-flavours in 'filled' milk products could be masked by the addition of flavours such as chocolate, fruits and fruit syrups. Lipolytic bacteria can accelerate the hydrolysis of fats and oils

Table 5.1 Quality control results of vegetable oils

Test		Oil		
	Olive	Groundnut	Sunflower	Corn
Moisture%	0.02	0.05	0.06	0.06
Peroxide value[a]	4.21	4	4.20	3.50
Total viable count (CFU g^{-1})	80	70	60	65
Plate count 55°C (CFU g^{-1})	<10[b]	<10	<10	<10
Enterobacteriaceace (CFU g^{-1})	<10	<10	<10	<10
Yeast and moulds (CFU g^{-1})	<10	<10	<10	<10
Lipolytic bacteria (CFU g^{-1})	<10	<10	<10	<10

[a] meq O_2 kg^{-1}.
[b] No growth at 10^{-1} dilution.

and their presence reflect the quality of these products. The results shown in Table 5.1 indicate that no lipolytic bacteria were detected at 10^{-1} dilution in the four types of oil used.

The water content in the oils ranged between 0.02 and 0.06% and comply with recommended specifications reported by Sjollema (1990), *i.e.* maximum 0.2%, in oils being used for recombination of milk products.

The spectrum of fatty acids contents (%) in AMF and vegetable oils were determined as described in section 3.5.3. Fourteen different fatty acids were separated by the GLC method and the results are shown in Figure 5.1 and Appendix VIII.

Saturated fatty acids (C4:0 to C12:0) were not detected in olive, groundnut, sunflower and corn oils, but were present in the AMF. The oils contained 0.25 to <1.0% of myristic and myrisoleic acids when compared with AMF containing 10% and 1.5% respectively. Sunflower oil and the AMF contained the lowest and highest values of palmitic acid respectively (Figure 5.1). Unsaturated fatty acids such as oleic in olive and groundnut oils, and linoleic in sunflower and corn oils constituted the highest proportion ˜55% to 63%. Similar fatty acids composition of fats and oils was reported by Pomeranz (1991) and Clark (1992).

5.2   Production of Set-Yoghurt

A total of three trials, *i.e.* fifteen batches of set-yoghurt using different types of vegetable oils were produced as described in section 3.3 and illustrated in Figure 2.2.

5.2.1 Stability of the Oil Emulsion

Due to the difference in the nature of vegetable oils when compared with AMF, Newstead *et al.* (1979) have reported that 'filled' milk may need to be processed at different homogenising conditions or require the use of lecithin or glycerol monostearate in order to stabilise the oil emulsion. However, no technical specifications were reported. Thus, in the present study a preliminary trial was carried out on the yoghurt bases (˜14% TS) containing 1.5% (w/w) olive, groundnut, sunflower or corn oil which were then homogenised at 60°C using three different pressures (17.3, 20.7 and 24.1 MPa respectively) and the yoghurt bases were then processed as shown in Figure 5.2.
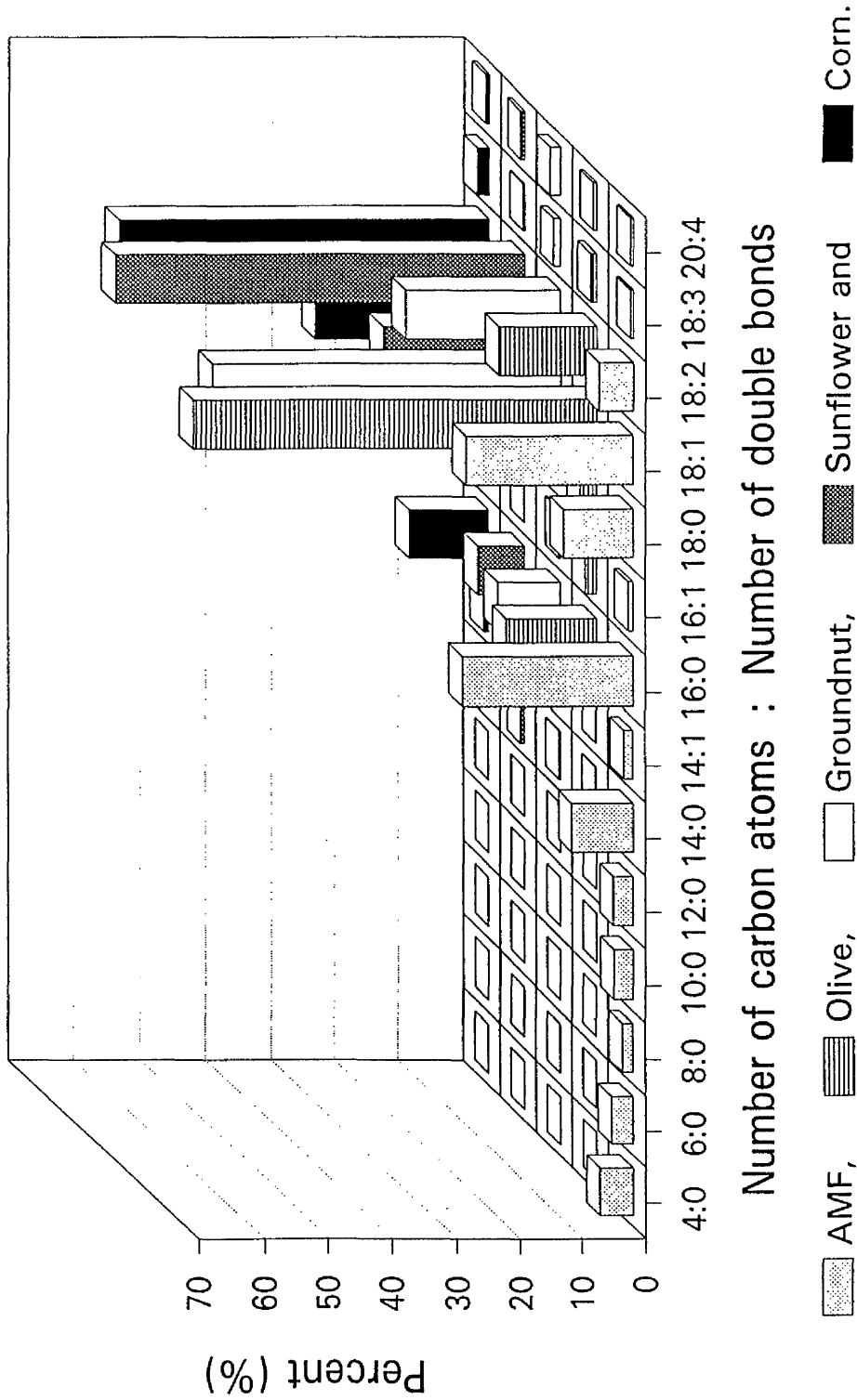
Figure 5.1   Fatty acids composition (%w/w) of anhydrous milk fat and four types of vegetable oils.

RSMP (˜14% T.S.)
↓
Addition of oil (1.5% w/w)
↓
Warm to 60°
↓
Homogenise at
↓

17.3 MPa   20.7 MPa   24.1 MPa

↓
Heat to 95°C for 5 min
↓
Cool to 45°C
↓
Dispense into cups
↓
Sample the yoghurt base from the top and bottom layers of the cup
↓
Place in an incubator at 42°C for 3 h
↓
Sample the yoghurt base from the top and bottom layers of the cup
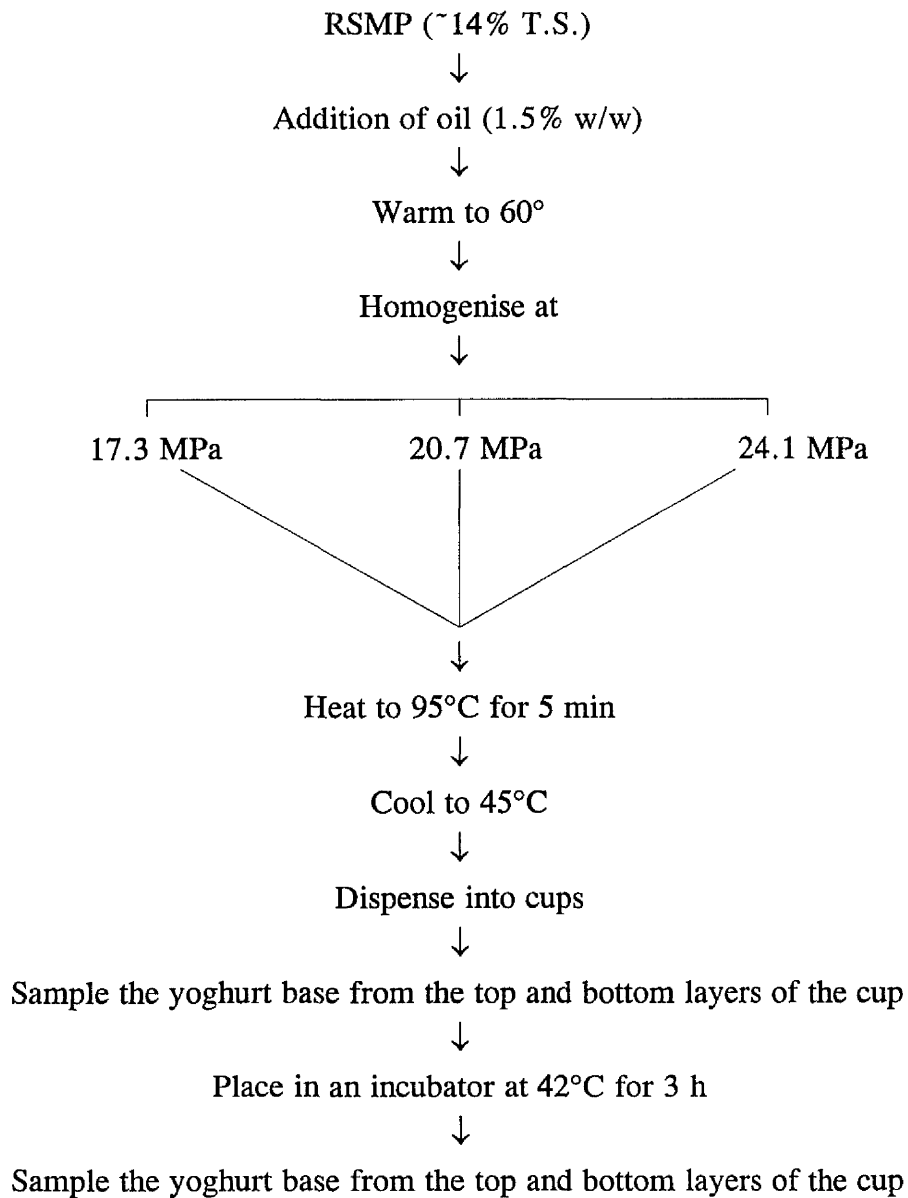
Figure 5.2   **Schematic preparation of yoghurt base for the measurement of oil separation.**

The oil content in all the yoghurt bases was analysed using Gerber technique on the top and bottom layers and any discrepancies in these determinations is indicative of unstable emulsion. The time chosen (*i.e.* 3 h) is sufficient because under normal yoghurt making the culture would have lowered the pH to a level where the gel starts to form thereby preventing oil droplets from rising to the surface.

Results of the four types of vegetable oils stability in yoghurt bases is shown in Table 5.2. The separation of the oil to the surface of the yoghurt base was not evident when using different homogenisation pressures. However, after 3 h duration at 42°C, slightly higher readings (no significant effect) for the oil content in the yoghurt base was observed (Table 5.2) which could be attributed to the analytical methods use (*i.e.* Gerber). It is evident from such trial that neither a stabiliser nor homogenising the yoghurt base at higher pressure was required during the production of yoghurt for obtaining a stable emulsion. Thus, it was decided to homogenise the yoghurt base at 17.3 MPa pressure which is similar to the homogenisation conditions that is used in the industry. Hefnawy *et al.* (1992) used the same homogenisation pressure during the manufacture of strained yoghurt (known as Labneh in the Middle East) made with different types of vegetable oils.

5.2.2 Viscosity of the Yoghurt Base

The pattern of viscosity for the five types of yoghurt base, after the heating stage, is shown in Figure 5.3 and Appendix IX. Since the protein content in the yoghurt bases were similar (Table 5.3), the viscosity in turn, has been influence by the type of fat or oil that was used. The highest viscosity in these trials (2.34 centipoise) was observed in the yoghurt base containing AMF. Analysis of variance of the viscosity results indicated that there was no significant difference between the trials, but there was only a marginal significant difference (variance ratio = 2.91; P> 0.093) between the type of fat/oils that were used.

5.2.3 Chemical Composition of Yoghurt Base

The chemical composition and acidity measurements of fifteen batches of yoghurt bases is shown in Table 5.3. The milk solids-not-fat were ˜14% and the total solids and protein contents ranged between 15.00 to 15.45% (SED = 0.11) and 5.30 to 5.41% (SED = 0.02) respectively. The fat contents were between 1.24 to 1.51% (SED = 0.07) and ash varied from 1.11 to 1.16% (SED = 0.02). The slight variation in these

Table 5.2   **Means of oil (%) distribution in reconstituted skim milk powder homogenized at different pressures and held at 42°C for 3 hours[a]**

	Olive	Groundnut	Sunflower	Corn
Homogenization pressure (MPa)				
17.24	1.79	1.50	1.59	1.59
20.69	1.56	1.28	1.68	1.62
24.14	1.58	1.36	1.59	1.65
Sampling position				
Top	1.64	1.37	1.62	1.61
Bottom	1.64	1.39	1.62	1.62
Duration (h) at 42°C				
0	1.62	1.40	1.60	1.60
3	1.66	1.36	1.63	1.64
SED[b]	0.012	0.072	0.027	0.058

[a] The yoghurt base was heated to 95°C for 5 min cooled to 45°C and the results are average of two determinations performed on the same sample.

[b] Standard Error of Difference.
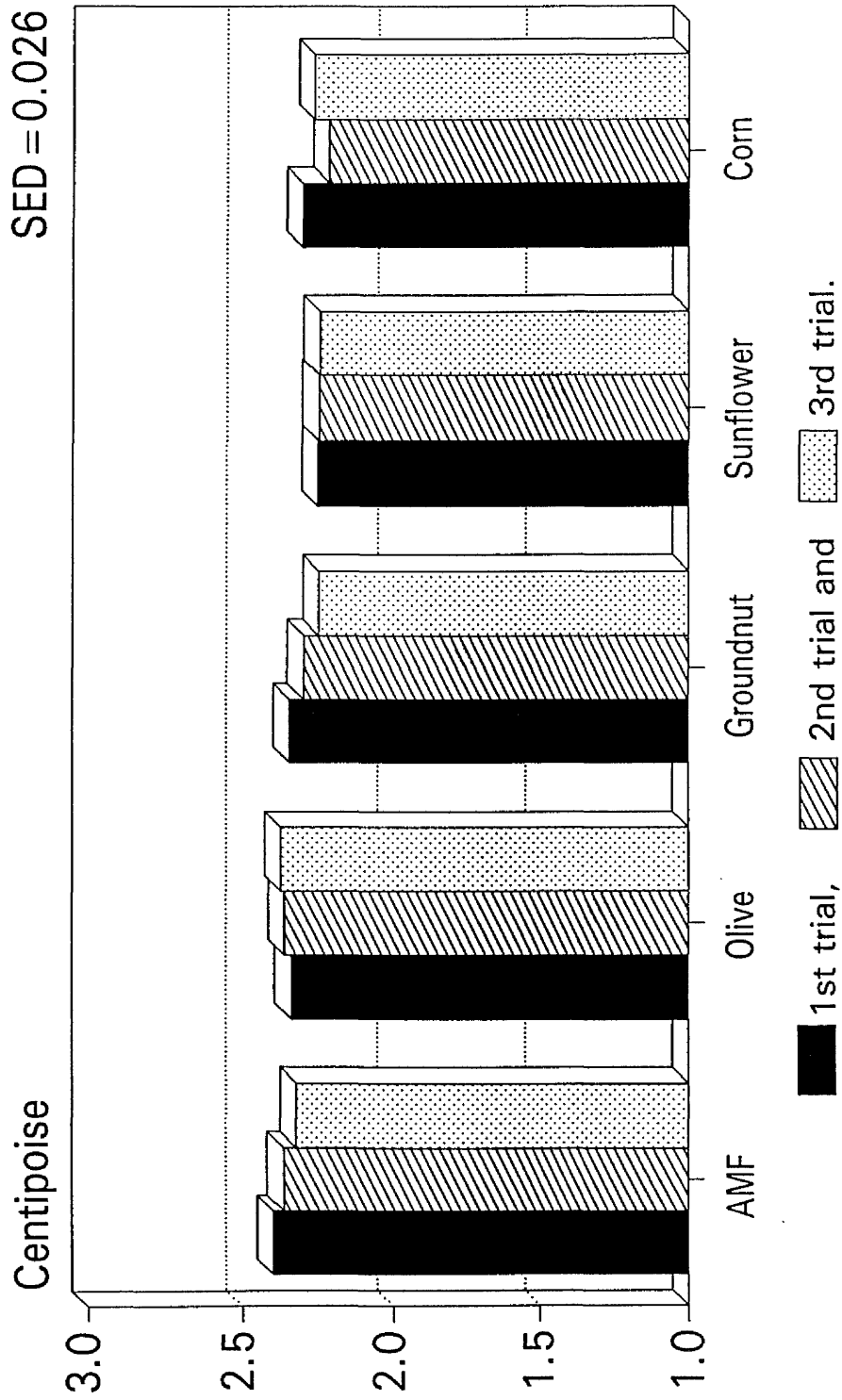
Figure 5.3   Influence of different types of vegetable oils on the viscosity of yoghurt bases.

Table 5.3   Chemical composition (%w/w) of yoghurt bases using different types of vegetable oils[a]

Product	Protein	Fat	Lactose	Ash	Total Solids	pH	Acidity T.A.[b]	HPLC[c]	Calorific value[d]
1st Trial									
AMF	5.28	1.47	7.18	1.11	15.41	6.22	0.25	0.22	63.70
Olive	5.32	1.38	7.16	1.12	15.36	6.15	0.23	0.22	63.00
Groundnut	5.31	1.49	7.32	1.12	15.53	6.28	0.21	0.21	64.60
Sunflower	5.28	1.30	7.37	1.13	15.17	6.58	0.24	0.23	63.00
Corn	5.30	1.39	7.21	1.12	15.39	6.33	0.25	0.25	63.31
2nd Trial									
AMF	5.38	1.47	7.23	1.12	15.36	6.46	0.19	0.28	63.63
Olive	5.41	1.24	7.15	1.12	15.00	6.42	0.22	0.25	62.33
Groundnut	5.35	1.24	7.16	1.12	15.01	6.45	0.20	0.27	63.06
Sunflower	5.37	1.44	7.18	1.13	15.19	6.50	0.21	0.28	61.80
Corn	5.33	1.29	7.18	1.13	15.01	6.46	0.20	0.29	64.29
3rd Trial									
AMF	5.36	1.51	7.21	1.13	15.44	6.29	0.22	0.17	64.38
Olive	5.39	1.32	7.21	1.15	15.04	6.32	0.23	0.17	62.80
Groundnut	5.30	1.47	7.18	1.15	15.15	6.26	0.26	0.18	63.70
Sunflower	5.38	1.27	7.24	1.16	15.11	6.48	0.27	0.18	62.46
Corn	5.34	1.37	7.20	1.15	15.34	6.48	0.27	0.18	63.04

[a] Results are average of two determinations performed on the same sample.
[b] Titratable acidity.
[c] Data compiled from Appendix XI.
[d] k cal 100 g^{-1}.

levels was mainly attributed to the preparation of the reconstituted skim milk powder and the added oils in each trial.

Owing to the problems found with the use of the enzymatic method mentioned in section 4.2.2 and the high cost of the sets of reagents and enzymes, it was decided to measure the lactose content by using the polarimeter method mentioned in section 3.10.3. As illustrated in Table 5.3 the lactose content of the different yoghurt bases were between 7.15 to 7.37% (SED = 0.05).

The acidity measurements *i.e.* pH, titratable acidity and total organic acids contents were similar in all batches and in the three trials, and averaged 6.38, 0.24% and 0.23% respectively.

