MICROPARTICULATED WHEY PROTEIN AS A FAT SUBSTITUTE IN FROZEN YOGHURT

BY

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I declare that this dissertation herewith submitted for the degree of Master of Science (Food Science) at the University of Pretoria, has not been submitted by me for a degree at any other university or institution of higher education.

DEDICATION

This dissertation is dedicated to my parents,
Claude and Aurette Seevathean,
who have spared no efforts whatsoever
to support me in all my endeavours.
Thank you mum and dad for everything
you have done for me.

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ABSTRACT

MICROPARTICULATED WHEY PROTEIN AS A FAT SUBSTITUTE IN FROZEN YOGHURT

by

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Microorganisms have traditionally been selected for fermentation of milk on their ability to grow in milk. However nowadays the trend is towards including probiotic bacteria, i.e. those bacteria that have been suggested to provide additional health benefits to the consumer. Probiotic microorganisms have beneficial effects when ingested such as lowered incidence of colon cancer, the suppression of putrefactive and pathogenic bacteria by competitive exclusion and the production of organic acids, diacetyl and bacteriocins and a hypocholesterolemic effect, to mention but a few.

To be more effective, the proposed microorganisms must be of human origin. *Lactobacillus acidophilus* and *Bifidobacterium bifidum,* which are two of the most successful probiotic bacteria used commercially, are normal inhabitants of the intestine of many animals including humans. They must however maintain their viability and activity in the bio-product in which they are formulated to be available at the right level at the time of consumption. A number of factors affect their viability, including low pH, the type of culture used and availability of essential amino acids. The choice of ingredients in the mix is crucial since it affects a number of mix properties such as pH and availability of nutrients.

The demand for low fat products is a very strong driving force on the market place. However, reducing the fat content of such complex products as frozen dairy desserts is very difficult, since fat forms an integral part of the product. Removing fat poses a number of challenges as to which other ingredients to add in its place. Microparticulated whey protein concentrates have been formulated by food technologists to mimic the functionalities of fat especially as far as creaminess is concerned.

Little information is available on fat replacement in frozen yoghurt, in terms of both its effects on the survival of probiotic bacteria and on some important quality parameters. Likewise, little research, if any, has been done on the perception of strawberry flavour in fat-free ice-creams and similar products. The purpose of the present study was thus to provide information on fat replacement by a microparticulated whey protein concentrate (Simplesse® 500) in frozen yoghurts and its influence on the survival of *L. acidophilus* and *B. bifidum*. Several quality parameters of the frozen yoghurts were also evaluated.

Frozen yoghurts were prepared by inoculating the mix with an ABT (*acidophilus, bifidum* and *thermophilus*) culture. Four mixes were formulated to contain 10% (m/m) milk fat, 5% (m/m) milk fat, 5% (m/m) of the microparticulated whey protein concentrate (WPC) and 3.4% (m/m) of the microparticulated WPC respectively. Decreasing milk fat from 10% to 5%, together with the addition of more milk solids-not-fat, did not lead to a significant increase ($p > 0.05$) in the acidity of the mix. Likewise the buffering capacity in both alkaline and acidic conditions were not significantly different ($p > 0.05$). Substituting fat with Simplesse® 500 at 3.4% level led to a significant decrease in the pH of the mix ($p < 0.05$), which was due to the presence of more weak acids. The addition of the microparticulated WPC also led to an increase in the buffering capacity of the mix, which was due to an increase in weak acids and other buffer systems present in milk such as the citrate and phosphate systems. The addition of more Simplesse® 500 did not result in a significantly higher acidity ($p > 0.05$) since whey proteins are only weak acids.

The viability of all three the bacteria types present in the ABT culture did not seem to be related to the presence or absence of the microparticulated WPC. The numbers of

Streptococcus salivarius subsp. *thermophilus*, *L. acidophilus* and of *B. bifidum* did not differ significantly ($p > 0.05$) between the four yoghurt mixes after incubation, ageing, whipping and freezing and three weeks of storage. However, only *S. salivarius* subsp. *thermophilus* and *L. acidophilus* increased in numbers during fermentation in all the yoghurt mixes and only *S. salivarius* subsp. *thermophilus* increased significantly ($p < 0.05$) as a result of ageing in all the yoghurt mixes. Although the addition of the microparticulated WPC led to an increase in the acidity of the yoghurt mixes it also led to an increase in the buffer capacity, which thus helped to maintain the numbers of *S. salivarius subsp. thermophilus* and *L. acidophilus* at the same level as in the mixes containing milk fat. While *S. salivarius subsp. thermophilus* and *L. acidophilus* grew to numbers greater than 10^7 cfu/g, *B. bifidum* did not grow at all in any of the yoghurt mixes and the results suggest that they might not be available at the right level to have any therapeutic benefits to the consumers.

Decreasing fat content led to an obvious increase in coarseness of frozen yoghurts ($p < 0.05$). The increasing perceived coarseness could be related to the amount of ice nuclei formed during whipping and freezing. The addition of the microparticulated whey protein concentrate could have resulted in a decrease in the freezing point, low enough to lead to a decrease in the amount of ice nuclei formed as a result of whipping and freezing. Therefore, the amount of unfrozen water available to freeze during hardening increased, thereby leading to an increase in the size of ice crystals. Fat is also known to decrease the size of ice crystals and the presence of more fat could also have led to an increased perception of smoothness.

Increasing fat content led to a decrease in the perception of strawberry flavour and an increased perception of an aftertaste ($p < 0.05$). Fat is known to bind lipophilic compounds as well as decrease the melting rate and mass transfer, both of which will decrease the release of flavour compounds.

UITTREKSEL

MIKROPARTIKEL-WEIPROTEÏEN AS VETSUBSTITUUT IN BEVRORE JOGHURT

deur

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Mikroörganismes is tradisioneel geselekteer vir fermentasie van melk as gevolg van hul vermoë om in melk te groei. Deesdae is die neiging egter om probiotiese bakterieë in te sluit, d.w.s. daardie bakterieë wat veronderstel is om addisionele gesondheidsvoordele vir die verbruiker in te hou. Probiotiese mikroörganismes het 'n voordelige effek wanneer dit ingeneem word, byvoorbeeld 'n afname in die voorkoms van kolonkanker, die onderdrukking van verrottings– en patogeniese bakterieë deur middel van kompeterende uitskakeling en deur produksie van organiese sure, diasetiel en bakteriosine asook 'n hipocholesterolemiese effek, om slegs 'n paar te noem.

Om meer effektief te wees, moet die betrokke mikroörganisme van menslike oorsprong wees. *Lactobaccillus acidophilus* en *Bifidobacterium bifidum*, twee van die suksesvolste probiotiese bakterieë wat kommersieël aangewend word, is normale bewoners van die dermkanaal van die mens en verskeie diersoorte. Dit is belangrik dat hulle lewensvatbaar en aktief bly in die bio-produk waarin hulle voorkom, sodat hulle beskikbaar sal wees in genoegsame getalle ten tye van inname van die produk. 'n Verskeidenheid van faktore beïnvloed hul lewensvatbaarheid, byvoorbeeld 'n lae pH, die tipe kultuur wat gebruik word asook die beskikbaarheid van essensiële aminosure. Van kardinale belang is die

keuse van bestanddele gebruik in die mengsel, aangesien dit eienskappe soos die pH en beskikbaarheid van voedingstowwe affekteer.

Daar is 'n sterk aanvraag na laevet produkte. Om die vetinhoud van komplekse produkte soos bevrore suiwelnageregte te verlaag, is egter ingewikkeld omrede vet so 'n integrale deel van die produk uitmaak. Om die vet te verwyder veroorsaak 'n aantal ander uitdagings, byvoorbeeld met watter bestanddele die vet vervang moet word. Mikropartikel-weiproteïenkonsentraat is geformuleer deur voedseltegnoloë om die funksionaliteit van vet, veral wat romerigheid betref, na te boots.

Beperkte inligting is beskikbaar oor die vetvervanging in bevrore joghurt, oor die effek daarvan op die oorlewing van probiotiese bakterieë en op sommige belangrike kwaliteitsparameters. Eweneens is min navorsing, indien enige, gedoen oor die waarneming van aarbeigeur in vetvrye roomys en soortgelyke produkte. Die doel van hierdie studie was dus om inligting te verky oor die vervanging van vet deur 'n mikropartikel-weiproteïenkonsentraat (Simplesse ® 500) in bevrore joghurt en die invloed daarvan op die oorlewing van *L. acidophilus* en *B.bifidum*. Verskeie kwaliteitsparameters is ook geëvalueer.

Bevrore joghurt is voorberei deur die mengsel met 'n ABT (*acidophilus, bifidum* and *thermophilus*) kultuur in te ent. Vier mengsels is geformuleer om onderskeidelik 10% (m/m) melkvet, 5% (m/m) melkvet, 5% (m/m) van die mikropartikel-weiproteïenkonsentraat (WPK) en 3,4 % (m/m) van die mikropartikel-WPK, te bevat. Die vermindering van melkvet vanaf 10% (m/m) tot 5% (m/m) en die byvoeging van meer vetvry-vaste stowwe van melk het nie 'n betekenisvolle verhoging ($p > 0.05$) in die suurheid van die mengsel veroorsaak nie. Eweneens was die bufferkapasiteit in beide alkaliese en suur konsisies nie betekenisvol verskillend nie ($p > 0.05$). Die vervanging van vet met Simplesse ® 500 teen 'n koers van 3.4% (m/m) het gelei tot 'n betekenisvolle afname in die pH van die mengsel ($p < 0.05$), wat 'n gevolg was van die teenwoordigheid van meer swak sure. Die byvoeging van die mikropartikel-WPK het aanleiding gegee tot 'n verhoging in die bufferkapasiteit van die mengsel, wat die gevolg

was van 'n toename in swak sure en ander buffersisteme teenwoording in melk, soos die sitraat– en fosfaatstelsels. Die byvoeging van meer Simplesse®500 het nie 'n betekenisvol hoër suurheid ($p > 0.05$) tot gevolg gehad nie, aangesien weiproteïene slegs swak sure is.

Die lewensvatbaarheid van aldrie die bakteriespesies teenwoordig in die ABT kulture is nie beïnvloed deur die teenwoordigheid of afwesigheid van die mikropartikel-WPK nie. Die aantal *Streptococcus salivarius* subsp. *thermophilus*, *L. acidophilus* and *B. bifidum* het nie betekenisvol verskil ($p > 0.05$) tussen die vier joghurtmengsels direk na inkubasie, veroudering, opklop en bevriesing en na drie weke se opberging nie. Alhoewel die byvoeging van die mikropartikel-WPK aanleiding gegee het tot 'n verhoging in die suurheidsgraad van die joghurtmengsels, het dit ook aanleiding gegee tot 'n verhoging in die bufferkapasiteit, wat gehelp het met die handhawing van die getal *S. salivarius* subsp. *thermophilus* en *L. acidophilus* op dieselfde vlak as dié in die mengsels wat melkvet bevat het. Terwyl *S. salivarius* subsp. *thermophilus* en *L. acidophilus* in al vier die joghurtmengsels gegroei het tot getalle groter as 10^7 kve/g, het *B. bifidum* nie in enige van die joghurtmengsels gegroei nie en dit lyk nie of hulle in voldoende getalle teenwoordig sal wees om enige terapeutiese voordele vir die verbruiker in te hou nie.

'n Afname in die vetinhoud het gelei tot 'n duidelike verhoging in die grofheid van die bevrore joghurt ($p < 0.05$). Die toename in grofheid mag verband hou met die aantal yskrystalkerne wat gevorm het gedurende opklop en bevriesing. Die byvoeging van die mikropartikel-weiproteïenkonsenstraat kon die vriespunt sodanig verlaag het dat minder yskrystalkerme gevorm het tydens opklop en bevriesing. Daar was dus meer onbevrore water beskikbaar wat kon vries gedurende verharding en groter yskristalle kon vorm. Dit is ook bekend dat vet die grootte van yskristalle beperk en die teenwoordigheid van meer vet kan lei tot 'n gladder voorkoms.

'n Vermeerdering in die vetinhoud het aanleiding gegee tot 'n afname in die waarneming van die aarbeigeur en 'n vermeerdering in 'n nasmaak ($p < 0.05$). Dit is bekend dat vet

lipofiliese komponente bind en die smelttempo en massa-oordrag vertraag en beide sal die vrystelling van geurkomponente verminder.

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ACH: Acid casein hydrolysate

BC: Buffering capacity

BSA: Bovine serum albumin

Cfu: Colony forming units

CMC: Carboxyl methyl-cellulose

CSS: Corn syrup solids

DE: Dextrose Equivalent

DMHF: 2,5-Dimethyl-4-hydroxy-2H furan-3-one

E_h: Redox potential

GRAS: Generally recognised as safe

HLB: Hydrophilic-Lipophilic Balance

IDF: International Dairy Federation

MRS: de Man, Rogosa & Sharpe

MSNF: Milk solids-not-fat

NPNL: Naladixic acid, Paramomycin sulphate, Neomycin sulphate and Lithium chloride

SA: South Africa

SE: Sucrose equivalent

SEM: Scanning electron microscopy

SMP: Skim-milk powder

TA: Titratable acidity

Tg`: Glass transition temperature

T_s: Storage temperature

TS: Total solids

UF: Ultra filtered

UHT: Ultra high temperature

VHMF: Very high melting point fat

WBC: Water binding capacity

WP: Whey protein

WPC: Whey protein concentrate

WPI: Whey protein isolate

CHAPTER 1

INTRODUCTION

Microorganisms have traditionally been selected for fermentation of milk on their ability to grow in milk. However, nowadays the trend is towards including also probiotic bacteria, i.e. those bacteria that have been suggested to provide additional health benefits to the consumer (Driessen, 1992).

It has been postulated that probiotic microorganisms can have certain beneficial effects in the body such as:

- improved lactose utilisation by lactose-intolerant consumers through the release of β-galactosidase (Kim & Gilliland, 1983; Gilliland, 1985),
- hypocholesterolemic effect (Gilliland, Nelson & Maxwell, 1985; Lin Ayres, Winkler & Sandani, 1989),
- production of vitamins,
- enhancement of mineral absorption (Hughes & Hoover, 1991),
- the suppression of putrefactive and pathogenic bacteria by competitive exclusion and the production of organic acids, hydrogen peroxide and bacteriocins (Mitsuoka, 1990; Gonzalez, Apela, Romero, Nader de Macias & Oliver, 1993), and
- modulating the host's immune system (immunopotentiating) in response to potentially harmful antigens (Mitsuoka, 1990; Perdigon, Alvarez, Macias, Rou & de Ruiz Holgado, 1990; Yasui & Ohwaki, 1991).

The importance of an intake of exogenous probiotic bacteria is also illustrated by the fact that, while the population of bifidobacteria in the intestinal tract decreases with age and other stress-related factors, the proportion of putrefactive and potential pathogenic bacteria tends to increase (Mitsuoka, 1990). These bacteria can produce a number of toxic compounds in the colon, which can be carcinogenic and harmful to the health. An optimal balance of microorganisms in the gastro-intestinal tract has thus been suggested to be an important aspect of maintaining good health (Hekmat & McMahon, 1992).

To be more effective, the proposed microorganisms must be of human origin (Ishibashi & Shimamura, 1993). *Lactobacillus acidophilus* and *Bifidobacterium bifidum,* which are two of the most successful probiotic bacteria used commercially, are normal inhabitants of the intestine of many animals including humans (Klaver, Kingma & Weerkamp, 1993; Shah, Lankaputhra, Britz & Kyle, 1995). The minimum level of probiotic bacteria in a bio-product to achieve the physiological active concentration of 10^6-10^7 cfu/ g of intestinal contents, and hence to have any beneficial effects, has been suggested as 10^7-10^8 cfu/g (Bouhnik, 1993). They must therefore maintain their viability and activity in the bio-product before consumption (Kim, 1988). A number of factors affect their viability, including low pH (Laroia & Martin, 1991) and availability of amino acids (Dave & Shah, 1997c). The choice of ingredients in the mix is crucial since it affects a number of mix properties such as buffering capacity, pH decrease and availability of nutrients.

According to Collins & Hall (1984) and Poch & Bezkavanany (1988), fermented milk and yoghurt are among the most useful media for administering bifidobacteria and the use of cultured dairy products for taking probiotic bacteria is well-known (Holocomb, Frank & McGregor, 1991). Frozen yogurt, which was introduced as a new product in the early 1970's (Lassus & Stelitzer, 1977; Knupp, 1979), is an interesting bio-product to the consumers as it offers the healthy image of yoghurt as well as the attractiveness of a dairy dessert (Inoue, Shiota & Ito, 1998). However, freezing temperatures of -2 to -10°C can be considered as being conducive to freezing injury of bacteria (Holocomb *et al.*, 1991).

There are many ways of manufacturing frozen yoghurt and each manufacturer goes about it in his own way (Westerbeek, 1995). Frozen yoghurt of the ice-cream type can be made by culturing a pasteurised and homogenised ice-cream mix and subsequent whipping and freezing of the cultured mix. From studies of Modler, McKellar, Goff & Mackie, 1990, Mashayekh & Brown, 1992 and Inoue *et al.*, 1998, it appears that these products have fat contents of 10-14%. However, concern about the impact of diet on health has led consumers to reduce the consumption of foods perceived as being high in fat (Bruhn, Cotter, Diaz-Knauf, Sutherlin, West, Wightman, Williamson, & Yaffee, 1992). Ingestion of some saturated fatty acids increases the concentration of plasma low density

lipoprotein (LDL) cholesterol in humans, which leads to increased risks of coronary heart disease. The core of milk fat globules is mainly composed of triglycerides and the majority of it is saturated (Jensen, Ferris, Lammikeefe & Heperson, 1990).

One way of manufacturing low-fat frozen yogurt is to freeze a cultured ice milk mix (Thompson & Mistry, 1994) or to formulate a mix of sugars and stabilisers to which will be added a certain ratio of yogurt (Westerbeek, 1995). However, reducing the fat content of a dairy dessert poses several other problems as the fat forms an integral part of the structure of the product (Marshall & Arbuckle, 1996). Fat contributes towards the flavour, the rich and creamy texture as well as heat shock stability of frozen dairy desserts (Anon, 1990a; Alexander, 1997). Formulating low-fat and non-fat dairy desserts is thus not an easy task as it usually results in a lowering of the total solids content of the mix, thereby causing a number of defects such as a coarse and crumbly texture (Tharp & Gottemoller, 1990; Anon, 1993). One alternative would be to indiscriminately increase the milk solids not fat (MSNF), but too high a MSNF level might lead to defects such as sandiness from lactose crystallisation (Arbuckle, 1977).

To meet the demand for well-textured non-fat dairy desserts, food technologists have developed a number of fat replacers, the main function of which is to mimic the sensory mouthfeel properties of fat (Singer & Dunn, 1990). Microparticulated proteins are but one of such fat replacers. The process by which they are made yields small spherical particles with a diameter ranging from 0.1-2.0 μm and which are expected to give mouthfeel properties similar to fat (Cheftel & Dumas, 1993). However, their incorporation into a frozen yoghurt of the ice-cream type, in replacing milk fat, has to be assessed since they can potentially affect a number of mix properties that will determine the quality of the final product in terms of texture, flavour as well as the survival of probiotic bacteria and their availability at the time of consumption.