5.2.4 Analysis of Fatty Acid in Yoghurt Bases

The fatty acid profile (Table 5.4 and Appendix X) in the yoghurt bases was determined by the method described in section 3.5.3, which is based on Gas Liquid Chromatography (GLC). The different fatty acids contents (mg 100g^{-1}) in yoghurt bases shown in Table 5.4 confirmed the following aspects: (a) the level of saturated fatty acid content in the yoghurt base made with AMF was the highest (834.4 mg 100g^{-1}) as compared with 201 to 274 mg 100 g^{-1} in vegetable oils yoghurt bases, (b) all the vegetable oil yoghurt bases contained very small quantities of saturated fatty acids (C4:0 to C12:0) originating from the skim milk powder, (c) the unsaturated fatty acid contents in all the experimental yoghurt bases were approximately twice the level present in AMF yoghurt base and (d) all the vegetable oil yoghurt bases had higher rations of poly- and mono-unsaturated to saturated fatty acids; linoleic and linolenic, which were high in vegetable oil yoghurt bases, have been identified as essential fatty acids (Gurr, 1992 b).

5.2.5 Compositional Quality of Yoghurts (Fresh and Stored)

The average chemical composition of fifteen yoghurts (fresh and stored) is shown in Table 5.5 while Appendix XI illustrates the results of each trial. After the fermentation period and storage of the yoghurts for 20 days, the chemical composition of all the products had similar values to those reported in Table 5.3 (variance ratio = 2.13; P > 0.45; SED = 0.045) with the exception of lactose which was metabolised by the starter culture organisms.

Table 5.4   **Means of fatty acid composition (mg 100 g^{-1}) in yoghurt bases containing AMF[a] and four different vegetable oils[b]**

Fatty Acids		AMF	Olive	Groundnut	Sunflower	Corn
Saturated						
Butyric	C4:0	88.7	3.9	3.8	4.0	3.5
Caprioc	C6:0	51.8	2.4	2.5	2.7	2.3
Caprylic	C8:0	24.7	1.7	1.8	1.6	1.5
Capric	C10:0	46.0	2.4	2.7	2.5	2.2
Lauric	C12:0	47.5	2.5	3.4	2.9	2.4
Myristic	C14:0	150.2	11.0	12.0	11.5	9.2
Palmitic	C16:0	414.4	205.7	182.0	107.2	146.3
Stearic	C18:0	11.1	44.0	61.6	69.0	36.0
	Total	834.4	273.6	269.8	201.4	203.4
Unsaturated						
Myrisoleic	C14:1	20.7	1.0	1.0	2.7	0.8
Palmitoleic	C16:1	167.4	5.2	3.8	3.4	2.2
Oleic	C18:1	389.7	816.8	760.2	286.7	362.5
Linoleic	C18:2	64.1	200.0	341.3	839.0	751.0
Linolenic	C18:3	3.8	3.7	7.7	2.0	17.8
Arachidonic	C20:4	3.0	9.8	16.7	5.0	12.4
	Total	648.7	1036.5	1130.7	1138.8	1146.7
P:S[c]		0.09:1	0.78:1	1.35:1	4.20:1	3.84:1
M:S[d]		0.7:1	3.0:1	2.8:1	1.5:1	1.8:1

[a]   Anhydrous milk fat.
[b]   Results are average of three trials.
[c]   Poly-unsaturated: saturated.
[d]   Mono-unsaturated: saturated.

Table 5.5   **Compositional quality (%w/w) of fresh and stored yoghurt made with different types of vegetable oils**

Product	Protein	Fat	Lactose	Ash	Total Solids
Fresh Yoghurt					
AMF	5.32	1.49	4.75	1.12	15.10
Olive	5.35	1.31	4.73	1.13	15.06
Groundnut	5.31	1.38	4.74	1.13	15.06
Corn	5.39	1.33	4.71	1.13	15.00
Corn	5.29	1.35	4.75	1.13	15.06
SED[b]	0.032	0.079	0.032	0.005	0.040
Stored Yoghurt					
AMF	5.30	1.48	4.50	1.12	15.01
Olive	5.33	1.32	4.54	1.12	15.00
Groundnut	5.33	1.36	4.55	1.12	15.00
Sunflower	5.31	1.33	4.51	1.12	14.98
Corn	5.27	1.34	4.54	1.12	14.98
SED	0.027	0.076	0.045	0.008	0.029

[a]   Results are average of single sample analysed in duplicate in each of the three trials.
[b]   Standard Error of Difference.

Using the polarimeter method (see section 3.10.3) for the determination of residual lactose in fresh and stored yoghurts, it was found that the lactose content was much higher than expected. Apparently, when measuring the optical rotation of lactose in the polarimeter, the method is not capable of distinguishing the residual lactose from free galactose present in the yoghurt sample; thus, giving a higher reading. Hence, in order to overcome this problem, a set of different solutions of lactose : galactose (*i.e.* 7.5:1; 6.5:1; 5.5:1; 4.5:1 and 4:1) were prepared and analysed for the determination of lactose content using the polarimeter. The lactose and the lactose plus 1% galactose contents in these different solutions is shown in Table 5.6 where higher amounts of sugars were observed in solutions containing galactose. The average difference was 1.42 (see Table 5.6) and such figure was used as a factor to be subtracted from the polarimetric measurement in order to calculate the amount of lactose in yoghurts. This approach was adopted based on the assumption that the residual galactose in the yoghurt was ∼ 1% (see Table 4.5) when using the Texel Rhône Poulenc starter culture (see Tamime, 1977; Marshall, 1987). Thus, the calculated lactose contents in vegetable oils yoghurt (fresh and stored) is shown in Table 5.7.

In these three trials the average consumption of lactose in fresh and stored yoghurts were 34% and 40% respectively (Table 5.7). The degree of lactose utilisation by the streptococci and lactobacilli starter organisms in vegetable oil yoghurts were similar to the yoghurt made with AMF. It is evident that the use of vegetable oils did not affect the metabolic activity of the microbial flora of yoghurt. These results confirm other published work where generally about a third of the lactose in milk is metabolised by *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Goodenough and Kleyn, 1976; Rasic and Kurmann, 1978; Tamime, 1977, 1978; Tamime and Robinson, 1985; Frank and Marth, 1988; Mahdi, 1990).

5.2.6 Acidification of Yoghurt Bases and Post-Acidification of Products

The incubation period of all the yoghurts in the three trials was 5½ to 6½ at 42°C. The increase in titratable acidity and total organic acid during the fermentation period and storage of yoghurts for 20 days at 5°C is shown in Appendix XI, while the decrease in pH measurements of the same yoghurts is illustrated in Figure 5.4.

Table 5.6   **Determination of lactose content (%) of different solutions containing galactose by the polarimeter method**[a]

Solutions	Results	Difference[b]
Lactose		
7.5	7.22	
6.5	6.45	
5.5	5.5	
4.5	4.57	
4.0	3.95	
Lactose + Galactose		
7.5 + 1	-	-
6.5 + 1	7.96	1.51
5.5 + 1	6.77	1.27
4.5 + 1	6.16	1.59
4.0 + 1	5.25	1.30
Average difference[b]		1.42

[a]   Results are average of two determinations performed on the same sample.

[b]   Difference between the results of lactose against the results of lactose + galactose solutions.

(-)   Not determined.

Table 5.7   Lactose content (%w/w) of yoghurt bases and different yoghurts.

Product	Yoghurt				
	Base	Fresh		Stored	
1st Trial					
AMF	7.18	4.80	(33)	4.55	(43)
Olive	7.16	4.77	(33)	4.58	(42)
Groundnut	7.32	4.80	(34)	4.57	(44)
Sunflower	7.37	4.78	(35)	4.46	(49)
Corn	7.21	4.77	(34)	4.55	(45)
2nd Trial					
AMF	7.23	4.69	(37)	4.51	(39)
Olive	7.15	4.65	(35)	4.45	(38)
Groundnut	7.16	4.68	(35)	4.55	(37)
Sunflower	7.18	4.71	(34)	4.52	(37)
Corn	7.18	4.78	(34)	4.50	(37)
3rd Trial					
AMF	7.21	4.76	(34)	4.43	(38)
Olive	7.21	4.78	(34)	4.60	(36)
Groundnut	7.18	4.75	(34)	4.54	(37)
Sunflower	7.24	4.65	(36)	4.54	(37)
Corn	7.20	4.74	(34)	4.48	(37)

Above results are average of single sample analysed in duplicate.
Figures in parenthesis represent % of lactose utilisation.

pH measurement
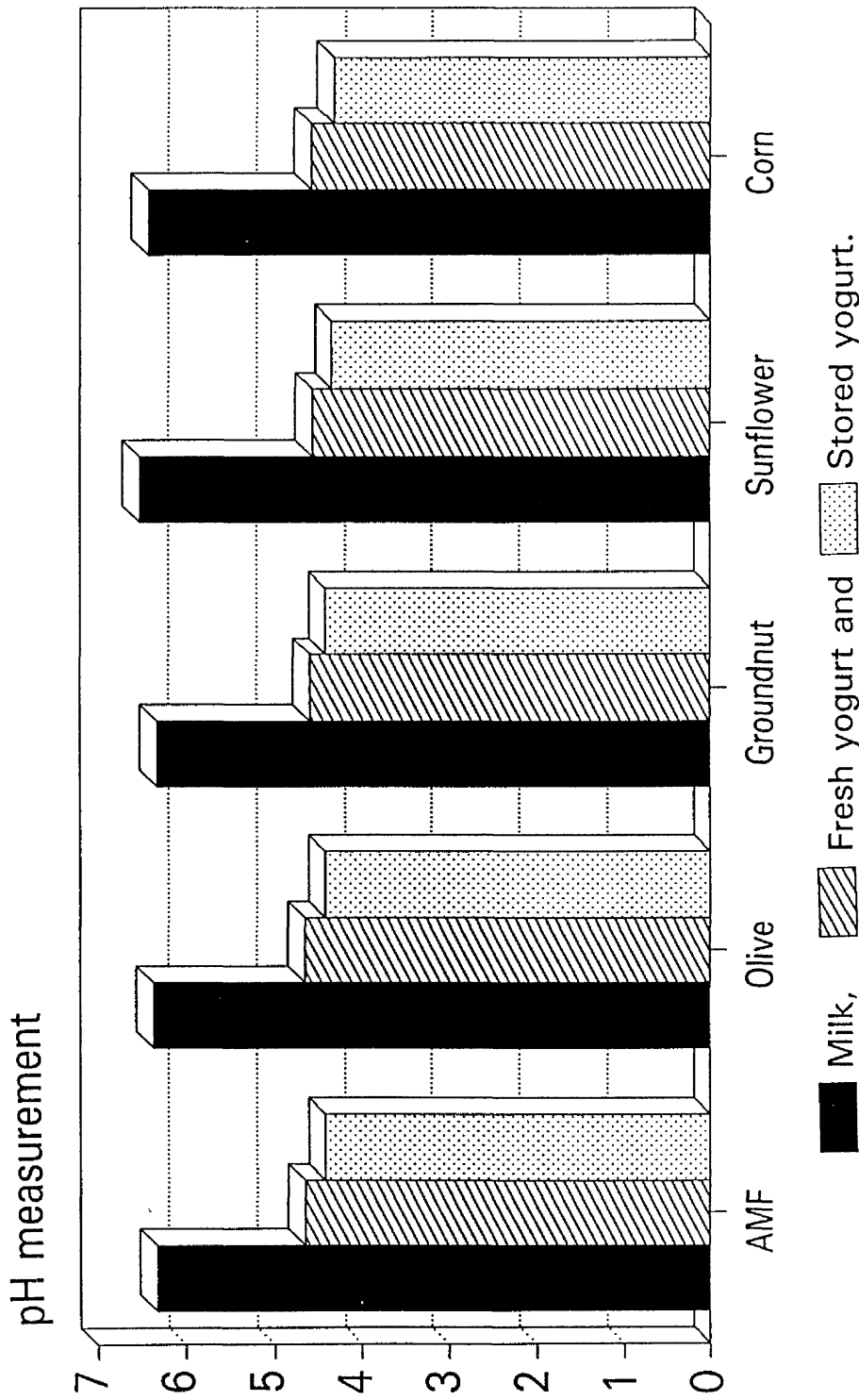
Figure 5.4   Influence of storage on post-acidification of yoghurts[a] after 20 days at 5°C.

[a] . Results are average of three trials and of two determinations on the same sample.

In the first trial the pHs of fresh and stored yoghurts ranged between 4.6 to 4.7 and 4.3 to 4.4 respectively. A similar pattern was also observed in the second and third trial. The mean pH decrease during the storage period of all the yoghurts was ~ 0.23 units which was due to the continued metabolic activity of the starter culture (Tamime *et al.*, 1987; Becker & Puhan, 1989). The use of vegetable oils during yoghurt making did not affect the activity of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*.

Statistical evaluation of the pH change of all the yoghurts show significant difference ($P < 0.05$) between trials and between fresh and stored yoghurts. No significant difference was observed between the yoghurts made with AMF and different vegetable oils in each trial.

5.2.7 Analysis of Organic Acids

The different organic acid contents ($\mu g\ g^{-1}$) in yoghurt bases and the fermented products (fresh and stored) is shown in Figure 5.5 and Appendix XII. The pattern of increase or decrease of organic acid contents in fresh and stored yoghurts was mainly influenced by the metabolic activity of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*. The main feature is a large increase in lactic and acetic acids after fermentation when compared with the yoghurt base.

Some data on the organic acids content of yoghurt has been published, but the age of the product was not reported (Marsili *et al.*, 1981; Ashoor and Wetty, 1984; Bevilacqua and Califano, 1989). No results are available on vegetable oils yoghurts, and in this present study the following observations could be made:-

a) A portion of the orotic and citric acids contents were utilised by the starter culture.

b) Although pyruvic acid was slightly metabolised during the incubation period, this acid was slightly increased in the stored yoghurts.

c) Lactic and acetic acids had increased by 35 and 120 fold respectively.

d) Hippuric acid had been slightly synthesised in fresh yoghurts and remained constant after 20 days storage.

e) Uric/formic acids had been slightly synthesised by the yoghurt cultures.

f) Propionic and butyric acids were not detected in the milks or any of the yoghurt samples in these trials.
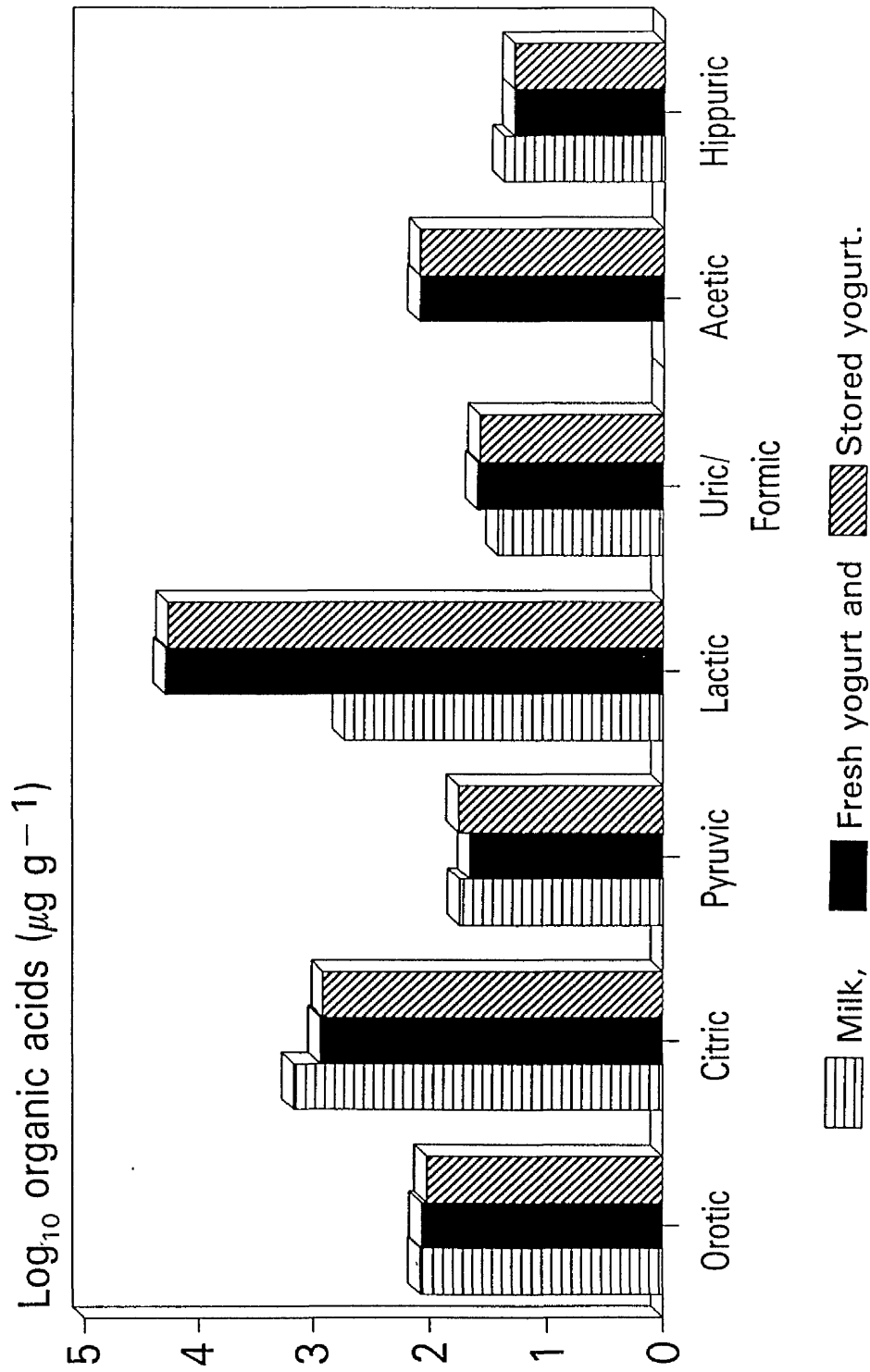
Figure 5.5   Organic acid contents (µg g⁻¹) of different types of yoghurt bases and fermented products.[a]

[a]   Results are average of three trials and two determinations performed on the same sample.

Acetic acid was not detected in some of the yoghurt samples (fresh and stored) using the standard technique. Such results were not observed for the same yoghurt starter culture MY 087 when seven different types of starch based fat-substitutes were studied (section 4.2.5). Thus, it was decided to use another HPLC column (Chrompak organic acids catalogue number 28350 - polymeric packing, *i.e.* cation exclusion resin in the hydrogen form) which was obtained from Chrompak International in the Netherlands. Detection and identification of acetic acid was evident, but some of the other organic acids present in the yoghurts co-eluted. Therefore, the data shown in Figure 5.5 represents results from two columns (a) the acetic acid values from the Chrompack column and (b) the remaining organic acids from the Bio-Rad column.

The organic acids contents in fresh and stored yoghurts (Appendix XII) were similar to those reported in the literature and the pattern in previous analysis on fat-substitute yoghurts (section 4.2.5 and Figure 4.3), including the higher amount of lactic acid content in fresh yoghurt compared with the stored samples (see Appendix XII). Analysis of variance showed no significant difference in the organic acids contents (increase or decrease) among all the different types of yoghurts.

5.2.8   Approximate Calculation of the Calorific Value

The data shown in Tables 5.3, 5.5 and Appendix XI was used to calculate the calorific value in different yoghurt bases and products, plus a calculated 1% of galactose that normally accumulates owing to the hydrolysis process of the lactose and the limited utilisation by the starter culture (Table 4.6)

The calculate calorific value in different types of yoghurt bases ranged between 62 and 64 k cal 100 g^{-1} (Table 5.3), and the energy values observed were similar because all the yoghurt bases contain the same amount of fat or oil ˜ 1.4%, protein 5.3% and lactose 7.2%.

The calorific content of the different types of yoghurts ranged between 60 and 64 k cal 100g^{-1} when fresh and 61 to 63 k cal 100g^{-1} for stored products similar to, or slightly lower than the calculated energy values reported for the different yoghurt bases (Table 5.3). This is for the following reasons:- firstly, the fat and the protein contents will be the same, and secondly, although 35 to 38% of the lactose content is utilised by the starter culture organisms (Table 5.7), the production of metabolites such as organic acids [1.5 - 2.0% (Marsili *et al.*, 1981)] - see also Figure 5.5 and the partial

accumulation of galactose in the yoghurt will compensate for the reduction in the lactose content. The only difference, which is evident when calculating the calorific content of yoghurt is the energy conversion factors [*e.g.* carbohydrates 4 as compared with 3 for organic acids (Holland *et al.*, 1991)]. Analysis of variance on the calculated calories showed no significant difference between the yoghurts.