CHAPTER 2

OBJECTIVES

2.1 Overall objective

The primary objective of this study was to assess the effects of replacing milk fat by a microparticulated whey protein concentrate (WPC) on several quality parameters of a frozen yoghurt of the ice-cream type and on the survival of *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum.*

2.1 Secondary objectives

The secondary objectives of the study were:

1. To determine the effect of fat replacement by a microparticulated WPC on the buffering capacity of the frozen yoghurt mix and pH decrease during fermentation.

2. To determine the effect of fat replacement by the microparticulated WPC on the viability of *S. salivarius* subsp. *thermophilus, L. acidophilus* and *B.bifidum* at various stages of manufacturing and storage of a frozen yoghurt of the ice-cream type and its ability to act as a bio-product that will provide the probiotic bacteria at the minimum required level.

3. To determine the effect of milk fat replacement by the microparticulated WPC on the viscosity, overrun, texture, flavour and microstructure of the frozen yoghurt.

CHAPTER 3

LITERATURE REVIEW

3.1 General description of bacteria used in yoghurt manufacture

3.1.1 *Streptococcus salivarius* subsp. *thermophilus*

Streptococcus salivarius subsp. *thermophilus* is a Gram positive, micro-aerophilic to aerobic bacterium (Dave & Shah, 1998). It is active even at temperatures as high as 45°C (Acolas, 1982). During fermentation, it mainly produces lactic acid with small amounts of diacetyl (Dellaglio, Torriani, Vlaeminck & Cornet, 1992). Some strains of *S. salivarius* subsp. *thermophilus* can ferment galactose (Oberg & Broadbent, 1993). The species has weak proteolytic activities (Oberg & Broadbent, 1993). It use in probiotic food is explained by the fact that they not only have certain technological benefits but they also have higher lactase activities than *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Sanders, Walker, Walker, Aoyama & Klaenhammer, 1996).

3.1.2 *Lactobacillus delbrueckii* subsp. *bulgaricus*

Lactobacillus delbrueckii subsp. *bulgaricus* is a Gram positive, rod-shaped, homofermentative bacterium that produces lactic acid as an end product of fermentation (Acolas, 1982) through the Embden-Meyerhof pathway (Dellaglio, 1988). It ferments a large range of sugars including lactose, fructose galactose and glucose (Acolas, 1982), but pentose sugars are not fermented and carbon dioxide is not produced (Dellaglio, 1988). It is anaerobic to facultative aerobic (Rasic & Kurman, 1978) and has a wide range of proteolytic activities (Oberg & Broadbent, 1993).

3.2 The so-called probiotic bacteria used in modern yoghurt

3.2.3 *Lactobacillus acidophilus*

Lactobacillus acidophilus is a Gram positive, rod-shaped bacterium with rounded ends (IDF, 1991; Shah *et al.,* 1995). It is micro-aerophillic to aerobic (Dellaglio *et al.,* 1992; Dave & Shah, 1998) and is known to have good proteolytic activities (Klaver *et al.,*1993; Dave & Shah, 1998). It is homofermentative and as such ferments hexose sugars, including galactose, (Oberg & Broadbent, 1993) to lactic acid through the Embden-Meyerhof pathway (Dellaglio, 1988). Pentose sugars are not fermented and carbon dioxide is not produced (Dellaglio, 1988).

3.2.2 *Bifidobacterium bifidum*

Bifidobacterium bifidum, first encountered in the faeces of breast fed infants (Yaeshima, 1996), is a Gram positive bacterium of variable morphology with an an optimum pH of 6-7 (Martin & Chou, 1992; Shah *et al.,* 1995). Little or no growth occurs at temperatures below 20°C and at pH values below 5.5 (Martin & Chou, 1992). It is an obligate anaerobe that can hardly proliferate in the presence of oxygen (Mizota, 1996), although the sensitivity of bifidobacteria to oxygen is species and strain related (Dellaglio *et al.,* 1992). *Bifidobacterium bifidum* degrades glucose to a theoretical acetic acid to lactic acid molar ratio of 3:2 without the production of carbon dioxide (Dellaglio *et al.,* 1992; Hunger & Peitersen, 1992; Dave & Shah, 1997a). It is unable to decompose proteins and as such grow poorly in milk without additives or when grown alone (Hunger & Peitersen, 1992). Given the various growth requirements of *B. bifidum*, its growth in milk without any supplementation with growth factors and /or reducing agents is very poor (Dave & Shah, 1998).

3.3. The use of an ABT (*acidophilus, bifidum* and *thermophilus)* culture

A more rapid rate of acid production is known to occur in mixed cultures of *L. delbrueckii subsp. bulgaricus* and *S. salivarius* subsp. *thermophilus* compared to single strain cultures due to the well-established symbiotic relationship between the two species (Morichi, 1992). For example, Dave & Shah (1997c) observed that, upon fermentation of yoghurt bases, the decrease in pH by starter culture containing *L. delbrueckii subsp. bulgaricus* in addition to *S. salivarius* subsp. *thermophilus, L. acidophilus* and *B. bifidum* was faster than ABT culture. However, *L. delbrueckii* subsp. *bulgaricus* also produces lactic acid during storage, referred to as post acidification, which is claimed to adversely affect the viability of probiotic bacteria (Laroia & Martin, 1991; Shat *et al.,* 1995; Dave & Shah, 1998). To avoid the problem of post acidification the present trend is towards the use of ABT starter cultures lacking *L. delbrueckii* subsp. *bulgaricus* but with *S. salivarius* subsp. *thermophilus* as the main fermenting organisms (Shah *et al.,* 1995; Dave & Shah, 1998).

3.4 The factors affecting the survival of probiotic and yoghurt bacteria in cultured dairy products

To be of any therapeutic value, it is recognised that the species and strains used in the bio-product must be available in sufficient numbers at the time of consumption. Therefore, as far as bio-products are concerned, the survival of probiotic bacteria is yet another criterion for shelf-life determination. The survival of probiotic bacteria in both yoghurt and frozen yoghurt is affected by a number of factors such as the species and strains of the probiotics used, the starter culture used in the manufacture of the yoghurt base, the type of inoculum used (freeze dried v/s bulk starter culture) as well as the formulation of the mix. The choice of ingredients in the formulation is known to affect a number of mix properties such as E_h, (Dave & Shah, 1997 c) buffering capacity and pH decrease (Ventry & Mistry, 1993) and availability of growth factors (Poch & Bezkoravainy, 1988; Dave & Shah, 1998), while some processes can also have an effect on some mix properties, e.g. pasteurisation on Eh (Driessen, 1984). Fig. 1 shows the

factors affecting the survival of probiotic bacteria in dairy desserts and the complexity of the factors depicted in the figure might suggest a high level of interaction between the factors. It has been found that, while some factors were beneficial to the bifidobactreia in in general, for e.g. bovine casein digests (factors that may originate from κ-casein) and yeast extracts, others were more species specific, for e.g. bovine serum albumin digest which supported only the growth of *B. infantis* and *B. brevis* (Poch & Bezkoravainy, 1988). Thus, it was in that case not only a question of availability of nutrients but also which organisms benefited from the supplementation.

A distinction can be made between yoghurt and frozen yoghurt regarding the survival of yoghurt and probiotic bacteria. The difference is technology related more than anything else. In yogurt, low pH and the extent of post-acidification during storage may become major limiting factors to the survival of the probiotic bacteria. For example, Shah *et al.* (1995) observed that the pH of yoghurts decreased by 0.07-0.42 pH units during a 5 weeks storage of yoghurt at 4°C. The decrease in the numbers of *B. bifidum* was attributed to the lower pH attained during storage. On the other hand, in frozen dairy desserts, the freezing step is usually accompanied by a decrease of up to 1-1.5 log units in the bacterial count and post-acidification is less of a problem, although some studies showed a small increase in the acidity during storage (Laroia & Martin, 1991). It should, however, be mentioned that in both cases the sum total of the factors determines the success of survival of the probiotics. In this literature review, more emphasis will be placed on the factors that are of direct interest to the present study.

3.4.1 The effect of acidity

The survival and viability of probiotic bacteria in both yoghurt (Martin & Chou, 1992; Shah *et al.*, 1995) and frozen yoghurt is strongly affected by low pH (Laroia & Martin, 1991; Hekmat & McMahon, 1992). *Bifidobacterium bifidum* is quite sensitive to low pH, while *L. acidophilus* is not as fastidious (Laroia & Martin, 1991; Shah *et al.*, 1995). The sensitivity or adaptability of bacteria to pH changes depend on the buffering capacity and hydrogen membrane conductance of the related species and strains (Rius, Sole, Francia &