5.2.9 Analysis of Fatty Acids on Yoghurts (Fresh and Stored)

The GLC results of total fatty acids in fresh and stored yoghurts are summarised in Table 5.8 (see Appendix XIII). Statistical analysis showed no significant change in the spectrum of fatty acids between the yoghurt bases and the fresh and stored products. Consequently, the overall pattern of fatty acids in these yoghurts is similar when compared with the original oils (Appendix VIII). This suggests that the yoghurt starter culture MY 087 used do not posses any lipolytic activity enzymes. Some authors have detected lipase activity in *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, but the level of fatty acids in the yoghurts was inconsistent while others reported that the final pattern did not changed significantly during cold storage (Tamime & Robinson, 1985).

When calculating the means of fatty acid composition in the fresh and stored yoghurts, it was found very similar levels (no statistical difference) to those obtained for the yoghurt bases (Table 5.4). Analysis of variance showed no significant difference between the three different trials when testing for each fatty acids.

5.3   Microbiological Quality of Yoghurt

5.3.1 Microbiological Analysis of Yoghurt

In this study, the coliform, yeasts and moulds counts of all the yoghurts (fresh and stored) tested were < 10 CFU g^{-1} (Table 5.9) These results indicate that the yoghurts were produced under good sanitary conditions and hygienic standards (Tamime, *et al.*, 1987; Barnes *et al.*, 1979). The total count of the non-lactic acid bacteria in all the yoghurt samples tested was < 100 CFU g^{-1} which is low and acceptable count (Tamime *et al.*, 1987).

No lipolytical activity on tributyrin agar was detected in all the yoghurts (fresh or stored) tested at 10^{-1} dilution (Table 5.9). This confirms that the MY 087 *Str. salivarius*

Table 5.8 Means of fatty acid composition (mg 100g⁻¹) in yoghurt (fresh and stored)

Yoghurt	Saturated								Unsaturated					
	C4	C6	C8	C10:0	C12:0	C14:0	C16:0	C18:0	C14:1	C16:1	C18:1	C18:2	C18:3	C20:4
Fresh														
AMF (1.49)	82.94	46.67	24.21	44.50	47.50	151.50	418.70	167.80	20.71	10.60	3.99	63.37	5.37	2.54
Olive (1.31)	3.30	2.14	1.42	2.16	2.52	9.61	203.35	42.46	0.90	7.50	828.20	197.26	8.45	6.73
Groundnut (1.38)	3.68	2.34	1.52	2.46	3.23	11.14	181.60	60.44	0.96	3.30	756.00	334.22	5.26	14.12
Sunflower (1.33)	4.00	2.50	1.70	2.56	3.01	13.24	111.55	67.94	1.04	2.50	288.30	825.70	2.86	4.00
Corn (1.34)	3.30	2.10	1.34	2.04	2.45	9.33	148.41	36.05	0.71	2.43	365.00	749.30	21.17	9.00
Stored														
AMF (1.48)	80.57	48.30	24.12	44.27	47.88	151.70	416.00	166.30	20.30	10.68	394.29	64.25	6.40	2.73
Olive (1.32)	3.38	2.15	1.51	2.20	2.58	10.32	205.00	43.60	1.03	10.65	820.00	201.30	9.02	7.60
Groundnut (1.23)	3.49	2.26	1.50	2.24	3.17	11.26	179.20	60.24	1.09	3.07	748.00	331.60	1.37	15.24
Sunflower (1.33)	3.47	2.30	1.36	2.38	2.86	12.23	108.40	66.36	1.08	2.53	288.50	828.90	2.86	4.16
Corn (1.34)	3.00	1.98	1.40	2.20	2.54	9.62	148.00	36.23	0.70	2.48	365.00	741.50	23.30	9.62

Results are an average of three trials.

Table 5.9: Microbiological quality of yoghurt made with vegetable oils (CFU g⁻¹) along the storage time

Sample	Fresh				Stored			
	Total count	Coliform	Yeast & moulds	Lipolytic	Total count	Coliform	Yeasts & moulds	Lipolytic
1st trial								
AMF	<100[a]	<10[b]	<10	<10	<100	<10	<10	<10
Olive	<100	<10	<10	<10	<100	<10	<10	<10
Groundnut	<100	<10	<10	<10	<100	<10	<10	<10
Sunflower	<100	<10	<10	<10	<100	<10	<10	<10
Corn	<100	<10	<10	<10	<100	<10	<10	<10
2nd trial								
AMF	<100	<10	<10	<10	<100	<10	<10	<10
Olive	<100	<10	<10	<10	<100	<10	<10	<10
Groundnut	<100	<10	<10	<10	<100	<10	<10	<10
Sunflower	<100	<10	<10	<10	<100	<10	<10	<10
Corn	<100	<10	<10	<10	<100	<10	<10	<10
3rd trial								
AMF	<100	<10	<10	<10	<100	<10	<10	<10
Olive	<100	<10	<10	<10	<100	<10	<10	<10
Groundnut	<100	<10	<10	<10	<100	<10	<10	<10
Sunflower	<100	<10	<10	<10	110	<10	<10	<10
Corn	<100	<10	<10	<10	<100	<10	<10	<10

a   No growth growth at 10^{-2} dilution.
b   No growth at 10^{-1} dilution.
Results are the average of two determinations perfomed on the same sample.

subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* do not posses any lipase activity.

5.3.2 Enumeration of Starter Culture

The viability of starter organisms in yoghurt has an important influence on the safety and health attributes of the product. Hence, in the three trials the effects of vegetable oils and storage on the counts of streptococci and lactobacilli were considered in detail (Appendix XIV) and are illustrated in Figure 5.6.

After the fermentation period the viable counts of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* had increased 2 $\log_{10}$ cycles (Figure 5.6). It is evident that the streptococci had dominated the microbial flora of the yoghurt, mainly as a consequence of the initial ratio of the inoculum (section 4.1.3). However, no significant effect on the viable counts of streptococci and lactobacilli were seen comparing the yoghurts made with AMF or vegetable oils, nor in different trials and during storage at 5°C. A similar pattern was observed for the same yoghurt starter culture when other types of fat-substitutes (see Figure 4.6 and Appendix V). The combined counts of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were high in all the yoghurts, thus ensuring microbiological safety. The high count of streptococci in the yoghurt culture used in the present study is probably designed for the production of low-acid mild yoghurt.

5.4 Rheological Properties of Yoghurt

5.4.1 Measurement of Serum Separation/Syneresis

The extent of serum separation of five different yoghurts was monitored according to the methodology mentioned in section 3.11.4 and the results are illustrated in Figure 5.7 and Appendix XV.

After two days storage the syneresis of each yoghurt ranged between 2.57 and 2.80 ml (Figure 5.7). Analysis of variance carried out on the average of the duplicate scores suggested no significant difference between the yoghurts. At day 5, most of the yoghurts (except AMF) increased. After that, the whey separation of each yoghurt decreased as storage time progressed; more rapidly for the corn oil than the others.
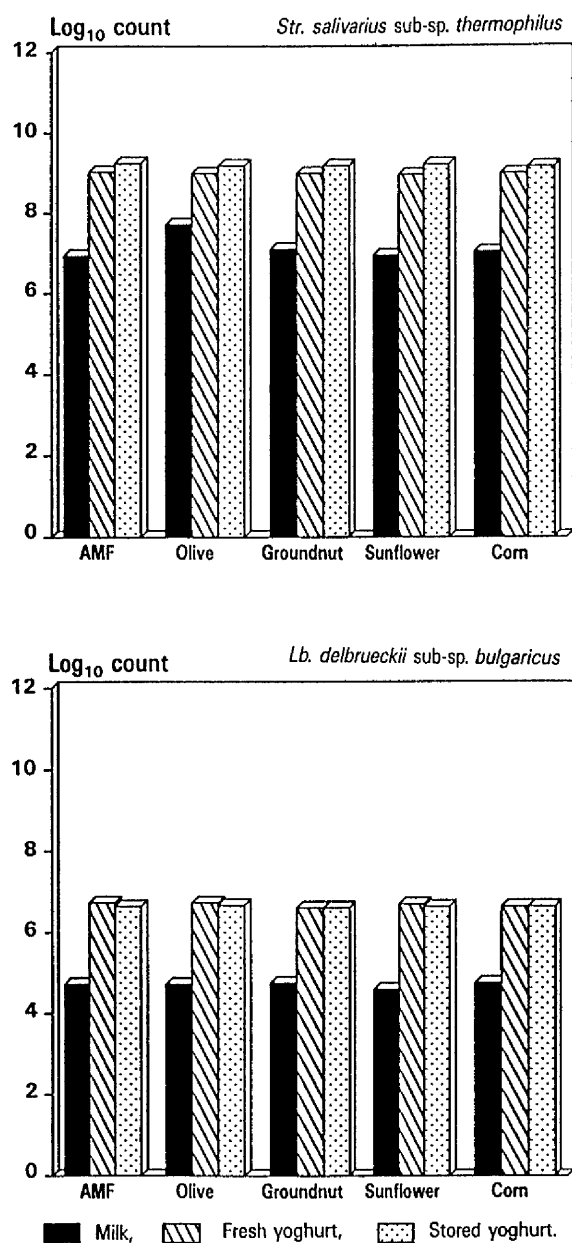
Figure 5.6   The enumeration of the starter cultures (CFU ml^{-1} milk or g^{-1} in yoghurt) during the production and storage of different yoghurts[a].

[a]   Results are average of three trials.
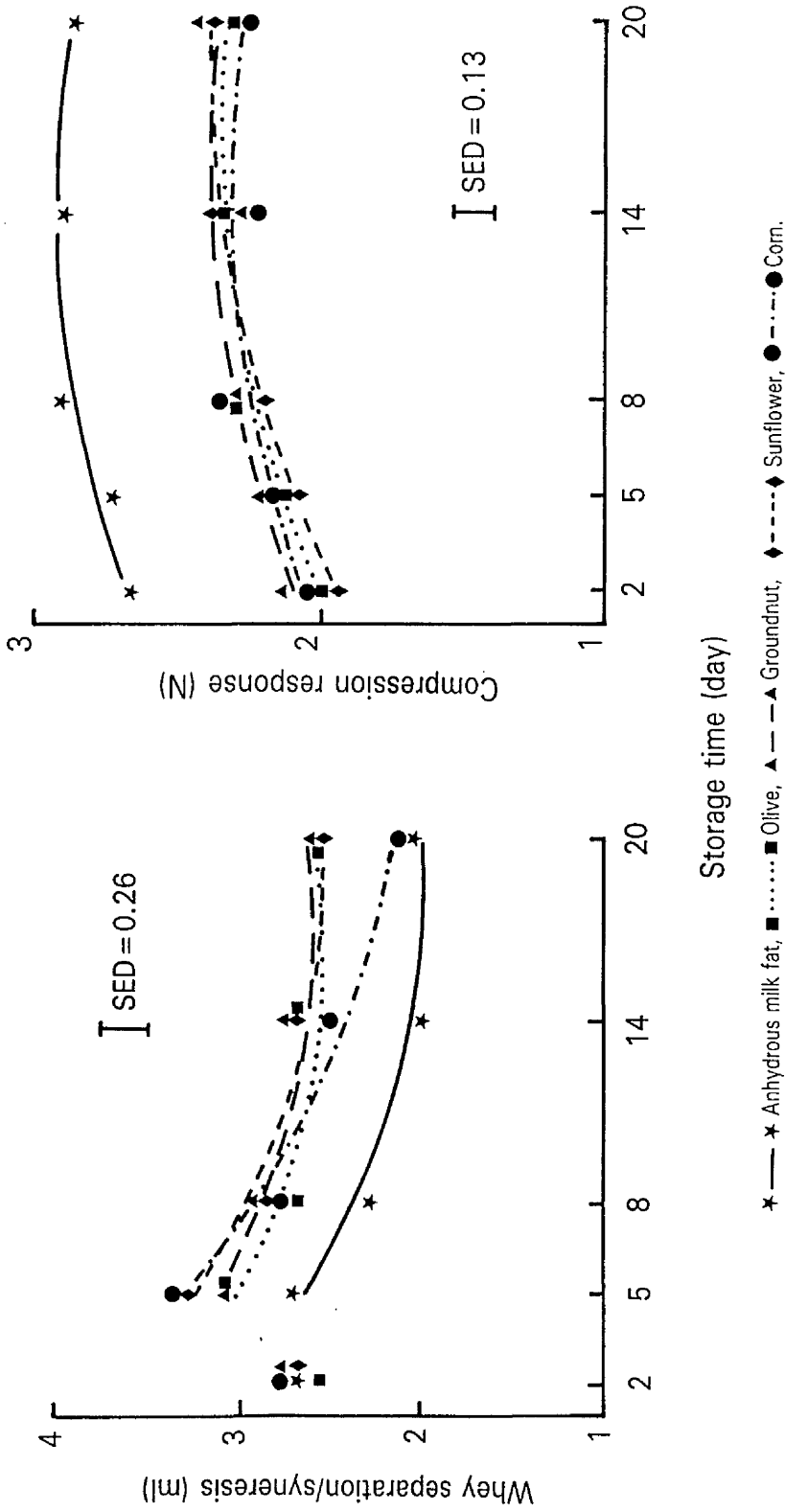
Figure 5.7   Changes in whey separation and firmness of different yoghurts[a] during 20 days storage at 5°C.

a   Results are average of three trials.

This decrease in whey separation between day 5 and day 20 was modelled using orthogonal polynominals of time by a model that allowed separated linear effects for each yoghurt plus a common quadratic effect. This modelled the different rates of decrease for each yoghurt and the change in the rates that occurred as time progressed (Figure 5.7). This model shows significant differences (P < 0.05) between yoghurts at day 5, 8 and 14. This was mainly due to the AMF yoghurt having lower values than the rest.

5.4.2 Measurement of Firmness

The firmness of different yoghurts made with vegetable oils and AMF was measured as specified in section 3.11.5. The results are illustrated in Figure 5.7 and Appendix XVI.

At the beginning of the storage period the firmness of the AMF yoghurt averaged 2.7 N while the yoghurts made with vegetable oils ranged between 1.9 and 2.1 N (Figure 5.7). The duplicate measurements taken at each sampling date were averaged and analysis of variance was carried out. This confirmed that the difference in firmness was greater than would be expected by chance (variance ratio = 22.05; P < 0.05; SED = 0.09). As the storage time progressed, the firmness of each yoghurt increased until day 8, when the level of firmness attained stayed relatively constant until the end of the storage period. The yoghurt containing AMF remained significantly firmer than the other yoghurts throughout the storage period (P < 0.05). Orthogonal polynominals were used to quantify the effect of storage time on the firmness of each yoghurt and an acceptable model was obtained by fitting a separate linear plus a single common quadratic polynomial of storage time for each yoghurt. The fitted curves are shown in Figure 5.7 along with the means of the original data.

5.4.3 Combined Analysis of Rheological Measurements

The firmness and whey separation measurements provided complementary information for the five types of yoghurts (correlation coefficient = -0.51). Both measurements indicated significant changes during the storage period and the yoghurt containing AMF was identified as being distinct from the others. However, these rheological properties of the experimental yoghurts are also acceptable. The differences in the firmness and whey separation of these yoghurts could be attributed to the protein matrix structure of the gel and the association or embeddedment of the vegetable oils in the casein micelle

chains and clusters. The AMF would also be more crystalline and this in itself could increase the firmness of the yoghurt.

5.5   Organoleptic Properties

5.5.1 Organoleptic Evaluation

The sensory data from the three trials was aggregated for a composite analysis. Residual Maximum Likelihood (REML) was used to fit a mixed model to the data. Random effects of judge, trial within judge, and storage time within trial within judge were estimated. Fixed effects of yoghurts by storage time by trial were estimated as well as the effect of order of presentation. None of the attributes were greatly affected by order of presentation or trial. The main effect of yoghurt and its interaction with trial were both significant for the majority of attributes, with the greatest evidence of a consistent effect on the flavour attributes (creamy, oxidised, unclean, aftertaste and overall flavour and aroma). The estimated means are given in Table 5.10. It shows the yoghurt made from the AMF scored significantly higher in the positive attributes and significantly lower in the negative attributes while the yoghurt made from the groundnut oil received significantly poorer scores for most of the flavour attributes. The extent of whey separation was significantly less for the AMF yoghurt than the others ($P < 0.05$). This was strongly negatively correlated ($r = -0.75$) with overall appearance and colour.

Storage of the yoghurts for 20 days significantly reduced whey separation ($P < 0.05$) and produced a more acidic yoghurt ($P < 0.05$) with a less oxidised ($P < 0.05$) and better overall flavour and aroma ($P < 0.05$) (Table 5.11). Neither of the two-factor interactions involving storage time was significant for any of the attributes.

It is difficult to be emphatic about the reason for the interaction between yoghurt and trial but there are two possible explanations: firstly, the appearance and colour, and body and texture of the first trial AMF yoghurt was superior to that of subsequent trials, and secondly, the cause may have related to the judges rather than to the manufactue of the yoghurts. It seems that the judges became more tolerant of the perceived 'off-flavours' of the vegetable oil yoghurts as the trials progressed.

In order to summarise the univariate results, Canonical Variates Analysis (CVA) was carried out on the yoghurt by storage time by trial table of means obtained from the REML analysis of each of the simple attributes; perceived whey separation, fat

TABLE 5.10 Summary of the sensory scores (0-100) of yoghurt in which the fat was replaced by four different types of vegetable oils

Sensory attribute	AMF[a]	Olive	Groundnut	Sunflower	Corn	SED[b]
Perceived whey separation[c]	**13.4**	**19.6**	**24.7**	**27.6**	**19.3**	**2.81**
Fat separation	13.9	9.4	11.6	11.2	9.8	2.52
Discolouration	8.2	6.7	9.4	9.1	10.0	1.79
Appearance & colour[c]	**72.5**	**69.1**	**57.0**	**69.1**	**68.3**	**3.22**
Firmness	74.5	72.2	70.0	72.7	74.1	1.65
Lumpy/coarse	25.2	27.7	30.7	27.6	29.2	2.92
Gummy	17.9	24.7	25.5	23.4	23.1	2.77
Body & texture[c]	**69.8**	**65.9**	**58.6**	**66.0**	**64.6**	**2.87**
Acidic	40.9	41.6	44.9	39.6	41.0	3.14
Creamy[c]	**48.6**	**39.0**	**34.8**	**37.6**	**44.1**	**2.84**
Oxidised[c]	**11.8**	**23.0**	**22.9**	**24.9**	**20.3**	**3.29**
Unclean[c]	**6.4**	**16.3**	**19.4**	**14.7**	**14.0**	**2.81**
After-taste[c]	**13.3**	**27.7**	**35.5**	**31.6**	**23.7**	**3.40**
Flavour & aroma[c]	**71.9**	**51.8**	**42.8**	**52.1**	**52.4**	**3.64**
Acceptability[c]	**72.1**	**51.6**	**43.5**	**47.9**	**57.4**	**3.91**

a　Anhydrous milk fat.
b　Standard Error of Difference.
c　Sensory attributes for which sample effects are significantly different.

Table 5.11   Summary of the effects of the average sensory scores (0-100) of different types of yoghurts

Sensory attribute	Fresh	Stored	SED[a]
Whey separation[b]	24.0	17.8	2.43
Fat separation	10.9	11.6	1.41
Discolouration	8.3	9.0	1.15
Appearance & colour	65.3	69.0	2.67
Firmness	73.0	72.3	1.68
Lumpy/coarse	28.7	27.4	1.63
Gummy	23.1	22.7	1.51
Body & texture	64.8	65.2	2.30
Acidic[b]	37.2	45.9	2.72
Creamy	41.2	40.4	1.81
Oxidised[b]	24.1	17.1	2.30
Unclean	16.5	11.9	2.18
After-taste	26.6	26.1	2.58
Flavour & aroma[b]	50.8	57.3	2.22
Acceptability	51.9	57.1	2.35

[a]   Standard Error of Difference.