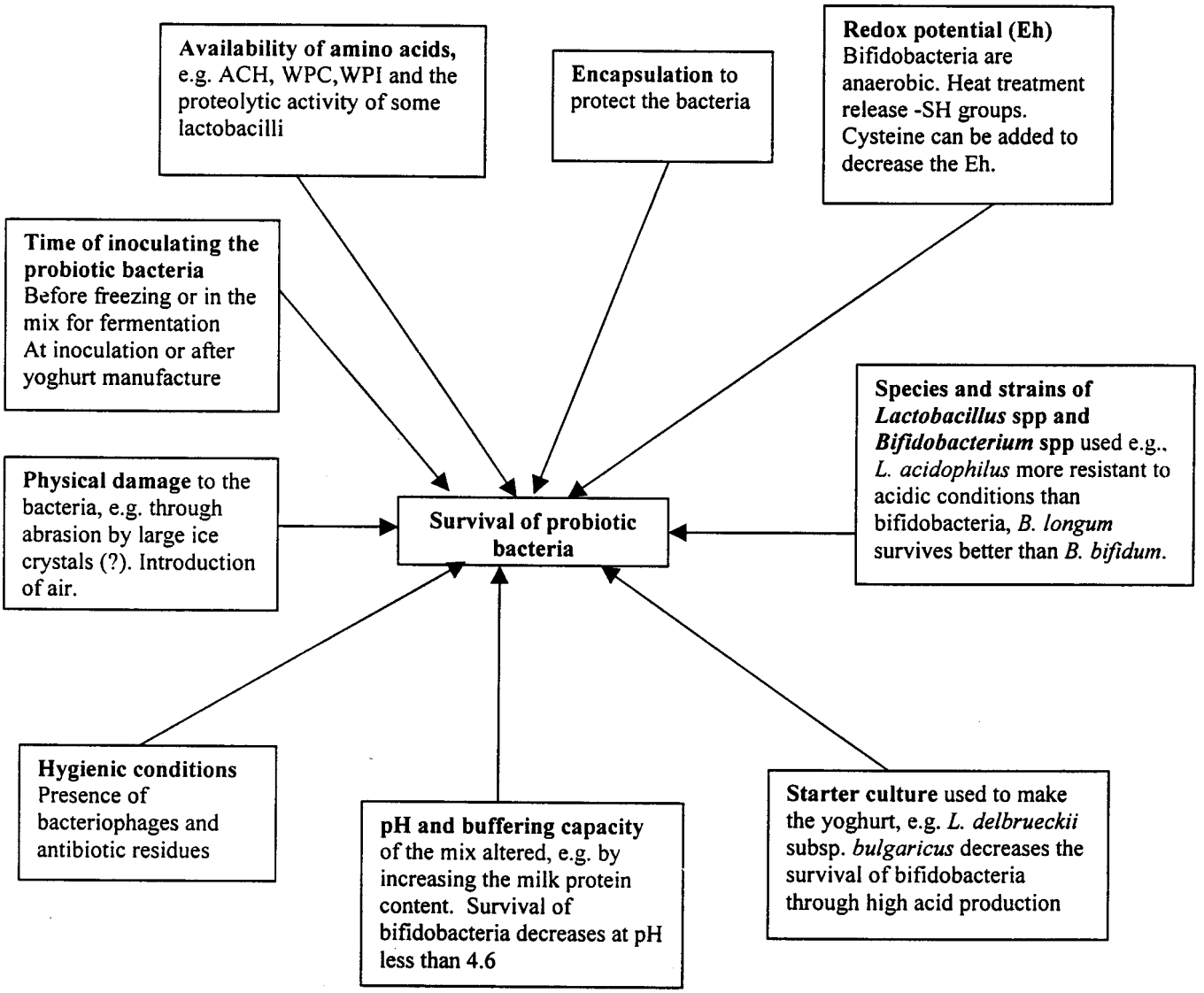


Figure 1 Factors affecting the survival of probiotics in cultured dairy desserts with emphasis on frozen yoghurt (ACH= Acid Casein Hydrolysate, WPI =Whey Protein Isolates, WPC= Whey Protein Concentrate)

Loren, 1995).Studies suggest that the effects of pH on the survival of probiotic bacteria may be related to both mix formulations (through the influence of proteins on buffering capacity and decrease in pH) as well as post-acidification or too fast acidification by the species or strains used in the culture. Laroia & Martin (1991) observed low survival of *B. bifidum* at low pH (pH 3.9-4.9) in frozen dairy desserts cultured by a yoghurt culture compared to a better survival of the species at high pH (pH 5.6-6.8) in frozen dairy desserts and frozen yogurts. As far as yoghurt is concerned, Martin & Chou (1992) observed a marked decrease in the cell count (of more than 3 log units) of *B. bifidum* in low-pH yoghurt during storage at 4-6°C for 56 days. Shah *et al.* (1995) also reported that the decrease in pH, during the 5 weeks storage, in their study on commercial yoghurts could have contributed to a lower viability of *B. bifidum*.

3.4.2. Availability of nutrients

3.4.2.1 The lack of appropriate proteolytic activity on the part of bifidobacteria and the need for co-culturing

Probiotic bacteria, especially the bifidobacteria, grow poorly in milk due to a lack of appropriate proteolytic activity and, therefore, the usual practice is to add yoghurt bacteria to reduce the fermentation time (Klaver *et al.*, 1993; Dave & Shah, 1998). Bifidobacteria can as such only be grown in milk by adding certain growth factors and/or by co-culturing with proteolytic species such as *L. acidophilus* (Gomes, Malcata & Klaver, 1993). According to Klaver *et al.* (1993), *L. acidophilus* has been reported to be more proteolytic than *S. salivarius* subsp. *thermophilus*. It can use peptides and free amino acids for its own growth and acidification activities, but also hydrolyses milk proteins using the proteinases to produce low molecular peptides and thus aid in the growth of bifidobacteria (Gomes *et al.*, 1993).

3.4.2.2 The addition of growth factors and the importance of an appropriate source of the nutrients

Lack of available nitrogen limits the growth of bifidobacteria in milk and unless some growth promoting elements are added to the milk, growth and well-controlled fermentation will be difficult (Gomes *et al.,* 1993). Certain growth promoters are often used in the bulk starter culture preparation of those organisms as well as in the media used for their enumeration. These growth promoters are specially formulated substances composed of yeast extracts, milk protein hydrolysates, vitamins, amino acids and mineral salts (Hunger & Peitersen, 1992). Hughes & Hoover (1995) reported that many of the bifidobacteria species grew better in MRS broth supplemented with lactose compared to skim milk. However, the addition of yeast extracts and some other nutrients are not allowed in the production of yoghurt in some countries (Driessen & Loones, 1992). Therefore new sources of available nitrogen prove essential in the case of consumer products. Unlike traditional yoghurt cultures where there is an established proto-coorperation (Driessen & Loones, 1992), ABT cultures might necessitate the incorporation of micronutrients available through whey proteins (WP), whey protein concentrate (WPC), acid casein hydrolysates (ACH) or tryptone to reduce the fermentation time. The nature and combination of the growth promoters to be used also depends on the species or strains involved since some bifidobacteria can produce an excess of vitamins that helps in their growth and as such those vitamins would not be the limiting factor. Thus *B. infantis* produces an excess of thiamine, nicotinic acid and folic acid compared to *B. adolescentis* (Desjardin, Roy & Goulet, 1990), while *B. bifidum* strain ATCC 29521 has a number of requirements in terms of vitamins such as riboflavin, nicotinic acid and folic acid (Dellaglio, 1988).

Lactobacillus acidophilus is proteolytic in nature and not that dependent upon exogenous available nitrogen supplementation (Gomes *et al.,* 1993), although amino acids such as arginine, glutamic acid, isoleucine, leucine, tryptophane, tyrosine and valine have been reported to be essential for some strains of *L. acidophilus* (Gonsalves, Nambudripad, Laxminarayana & Iya, 1957).

3.4.3 The freezing process of frozen yoghurt

Although compounds such as lactose and casein are known to be cryoprotectants, the freezing process causes a decrease in the counts of the lactobacilli of frozen dairy desserts. Bielecka, Przewozna & Kowalczuk (1988) suggested that protective agents such as carbohydrates, proteins, emulsifiers and stabilisers might help in protecting the bacteria against freezing injury. Sucrose (Bielecka *et al.*, 1988), glycerol and mannitol (Sheu, Marshall & Heymann, 1991) are also expected to act as good cryoprotectants. However, studies have revealed a decrease in the lactobacilli count at the freezing step. From a study by Thompson & Mistry (1994), it appears that whipping and freezing have deleterious effects on lactobacilli due to incorporation of air and/or freezing injury. Modler *et al.* (1992) observed a decrease of slightly less than one log unit in the counts of *B. longum*, *B. brevis* and *B. infantis* after batch freezing of frozen yoghurt at –5°C, hardening and subsequent storage at –17°C. They attributed the decrease in the viability of the various species to the incorporation of air. Freezing temperatures of -2 to -10°C can be considered as being optimum for freezing injury (Holocomb *et al.*, 1991). And according to Goff (1992), slow freezing may destroy vegetative cells due to ice crystal damage rather than low temperature. This may be of concern when lowering the fat content of frozen dairy desserts since more freezable water would be made available. Mashayek & Brown (1992) reported a decrease of 1 log unit in the counts of yoghurt bacteria while Laroia & Martin (1991) found a decrease of slightly less than one log unit, as far as *B. bifidum* and *L. acidophilus* are concerned at a 100% overrun. Hekmat & McMahon (1992) obtained a similar decrease in numbers of *L. acidophilus* and *B. bifidum*. Some other authors have reported a decrease of 1.5 log units in the number of yoghurt and probiotic bacteria (Thompson & Mistry, 1994; Inoue *et al.*, 1998).

3.4.3.1 Encapsulation

Entrapment of cells, e.g. in calcium alginate, can provide additional protection to the bacterial cells both during freezing and storage (Sheu, Marshall & Heymann, 1991).

3.4.4 Species and strains

Laroia & Martin (1991) suggested that the low survival of *B. bifidum* in a low pH frozen yoghurt could be attributed to a strain specificity. Modler *et al.* (1990) reported that *B. infantis* had a higher survival rate than *B. longum* and *B. brevis*. Desjardin *et al.* (1990) observed that the *B. bifidum* strain 15696 had a better growth rate in skim milk than strain 11863. Furthermore, Martin (1996) reported that, compared to *B. bifidum* and *B. breve,* some strains of *B. adolescentis* and *B. longum* survived well during storage at 4°C when added to yoghurt with a pH of 4.2. The same effect was observed in high pH (5.5-5.6) yoghurt, thus suggesting a strong and significant species or strain difference. Martin & Chou (1992) observed that, compared to *B. bifidum,* which is very sensitive to low pH, the sensitivity of *B. adolescentis* and *B. longum* was more strain dependent. Their study thus showed that the viability of *Bifidobacterium* spp. was species and strain dependent. Misra & Kuila (1991) also reported that different strains of *B. bifidum* had different growth rates. Dave & Shah (1997a) attributed the loss of viability of *B. bifidum* in a certain ABT culture to the type of bifidobacterial strain used in their studies. Similar strain differences have been observed for *L. acidophilus* during the storage of yoghurt at 7°C (Brashears & Gilliland, 1995).