[b]   Sensory attributes for which sample effects are significantly different.

separation, discolouration, firmness, lumpy/coarse, gummy, acidic, creamy, oxidised, unclean and after-taste. The attributes; overall appearance and colour, body and texture, flavour and aroma, and acceptability have been omitted as they represent a summary judges' scores of the constituent parts. The grouping factor used in the CVA was the combination of five yoghurts and two storage times. Figure 5.8 displays the yoghurt means plotted on the first two CV axes and highlights the separation of the five different yoghurts (fresh and stored). The first CV axis accounts for 54% of the total variability and comprised one large negative loading (after-taste) and one large positive loading (creamy flavour). Table 5.12 lists the loadings of all attributes. This first axis orders the five yoghurts; AMF, corn, olive, sunflower and groundnut from good to bad. It should be noted that the separation of the yoghurts is larger after storage than before. The second CV axis accounts for a further 22% of the variability and successfully, separates the yoghurts before and after storage. The main attributes responsible for this separation are the defect attributes. Yoghurts appear to have a stronger oxidised and more unclean flavour, but have less after-taste when fresh than after storage.

5.6 Consumer Acceptability of Strawberry Yoghurts Using Four Different Types of Vegetable Oils

As mentioned elsewhere (section 4.7) fruit flavoured yoghurts are among the most important in the market and strawberry yoghurt is a popular fruit flavour with consumers.

In order to know the acceptability of vegetable oils in yoghurt among local consumers, strawberry yoghurt was made using four different types of vegetable oils (olive, groundnut, sunflower and corn).

The yoghurt was produced from reconstituted SMP containing ~14% total solids and 1% sugar as described in Figure 2.2. A control batch, *i.e.* fortified with AMF was produced along with four batches containing vegetable oils at a rate of 1.5% (w/w). The freeze dried starter culture MY 087 was used as direct-to-vat inoculation of the milk at a rate of 16 g 100 1^{-1}. The processed strawberry fruit was 187.6 k cal 100 g^{-1} (see section 3.10.11). The yoghurts were organoleptically evaluated by students and personnel at SAC - Auchincruive. The acceptability score, was awarded on a nine-point Hedonic scale (Appendix I - B).
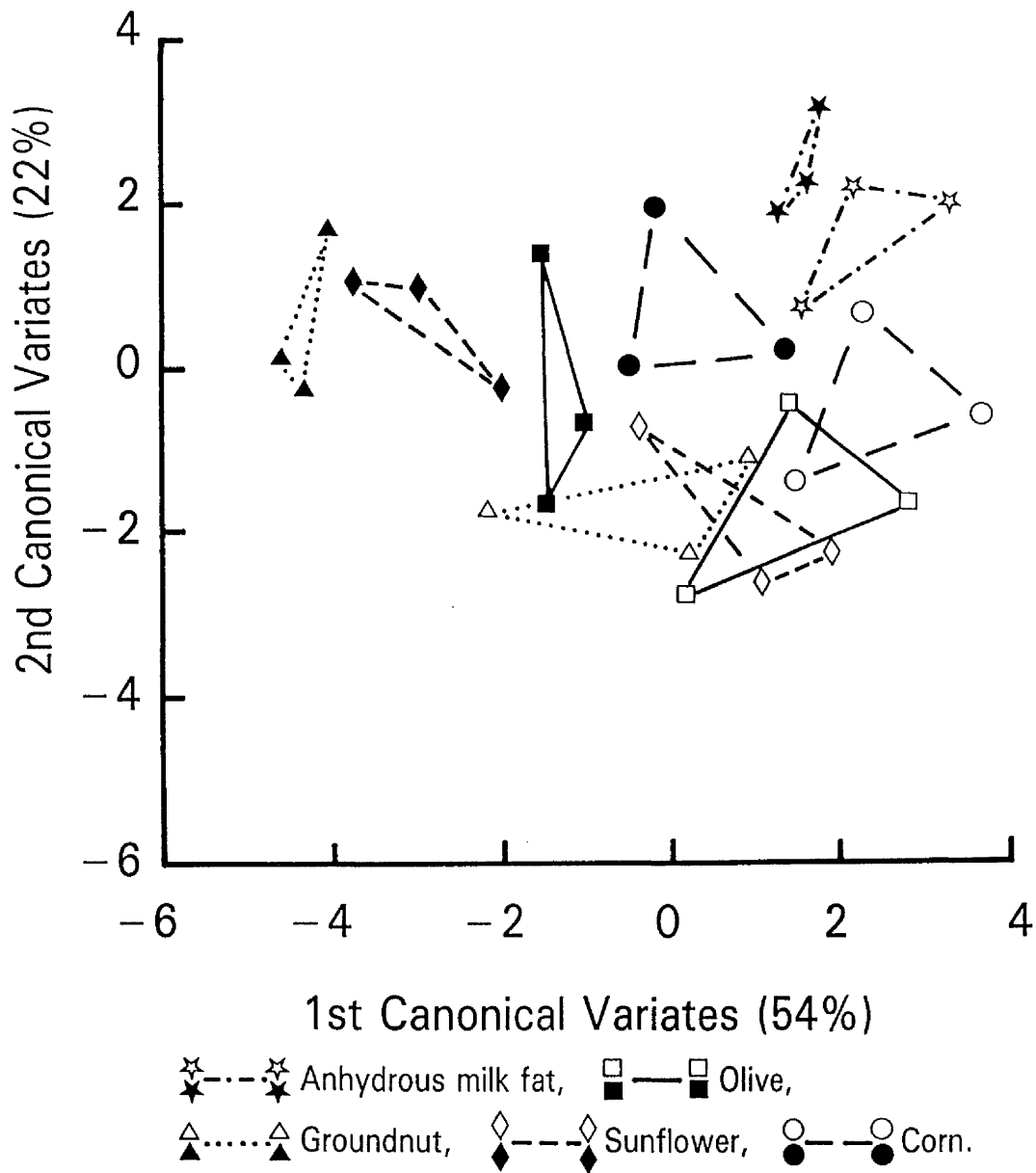
Figure 5.8   Canonical Variates Analysis of the organoleptic attributes assessments of different yoghurts.

Results are average of three trials.

Open and solid symbols represent fresh and stored yoghurts respectively.

Table 5.12   Coefficients of first and second Canonical Variates

Sensory attribute	Canonical Variates	
	First	Second
Perceived whey separation	0.11	-0.59
Fat separation	-0.65	0.35
Discolouration	0.66	**0.50**
Firmness	-0.95	0.11
Lumpy/coarse	-0.34	**0.58**
Gummy	0.71	-0.72
Acidic	**-1.28**	-0.03
Creamy	**1.33**	0.06
Oxidised	0.77	-0.97
Unclean	0.98	-0.81
After-taste	**-2.87**	0.67

Attributes in bold type believed to be important.

5.6.1 Compositional Quality of Yoghurt

The compositional quality of the natural yoghurts were similar to those reported in Table 5.5 (*i.e.* ~14% solids-not-fat, 5.3% protein, 4.7% lactose, 1.45% fat, 1.2% total organic acids and pH ~4.6%). Thus, the energy value of the strawberry yoghurts was calculated using the conversion factors reported by Holland *et al.* (1991). These values are shown in Table 5.13 and the calorific values were higher when compared with set-yoghurt because of the added sucrose (1% w/w) and strawberry flavour (15% w/w).

5.6.2 Microbiological Quality of Yoghurt

In this study, the coliforms, yeasts and moulds and lipolytic counts of the yoghurts were <10 CFU g^{-1}. The level of total count of non-lactic acid bacteria in all the yoghurts tested was low (*i.e.* <100 CFU g^{-1}). These results, for example, the virtual absence of coliform and the total count of non-lactic acid bacteria in 10^{-1} and 10^{-2} dilutions respectively from all the yoghurts analysed, indicate that the production of these samples were carried out under good hygienic standards and sanitary conditions (Tamime *et al.*, 1987; Barrantes and Tamime, 1992).

5.6.3 Organoleptic Evaluation

Eighty consumers of yoghurts were (self) selected from staff and students at SAC - Auchincruive to sample five types of yoghurts and score each for overall acceptability using the nine-point Hedonic scale. The yoghurts were presented in a fixed sequence, determined by a design based on Williams' squares (Macfie *et al.*, 1989) to balance any bias from order of presentation and carry-over effect.

Four personal characteristics were recorded for each consumer: age, sex, weekly consumption of yoghurt and permanent address. For the purposes of the analysis the personal details were categorised in the following ways. Age was coded as: (a) less than 20 years, (b) between 20 and 30 years, and (c) greater than 30 years. Permanent address was reclassified into three regions: (a) living in Strathclyde, (b) not living in Strathclyde but within the U.K., and (c) living outwith the U.K. Weekly consumption was divided into: (a) less than or equal to 3 pots per week, (b) 4 or 5 pots per week, and (c) more than 5 pots per week. Sex was retained as male or female. The overall scores of the consumer tasters is shown in Figure 5.9. It can be observed that the majority of consumers scored the yoghurts between 5 and 7 points (*i.e.* 'liked').

Table 5.13 Approximate calculation of the calorific content (k cal 100 g⁻¹) of strawberry flavoured yoghurts containing vegetable oils

Product	Yoghurt	
	Set/Natural[a]	Stirred/Strawberry[b]
AMF	63.6	88.5
Olive	62.3	86.0
Groundnut	63.1	85.9
Sunflower	61.8	86.0
Corn	64.3	86.2

[a] Data compiled from Table 5.3 and 5.5.

[b] The yoghurts contain sugar and strawberry flavour at a rate of 1% and 15% w/w respectively.
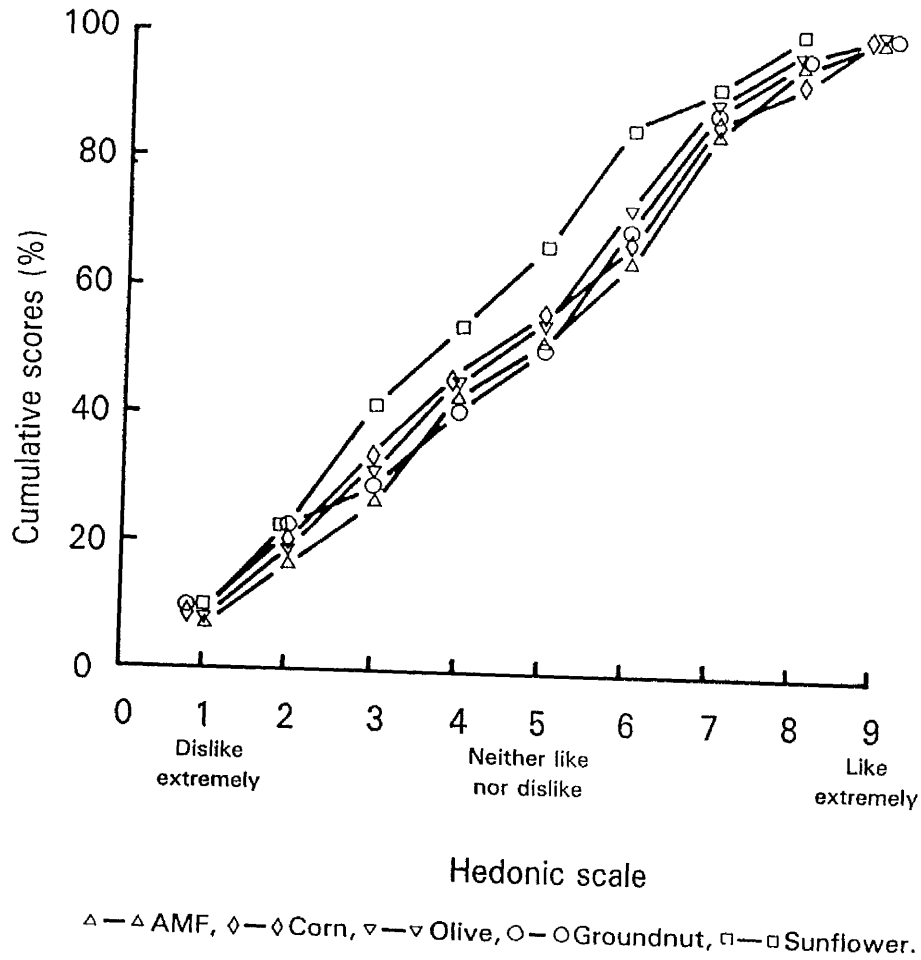
Figure 5.9   Cumulative scores for organoleptic acceptability of different types of yoghurts.

The scores were analysed for the effects of yoghurt, order of presentation and personal characteristics by the Residual Maximum Likelihood (REML) technique. Yoghurt mean scores ranged between 3.2 (sunflower) and 6.6 (AMF) with SED = 0.39. The AMF yoghurt was significantly superior (P< 0.05) to each of the other yoghurts (Figure 5.10). Yoghurts sample first averaged a score of 4.1. This was significantly lower than yoghurts tasted later on in the session where scores ranged between 4.8 and 5.2, with SED = 0.25 (Figure 5.11). There was no evidence to suggest that either of the categorised personal factors; consumption or permanent address had any influence on yoghurt preference. However, there was significant evidence of an interaction between yoghurt scores and sex of consumer: yoghurt made from groundnut oil was rated significantly higher by the females than the males. Similarly, age-group influenced yoghurt preference. Although all three groups rated the AMF yoghurt highest and the sunflower the lowest, only the 20-30 year old group believed there was an appreciable difference between the remaining three yoghurts (Figure 5.12).

A similar pattern of order of the yoghurts was found (r = 0.915) between the panellists and the consumers showing no major effect of the addition of fruit and sugar on masking the flavours coming from the oils.

5.7   Microscopic Analysis

Microscopic analysis on the structure of yoghurts made with vegetable oils is carried out in Canada and are not ready by the time these results are presented. Samples preserved in gluteraldehyde are being analysed and the results will be published separately.

5.8   Conclusion

The use of different types of vegetable oils offer new possibilities for the manufacture of healthy yoghurt *i.e.* high in mono- and poly-unsaturated fatty acids. Although the calorific value could not be reduced because of the content of oils; however, the energy value of the product could be further reduced by adopting one or combinations of the following methods: (a) lowering the level of milk solids-not-fat in the yoghurt bases, (b) using low-calorie bulking agent in order to maintain the rheological properties of the product, (c) using sweetener to replace the added sugar in the yoghurt base, and (d) adding fruit flavours high in fructose rather than sucrose.
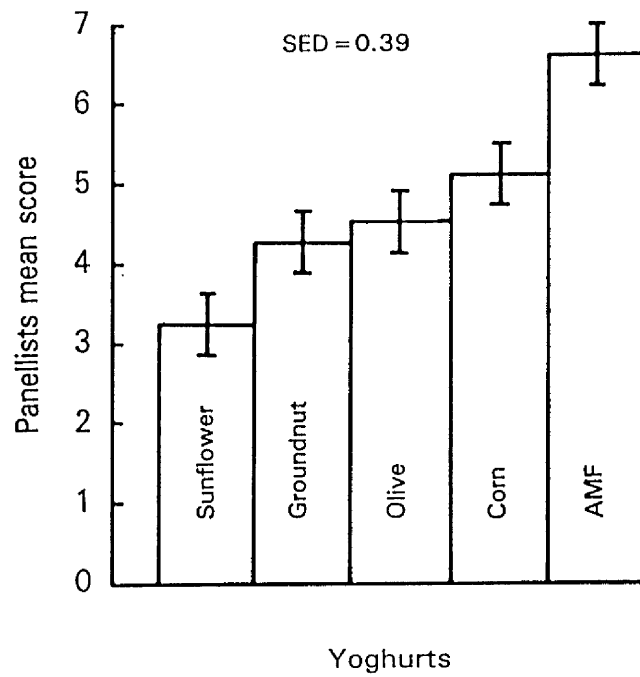
Figure 5.10   Yoghurt means scores of the consumer panel.
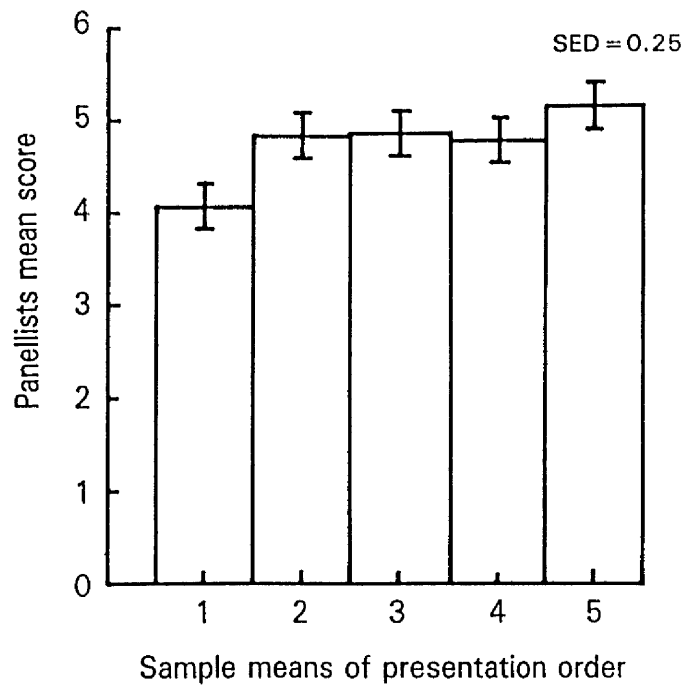
Figure 5.11   Effect of order of presentation of yoghurt samples on the consumer's scores.
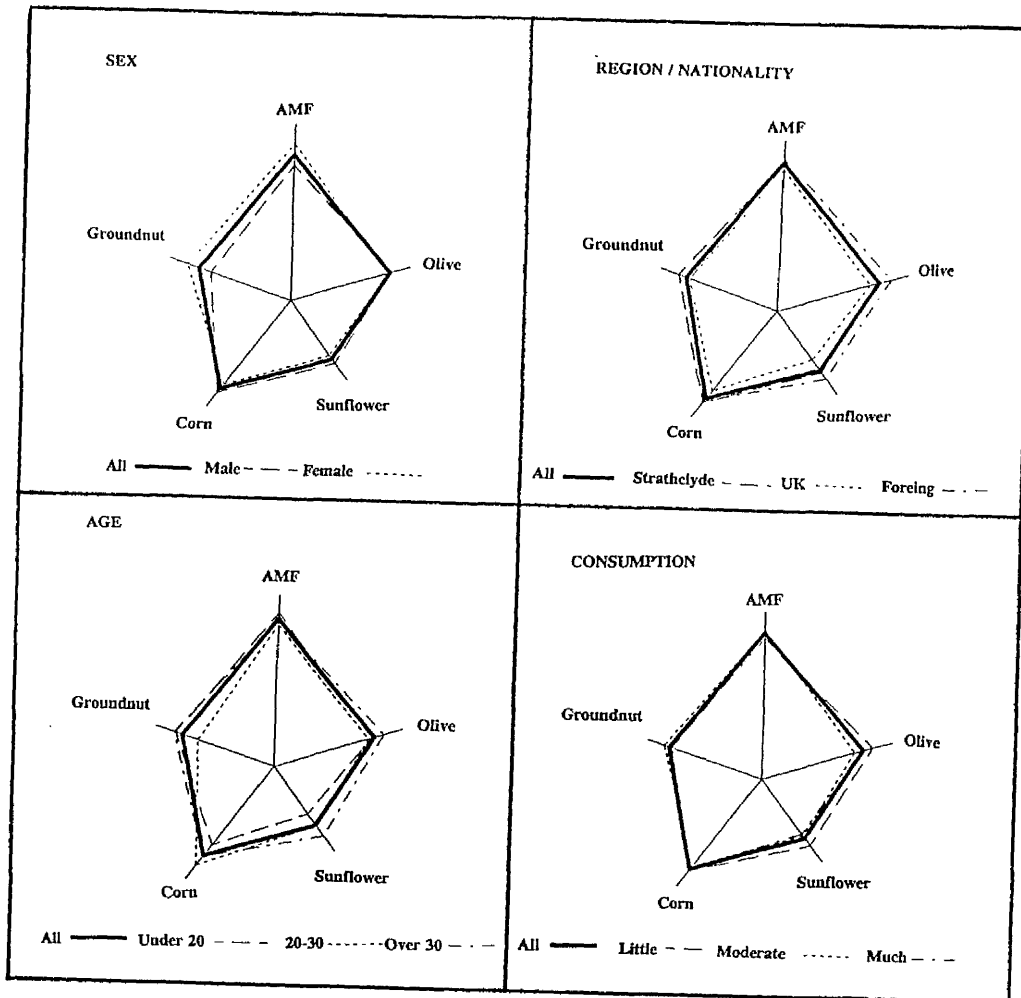
Figure 5.12   Web diagram illustrating the organoleptic acceptability of vegetable yoghurts.

No processing difficulties were experienced for the production of vegetable oil and yoghurts and the homogenisation of the yoghurt base caused enough emulsification of the oils in the milk. The use of stabilisers were not necessary to avoid oil separation during the production and storage of yoghurt.

The compositional quality of the vegetable oil yoghurts and the control were similar. The only difference was the fatty acids content which affected the ratios of poly- and mono- unsaturated to saturated. Saturated fatty acids were reduced by ~50% when compared with AMF.

The microbiological quality of all the yoghurts was excellent. The coliforms, yeasts and moulds were <10 CFU g^{-1} in fresh and stored products. The yoghurt starter organisms were recovered in high numbers (streptococci x 10^8 CFU g^{-1} and lactobacilli x 10^5 CFU g^{-1}), and the substitution of AMF with vegetable oils did not affect the starter culture activity during the fermentation stage. There was no apparent lipolytic activity in the yoghurts and the fatty acid composition did not change significantly along the storage time; hence, the use of antioxidants is not required at the level of 1.5% (w/w) of vegetable oils.

The rheological properties of vegetable oils yoghurt were different to the yoghurt made with AMF. Syneresis and texture showed higher and lower figures respectively when compared with the control. For all the yoghurts, whey separation decreased with storage time, while firmness increased with time.

Quantitatively the polarimeter and HPLC methods for the determination of lactose and lactic acid have to be evaluated further in order to overcome some of the analytical difficulties experienced in the yoghurts (fresh and stored). The pattern of acidification of milk and organic acids production/consumption by the starter organisms was similar to those reported in the literature.

Few sensory attributes (based on flavour and aroma) were identified by the judges to be significantly different to yoghurt made with AMF. This could be attributed to the 'typical' flavours associated with these vegetable oils. When adding fruit and sugar to the yoghurts, the scores for acceptability were similar to those of the panellists evaluating natural yoghurts. Similarly the panellists and consumers in U.K. are not used to flavours coming from vegetable oils, as other places (*i.e.* Mediterranean and

Latin America) where the use of these oils is more common in their diets. Foreign consumers scored slightly higher the yoghurts containing vegetable oils.

CHAPTER SIX

PRODUCTION OF SET-TYPE YOGHURT USING MICROPARTICULATED WHEY PROTEIN

CHAPTER SIX: PRODUCTION OF SET-TYPE YOGHURT USING

MICROPARTICULATED WHEY PROTEIN

6.1   Preliminary Analysis

6.1.1   Quality of the Skim Milk Powder (SMP), Anhydrous Milk Fat (AMF) and Starter Culture

The SMP, AMF and starter culture utilised in this study were of the same batches as the raw materials used in the production of fat-substitute and vegetable oil yoghurts. Consequently, chemical, physical and microbiological results are presented in sections 4.1.1, 4.1.2, 4.1.3 and Table 4.1.   All the raw ingredients were suitable for the recombination and the production of yoghurt.

6.1.2   Compositional Quality of Microparticulated Whey Protein

Low-calorie yoghurt was produced by replacing the milk fat with a microparticulated whey protein ingredient (Simplesse® 100) derived from the milk (see section 2.5.2 and Table 2.5).

The preparation was available as a concentrated suspension (~39% solids with a shelf-life of 4 weeks at 5°C) or in the more stable dried form (shelf-life ~10 months stored at <30°C and 65% relative humidity).   Chemical analysis were prepared as specified in section 3.7.1 and physical (i.e. scorched particles) was carried out as described in section 3.4.8.   Microbiological analysis were done as in sections 3.4.10, 3.4.12 and 3.4.13. Results are shown in Table 6.1.   All the results were similar to those reported by the independent laboratory (i.e. Netherlands Controlling Authorities for Milk and Milk Products (C.O.Z) according to the supplier standards.

Table 6.1   Chemical, physical and microbiological quality of Simplesse® 100 wet and dry

Component	Simplesse® 100	
	Wet	Dry
Chemical		
Moisture (%)	60	4.0
Protein (%)	21.6	49.6
Ash (%)	2.7	6.8
Lactose (%)[a]	11.6	30.1
Fat (%)	1.7	4.1
Scorched particles (mg)	-	27.5 (Disc A)
Medium size particle diameter (μm)[a]	1.2 ± 0.3	1.2 ± 0.3 (reconstituted)
Microbiological		
Total viable count (CFU g^{-1})	200	180
Coliforms (CFU g^{-1})[b]	<10	<10
Yeasts and moulds (CFU g^{-1})	<10	<10

[a]   Date provided by the supplier (Anon., 1993 a, b).

[b]   No growth at 10^{-1} dilution.

6.2   Production of Set-Yoghurt

Three trials, *i.e.* twelve batches of set-yoghurt using microparticulated whey protein Simplesse® 100 were produced as described in section 3.3 and as illustrated in Figure 2.2. Simplesse® 100 (dry and wet) fat-substitutes were added to two batches of milk at a rate of 1.5% (w/w), and the remaining batch was made with Simplesse® 100 (wet) to yield an increase in dry matter (DM) content equivalent to 1.5%.

6.2.1   Viscosity of the Yoghurt Base

The pattern of viscosity for the four types of yoghurt base, after the heating stage, in the three trials were similar (Table 6.2). Viscosity was influenced by the level of the fat-substitute used which, in turn, has influenced the protein content in the yoghurt base (Tables 6.3 and 6.4). The highest viscosities in these trials ($^-$3 centipoise) were observed in yoghurt bases containing S-100 (W-DM) and S-100 (D).

Analysis of variance of the viscosity results indicated that there was no significant difference between the trials, but there was a significant difference (P< 0.001) between the way the fat-substitutes were used.

6.2.2   Chemical Composition of Yoghurt Bases

The chemical composition and acidity measurements of twelve batches of yoghurt bases is shown in Table 6.3. In general the milk solids-not-fat contents were $^-$14% and the total solids ranged between 14.34 to 15.46% (SED = 0.07) and the protein ranged between 5.22 to 6.41% (SED = 0.06). The fat contents were around 0.12 and 0.20% with the exception of the batch which contained AMF ($^-$1.45%, see Table 6.3). Ash content varied between 1.13 to 1.27% (SED = 0.02) and the lactose ranged from 7.15 to 7.41%. The variation in these levels was mainly attributed to the ingredients which were added to the reconstituted skim milk powder. For example, S-100 (W) had the lowest level of total solids content and this had influenced the protein percentage in the milk when compared with other Simplesse® batches. The protein content was increased by $^-$1% in the products containing concentrated Simplesse® 100. Furthermore, the ratio of casein to non-casein nitrogen was calculated and the results are shown in Table 6.4. A higher content of non-casein proteins in the Simplesse® 100 products is noticeable since the principal component is whey protein.

Table 6.2   **Viscosity measurement (centipoise) of different yoghurt bases made with microparticulated protein and AMF**

Product	Trial		
	1	2	3
AMF	2.45	2.49	2.53
Simplesse® 100			
Wet - DM	2.92	2.78	2.89
Wet	2.54	2.49	2.54
Dry	2.80	2.78	2.85

Above results are average of two determinations performed on the same sample.

Table 6.3   **Means of chemical composition (%w/w) of different types of yoghurt bases using Simplesse® 100**

Product	Protein	Fat	Ash	Lactose	Total solids	pH	Acidity T.A.
1st Trial							
AMF[a]	5.22	1.45	1.17	7.22	15.14	6.67	0.23
Simplesse® 100							
Wet - DM[b]	6.29	0.19	1.26	7.10	15.37	6.66	0.29
Wet	5.52	0.12	1.17	7.15	14.34	6.64	0.20
Dry	6.21	0.15	1.27	7.31	15.27	6.66	0.29
2nd Trial							
AMF	5.34	1.49	1.15	7.20	15.36	6.13	0.25
Simplesse® 100							
Wet - DM	6.41	0.20	1.27	7.14	15.46	6.62	0.30
Wet	5.53	0.14	1.19	7.28	14.44	6.65	0.27
Dry	6.00	0.17	1.24	7.34	15.20	6.72	0.29
3rd Trial							
AMF	5.25	1.55	1.13	7.19	15.21	6.40	0.25
Simplesse® 100							
Wet - DM	6.26	0.19	1.22	7.63	15.22	6.45	0.28
Wet	5.60	0.13	1.16	7.17	14.34	6.30	0.26
Dry	6.13	0.16	1.23	7.40	15.18	6.25	0.27
SED[c]	0.06	0.02	0.02	0.04	0.07	0.03	0.05

[a]   Anhydrous Milk Fat.

[b]   Simplesse® 100 wet was fortified based on dry matter (DM) content.

[c]   Standard Error of Difference.

Table 6.4   Protein, casein and non-casein nitrogen contents (% w/w) in yoghurt bases[a]

Yoghurt base	Protein	Non-casein nitrogen	Casein	Ratio of casein to non-casein
AMF	5.27	0.96	4.31	4.47
Simplesse® 100				
Wet - DM	6.32	1.46	4.86	3.33
Wet	5.55	1.13	4.42	3.91
Dry	6.11	1.32	4.66	3.53
SED[b]	0.060	0.013	0.060	0.036

Note:Above results are average of three trials.

[a]   The various protein fractions in milk were determined before the heat treatment stage.

[b]   SED: Standard Error of Difference.

The fat contained in all the experimental batches originated from Simplesse® products and the SMP (see Table 6.1).

The acidity measurements *i.e.* pH, and titratable acidity were similar in all batches and in the three trials and averaged 6.5 and 0.25% respectively (Table 6.3 and Appendix XVII).

6.2.3 Compositional Quality of Yoghurt (Fresh and Stored)

The average composition of twelve yoghurts (fresh and stored) is shown in Table 6.5 while Appendix XVII illustrates the results of each trial. The protein, fat, ash and total solids contents in fresh and stored yoghurts were similar to the yoghurt bases (see Table 6.3). Analysis of variance showed no significant difference for these constituents compared to the yoghurt bases (variance ratio = P >0.543).

As mentioned in section 5.2.5 there were some problems on the determination of the residual lactose in yoghurts. Hence, the results from the polarimeter measurement were adjusted by subtracting the factor 1.42 (for detail refer to section 5.2.5).

In comparing the yoghurt bases with the yoghurts, the only change, which was evident, was the reduction in the lactose content in yoghurt (fresh and stored) due to the metabolic activity of the starter culture. The amount of lactose utilised by the starter culture MY 087 amounted to ~35-38% (Table 6.6) this is similar to values reported elsewhere. The results were used for comparative purposes and also to facilitate the approximate calculations of the calorific value of these yoghurts.

6.2.4 Acidification of Yoghurt Bases and Post-Acidification of Products

The incubation period of all the yoghurts in the three trials was for 6-7 h at 46°C. The increase in titratable acidity and reduction in pH during the fermentation period and storage of yoghurts for 20 days at 5°C is shown in Tables 6.3, 6.5 and Appendix XVII.

For example, in all the trials, the pHs of fresh and stored yoghurts ranged between 4.44 to 4.69 and 4.31 to 4.46 respectively (see Appendix XVII). The mean pH decrease during 20 days storage of all yoghurts was ~0.19 units. The tendency to lower post-

Table 6.5   **Compositional quality (% w/w) of fresh and stored yoghurt made with microparticulated protein[a]**

Product	Protein	Fat	Ash	Lactose	Total solids	pH	Acidity T.A.
Fresh Yoghurt							
AMF[b]	5.22	1.47	1.16	4.67	14.88	4.57	1.18
Simplesse® 100:							
Wet - DM[c]	6.30	0.18	1.26	4.60	15.10	4.56	1.27
Wet	5.55	0.13	1.18	4.69	14.10	4.53	1.24
Dry	6.10	0.15	1.25	4.84	14.94	4.56	1.29
SED[d]	0.042	0.024	0.011	0.002	0.17	0.022	0.042
Stored Yoghurt							
AMF	5.21	1.46	1.18	4.46	14.85	4.35	1.30
Simplesse® 100							
Wet - DM	6.28	0.19	1.24	4.50	14.92	4.39	1.44
Wet	5.56	0.13	1.18	4.36	13.86	4.37	1.46
Dry	6.12	0.15	1.24	4.58	14.82	4.39	1.46
SED	0.033	0.012	0.029	0.093	0.14	0.015	0.054

[a]   Above results are average of a single sample analysed in duplicate in each of the three trials.

[b]   Anhydrous Milk Fat.

[c]   Simplesse® 100 wet was fortified based on dry matter (DM) content.

[d]   Standard Error of Difference.

Table 6.6   Lactose content (% w/w) of yoghurt bases and products

Product	Yoghurt				
	Base	Fresh		Stored	
1st Trial					
AMF	7.22	4.73	(34)	4.38	(39)
Simplesse® 100					
Wet - DM	7.10	4.58	(35)	4.53	(36)
Wet	7.15	4.69	(34)	4.38	(39)
Dry	7.31	4.76	(35)	4.52	(38)
2nd Trial					
AMF	7.20	4.62	(36)	4.42	(39)
Simplesse® 100					
Wet - DM	7.14	4.57	(36)	4.49	(37)
Wet	7.28	4.66	(36)	4.42	(39)
Dry	7.34	4.83	(37)	4.47	(39)
3rd Trial					
AMF	7.19	4.66	(35)	4.59	(36)
Simplesse® 100					
Wet - DM	7.63	4.71	(38)	4.43	(41)
Wet	7.17	4.73	(34)	4.29	(40)
Dry	7.40	4.92	(34)	4.74	(36)

Figures in parenthesis represent % of lactose utilisation.

acidification in yoghurt after storage was evidently due to the continued metabolic activity of the starter culture (Tamime *et al.*, 1987; Becker and Puhan, 1989).

Statistical evaluation of the pH and titratable acidity change of all the yoghurts showed significant difference (P <0.001) between trials and between fresh and stored yoghurts (see Appendix XVII). However, no significant difference was observed between the yoghurts made with different vegetable oils in each trial.

The yoghurt starter organisms utilise the available chemical energy (*i.e.* lactose) in milk for respiration and/or cell division. In the three trials the average consumption of lactose in fresh and stored yoghurts were 35% and 38% respectively (Table 6.6). The degree of lactose utilisation by the streptococci and lactobacilli starter organisms in the experimental yoghurts were similar to the yoghurt made with AMF and it is evident that the Simplesse® 100 fat-substitute did not affect the metabolic activity of the microbial flora of yoghurt. These results confirm other published work where generally about a third of the lactose in milk is metabolised by *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Goodenough and Kleyn, 1976; Rasic and Kurmann, 1978; Tamime, 1977, 1978; Tamime and Robinson, 1985; Frank and Marth, 1988; Mahdi, 1990, see also sections 4.2.4 and 5.2.6).

Lactose is metabolised after hydrolysis to glucose and galactose, of these glucose is metabolised to a much greater extent and galactose to a lesser extent where ~1% is usually excreted as such by the microbial cells of the starter organisms and accumulate in the yoghurt. Thus, the residual galactose in the yoghurts had to be accounted for when calculating the energy values of these products.

6.2.5 Approximate Calculation of the Calorific Values

The calculated calorific value in different types of yoghurt base are expressed in k cal 100 g^{-1} and were obtained using energy conversion factors as described by Holland *et al* (1991) (see Section 3.10.11). The data shown in Table 6.2 and Appendix XVII was used to calculate the calorific value in different types of yoghurt bases and products.

The galactose and organic acid contents were not determined in this study for the following reasons:

a)   The enzymatic method used previously (Chapter 4) showed difficulties in its application. Additionally it is an expensive methodology and other methods such

as the measurement of sugars by HPLC was not available. Thus for approximate calculation of the energy values in yogurts, it was decided to calculate the amount of residual galactose of ˜1%, based on the analysis done on fat-substitute yoghurts which comply with what had been reported (Marshall, 1987) and the fact that the acidification process and lactose consumption (*i.e.* ˜35-40%) are similar in all the studies carried out on fat-substitutes (Chapter 4) and vegetable oils (Chapter 5). Furthermore, the same starter culture was used and the protein and lactose contents of the products were very similar.

b)   The determination of organic acids was not possible owing to a breakdown of the freezer and loss of the samples. However, for the approximate calculation of the calorific value of microparticulated whey protein yoghurts, the organic acid contents shown in Appendix III were used for comparative purposes.

The calculated calorific value in different types of yoghurt base ranged between 52 and 63 k cal 100 g^{-1} (see Table 6.7). The highest energy value was observed in the milk containing ˜1.5% fat and the lower values were for the experimental batches.

The calorific content of the different types of yoghurts were similar to, or slightly lower than the calculated energy values reported for the different milks (see Table 6.6). This is for the following reasons:- firstly, the fat and the protein contents in milk and yoghurts will be the same, and secondly, although 35 to 38% of the lactose content is utilised by the starter culture organisms (see Table 6.6) the production of metabolites such as organic acids [1.5-2.0% (Marsili *et al.*, 19871; Madhi, 1990 and sections 4.2.5 and 5.2.7)] and the partial accumulation of galactose in the yoghurt (see Table 4.5) will compensate for the reduction in the lactose content. The only difference, which is evident when calculating the calorific content of yoghurt is the energy conversion factors [*e.g.* carbohydrates 4 as compared with 3 for organic acids (Holland *et al.*, 1991)].

6.3   Microbiological Quality of Yoghurt

6.3.1 Microbiological Analysis of Yoghurt

In this study, the coliform, yeasts and moulds counts of all the yoghurts (fresh and stored) tested were <10 CFU g^{-1} (see Table 6.8). These results indicate that the yoghurts were produced under good sanitary conditions and hygienic standards

Table 6.7   **Calculated calorific content (k cal 100 g⁻¹) in yoghurt bases and products (fresh and stored)**

Product	Yoghurt		
	Milk	**Fresh**	**Stored**
1st Trial			
AMF	63.41	62.32	61.13
Simplesse® 100			
Wet - DM	55.87	54.86	55.27
Wet	52.36	51.97	50.71
Dry	56.03	54.87	54.06
2nd Trial			
AMF	64.16	62.71	61.96
Simplesse® 100			
Wet - DM	56.57	55.52	54.77
Wet	53.14	52.15	51.28
Dry	55.48	54.98	53.56
3rd Trial			
AMF	64.35	63.27	62.76
Simplesse® 100			
Wet - DM	57.90	57.64	54.45
Wet	52.87	52.48	50.60
Dry	56.21	55.69	54.93

Table 6.8 Microbiological quality (CFU g^{-1}) of different yoghurts

	Fresh yoghurt			Stored yoghurt		
	Total count	Coliform	Yeasts & moulds	Total count	Coliform	Yeasts & moulds
1st Trial						
AMF	<100[a]	<10[b]	<10	<100	<10	<10
Simplesse® 100						
Wet - DM	<100	<10	<10	<100	<10	<10
Wet	<100	<10	<10	<100	<10	<10
Dry	<100	<10	<10	<100	<10	12
2nd Trial						
AMF	<100	<10	<10	<100	<10	<10
Simplesse® 100						
Wet - DM	<100	<10	<10	<100	<10	<10
Wet	<100	<10	<10	<100	<10	<10
Dry	<100	<10	<10	<100	<10	<10
3rd Trial						
AMF	<100	<10	<10	<100	<10	<10
Simplesse® 100						
Wet - DM	<100	<10	<10	<100	<10	<10
Wet	<100	<10	<10	<100	<10	<10
Dry	<100	<10	<10	<100	<10	<10

[a] No growth at 10^{-2} dilution.
[b] No growth at 10^{-1} dilution.
Results are the average of two determinations performed on the same sample

(Tamime *et al.*, 1987; Barnes *et al.*, 1979). Furthermore, the total count of the non-lactic acid bacteria in all the yoghurt samples tested was < 100 CFU g^{-1} which is a low and acceptable count (Tamime *et al.*, 1987).

6.3.2 Enumeration of Starter Organisms

The viability of starter culture organisms in the yoghurt bases and products was estimated in the three trials and along the storage time (see Figure 6.1 and Appendix XVIII).

After the fermentation period the viable counts of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* had increased 2 log$_{10}$ cycles (see Figure 6.1). It is evident that the streptococci had dominated the microbial flora of the yoghurt, mainly as a consequence of the initial ratio of the inoculum. However, no significant effect on the viable counts of streptococci and lactobacilli were seen comparing the yoghurts made with AMF or Simplesse® 100 (wet or dry), nor in different trials and during storage at 5°C. A similar pattern was observed for the same yoghurt starter culture when other types of fat-substitutes and vegetable oils were studied (see section 4.3.2 and 5.3.2). The combined counts of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were high in all the yoghurts, (*i.e.* 10^5 and 10^8 CFU g^{-1}) thus ensuring microbiological safety.