Moreover, the degree of proteolysis of *S. salivarius* subsp. *thermophilus* can also be strain specific (Dave & Shah, 1997a) and this can as such affect the amount of free amino acids available for the bifidobacteria. The proteolytic activity of lactobacilli was also found to be species and strains dependent by Sasaki, Bosman & Tan (1995).

3.4.5 Redox potential

Bifidobacteria are anaerobic microorganisms that prefer a low redox potential and oxygen content (Driessen, 1984; Martin & Chou, 1992; Klaver *et al.,* 1993; Dave & Shah, 1998). High oxygen contents may, therefore, affect their growth and viability (Klaver *et al.,* 1993; Hughes & Hoover, 1995; Dave & Shah, 1998) and in fact both homo and heterofermentative lactic acid bacteria are sensitive to oxygen contents greater than 4

mg/l (Driessen & Puhan, 1988). Heat treatment, cysteine and whey proteins are all expected to reduce the Eh of the mix; the heat treatment liberating -SH groups from amino acids, cysteine being a strong reducing agent and the whey proteins and WPC being rich in -SH group-containing amino acids and minerals (Dave & Shah, 1998). However, too high a cysteine content proved to be unfavourable to *S. salivarius* subsp. *thermophilus* (Dave & Shah, 1997c). Low Eh also tends to favour the growth and survival of *L. acidophilus*. The Eh of yoghurt increases during storage as a result of an increase in the oxygen content and as such oxygen scavengers could be added to decrease the oxygen content (Dave & Shah, 1997b). The addition of a WPC would most probably result in a decrease in the redox potential of the frozen yoghurt through the contribution of mineral salts and the release of –SH groups from amino acids (Dave & Shah, 1998).

3.5 Quality aspects of ice-creams and related frozen dairy desserts

Ice-cream or frozen dairy desserts can be defined as a mixture of various dairy and non-dairy products which is frozen with the incorporation of some air. Some substances occur in true solution (sugar and salts), others in colloidal suspension (casein, stabilisers, and some of the Ca and Mg phosphates) and the fat globules in a coarse dispersion (Marshall & Arbuckle, 1996).

Alternatively ice-cream can be defined in terms if its microstructure. The microstructure of ice-cream has been reported by a number of authors to consist of four main components: (1) air bubbles that are introduced during whipping, (2) ice crystals resulting from freezing, (3) fat globules if the mix is formulated with fat, and (4) a serum phase which is in fact the unfrozen phase of the ice-cream (Caldwell *et al.*, 1992a). Lactose crystals would be part of the microstructure of the product whenever the driving forces for lactose crystallisation come into play.

The serum phase, which contains dissolved (sugars and salts) and/or colloidal substances (proteins and stabilisers) surrounds and separates the ice crystals from the air bubbles (Berger, 1997). The latter are spherical in shape, ranging in diameter from 10-60 μm and are coated by the fat globules. The ice crystals are much more rectangular in shape with a

network structure (Caldwell *et al.*, 1992a). When viewed after etching, the ice crystals are characterised by a reticulate network and the presence of small spheres of approximately 0.2-0.4μm diameter can be observed within the network (Caldwell *et al.*, 1992a). Yet another characteristic of the microstructure of ice-cream is the presence of polyhedral ice crystals within or at the interface of the air bubbles (Caldwell *et al.*, 1992a). No relevant information is available as to the influence of those crystals on the texture as well as to the driving forces for their formation. The fat globules, ranging in size from 0.5-1.5 μm, are coated by an emulsifier/protein layer (Caldwell *et al.*, 1992a). They are usually disproportionately distributed at the air bubble/serum interphase and are present to a lesser extent throughout the serum. With regard to the size of the fat globules, whipping and freezing of the homogenised and aged mix leads to the partial disruption of the fat globule membrane and thereafter leading to partial coalescence of the fat globules (Lin & Leeder, 1974; Goff & Jordan, 1989). The homogeneity in size of the fat globules, achieved at the homogenisation step, is somehow therefore lost.

Inoue *et al.* (1998) found that the ice crystals of a frozen yoghurt of the ice-cream type with a fat content of 10% were larger than the reported sizes for ice-cream after long term storage at -35°C. They observed that the microstructure was similar to that of ice-cream but the fat and ice crystals' sizes were larger. However, they did not report on the total solids of the mix they used.

The quality of frozen dairy desserts is a function of several parameters, i.e. mix composition and its effects on mix properties, pre-freezing processing (e.g. homogenisation and ageing), whipping and freezing, post freezing and the effects of the various processing conditions on the mix properties. These factors are schematically represented in Fig. 2. Removing the fat from a frozen dairy dessert mix not only removes a constituent of the total solids (% TS) but also removes the various effects and interactions of processing on the fat as well as the influence of fat on a number of mix properties. It poses a number of challenges as to which of the ingredients to increase at the expense of fat. Removing the fat altogether will most certainly cause a decrease in the % TS that can result in product defects such as a crumbly texture and a fluffy and weak

body (Anon, 1993). The replacement of fat might also offer less protection towards the heat shock stability of frozen dairy desserts by influencing the melting behaviour of the product (Tharp & Gottemoller, 1990; Anon, 1993). A fat replacer should therefore match the texture, mouthfeel and functionality contributed by the fat in a food product and should convey the desired flavour profile if it is to successfully replace fat (Ohmes, Marshall & Heymann, 1998). Removal of fat, therefore, creates a void that must be adequately filled by proper reformulation of the mix. The latter process is bound to affect a number of mix properties that must be accounted for and monitored.

The quality of frozen dairy desserts is affected by the following factors.

3.5.1 Mix composition.

Proper mix formulation is a very important aspect affecting the quality of frozen dairy desserts. Different components of the mix will have a number of effects on some mix properties such as the ratio of water to solids, viscosity of the mix, mix stability, freezing point depression and the glass transition temperature (Tg`). For example, the addition of more small molecular weight carbohydrates will depress the freezing point and thus influence the amount of unfrozen water available during hardening.

3.5.1.1 The total solids content (% TS)

The total solids content of a mix is a very important factor determining the texture of frozen dairy desserts (Marshall & Arbuckle, 1996) and as a general rule the higher the level of TS, the lower will be the proportion of water in the ice phase (Hartel, 1996). This would most certainly be due to the fact that increasing total solids is very often to the detriment of water. Too low total solids content can lead to an icy product while too much TS causes a heavy and soggy product (Marshall & Arbuckle, 1996). However, the effect of total solids on many mix properties will depend on how the specific components

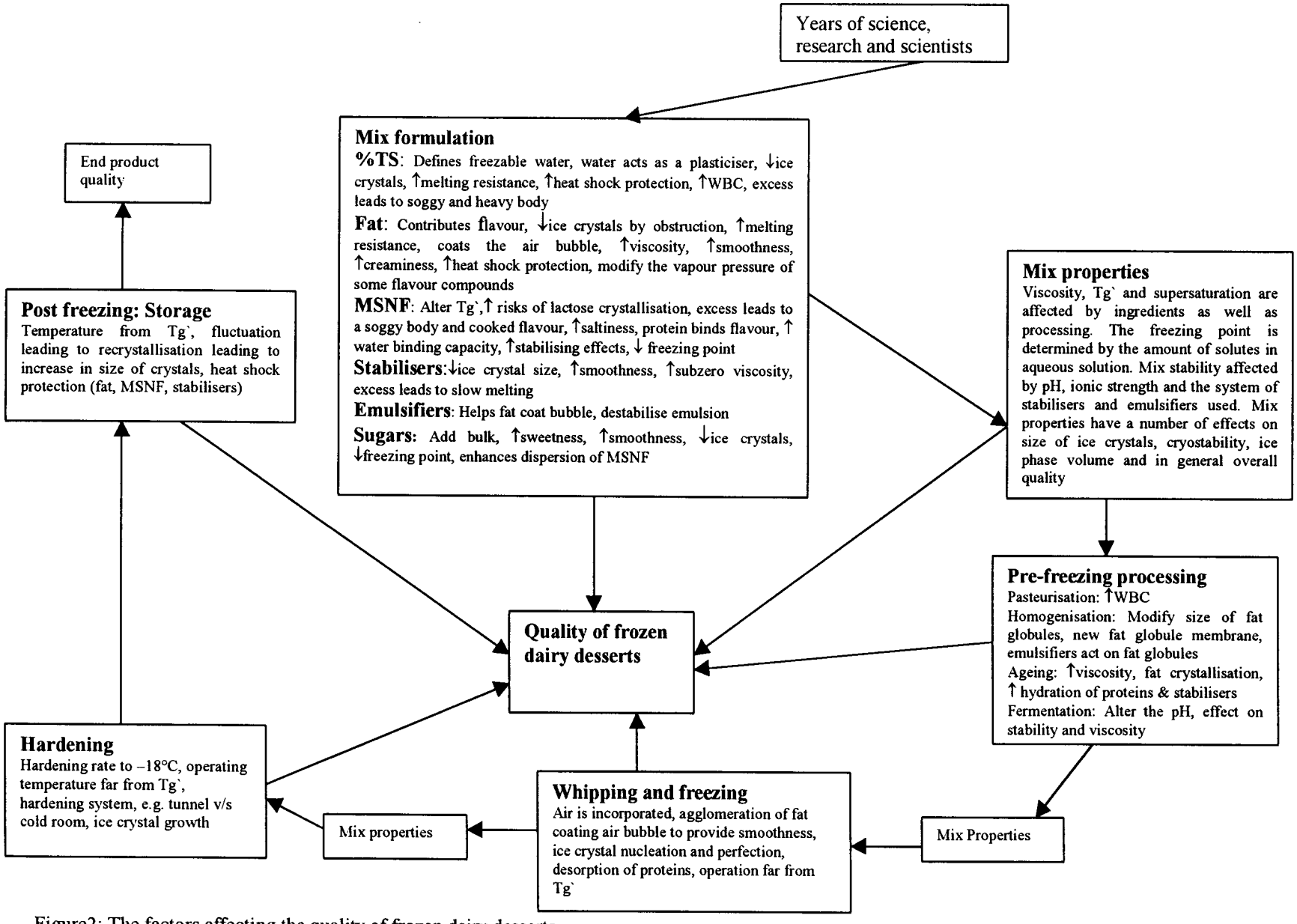


Figure2: The factors affecting the quality of frozen dairy desserts
(↑= increase,↓=decrease, Tg`=glass transition temperature, MSNF= milk solids not fat, WBC= water binding capacity, TS= total solids)

will influence some of those properties. For example, solutes such as sugars will depress the freezing point of the mix and alter the glass transition temperature (Tg`) of the frozen product, while denatured proteins will increase the water binding capacity of the mix. Therefore, Donhowe, Hartel & Bradley (1991) observed that increasing the %TS decreases the freezing point of frozen dairy desserts. On the other hand, increasing the % TS was also found to increase the critical supercooling required for secondary ice crystal nucleation in some model dairy systems (Hartel & Chung, 1993).