6.4   Rheological Properties of Yoghurt

The rheological properties of five different types of yoghurt were assessed by monitoring the rate of serum separation/syneresis and firmness of the product as described in sections 3.11.4 and 3.11.5. The following intervals during storage at 5°C: 2, 5, 8, 14 and 20 days were followed to measure these properties.

6.4.1 Measurement of Serum Separation/Syneresis

After 2 days storage at 5°C, syneresis from the AMF yoghurt averaged 2.75 ml when compared with all the yoghurts made with microparticulate whey protein where the extent of serum separation ranged between 3.0 and 3.3 ml (Figure 6.2). Appendix XIX shows the results in each trial.
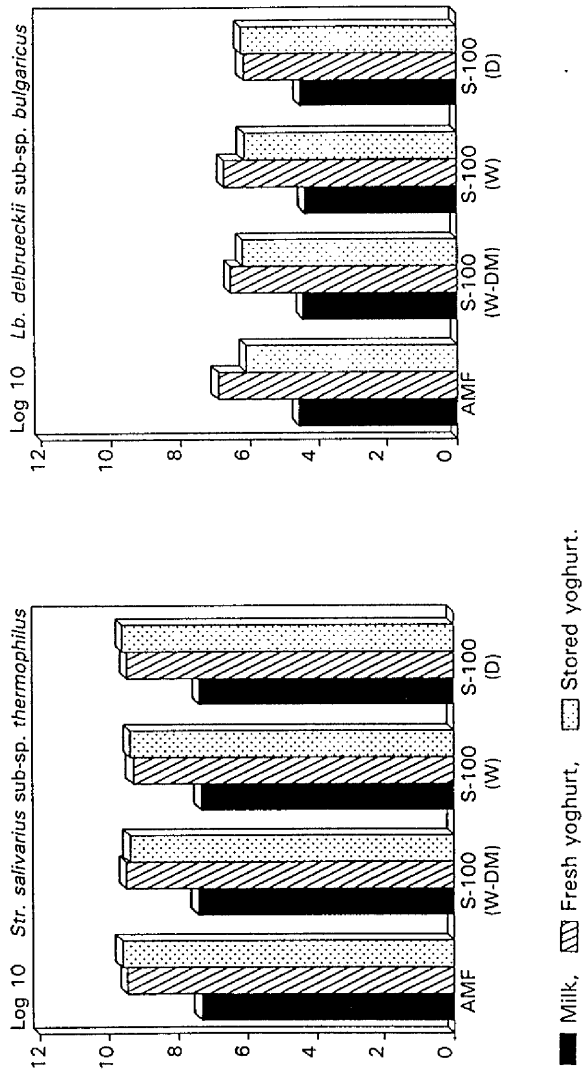
Figure 6.1 The enumeration of starter culture (CFU ml⁻¹ in milk or g⁻¹ in yoghurts) during the production and storage of different yoghurts[a].

Results are average of three trials.
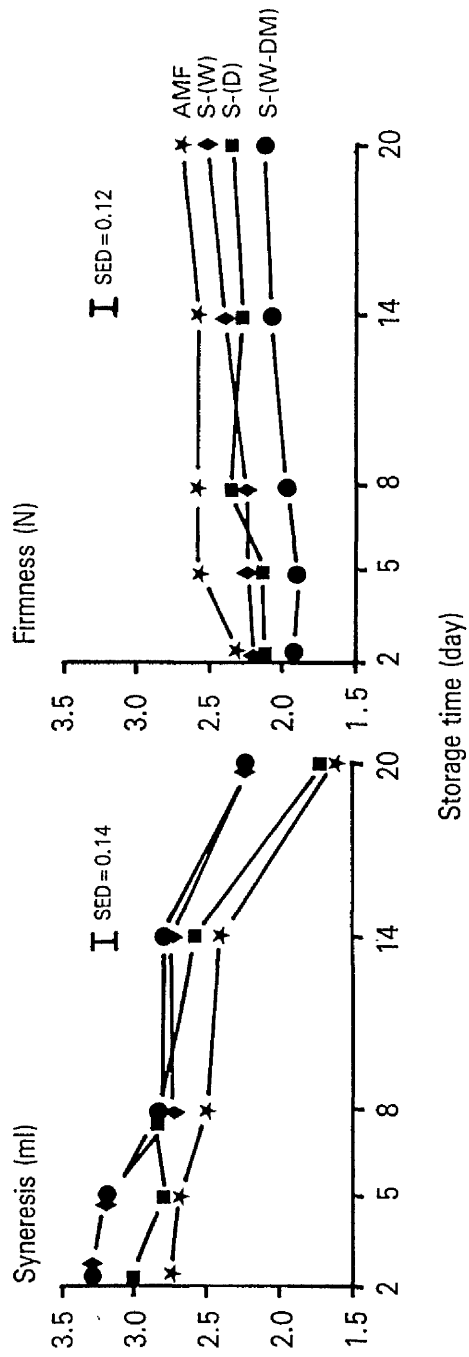
Figure 6.2   Changes in (a) syneresis and (b) firmness of yoghurts during 20 days storage at 5°C.

Analysis of variance was carried out on the syneresis measurements made after 2, 5, 8, 14 and 20 days of storage at 5°C. The duplicate measurements taken on each sampling date were averaged and significant differences (variance ratio test; $(P < 0.05)$ were detected between the yoghurts on each date. Also, the yoghurt made with AMF showed least syneresis at each sampling date.

Using orthogonal polynominals to quantify the effect of storage, analysis of variance was carried out on the total data set. The behaviour of the yoghurts was significantly different $(P < 0.05)$ and the effect of time was satisfactorily modelled by a linear relation.

It is possible to expect that the increase in protein content will enhance the formation of a firmer matrix complex after the fermentation period and more free water will be bound. It seems to have happened in the yoghurts containing Simplesse® 100 at day 14 of storage (see Figure 6.2), although the AMF yoghurt showed the same pattern. No significant difference was found between the use of Wet or Wet - DM Simplesse® 100 in the reduction of serum separation.

6.4.2 Measurement of Firmness

The firmness measurement of different yoghurts is shown in Figure 6.2 and Appendix XX illustrates the results in each trial.

At the beginning of the storage period the firmness of all yoghurts ranged between 1.75 to 3.67 N (see Figure 6.2). The duplicates taken on each sampling date were averaged and analysis of variance was carried out on each sampling date in turn. There was no significant difference between the yoghurts after 2 days storage. However, on subsequent days (5, 8, 14 and 20) the firmness score of the yoghurts made from Simplesse® 100 (wet and dry) were significantly lower than the AMF yoghurt, while the yoghurt made from Simplesse® 100 Wet - DM was lower still $(P < 0.05)$, possibly owing to the higher amount of non-casein (see Table 6.4).

Analysis of variance on the total data set using orthogonal polynomials to model the effect of storage revealed that the yoghurts were significantly different $(P < 0.05)$ and that the firmness measurements increased linearly with time for all the yoghurts. There was no interaction between yoghurt type and time.

6.4.3 Combined Analysis of Serum Separation and Firmness

The syneresis and firmness measurements provided complementary information for the four types of yoghurt (correlation coefficient = -0.672). In particular, both indicated significant changes with length of storage and identified the yoghurt containing AMF as being distinct from the others. However, the structure of yoghurt consists of a protein matrix composed of casein micelle chains and clusters, with fat particles embedded in it due to the homogenisation of the milk before the heat treatment stage (Kalab, 1979; Tamime *et al.*, 1984, 1989). The observed pattern of syneresis and firmness measurements (*i.e.* decrease and increase respectively) during the storage period may have been the result of functional differences of the proteins in the yoghurt bases. For example, the protein matrix of the yoghurt gel is influenced by the casein to non-casein protein ratio (Tamime *et al.*, 1984; Mottar *et al.*, 1987, 1989). The yoghurt made with AMF had the highest ratio of casein to non-casein protein (Table 6.4) and had a high firmness measurement after storage. Conversely, the yoghurt containing AMF had the lowest milk protein content and a lower amount of serum separation. This applied to both fresh and stored yoghurts. Currently, microstructure (*e.g.* scanning electron microscopy and transmission electron microscopy) studies of these yoghurts are planned to be done and the nature of the protein matrix may explain differences in firmness and serum separation. This microscopic analysis could not be ready by the time of submission of this thesis, but samples are still preserved in glutaraldehyde for analysis in Canada.

6.5   Organoleptic evaluation

The four different yoghurts were assessed at intervals of seven days (*i.e.* 1, 7 and 14) by ten trained panellists according to the scheme described in Appendix I (D), see also section 3.11.7. In this study, the organoleptic evaluation was done in a different experimental model as compared with the previous assessments including fat-substitutes and vegetable oils (*i.e.* when fresh and stored after 20 days). The reason for the change was to use the latest facilities available at the Hannah Research Institute which was not ready when the fat-substitutes and vegetable oils yoghurts were studied. Also due to the limited time factor available if the extended storage time was used (*i.e.* 20 days) then the panellists will be assessing the yoghurts from different trials.

The sensory data from the three trials was aggregated for a composite analysis. Residual Maximum Likelihood (REML) was used to fit a mixed model to the data. Random effects of judge, trial within judge, and storage time within trial within judge

were estimated. Fixed effects of yoghurt by storage time by trial were estimated as well as the effect of order of presentation. None of the attributes was greatly affected by order of tasting. The only attributes which showed significant differences in order were sour odour and sour flavour. The first yoghurt tasted was perceived to have a more intense sour odour and flavour than the subsequent yoghurts.

For the majority of the attributes (Table 6.9), no practical or statistically significant differences were detected between the 4 yoghurts under test. The two exceptions were sour odour and perceived serum separation. Sour odour was significantly higher ($P < 0.05$) for the yoghurt sample containing AMF than for the other products. The difference was not substantial. However, the differences in perceived serum separation were both statistically significant ($P < 0.05$) and substantive. The control sample (AMF) had the lowest score (37.7) and yoghurt (Simplesse® 100 Dry) had the highest score (52.1).

The yoghurts were assessed by the judges when fresh and after 7 and 14 days of storage at 5°C. There were statistically significant differences between the 3 storage dates for several of the attributes namely, creamy odour, sweet odour, sour aftertaste, chalky aftertaste and perceived serum separation (Table 6.10).

However, it is unwise to expect that each judge would be able to reference the results of one day's tasting with another. What is possible, though, is that a judge should be expected to be consistent in is/her preference. This means that if there is a significant interaction between day of tasting and yoghurt for any of the attributes then a particular yoghurt will change its rank order with time of storage. There were no significant interactions.

From the univariate analysis it is possible to select an attribute of interest and the rank the four yoghurts after having adjusted for effects of order of tasting and judge. Multivariate techniques allows us to combine the information on rankings and similarity of yoghurts from several attributes. However, the adjustment for any undesirable effects must usually be made explicitly before the analysis is carried out. There are several ways in which this adjustment can be made and the choice is critical.

What is of interest is the judges' preferences, but sometimes judges may use the scales differently. They may either use different parts of the scale (location) and /or that they

Table 6.9 Summary of the sensory scores (0-100) properties of yoghurt in which the fat was replaced by a protein-based fat-substitute

Sensory attribute	Control[1]	Simplesse			SED[2]
		Wet - DM	Wet	Dry	
Sour odour	**42.1**	**37.3**	**36.9**	**35.1**	**1.98**
Creamy odour	18.8	20.3	19.0	18.6	1.27
Sweet odour	12.5	11.7	13.3	11.8	1.03
Sour flavour	54.9	53.4	56.5	52.6	1.83
Creamy flavour	18.9	17.2	15.6	18.5	1.38
Sweet flavour	12.6	11.4	11.2	12.4	1.09
'Other' flavour	2.7	2.3	1.6	3.6	0.99
Sour after-taste	38.8	38.3	40.9	38.1	1.98
Chalky after-taste	27.3	27.9	27.6	28.0	1.39
Viscosity	62.4	59.6	58.8	59.8	1.35
Chalky texture	36.4	36.6	35.7	36.0	1.28
Perceived serum separation[3]	**38.5**	**40.7**	**48.8**	**51.9**	**2.66**
Acceptability	33.8	31.7	31.5	30.3	1.79

Note: [1]   Containing AMF.

[2]   SED: Standard Error of Difference.

[3]   Sensory attributes for which sample effects are significantly different.

Table 6.10   Summary of storage effects of the average sensory scores (0-100) of yoghurts

Sensory attribute	Day			SED[1]
	1	7	14	
Sour odour	38.6	36.6	38.31	1.96
Creamy odour[2]	**21.6**	**17.9**	**18.0**	**1.56**
Sweet odour[2]	**14.4**	**11.0**	**11.5**	**1.47**
Sour flavour	51.8	56.0	55.3	2.34
Creamy flavour	19.3	16.4	16.9	1.58
Sweet flavour	12.9	11.6	11.3	1.83
'Other' flavour	2.8	3.4	.1.5	1.21
Sour after-taste[2]	**34.8**	**40.9**	**4.4**	**2.30**
Chalky after-taste[2]	**24.6**	**27.8**	**30.7**	**2.27**
Viscosity	58.3	60.8	61.3	1.90
Chalky texture	35.3	37.1	36.0	1.98
Perceived serum separation[2]	**51.1**	**45.0**	**38.7**	**2.25**
Acceptability	32.2	33.0	30.3	2.70

Note: [1] SED: Standard Error of Difference.

   [2] Sensory attributes for which sample effects are significantly different.

may differ in how much of the scale they use (spread). If these factors are not removed then, for example, the first axis of a Principal Components (PCA) or Canonical Variates Analysis (CVA) may be dominated by differences between judges rather than true differences between samples. It was decided to remove the location effect by simply subtracting each judges' attribute mean from the scores. The influence of spread was removed by dividing the location adjusted scores by each judges' average Standard Deviation (SD). Using the average SD for each judge in preference to the judge's individual attribute, SD retains information concerning how important each taster believes a particular attribute to be in discriminating between the yoghurts. The adjusted scores were analysed by CVA.

From the REML analyses it has been possible to identify differences and similarities between the yoghurts for each of the eleven sensory attributes. Scatter plots of each pair of attributes illustrate the relationship between any two attributes and identify any two-dimensional groupings. Canonical Variates Analysis (CVA) of the yoghurt by storage time by trial tables provides a low dimensional representation of the eleven attributes which highlights differences between the four yoghurts. The first, second and third canonical variate axes accounted for 61%, 24% and 15% of the variance respectively. The yoghurt mean scores have been plotted on the first two canonical variate axes in Figure 6.3 and the canonical variate coefficients are displayed in Table 6.11. There is a large degree of overlap of the canonical variate scores for the different yoghurts; the yoghurts do not form disjoint sets. The implication is that the yoghurts are not distinctly different.

6.6   Conclusion

The use of microparticulated whey protein (Simplesse® 100), offer new possibilities for the manufacture of diet yoghurt *i.e.* very low in fat and higher in protein.

No processing difficulties were experienced in the production of yoghurts containing Simplesse® 100. The compositional and microbiological qualities of low-calorie yoghurts including the different types of fat-substitutes based on the microparticulated whey protein were excellent. The coliforms, yeasts and mould counts were <10 CFU g^{-1} in fresh and stored products. The yoghurt starter organisms were recovered in high numbers (streptococci x 10^8 CFU g^{-1} and lactobacilli x 10^5 CFU g^{-1}) and these fat-substitutes did not affect the starter culture activity during the fermentation period.
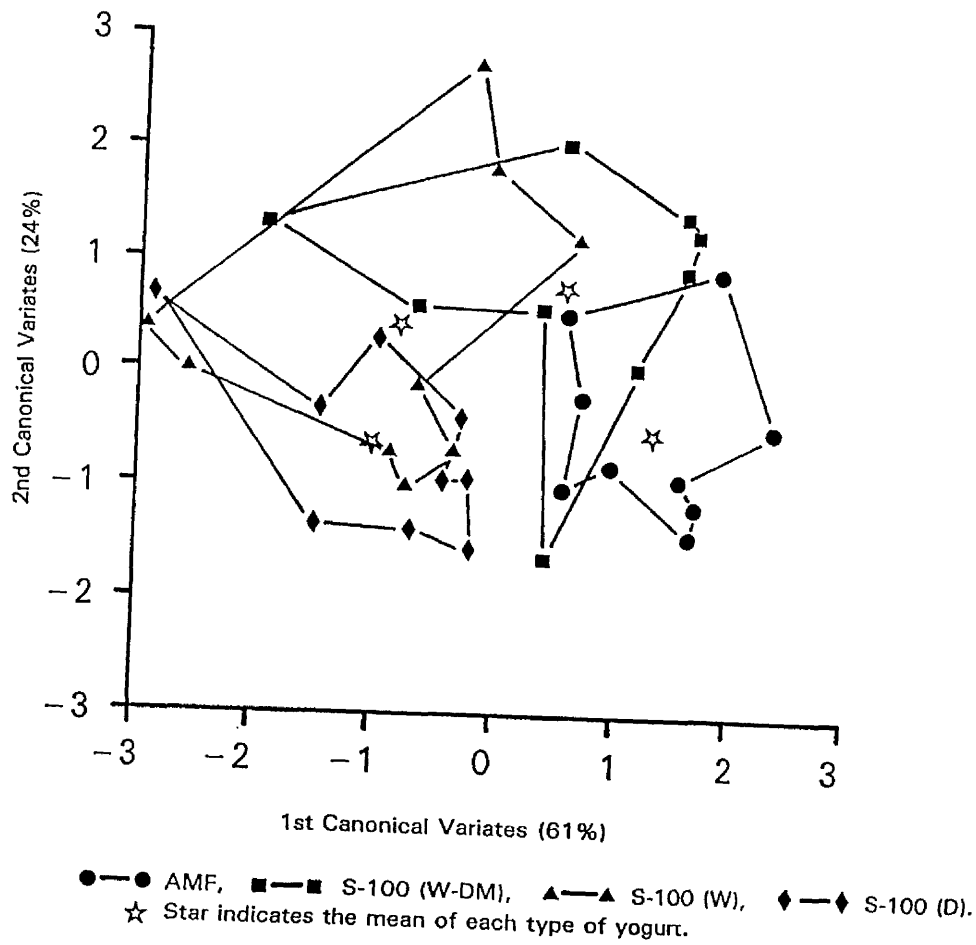
Figure 6.3   Canonical Variates Analysis of the organoleptic attributes assessments of different yoghurts.

*   Star indicates the mean of each type of yoghurt.

Table 6.11   Coefficients of first, second and third Canonical Variates

Sensory attributes	Canonical Variates		
	First	Second	Third
Sweet odour	**-0.15**	0.00	**-0.32**
Chalky after-taste	-0.09	0.03	-0.03
Perceived serum separation	-0.08	0.06	0.01
Sour after-taste	0.00	0.03	0.06
Viscosity	0.01	**-0.22**	0.02
Sour flavour	0.03	0.05	-0.16
Creamy flavour	0.08	0.07	0.04
Sweet flavour	0.12	**0.19**	0.02
Chalky texture	0.12	0.11	0.05
Sour odour	0.13	-0.03	-0.03
Creamy odour	**0.18**	0.02	**0.19**

Attributes in bold type believed to be important.

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Yoghurts made with wet or dry Simplesse® 100 at 1.5% addition were similar in rheological properties to the yoghurt made with AMF. Fortification with Simplesse® 100 (wet) on the basis of dry matter content produced a yoghurt with weak gel prone to serum separation. For all yoghurts, serum separation decreased with time; while firmness increased with time. The rate of change was lower for both firmness and serum separation, in the initial days of storage than at the end of storage life.

Only two sensory attributes (sour odour and perceived serum separation) were identified by the sensory judges to be significantly different to yoghurt made with AMF. Although, serum separation was perceived to decrease significantly along the storage time.

The calorific value in set-type yoghurt using microparticulated whey protein was reduced significantly when compared with AMF and the protein level was increased by ˜1%, improving the nutritional value of the fermented milk.

The evidence from the present study suggests that the fat-substitutes Simplesse® 100 (wet and dry), at 1.5% fortification could be used successfully for the manufacture of low-calorie yoghurts.

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APPENDICES

Appendix I   Illustrations of different organoleptic schemes used for the evaluation of yoghurt

A.   Five-Point Hedonic Scale (Fat-Substitutes Yoghurt)

Organoleptic Evaluation

Date: Tester:

Sample No./Code	Appearance and Colour		Body and Texture		Flavour and Aroma		Overall Score	Acceptability
	Score	Comments	Score	Comments	Score	Comments		

INSTRUCTIONS

Assess the above characteristics on the 1-5 Scale

5   Excellent
4   Very good
3   Good
2   Fair
1   Poor

Defects are:

Appearance and Colour: Lack of uniformity, unnatural colour, surface discolouration, wheying off, fat separation, gassiness.

Body and Texture: Too thin, too thick, lumpy, course or granular, slimy, gummy, clawy.

Flavour and Aroma: Excess acid, excess milk powder, yeasty, oxidised, unclean, lacking in flavour, mild.

B. **Nine-Point Hedonic Scale (Consumer Acceptance Survey)**

YOGHURT

Age: _____   Sex: _____   Yoghurt consumption: _____   pots/week

Home town: _____

Please give your score of **acceptability** to each yoghurt, (matching X) according to the table below.

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

YOGHURT _____ 1   2   3   4   5   6   7   8   9

YOGHURT _____ 1   2   3   4   5   6   7   8   9

YOGHURT _____ 1   2   3   4   5   6   7   8   9

YOGHURT _____ 1   2   3   4   5   6   7   8   9

YOGHURT _____ 1   2   3   4   5   6   7   8   9

C.   Unstructured/Graphic Scale (Vegetable Oils Yoghurt)

Organoleptic Evaluation of Yoghurt

Name: Sample No: Date:

Appearance and Colour*

Wheying off

    None   Extreme

Fat Separation

    None   Extreme

Discolouration

    None   Extreme

*Overall Rating

    Poor   Excellent

Body and Texture*

Firmness

    None   Extreme

Lumpy/Coarse

    None   Extreme

Smooth

    None   Extreme

Gummy

    None   Extreme

*Overall Rating

    Poor   Excellent

Flavour and Aroma*

Acidic

    None   Extreme

Creamy

    None   Extreme

Oxidised

    None   Extreme

Unclean

    None   Extreme

After- taste

    None   Extreme

*Overall Rating

    Poor   Excellent

Acceptability

    Poor   Excellent

D.   Unstructured/Graphic Scale (Microparticulated Protein Yoghurt)

Assessment of Natural Yoghurts

Please evaluate the yoghurt samples, strictly in the order in which they are presented.

Name:	Sample code:	Date:

1.   **Odour** (Carefully, sniff the yoghurt in the container and record your scores).

         |_____|

Undetectable   Very **Sour**

         |_____|

Undetectable   Very **Creamy**

         |_____|

Undetectable   Very **Sweet**

2.   **Flavour**   (You may taste as much or as little as you like, either swallowing it or spitting it out. Before re-tasting, it is advisable to consume a little biscuit and some water.)

         |_____|

Undetectable   Very **Sour/Acid**

         |_____|

Undetectable   Very **Creamy**

         |_____|

Undetectable   Very **Sweet**

(If you detect **Another** flavour, which you cannot describe in the terms above, write its name here and score as usual:

Name of **Other** flavour ....)

         |_____|

Undetectable   Very **Strong**

3.   **After-Taste** (Some of the products you taste may have a definite after-taste. Record your preception of this quality of he yoghurt in this section.)

         |_____|

Undetectable   Very **Acid/Sour**

         |_____|

Undetectable   Very **Chalky**

4.   **Texture**

Very thin   Very **Viscous**

         |_____|

Undetectable   Very **Chalky**

         |_____|

Undetectable   Very marked

         |_____| **Serum Separation**

5.   **Overall Acceptability** (Consider all aspects of this product, how much do you enjoy eating it?)

         |_____|

Dislike intensely   Enjoyed very much

Appendix II Viscosity measurement (centipoise) of yoghurt bases[a] containing different types of fat-substitutes

Product	Trial	
	First	Second
RSMP	2.7	2.4
AMF	2.3	2.3
Litesse™	2.8	2.6
Lycadex® 100	3.4	3.4
200	6.7	5.6
N-Oil® II	3.9	3.3
Paselli® SA2	2.8	2.7
P-150 C	5.1	4.7
285 F	2.6	2.1

[a]   Results are average of two determinations performed on the same sample.

Appendix III   Compositional quality (%w/w) of yoghurt (fresh and stored) made with different types of fat-substitutes[a]

Trial/ product	Protein	Fat	Ash	Carbohydrates Lactose[b]	Galactose[b]	Others[c]	Fibre[c]	Total solids	T.A.[d]	Organic acids[e]
1st Trial										
Fresh Yoghurt										
RSMP	5.34	0.10	1.14	4.56	1.02	-	-	14.01	1.24	1.90
AMF	5.31	1.51	1.10	4.48	1.05	-	-	15.08	1.20	1.97
Litesse™	5.28	0.10	1.13	4.86	0.90	1.40	-	15.07	1.22	1.80
Lycadex® 100	5.31	0.10	1.11	4.74	1.05	1.40	-	15.28	1.14	1.98
200	5.31	0.10	1.14	4.23	1.07	1.40	-	15.26	1.18	1.89
N-Oil® II	5.30	0.10	1.15	4.88	0.97	1.40	-	15.41	1.19	1.89
Paselli® SA2	5.27	0.10	1.10	4.61	1.01	1.40	-	15.32	1.17	1.95
P-150 C	5.40	0.10	1.16	4.86	1.04	0.70	0.50	15.51	1.26	1.96
285 F	5.27	0.10	1.15	4.78	0.95	0.10	1.10	15.22	1.22	1.89
Stored Yoghurt										
RSMP	5.33	0.10	1.14	4.36	0.82	-	-	14.00	1.28	1.85
AMF	5.31	1.50	1.16	4.43	0.73	-	-	15.01	1.30	1.82
Litesse™	5.20	0.10	1.14	4.36	0.88	1.40	-	15.22	1.29	1.50
Lycadex® 100	5.35	0.10	1.12	4.39	1.09	1.40	-	15.15	1.23	1.72
200	5.28	0.10	1.14	4.18	0.49	1.40	-	15.36	1.27	1.77
N-Oil® II	5.29	0.10	1.15	4.49	0.62	1.40	-	15.36	1.27	1.81
Paselli® SA2	5.28	0.10	1.11	4.41	0.49	1.40	-	15.30	1.29	1.83
P-150 C	5.39	0.10	1.17	4.58	0.53	0.70	0.50	15.14	1.40	1.90
285 F	5.27	0.10	1.15	4.48	0.54	0.10	1.10	15.14	1.29	1.89

Appendix III (continued)

Trial/product	Protein	Fat	Ash	Carbohydrates			Fibre[c]	Total solids	T.A.[d]	Organic acids[e]
				Lactose[b]	Galactose[b]	Others[c]				
2nd Trial										
Fresh Yoghurt										
RSMP	5.36	0.10	1.13	4.77	0.94	-	-	14.00	1.16	1.55
AMF	5.28	1.50	1.14	4.70	0.92	-	-	15.00	1.19	1.53
Litesse™	5.26	0.10	1.14	4.62	0.95	1.40	-	15.30	1.21	1.86
Lycadex® 100	5.25	0.10	1.13	4.42	0.99	1.40	-	15.07	1.19	1.60
200	5.29	0.10	1.15	4.78	0.97	1.40	-	15.22	1.13	1.60
N-Oil® II	5.18	0.10	1.14	4.65	0.99	1.40	-	15.47	1.10	1.45
Paselli® SA2	5.28	0.10	1.11	4.70	0.96	1.40	-	14.91	1.15	1.90
P-150 C	5.57	0.10	1.15	4.78	1.02	0.70	0.50	15.16	1.16	1.12
285 F	4.95	0.10	1.13	4.13	0.86	0.10	1.10	14.74	1.13	1.99
Stored Yoghurt										
RSMP	5.35	0.10	1.14	4.36	1.06	-	-	14.06	1.27	1.86
AMF	5.33	1.50	1.15	4.27	1.01	-	-	15.04	1.26	1.68
Litesse™	5.26	0.10	1.15	4.43	0.94	1.40	-	15.18	1.27	1.46
Lycadex® 100	5.30	0.10	1.13	4.29	1.06	1.40	-	15.18	1.27	1.49
200	5.27	0.10	1.16	4.60	0.91	1.40	-	15.14	1.27	1.70
N-Oil® II	5.21	0.10	1.15	4.42	0.92	1.40	-	14.75	1.27	1.67
Paselli® SA2	5.28	0.10	1.12	4.41	0.99	1.40	-	15.10	1.25	1.69
P-150 C	5.55	0.10	1.13	4.32	1.10	0.70	0.50	15.00	1.31	1.73
285 F	5.35	0.10	1.14	4.52	1.04	0.10	1.10	14.72	1.26	1.60

a   Results are average of two determinations performed on the same sample.
b   Lactose and galactose respectively.
c   Calculated on the basis of the technical data shown in Table 4.2.
d   Titratable acidity.
e   Data compiled from Appendix IV.
(-) Not reported.

Appendix IV   **Organic acids content (μg g^{-1}) of different yoghurt bases and yoghurts**

Product	Orotic	Citric	Pyruvic	Lactic	Uric/ Formic	Acetic	Hippuric
1st Trial							
Yoghurt Base							
RSMP	149	1393	15	812	47	0	24
AMF	131	1025	12	647	41	0	20
Litesse™	114	741	11	491	35	0	20
Lycadex® 100	121	808	10	565	36	0	21
200	121	807	11	556	36	0	21
N-Oil® II	118	770	10	538	35	0	21
Paselli® SA2	121	780	10	541	34	0	21
P-150 C	131	954	12	670	39	0	22
285 F	124	755	12	565	34	0	22
Fresh Yoghurt							
RSMP	108	481	24	0801	53	170	17
AMF	100	379	19	9032	49	154	18
Litesse™	87	277	16	7507	46	130	16
Lycadex® 100	102	424	21	9046	51	170	17
200	96	346	21	8269	48	124	17
N-Oil® II	99	354	21	8280	48	149	17
Paselli® SA2	103	388	20	8255	50	148	17
P-150 C	100	396	22	8880	50	154	16
285 F	101	356	22	8978	46	133	17
Stored Yoghurt							
RSMP	85	304	26	7937	45	182	16
AMF	83	279	25	7764	45	140	15
Litesse™	65	154	19	5669	37	107	16
Lycadex® 100	76	225	22	6713	48	173	13
200	80	262	24	7112	43	138	17
N-Oil® II	80	236	24	7189	42	127	17
Paselli® SA2	83	250	24	7493	45	150	16
P-150 C	82	245	26	7726	44	146	16
285 F	86	269	28	8484	43	116	17
2nd Trial							
Yoghurt base							
RSMP	112	906	15	633	24	0	22
AMF	118	931	18	662	26	0	23
Litesse™	115	824	16	556	25	0	22
Lycadex® 100	115	724	15	572	24	0	22
200	113	752	16	478	24	0	23
N-Oil® II	112	828	15	584	24	0	21
Paselli® SA2	116	750	17	642	25	0	23
P-150 C	96	452	12	354	20	0	20
285 F	112	847	24	558	24	0	20
Fresh Yoghurt							
RSMP	88	304	20	7215	32	81	16
AMF	81	242	25	6765	30	89	15
Litesse™	107	377	34	2190	36	94	15
Lycadex® 100	84	276	26	7795	32	120	15
200	82	251	24	7036	32	133	15
N-Oil® II	75	207	20	5893	29	123	15
Paselli® SA2	106	343	38	1360	36	120	16
P-150 C	79	251	23	6989	30	125	16
285 F	92	368	48	8536	31	110	16
Stored Yoghurt							
RSMP	88	327	39	7459	33	114	15
AMF	78	240	38	5691	30	93	15
Litesse™	65	169	26	3557	26	109	13
Lycadex® 100	71	192	33	4889	28	69	14
200	77	245	35	5841	30	96	15
N-Oil® II	76	227	37	6154	30	89	15
Paselli® SA2	79	265	36	6123	31	108	15
P-150 C	75	226	38	6356	30	102	16
285 F	87	317	26	7262	30	100	15

[a]   Above results are average of two determinations performed on the same sample.

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Appendix V   Enumeration and viability of starter organisms(CFU ml^{-1} or g^{-1})during the production and storage of different yoghurts[a].

Product	Streptococci			Lactobacilli		
	Inoculated milk (x 10^5)	Yoghurt Fresh (x 10^8)	Yoghurt Stored (x 10^8)	Inoculated milk (x 10^3)	Yoghurt Fresh (x 10^5)	Yoghurt Stored (x 10^5)
1st Trial						
RSMP	44	90	92	12	49	85
AMF	66	56	58	28	41	43
Litesse™	12	57	65	31	40	47
Lycadex® 100	58	72	75	37	54	59
200	39	57	59	27	53	93
N-Oil® II	55	58	60	22	41	60
Paselli® SA2	11	80	100	29	27	37
P-150 C	20	79	80	33	31	49
285 F	23	43	51	32	56	58
2nd Trial						
RSMP	34	81	87	22	52	85
AMF	54	67	69	36	39	49
Litesse™	29	54	56	32	36	72
Lycadex® 100	41	83	83	16	57	65
200	26	63	64	33	63	74
N-Oil® II	27	35	50	32	45	75
Paselli® SA2	10	104	106	35	31	37
P-150 C	36	78	79	50	33	36
285 F	12	43	52	33	48	50

a   Results are the average of single sample plated in duplicate.

Appendix VI   Serum separation (syneresis) of different yoghurts (ml)[a]

Product	Day				
	2	5	8	14	20
1st Trial					
RSMP	3.5	2.6	2.9	2.2	2.9
AMF	3.6	2.7	2.5	2.3	2.2
Litesse™	2.9	2.9	2.7	2.5	2.6
Lycadex® 100	1.9	2.9	3.2	2.5	2.8
200	2.6	2.8	2.8	1.8	2.6
N-Oil® II	3.5	2.5	2.6	1.6	2.7
Paselli® SA2	3.5	3.0	2.8	2.3	2.6
P-150 C	2.3	2.5	2.0	1.1	1.4
285 F	3.2	3.0	2.6	1.7	1.6
2nd Trial					
RSMP	3.2	3.4	3.3	3.3	3.6
AMF	3.8	2.4	1.8	2.3	2.7
Litesse™	3.9	3.5	2.8	3.5	3.3
Lycadex® 100	4.0	3.4	2.6	1.4	2.3
200	3.7	3.5	2.8	2.9	2.9
N-Oil® II	3.5	2.7	2.9	2.1	1.4
Paselli® SA2	3.4	3.2	3.4	3.1	1.3
P-150 C	1.2	2.3	1.7	2.3	1.2
285 F	4.5	4.2	3.8	3.6	1.9

[a] Results are average of two determinations performed on the same sample.

Appendix VII   **Firmness measurements (N) of different yoghurts[a]**

Product	Day				
	2	**5**	**8**	**14**	**20**
1st Trial					
RSMP	2.21	2.55	2.23	2.10	2.33
AMF	2.16	2.20	2.21	2.43	2.45
Litesse™	1.55	2.00	2.00	2.05	2.03
Lycadex® 100	1.64	1.92	2.12	2.04	2.12
200	1.81	2.19	2.09	2.36	2.38
N-Oil® II	1.58	2.30	1.91	2.19	2.29
Paselli® SA2	2.06	2.06	2.12	2.26	2.37
P-150 C	2.00	2.25	1.85	2.35	2.55
285 F	2.01	2.30	2.58	2.58	2.74
2nd Trial					
RSMP	1.81	1.94	2.02	2.06	2.02
AMF	2.23	2.56	2.38	2.47	2.41
Litesse™	1.99	2.02	1.99	2.03	1.98
Lycadex® 100	2.00	2.27	2.27	2.36	2.23
200	2.14	2.24	2.30	2.41	2.52
N-Oil® II	2.29	1.98	2.18	2.34	2.36
Paselli® SA2	1.41	1.82	1.81	2.00	1.85
P-150 C	1.83	2.30	2.18	2.47	2.48
285 F	2.27	2.24	2.37	2.56	2.63

[a]   Results are average of two determinations performed on the same sample.

Appendix VIII   **Fatty acids composition (%w/w) in AMF and four fifferent vegetable oils[a]**

Fatty acids		AMF	Olive	Groundnut	Sunflower	Corn
Saturated						
Butyric	C4:0	5.2	0.0	0.0	0.0	0.0
Caprioc	C6:0	3.4	0.0	0.0	0.0	0.0
Caprylic	C8:0	1.7	0.0	0.0	0.0	0.0
Capric	C10:0	3.0	0.0	0.0	0.0	0.0
Lauric	C12:0	3.1	0.0	0.0	0.0	0.0
Myristic	C14:0	9.8	0.2	0.4	0.7	0.2
Palmitic	C16:0	27.4	14.6	12.3	7.6	10.2
Stearic	C18:0	11.2	2.6	3.9	4.7	2.0
Unsaturated						
Myristoleic	C14:1	1.5	0.2	0.8	0.1	0.9
Palitoleic	C16:1	0.7	1.8	0.3	0.2	0.2
Oleic	C18:1	26.8	63.3	54.4	22.1	27.1
Linoleic	C18:2	5.5	15.8	24.5	63.6	57.2
Linolenic	C18:3	0.5	1.0	1.2	0.3	1.9
Arachionic	C20:4	0.5	0.6	1.7	0.7	0.6

[a]   Results are average of two determinations performed on the same sample.

Appendix IX   Viscosity measurement (centipoise) of yoghurt bases containing different types of vegetable oils[a] (at 20°C)

Product	Trial		
	First	Second	Third
AMF	2.3	2.3	2.3
Olive	2.3	2.2	2.3
Groundnut	2.3	2.3	2.3
Sunflower	2.3	2.4	2.3
Corn	2.4	2.4	2.3

[a] Results are average of two determinations performed on the same sample.

Appendix X   Means of fatty acids composition (mg 100 g^{-1}) in yoghurt bases

Yoghurt Base	Saturated								Unsaturated					
	C4	C6	C8	C10:0	C12:0	C14:0	C16:0	C18:0	C14:1	C16:1	C18:1	C18:2	C18:3	C20:4
1st Trial														
AMF (1.47)	91.20	51.00	24.87	44.00	45.70	144.60	398.50	167.00	18.52	9.17	389.70	71.80	6.40	5.15
Olive (1.38)	3.69	2.50	2.20	2.90	2.71	13.46	215.25	54.50	1.10	3.50	821.20	232.40	11.70	12.67
Groundnut (1.49)	4.76	3.10	2.54	3.60	4.10	14.50	195.60	70.90	1.90	3.80	777.80	364.20	20.67	22.03
Sunflower (1.30)	2.70	2.00	1.40	2.07	2.30	10.50	103.50	65.60	1.40	2.60	272.80	826.40	2.34	4.84
Corn (1.39)	3.80	2.50	1.70	2.50	2.70	9.90	152.00	39.70	0.95	2.03	370.12	762.00	28.96	10.57
2nd Trial														
AMF (1.47)	77.50	49.06	24.42	44.37	48.67	150.00	418.00	171.00	18.10	9.23	388.40	64.23	5.07	1.50
Olive (1.25)	3.52	2.04	1.30	2.04	2.40	10.00	196.00	37.00	1.30	2.74	793.20	179.40	0.10	8.42
Groundnut (1.24)	2.81	1.80	1.40	1.93	2.80	9.60	162.11	57.20	0.60	1.40	682.60	305.20	1.14	12.54
Sunflower (1.44)	3.84	2.40	1.70	1.87	2.90	12.40	113.30	73.40	3.62	3.42	315.80	893.10	3.80	7.90
Corn (1.29)	3.00	1.90	1.30	2.35	2.20	8.18	136.24	34.00	0.61	1.34	347.30	720.80	24.10	7.10
3rd Trial														
AMF (1.51)	98.30	53.04	24.94	45.64	48.63	157.30	422.00	165.70	25.51	15.06	394.00	56.74	5.22	8.80
Olive (1.33)	4.04	2.40	1.61	2.18	2.61	9.77	207.42	40.00	0.85	9.41	839.20	191.21	0.10	15.77
Groundnut (1.47)	4.80	2.40	1.62	2.36	3.23	10.63	190.30	59.70	0.75	6.50	818.00	354.35	1.35	19.91
Sunflower (1.27)	5.25	3.10	1.83	2.99	3.46	12.32	103.20	68.17	2.80	3.96	270.11	790.38	3.30	2.40
Corn (1.37)	3.80	2.21	1.42	2.18	2.48	8.90	151.30	34.18	0.73	2.98	369.90	770.06	25.60	19.91

Above results are an average of two analysis performed on the same sample.

Figures in parenthesis represent fat content in the yoghurt base.