Water acts as a plasticiser in food systems and decreases their glass transition temperature (Tg`) to below ambient temperature (Roos & Karel, 1991a,b; Roos, 1995). At temperatures above the glass transition temperature (Tg`) of the maximally freeze concentrated serum phase, the constituents in the frozen system increase in mobility thereby leading to a number of deteriorative changes in frozen products (Slade & Levine, 1991). Mashayekh & Brown (1992) argued that, since frozen yoghurt contains less solids than ice-cream, the ice crystals are larger when frozen.

The specific components of the total solids will be discussed in the following sections.

3.5.1.1 Fat content

The fat globules in frozen dairy desserts are known to coat the air bubbles, thus giving a sensation of smoothness. The amount of fat affects the size of ice crystals by occlusion while the amount of solidified fat is important as far as melting resistance is concerned. According to Singer, Wilcox, Podolski, Chang, Pookote, Dunn & Hatchwell (1989), increases in the milk fat content for virtually any given frozen dessert formulation will thus decrease the size of ice crystals. This would most probably be due to the fact that an increase in the fat content of the mix is usually accompanied by a decrease in the amount of water. There is thus a decrease in the amount water to freeze. And according to Arbuckle (1977), fat reduces ice-crystal size by obstruction, i.e. by interfering with the movement of water molecules to the crystals lattice. Table 1 illustrates this point.

Table 1: The effect of fat on the size of ice crystals (Arbuckle, 1977)

Fat Content (%)	Ice crystals size (μm)
10	82.6 x 60.8
16	47.2 x 38.0

Moreover, an increased level of fat can also lead to an increase in the viscosity of the frozen product at sub-zero temperature. And according to Goff, Caldwell, Stanley & Maurice (1993) increased viscosity at sub-zero temperature can impede the movement of water molecules to the growing ice crystal surface. This would be very important during temperature abuse. The viscosity of the mix is also affected by the amount as well as the source of fat (Abd El-Rahman, Madkor, Ibrahim & Kilara, 1997).

The physical properties of the fat have a significant influence on both the behaviour of the mix during whipping and on the formation of the ice-cream structure. According to Berger & White (1976), increased amounts of solidified fat in the mix result in better whipping properties as well as melting characteristics in the mouth. Berger (1997) claimed that the two factors of interest with regard to fat are the proportion of liquid fat present at the time of entry into the freezer and the way in which the fat has been crystallised, especially the extent to which subcooling was possible. According to the same author, the exclusive use of liquid oil in the mix can, therefore, result in an unstable structure with no agglomerated fat globules. Moreover, the viscosity of the mix decreases with the use of vegetable oil, probably due to the decreased amount of solid fat at any given temperature compared to milk fat (Marshall & Heymann, 1994). The presence of a larger amount of solidified fat will lead to an increase in the viscosity of the mix (Abd El-Rahman *et al.*, 1997).

Studies carried out by Abd El-Rahman *et al.* (1997) indicated that the source of fat has an important role in determining the amount of solidified fat. Ice-creams produced from cream and very high melting point (VHMP) fat had higher solidified fat content than those produced from anhydrous milk fat. Cream contains phospholipids and other

constituents of the milk fat globule membrane that act as emulsifiers and hence enhancing fat crystallisation. VHMP fat, due to the high melting range of the fat profile, has a higher amount of solid fat at any given temperature.

The melting properties of milk fat are also all-important in determining the melting characteristics of frozen dairy desserts since milk fat is a relatively complex mixture of triglycerides and other lipids with different melting points and emulsifying properties (e.g. phospholipids). Milk fats do not normally melt at any sharp point but rather over a temperature range, over which they gradually soften (Hyde & Rothwell, 1973) and as such, they also determine the melting rate of the ice-cream. As far as milk fat is concerned, melting starts at about -40°C and is complete at 40°C (Banks, 1991), while Boudreau & Arul (1991) quoted figures of -30°C and 37°C respectively. At the final melting point of milk fat, the higher melting triglycerides dissolve in the liquid fat (Walstra & Jenness, 1984). At any intermediate temperature, one can expect a mixture of both solid and liquid fat, the latter helping the fat globules to agglomerate (Abd El-Rahman *et al.*, 1997). Fat agglomeration helps to produce a stiffer product with slower meltdown (Marshall & Arbuckle, 1996). The agglomerated fat forms a network around the air cells thereby providing an insulating effect and increased resistance to melting (Abd El-Rahman *et al.*, 1997). The melting properties of the final product also depends on the type of the fat used in the formulation, since ice-creams formulated with VHMP fat had a slower melting rate (Abd El-Rahman *et al.*, 1997). The slower melting rate can be ascribed to a higher amount of solid fat present (Marshall & Heymann, 1994). According to Banks (1991), the ratio of solid : liquid fat influences certain rheological properties of dairy products. Therefore, ice-creams formulated with very high melting point (VHMP) fats having more solid fat required more time for the first drop to fall during melting (Abd El-Rahman *et al.*, 1997).

The melting behaviour of fats in ice-creams is also a reflection of the conditions under which they are crystallised. The process of fat crystallisation involves nucleation and growth. A certain degree of subcooling is required to start nucleation and growth occurs by growth units fitting into the crystal lattice. During cooling and ageing the fat will first

crystallise as α-crystals that will be transformed to the β` form (Banks, 1991). The β`-crystals have higher melting ranges than the α-crystals. The final melting point of the α-crystals is about 22°C, while that of the β`-crystals is around 30°C (Mulder & Walstra, 1974). However, much more work is required on fat crystallisation to reach more complete conclusions (Banks, 1991). The change from the α-crystal form to the β`-crystal form is expected to distort the shape of the fat globules, hence enhancing fat agglomeration.

The amount of fat incorporated into the ice-cream base as well as the type of fat, be it dairy or non-dairy fat, will also have a significant effect on the flavour characteristics of the final product obtained (Johnson, 1997). Moreover, many flavour compounds are lipo-soluble and as such dissolve in the lipid portion of the food (Li, Marshall, Heymann & Fernando, 1997). When replacing fat in a food formulation the flavour associated with fat is decreased and some flavour notes can at times be perceived as being too intense since fat is not there to mediate their vapour pressure (Schirle-Keller, Reineccius & Hatchwell, 1994; Leland, 1997).

According to de Roos (1997), lipids influence flavour through their effects on flavour perception, flavour stability and flavour generation. In addition, dairy fat provides a flavour source in itself and for these reasons the rate of flavour addition may need to be adjusted when substituting dairy fat (Johnson, 1997). Among the most important factors affecting flavour release into the saliva and the nasal cavity are the product-to-water and the product-to-air coefficients (de Roos, 1997; Leland, 1997). When a flavour compound is added to water and allowed to equilibrate in a closed system between water and air, it distributes over the air and water phases according to the air-to-water partition coefficient (P_{aw}):

$$P_{aw} = C_a / C_w \qquad (1)$$

Where P_{aw} is a measure of the volatility of a flavour compound in water

C_a represents the concentration of the flavour in air and C_w the concentration of the flavour compound in water.

A corresponding air/oil partition coefficient, $P_{a/o}$, describes how flavour molecules distribute between air and oil:

$$P_{a/o} = C_a / C_o \qquad (2)$$

Where $P_{a/o}$ is a measure of the volatility of a flavour compound in oil. The more lipophilic the flavour compound, the lower will be the value of $P_{a/o}$ and hence the lower the relative vapour pressure.

C_o represents the concentration of flavour in oil and C_a the concentration of flavour in air.

In products containing aqueous and lipid phases such as frozen dairy desserts, a flavour compound distributes over three phases: fat, water and air. The distribution of the flavour compounds over the oil and water phases after equilibration is given by the oil-to-water partition coefficient (P_{ow}):

$$P_{ow} = C_o / C_w \qquad (3)$$

Where P_{ow} is a measure of the solubility of a flavour compound in oil and water.

C_o represents the concentration of the flavour compound in oil and C_w the concentration of the flavour compound in water (de Roos, 1997).

A value of P_{ow} =1 indicates equal concentrations in both oil and water, while the higher the value of P_{ow}, the more lipophilic or hydrophobic is the flavour compound and hence the more will the compound be in the fat phase. The concentration and odour impact of the flavour compound is thus low in the aqueous and vapour phases (de Roos, 1997). Fat will have little direct influence on the relative vapour pressure (rvp) of flavour compounds with limited solubility in oil because they would not be bound. On the other hand, the rvp of lipophilic flavour compounds will be affected by the fat content. For example, ethyl-benzene, limonene and styrene are lipophilic and as such their partitioning ratio in water : oil is small (Schirle-Keller, Chang & Reineccius, 1992; Schirle-Keller *et al.*, 1994). The vapour pressure of water-soluble flavour compounds (diacetyl, pentanone, pentanol, hexanal and acetaldehyde) are relatively unaffected by fat content (Schirle Keller *et al.*, 1992; Schirle-Keller *et al.*, 1994). Fat-soluble flavour compounds dissolve in the fat and as such their vapour pressure and their perception decrease. Little quantitative and qualitative information is available on binding of flavour compounds by the milk fat globules. Flavour binding and release (i.e. how the flavour molecules become

available to the receptor sites in the olfactory epithelium) are very important because flavour perception is the ultimate criterion in determining food acceptability (Stevenson, Chen & Mills, 1996). Due to the dynamic nature of flavour release in the mouth, the maximum headspace conditions predicted by the vapour pressure and partition coefficient are however, never actually achieved during eating (de Roos, 1997). The vapour pressure and partitioning theories cannot, therefore, predict the entire relationship between fat content and aroma perception but are useful tools for understanding flavour behaviour.

Fat (level as well as presence) will also affect the time intensity profile of flavour release since, in general, flavour release from the oil/fat phase of a food product proceeds at a much lower rate than from the aqueous phase. The whole phenomenon is attributed to (1) the higher resistance towards mass transfer in fat compared to water and (2) the fact that with oil in water emulsions, flavour compounds have first to be released from the fat to the aqueous phase before they can be released from the aqueous phase to the headspace (de Roos, 1997). As a consequence, quick disappearance of flavour compounds is a common defect of low fat foods.

3.5.1.3 Milk Solids Not Fat (MSNF)

The MSNF content affects the Tg`, the freezing point, lactose crystallisation, and stabilising properties of the mix. The proteins of the MSNF help to make ice-creams more compact and smooth and thus prevent weak body and coarse texture. They also increase the viscosity and resistance to melting (Marshall & Arbuckle, 1996). Lactose and salts decrease the freezing point (Hartel, 1996), while the proteins might act as stabilisers since they have high water binding capacity through denaturation (Petersen & Smith, 1991; Marshall & Arbuckle, 1996). Due to the latter characteristic and the higher viscosity, increasing the MSNF helps to reduce the size of ice crystals. There is, however, a limit to the use of MSNF since an excess might lead to lactose crystallisation, reduce the freezing point excessively and impart a cooked, salty and whey protein flavour (Anon, 1993). Lactose crystallisation in ice-cream is one of quality loss and depends on both the mix formulation and processing conditions (Livney, Donhowe & Hartel, 1995).

Arbuckle (1977) pointed out that lactose crystallisation, and hence sandiness, can be a problem whenever the water portion of the mix contain as much as 9% lactose and as such the use of whey protein concentrate (WPC) should be restricted to only 25% of the MSNF as they are rich in lactose. However, lactose crystallisation is also dependent on other factors such as the storage temperature and supersaturation (Livney *et al.*, 1995). For example, the rate of crystallisation of lactose increases as a result of an increase in the difference between the storage temperature and the glass transition temperature (Tg`) of the freeze-concentrated serum phase (Roos & Karel, 1991a). Lactose nuclei can also form at low supersaturation through secondary nucleation (Shi, Hartel & Liang, 1989).

The interactions of flavour compounds in fat-free systems is not only a question of fat-to-water partitioning since proteins can also bind some flavour compounds and thus make them also unavailable to the headspace. For example, aldehydes can reversibly bind to some proteins and ketones can also bind to some bovine serum albumin (BSA) or egg albumin. The interactions of proteins with flavour compounds depend on a number of factors such as physicochemical conditions and composition of the food system (pH, ionic strength, temperature) that would have an effect on the conformational structure of the proteins (Lubbers, Landy & Voilley, 1998). Two types of interactions can occur:

1. reversible through van der Waal interaction and
2. irreversible chemical reaction through covalent and electrostatic linkages (Fischer & Widdler, 1997).

The former is expected to have little influence on the sensory profiles compared to the latter (Leland, 1997).

The hydrophobic region of certain proteins can constitute binding sites for some non-polar aroma compounds, while hydrogen binding sites will be available for more polar groups, thus making their perception less evident (Lubbers *et al.*, 1998). According to Duffour & Haertle (1990), β-lactoglobulin has a hydrophobic region that can act as binding sites for certain non-polar aroma compounds (O'Neil & Kinsella, 1987). Proteins will therefore have an effect on aroma perception depending on the nature of the aroma compound, the binding sites of the proteins as well as the protein concentration (through

its effect on viscosity) (Fisher & Widdler, 1997). Since frozen yoghurt is a fermented product its flavour profile could be different to that of ice-creams since some studies have shown that pH has an effect on binding of aroma compounds through their effect on the structure of proteins (Lubbers *et al.*, 1998). For example, the structure and binding properties of bovine serum albumin (Druaux, Lubbers, Charpentier & Voilley, 1995) were altered by pH.

3.5.1.4 The system of stabilisers

Stabilisers are usually added to ice-cream mixes to give body and stiffness during freezing and to impart smoothness of texture (Marshall & Arbuckle, 1996).

Buyong & Fennema (1988) suggested that stabilisers exert a desirable influence on the sensory quality of frozen dairy desserts by influencing the organoleptic perception of texture. They have virtually no influence on the freezing point of the ice-cream mix (Budiaman & Fennema, 1987) and in general barely affect the Tg` of the mix since thay are used at low concentrations (Goff, Caldwell, Stanley & Maurice, 1993). They moreover provide resistance to thermal deformation by decreasing the rate of flow of the serum phase (Goff *et al.*, 1993).

Xanthan gum, locust bean gum, carrageenan, CMC, sodium alginate and gelatin are among the most used stabilisers in frozen dairy desserts. Commercial blends containing two or three of those stabilisers in combination with emulsifiers are available for use in ice-cream manufacture. The type of stabilisers used highly influences some processing parameters, e.g. gelatin requires a longer ageing period. Stabilisers have an effect on the size of ice crystals both at manufacture (Caldwell, Goff & Stanley, 1992b) and storage (Caldwell *et al.*, 1992b; Goff *et al.*, 1993). Studies by Caldwell *et al.* (1992b) and Goff *et al.* (1993) showed that stabilised ice-cream mixes have smaller ice crystals at manufacture and that these products showed greater resistance to ice crystal growth. Goff *et al.* (1993) thus concluded that the stabilisers act within the concentrated serum phase to

control the ice crystallisation process. The exact mechanism(s) is still the subject of much research and debate and may eventually depend on the type of stabilser.

The stabilising system is all-important as far as heat shock is concerned and it has been shown that stabilisers have an effect on ice crystal growth of temperature-abused samples (Donhowe *et al.*, 1991; Goff *et al.*, 1993). The increase in ice crystals' size of unstabilised samples was due to migration of water to the ice crystals as well as fusion of several ice crystals at the interface because of weak lamellae surrounding them. Stabilisers act within the concentrated serum phase to control the ice crystallisation process by increasing the resistance to flow of the serum phase and hence reduce the rate of thermal deformation during heat shock (Goff *et al.*, 1993).

Stabilisers also increase the viscosity of the unfrozen phase at subzero temperatures, decrease the linear rate of ice crystallisation and hence decrease the size of the ice crystals (Goff *et al.*, 1993). According to Carrington, Goff & Stanley (1996), stabilisers (in that case CMC) may either be physically incorporated in the ice crystal lattice or weakly adsorb to the ice crystal interface, therefore inhibiting further growth (Sutton & Wilcox, 1998). According to Goff *et al.* (1993), stabilisers have an effect on ice crystallisation but their exact effect on the thermodynamics of nucleation and growth is difficult to understand. These theories, however, still need further investigation.

Stabilisers also affect lactose crystallisation by increasing the viscosity of the unfrozen phase of the frozen dairy desserts (Hartel & Shastry, 1991), but they may also have a certain specific inhibitory effect on lactose crystallisation (Livney *et al.*, 1995). Gelatin, for example, impedes lactose crystallisation by interfering with the adsorption layer where the crystals grow (Hartel & Shastry, 1991).

Depending upon their interactions with certain milk components, e.g. κ-carrageenan with κ-casein, stabiliers can prevent wheying off during melting (Marshall & Arbuckle, 1996; Berger, 1997).

When fat is removed, the amount of stabilisers can be increased, especially the cellulosic products (Marshall & Arbuckle, 1996). However, overstabilisation and overemulsification might lead to a crumbly, chewy or gummy body. Too much an increase in the stabilising system can also have deleterious effects on the melting rate, as will do a high overrun. Moreover, the flavour contribution of gums cannot be ruled out (Anon, 1993).

3.5.1.5 Emulsifiers

Emulsifiers have little direct effect on the viscosity of the mix but reduce surface tension at the interface of two immiscible phases which will then mix and form an emulsion (Baer, Wolkow & Kasperson, 1997). During the manufacture of ice-creams and other related dairy desserts, they have an effect on the stability of the fat emulsion to yield an optimum product quality. They promote crystallisation of the fat (Abd El-Rahman *et al.*, 1997) as well as fat globules agglomeration (Govin & Leeder, 1971; Goff & Jordan, 1989). Because both caseins and whey proteins provide fat globules with excellent stability against aggregation, low molecular weight emulsifiers are included to reduce the amount of adsorbed milk proteins at the interface of fat globules during ageing, thus improving partial coalescence during whipping (Westerbeek, 1996; Goff, 1997). A certain degree of deemulsification is necessary for increased melting resistance (Govin & Leeder, 1971). Emulsifiers with a hydrophillic-lipophilic balance (HLB) value of 4 to 16 have been shown to be more effective in promoting fat agglomeration and hence the desired structure of ice cream (Govin & Leeder, 1971; Lin & Leeder, 1974). Emulsifiers of higher HLB are only loosely anchored to the fat globule and thus are swept away during agitation, removing the protection of the globule to stresses and thus promoting consequent coalescence and churning of the fat (Goff & Jordan, 1989).