Appendix XI   Compositional quality (%w/w) of fresh and stored yoghurt made with different types of vegetable oils and AMF[a]

Yoghurt	Protein	Fat	Lactose	Ash	Total solids	pH	Acidity T.A.	HPLC
1st trial								
Fresh Yoghurt								
AMF	5.21	1.48	4.80	1.12	15.11	4.68	1.00	2.08
Olive	5.26	1.36	4.77	1.12	15.07	4.67	0.98	2.20
Groundnut	5.30	1.48	4.80	1.12	15.00	4.62	0.96	2.19
Sunflower	5.23	1.28	4.78	1.11	14.94	4.64	0.98	1.99
Corn	5.24	1.38	4.77	1.12	15.05	4.58	1.10	2.09
Stored Yoghurt								
AMF	5.18	1.48	4.55	1.12	15.00	4.44	1.31	2.08
Olive	5.25	1.36	4.58	1.12	15.00	4.43	1.27	2.05
Groundnut	5.30	1.48	4.57	1.12	14.98	4.38	1.35	2.04
Sunflower	5.23	1.28	4.46	1.11	14.92	4.38	1.35	2.11
Corn	5.22	1.38	4.55	1.12	14.95	4.32	1.34	2.06
2nd trial								
Fresh Yoghurt								
AMF	5.38	1.47	4.69	1.11	15.01	4.66	0.96	2.17
Olive	5.40	1.25	4.65	1.14	14.92	4.62	0.95	2.19
Groundnut	5.34	1.18	4.68	1.13	14.95	4.60	1.10	2.18
Sunflower	5.34	1.44	4.71	1.12	14.95	4.55	0.98	2.14
Corn	5.33	1.29	4.73	1.12	14.96	4.60	1.10	2.10
Stored Yoghurt								
AMF	5.37	1.47	4.51	1.13	14.96	4.48	1.33	2.07
Olive	5.37	1.25	4.45	1.12	14.89	4.41	1.34	2.06
Groundnut	5.34	1.18	4.55	1.11	14.90	4.39	1.33	2.04
Sunflower	5.33	1.44	4.52	1.13	14.92	4.33	1.35	2.08
Corn	5.30	1.29	4.50	1.12	14.95	4.34	1.29	2.10
3rd trial								
Fresh Yoghurt								
AMF	5.36	1.51	4.76	1.13	15.19	4.59	1.10	2.10
Olive	5.39	1.33	4.78	1.14	15.19	4.60	0.98	1.89
Groundnut	5.30	1.48	4.75	1.14	15.24	4.56	1.00	1.85
Sunflower	5.36	1.27	4.65	1.13	15.03	4.49	1.05	1.92
Corn	5.30	1.37	4.74	1.14	15.17	4.52	0.95	1.97
Stored Yoghurt								
AMF	5.35	1.48	4.43	1.11	15.08	4.32	1.26	1.83
Olive	5.38	1.34	4.60	1.12	15.12	4.36	1.20	1.78
Groundnut	5.34	1.43	4.54	1.14	15.10	4.35	1.37	1.82
Sunflower	5.38	1.26	4.54	1.12	15.00	4.33	1.39	1.72
Corn	5.30	1.35	4.58	1.13	15.03	4.30	1.38	1.85

[a]   Results are average of two determinations per sample.

Appendix XII   Organic acids content (ug g^{-1}) of yoghurt bases and yoghurts made with different vegetable oils

Product	Orotic	Citric	Pyruvic	Lactic	Uric/ Formic	Acetic	Hippuric
1st Trial							
Yoghurt base							
AMF	125	1383	45	580	27	0	24
Olive	112	1410	51	535	27	0	25
Groundnut	114	1230	47	645	25	0	26
Sunflower	124	1448	46	613	27	0	24
Corn	125	1630	50	635	27	0	25
Fresh yoghurt							
AMF	118	858	36	19697	39	120	19
Olive	114	827	32	18717	36	118	18
Groundnut	123	866	43	20671	44	135	20
Sunflower	116	884	32	19561	37	140	19
Corn	124	956	39	20775	39	125	21
Stored yoghurt							
AMF	116	760	43	19508	37	121	19
Olive	119	811	49	19931	39	101	20
Groundnut	115	834	47	19278	39	98	17
Sunflower	116	830	50	19657	38	118	20
Corn	116	814	48	19359	39	113	20
2nd Trial							
Yoghurt base							
AMF	126	1879	74	658	29	0	23
Olive	123	1819	62	495	27	0	24
Groundnut	125	1872	65	610	29	0	25
Sunflower	124	1920	72	645	29	0	24
Corn	126	1944	86	679	30	0	23
Fresh yoghurt							
AMF	108	957	53	19676	40	154	20
Olive	112	916	64	20074	41	138	18
Groundnut	117	1075	54	20385	41	146	19
Sunflower	115	978	55	20379	41	140	18
Corn	117	1042	64	20436	41	144	16
Stored yoghurt							
AMF	110	931	70	19728	40	107	19
Olive	109	898	58	19603	40	128	18
Groundnut	107	872	63	19204	39	108	18
Sunflower	109	939	67	19414	39	156	19
Corn	110	919	77	19380	40	118	19
3rd Trial							
Yoghurt base							
AMF	104	1086	50	376	24	0	25
Olive	109	1095	51	426	24	0	27
Groundnut	103	1183	50	391	24	0	26
Sunflower	111	1218	54	405	25	0	24
Corn	100	1218	54	409	23	0	22
Fresh yoghurt							
AMF	100	810	42	18553	38	106	20
Olive	99	655	36	18194	38	114	25
Groundnut	95	724	38	17511	36	96	21
Sunflower	112	891	37	21445	43	118	25
Corn	97	676	41	17861	39	99	24
Stored yoghurt							
AMF	93	712	40	17397	37	162	21
Olive	89	678	45	16183	37	157	20
Groundnut	94	750	43	17180	40	147	22
Sunflower	92	708	39	17319	38	125	23
Corn	101	741	44	16776	38	114	22

Results are an average of two determinations performed on the same sample.

Appendix XIII   Fatty acids composition (mg 100 g^{-1}) in yoghurt (fresh and stored)

Yoghurt	Saturated								Unsaturated					
	C4	C6	C8	C10:0	C12:0	C14:0	C16:0	C18:0	C14:1	C16:1	C18:1	C18:2	C18:3	C20:4
1st Trial														
Fresh														
AMF (1.48)	84.67	51.00	24.50	44.06	46.30	145.70	405.94	168.70	18.24	9.23	401.14	70.61	5.97	3.81
Olive (1.36)	3.90	2.50	1.70	2.50	2.84	11.41	213.30	48.47	1.23	1.66	837.50	215.87	9.60	7.46
Groundnut (1.48)	4.60	3.10	2.00	3.40	4.02	14.31	200.00	70.00	1.44	1.97	785.70	360.00	14.34	16.43
Sunflower (1.28)	2.91	2.00	1.50	2.30	2.42	12.33	108.00	66.77	1.00	1.41	279.73	791.30	3.10	6.06
Corn (1.38)	3.60	2.50	1.60	2.40	2.84	1.90	154.10	41.03	0.80	1.75	374.68	753.75	23.92	6.35
Stored														
AMF (1.48)	71.44	47.32	24.06	44.42	48.45	150.10	420.10	172.40	18.94	9.72	398.30	65.34	6.00	3.23
Olive (1.36)	3.60	2.45	1.74	2.43	2.94	11.76	211.00	49.28	1.20	1.86	832.00	221.16	10.16	8.60
Groundnut (1.48)	4.30	2.95	1.98	2.89	3.95	14.68	167.00	72.13	1.62	2.10	785.00	368.48	3.04	19.50
Sunflower (1.28)	2.81	1.90	1.40	2.00	2.34	11.05	104.00	66.00	0.90	1.60	281.00	790.50	3.73	6.70
Corn (1.38)	3.74	2.39	1.68	2.63	2.97	11.13	151.50	40.78	0.70	1.50	374.00	753.20	25.79	7.20
2nd Trial														
Fresh														
AMF (1.47)	73.70	47.28	23.78	43.50	46.80	149.80	416.30	170.00	21.60	11.08	396.40	65.90	5.00	1.81
Olive (1.25)	2.20	1.66	1.14	1.80	2.20	7.80	188.50	38.15	0.61	12.40	800.00	180.52	7.60	4.46
Groundnut (1.18)	2.61	1.67	1.16	1.70	2.50	8.52	153.23	51.60	0.61	1.60	658.00	284.87	0.60	11.20
Sunflower (1.44)	3.68	2.40	1.65	2.40	3.10	13.47	117.54	73.70	1.10	2.30	310.50	900.10	2.90	5.00
Corn (1.29)	2.56	1.65	1.10	1.73	2.10	7.80	137.40	32.80	0.66	1.72	358.90	728.10	19.20	4.28
Stored														
AMF (1.47)	78.60	47.49	24.20	43.30	46.60	149.00	408.00	167.00	21.35	10.60	395.60	68.70	6.60	2.65
Olive (1.25)	2.90	1.90	1.36	1.90	2.35	8.80	195.00	40.70	0.60	1.80	792.50	187.27	8.15	5.00
Groundnut (1.18)	2.50	1.60	1.15	1.70	2.50	8.40	154.00	51.70	0.70	1.70	660.70	280.70	0.50	12.20
Sunflower (1.44)	2.70	1.92	1.36	2.10	2.70	11.20	111.00	70.80	1.26	2.03	310.00	914.60	2.60	5.30
Corn (1.29)	1.62	1.37	1.00	1.65	2.10	7.80	137.80	34.10	0.80	1.85	3519.00	720.80	21.60	5.80
3rd Trial														
Fresh														
AMF (1.51)	90.47	50.14	24.37	45.90	49.92	159.00	433.80	164.30	22.25	11.50	400.00	56.60	5.12	2.00
Olive (1.33)	3.80	2.20	1.40	2.14	2.54	9.70	208.20	40.75	0.90	8.40	846.15	195.40	8.10	8.27
Groundnut (1.47)	3.80	2.30	1.40	2.24	3.15	10.60	192.00	59.72	0.80	6.30	824.34	358.70	0.80	14.70
Sunflower (1.27)	5.28	3.07	1.90	2.97	3.60	13.90	110.00	63.30	1.20	3.70	274.50	785.70	2.57	0.97
Corn (1.37)	3.61	2.13	1.35	2.01	2.42	9.30	154.00	34.30	0.70	3.80	374.00	766.00	20.40	6.40
Stored														
AMF (1.48)	91.64	50.15	24.10	45.10	48.60	155.60	420.00	160.00	21.60	11.74	389.00	58.70	6.61	2.30
Olvie (1.32)	3.60	2.16	1.42	2.10	2.40	10.40	209.20	39.10	1.10	28.30	835.00	196.00	8.74	9.20
Groundnut (1.43)	3.70	2.16	1.36	2.20	3.03	10.70	187.00	56.90	0.96	5.47	797.00	346.00	0.60	14.02
Sunflower (1.27)	4.90	2.96	1.30	3.04	3.52	14.42	110.00	62.30	1.10	4.00	275.00	777.90	2.30	0.40
Corn (1.35)	3.70	2.20	1.50	2.30	2.60	9.97	154.50	33.87	0.74	4.10	368.40	750.27	22.60	6.00

Appendix XIV   Enumeration and viability of starter organisms (CFU ml^{-1} or g^{-1})^a during the production and storage of yoghurt^b

Product	Streptococci				Lactobacilli			
	Inoculated Milk (x 10^5)	Fresh (x 10^7)	Yoghurt Store (x 10^7)	Inoculated Milk (x 10^3)	Fresh (x 10^5)	Yoghurt Stored (x 10^5)
1st Trial						
AMF	44	119	177	48	60	62
Olive	68	106	127	49	46	60
Groundnut	87	66	112	46	18	29
Sunflower	52	81	127	50	39	47
Corn	58	106	127	41	36	53
2nd Trial						
AMF	105	81	155	61	24	35
Olive	128	82	178	54	31	37
Groundnut	140	93	128	64	34	54
Sunflower	108	89	157	41	39	45
Corn	136	77	118	59	37	53
3rd Trial						
AMF	136	136	195	50	91	39
Olive	117	118	171	54	98	45
Groundnut	178	123	161	60	104	38
Sunflower	118	106	223	31	82	34
Corn	170	121	212	69	62	28

^{a} CFU ml^{-1} for milks and g^{-1} for yoghurts.
^{b} Results are an average of two determinations performed on the same sample.

Appendix XV   Whey separation measurements (ml) of yoghurts made with vegetable oils[a]

Product	Day				
	2	**5**	**8**	**14**	**20**
1st Trial					
AMF	2.95	3.05	2.25	1.90	2.10
Olive	2.65	3.05	2.40	2.40	2.95
Groundnut	3.00	2.85	2.70	2.80	2.20
Sunflower	2.60	3.25	2.60	2.80	2.80
Corn	2.80	3.50	2.50	2.50	1.85
2nd Trial					
AMF	2.80	2.60	2.00	2.30	2.10
Olive	2.65	3.37	2.60	3.25	2.90
Groundnut	3.25	3.50	2.80	3.00	3.25
Sunflower	2.60	3.62	3.15	3.25	3.00
Corn	2.90	3.50	2.70	2.60	2.80
3rd Trial					
AMF	2.30	2.50	2.60	1.80	1.90
Olive	2.40	2.80	3.00	2.30	1.90
Groundnut	2.20	2.90	3.10	2.40	2.30
Sunflower	2.80	3.00	2.85	2.00	1.80
Corn	2.70	3.05	3.20	2.45	1.70

[a]   Results are an average of two determinations performed on the sample pot.

Appendix XVI   Firmness measurements (N) of yoghurt made with vegetable oils[a]

Product	Day				
	2	5	8	14	20
1st Trial					
AMF	2.41	2.51	2.87	2.76	2.76
Olive	1.80	2.04	2.16	2.30	2.30
Groundnut	2.10	2.11	2.46	2.30	2.27
Sunflower	1.92	2.22	2.33	2.40	2.56
Corn	1.93	2.31	2.33	2.30	2.52
2nd Trial					
AMF	2.74	2.87	2.94	2.95	2.94
Olive	1.95	2.06	2.30	2.40	2.33
Groundnut	2.02	2.09	2.21	2.19	2.62
Sunflower	1.74	1.88	1.97	2.07	2.05
Corn	1.86	1.91	2.07	2.17	1.99
3rd Trial					
AMF	2.84	2.82	2.89	2.98	2.86
Olive	2.17	2.27	2.42	2.32	2.29
Groundnut	2.28	2.38	2.20	2.34	2.38
Sunflower	2.18	2.18	2.71	2.55	2.55
Corn	2.30	2.22	2.65	2.26	2.35

[a] Results are an average of two determinations on different pot.

Appendix XVII Compositional quality (%w/w) of yoghurt made with AMF and Simplesse® 100

Product	Protein	Fat	Ash	Lactose	Total solids	pH	Titratable acidity
1st Trial							
Fresh yoghurt							
AMF	5.20	1.45	1.16	4.73	14.55	4.69	1.18
Simplesse® 100							
Wet - DM	6.29	0.19	1.25	4.53	14.94	4.63	1.30
Wet	5.50	0.12	1.19	4.69	14.15	4.60	1.29
Dry	6.11	0.15	1.25	4.76	15.03	4.67	1.36
Stored yoghurt							
AMF	5.22	1.41	1.13	4.38	14.61	4.41	1.42
Simplesse® 100							
Wet - DM	6.32	0.18	1.19	4.58	14.91	4.43	1.58
Wet	5.52	0.12	1.18	4.38	13.82	4.44	1.51
Dry	6.14	0.15	1.24	4.52	15.02	4.46	1.58
2nd Trial							
Fresh yoghurt							
AMF	5.22	1.48	1.15	4.62	15.12	4.56	1.19
Simplesse® 100							
Wet - DM	6.36	0.19	1.26	4.57	15.42	4.56	1.29
Wet	5.55	0.14	1.19	4.66	14.04	4.52	1.31
Dry	6.04	0.16	1.25	4.83	14.89	4.58	1.37
Stored yoghurt							
AMF	5.20	1.48	1.16	4.42	15.00	4.31	1.26
Simplesse® 100							
Wet - DM	6.27	0.19	1.25	4.49	15.00	4.38	1.30
Wet	5.57	0.14	1.19	4.42	13.84	4.33	1.55
Dry	6.07	0.15	1.24	4.47	14.60	4.33	1.51
3rd Trial							
Fresh yoghurt							
AMF	5.23	1.52	1.16	4.66	14.97	4.47	1.16
Simplesse® 100							
Wet - DM	6.26	0.19	1.28	4.71	14.13	4.49	1.21
Wet	5.61	0.12	1.16	4.73	14.00	4.46	1.13
Dry	6.16	0.15	1.25	4.92	14.90	4.44	1.14
Stored yoghurt							
AMF	5.21	1.50	1.25	4.59	14.93	4.32	1.23
Simplesse® 100							
Wet - DM	6.25	0.19	1.28	4.43	14.92	4.35	1.35
Wet	5.58	0.12	1.16	4.29	13.93	4.35	1.31
Dry	6.14	0.15	1.24	4.74	14.85	4.37	1.28

Above results are average of two determinations performed on the same sample.

Appendix XVIII Enumeration and viability of starter organisms (CFU ml^{-1} or g^{-1})[a] during the production and storage of yoghurt[b]

Product	Streptococci			Lactobacilli		
	Inoculated Milk (x 10^6)	Yoghurt Fresh (x 10^8)	Yoghurt Store (x 10^8)	Inoculated Milk (x 10^3)	Yoghurt Fresh (x 10^5)	Yoghurt Stored (x 10^5)
1st Trial						
AMF	16	16	50	40	48	14
Simplesse® 100						
Wet - DM	16	19	58	23	49	33
Wet	16	12	38	32	55	13
Dry	15	16	56	32	57	16
2nd Trial						
AMF	28	45	43	40	50	15
Simplesse® 100						
Wet - DM	51	42	40	48	18	6
Wet	26	36	30	24	62	13
Dry	32	43	38	35	92	14
3rd Trial						
AMF	21	38	31	33	60	10
Simplesse® 100						
Wet - DM	22	40	65	21	54	20
Wet	26	18	12	22	52	18
Dry	25	37	27	33	83	25

a   CFU ml^{-1} for milks and g^{-1} for yoghurts.

b   Above results are an average of two determinations performed on the same sample.

Appendix XIX   **Syneresis/whey separation measurements (ml) of yoghurts made with microparticulated whey protein**

Yoghurt	Day				
	2	5	8	14	20
1st Trial					
AMF	2.85	2.80	2.85	2.65	2.10
Simplesse® 100					
Wet - DM	3.25	2.90	3.00	2.80	1.90
Wet	3.35	3.25	2.90	3.00	2.50
Dry	3.25	3.65	2.80	3.00	2.85
2nd Trial					
AMF	2.70	2.40	2.20	2.10	1.40
Simplesse® 100					
Wet - DM	3.25	3.00	2.65	2.80	1.75
Wet	3.00	2.85	2.60	2.60	1.40
Dry	3.00	2.65	2.60	2.60	1.40
3rd Trial					
AMF	2.70	2.80	2.40	2.40	1.45
Simplesse® 100					
Wet - DM	3.25	3.25	2.80	2.50	2.40
Wet	3.63	3.00	2.80	2.60	2.40
Dry	2.80	2.80	2.80	2.30	1.80

Above results are average of two readings performed on the same pot.

Appendix XX **Firmness measurements (N) of yoghurts made with microparticulated whey protein**

Yoghurt	Day				
	2	5	8	14	20
1st Trial					
AMF	1.79	2.16	2.12	2.18	2.22
Simplesse® 100					
Wet - DM	1.75	1.81	1.81	1.88	1.94
Wet	1.87	2.00	2.02	2.12	2.25
Dry	1.84	1.84	2.00	1.88	1.90
2nd Trial					
AMF	2.67	1.90	2.97	2.86	2.99
Simplesse® 100					
Wet - DM	2.00	2.02	2.11	2.16	2.19
Wet	2.48	2.36	2.42	2.46	2.70
Dry	2.51	2.43	2.64	2.66	2.65
3rd Trial					
AMF	2.39	2.60	2.59	2.62	2.78
Simplesse®100					
Wet - DM	2.03	1.78	1.95	2.14	2.20
Wet	2.19	2.28	2.35	2.43	2.51
Dry	2.02	2.10	2.26	2.26	2.41

Above results are average of two readings performed on different pots.