Emulsifiers increase stiffness, reduce weak body and impart a smooth texture to the final product (Marshall & Arbuckle, 1996; Baer *et al.*, 1997) by promoting fat agglomeration. They also reduce the rate of melting and help to produce smaller ice crystals that are more evenly distributed and smaller air cells that result in a smoother ice-cream

(Arbuckle, 1977). Emulsifiers also increase the solidification rate of fat and the amount of solidified fat by acting as nuclei for fat crystals (Abd El-Rahman *et al.*, 1997).

3.5.1.6 Type of sweeteners used

Sugar is a major component of the %TS, increase the viscosity of the mix (Donhowe *et al.*, 1991) and hence enhance the creamy texture of frozen dairy desserts. However, with excess sugar, the product tends to become soggy, sticky and the decrease in the freezing point is such that lower temperatures are needed for proper hardening (Marshall & Arbuckle, 1996).

The level as well as type of sugar used in the formulation of the mix affect ice crystallisation primarily by depressing the freezing point. Sugars can also affect the viscosity, the Tg` and the degree of supersaturation (Hagiwara & Hartel, 1996; Goff *et al.*, 1993). However, increased levels of sugar must also be balanced with increased sweetness and the freezing point depression effect. On freezing the mix one wants to achieve a maximum amount of small ice crystals below the freezing point of the mix. Therefore depressing the freezing point too much may interfere with ice crystal formation. This would result in less water being frozen at any given temperature and hence increased freezing point depression can increase the size of the ice crystals (Harper & Shoemaker, 1983). This would most probably be due to more water molecules being available for ice crystal growth during hardening and storage. However, the presence of solutes can also act as impurities for ice crystal nucleation by heterogeneous mechanisms (Goff, 1992). Although sweeteners affect the ice crystal size through freezing point depression, increased viscosity and the glass transition temperature, they can also exhibit certain specific effects on ice crystals. Budiaman & Fennema, (1987) reported on the beneficial effect of sucrose in decreasing the rate of ice crystallisation . As a general rule increasing the sugar level therefore decrease the ice crystal size (Hartel, 1996; Marshall & Arbuckle, 1996), given that the effect on the freezing point depression and ice volume phase must be properly managed.

3.5.2 Mix properties

The most important mix properties are: mix stability, (which includes both the emulsion and colloidal state), viscosity, pH, freezing point, water binding capacity, and Tg`. They are affected by a number of factors including both the mix formulation and processing as well as their interactions.

3.5.2.1 Mix stability

Mix stability refers to the resistance to separation of milk proteins in colloidal suspension and of the milk fat in emulsion. Several key processes, such as the heat treatment, homogenisation, ageing and freezing, affect the stability of the mix. Mix stability also depends on certain mix properties such as pH, the size of the fat globules and the ratio of fat to total milk solids (Karleskind, Laye, Morr & Schenz, 1996; Marshall & Arbuckle, 1996). Oil in water emulsions are inherently unstable due to the high surface tension between fat and water (Goff, 1997). During homogenisation, milk proteins acting as emulsifiers, become adsorbed at the surface of the newly formed oil droplets. This layer helps in maintaining the emulsion stability by decreasing risks of aggregation and coalescence (Berger, 1997; Dickinson, 1997). In the manufacture of ice-cream a certain degree of de-emulsification and aggregation of the fat globules have been shown to be important to yield an optimum foam structure. However, early aggregation of the fat globules is undesirable for the production of frozen dairy desserts as it leads to excessive clumping of fat globules and eventually excessive churning at the freezing step, which can be detected as butter granules in the final product. Therefore, optimum stability of the mix upon enterring the freezer is crucial to the development of an appropriate structure.

As the acidity of the mix increases and reaches the iso-electric point of the fat globule membrane, the net charge on the proteins is reduced and the emulsion becomes unstable (Kanno, Schimomura & Takano, 1991). The relative amounts of proteins to fat can also have an effect on the emulsion's stability. When the amount of proteins available during emulsification is not sufficient to cover fully all the newly created fat globule surfaces,

two fat globules can share the same protein molecules and hence aggregation is enhanced (Dickinson, Murray & Stanby, 1988; Dickinson, 1997). The emulsion stability will be discussed further in sections 3.5.3.3 and 3.5.3.5.

3.5.2.2 Viscosity

The viscosity of a frozen dairy dessert mix is a very important rheological property that is influenced by many of the ingredients used, such as fat, stabilisers and MSNF. Other parameters affecting the viscosity include some processing steps such as pasteurisation, homogenisation and ageing (through hydration of stabilisers, the denaturation and hydration of proteins and crystallisation of fat) (Marshall & Arbuckle, 1996). It is a well-known fact that a reduction in the temperature of a mix will result in a reduction in the flow, hence leading to an increase in viscosity. This is in fact the rationale behind cooling and ageing.

Acidity is yet another factor influencing the viscosity of the mix and the lower the pH the higher the viscosity. However, an excess viscosity may impair proper whipping (Marshall & Arbuckle, 1996). The effect of pH could be very important as far as frozen yoghurt is concerned since there is no legal standards regarding the final pH of the product.

Viscosity is a property that accompanies good whipping abilities, body and texture (Marshall & Arbuckle, 1996). It is recognised that a certain level of viscosity is essential for proper whipping and retention of air (Baer *et al.*, 1997). According to Govin & Leeder (1971), increased viscosity may prevent wheying off and butter granule development by preventing excessive flocculation and coalescence of the fat. This would probably be due to less agitation of a mix with high viscosity. According to Thompson, Reneirs, Baker & Siu (1993) and Marshall & Arbuckle (1996), the resistance to melting and the smoothness of the body increases as the viscosity increases, but the rate of whipping decreases. The rate of secondary nucleation in some dairy fluid products decreased with increasing viscosity due to reduced frequency of collisions between ice crystals and freezer wall or impeller (Shirai, Nakanishi, Matsuno & Kamikubo, 1985).

Moreover, as a result of the freeze concentration process, the polysaccharides become more concentrated and entangled, thus increasing the sub-zero viscosity of the unfrozen continuous phase surrounding the ice crystals. The high sub-zero viscosity helps to prevent migration of water molecules to the ice crystals during temperature abuse (Goff *et al.*, 1993).

3.5.2.3 Freezing point and degree of subcooling

The freezing point is determined by the percentage of solutes in an aqueous solution and is therefore dependent on the % TS, % MSNF and the type and amount of sugars used. Different sweeteners depress the freezing point of water to different extents, depending upon the number of small molecules present, e.g. high fructose corn syrup will decrease the freezing point to a lower point than low DE corn syrup and sucrose owing to the higher number of small molecules present in solution (Hagiwara & Hartel, 1996). In addition to balancing the mix for sweetness, the level of sweeteners used should therefore also be balanced for the ice phase volume formed. The lower the freezing point the higher will be the amount of unfrozen water at any given temperature and the softer will be the product formed on freezing, but the higher the growth rate of ice crystals during hardening. The rate of ice recrystallisation increases with larger amounts of unfrozen water (Hawigara & Hartel, 1996).

The degree of subcooling primarily depends on the amount of solutes in the aqueous phase. Solutes decrease the degree of subcooling due to lowered freezing point (Goff, 1992). The degree of supersaturation and the crystals' radii determine whether or not an ice crystal grows or shrink (Sutton, Evans & Crilly, 1994). The linear rate of ice crystallisation increases with increasing subcooling. The degree of subcooling below the initial freezing point determines the equilibrium ice-phase volume. If most ice is formed during the initial freezing then small ice crystals are formed. As the temperature of the mix is lowered, two conflicting mechanisms come into play:

1. the independent effect of the lowered temperature causing an increase in the viscosity, decrease in mass transfer and inhibiting the growth of ice crystals, and

2. increased supercooling increases the rate of heat transfer which facilitates the growth of ice crystals (Budiaman & Fennema, 1987).

3.5.2.4 Glass transition temperature (Tg`) of the freeze-concentrated serum phase

During dehydration or freezing the viscosity of a food system becomes so high that a metastable glass forms (Goff, 1994). The temperature at which such a phenomenon takes place is referred to as the glass transition temperature (Tg`). Water in food acts as a plasticiser and the glass transition temperature of such systems decreases with increasing water content (Roos & Karel, 1991a; Roos, 1995). Freezing separates water from the dissolved solids by ice formation leaving behind a freeze-concentrated unfrozen matrix. The decreasing temperature and increasing solute concentration increase the glass transition temperature and viscosity of the freeze-concentrated phase (Roos & Karel, 1991b; Roos, 1995). The glass transition temperature of such a freeze-concentrated phase is denoted by Tg` (Slade & Levine, 1991). The Tg` of ice-creams has been quoted by Caldwell *et al.* (1992b) to be around –35°C. However, both processing and storage temperatures are far from the Tg`. Data from Hagiwara & Hartel (1996) suggest that the Tg` of ice-creams decreases with decreasing freezing point. Ice-creams made with high fructose corn syrup solids had a freezing point of –4.36°C and Tg` of -42.6°C compared to –2.93° C and –35.9° C for sucrose. The glass transition temperature therefore decreases with decreasing molecular weight (Goff, 1992). Likewise, the freezing point of frozen dairy desserts also decreases with decreasing molecular weight of the sweetener used.

At temperatures below the Tg`, water in the concentrated serum phase becomes kinetically immobilised and as such unavailable for reactions (Slade & Levine, 1991). Above the Tg` molecular mobility is greatly increased and many amorphous compounds crystallise (Roos & Karel, 1991a). The rate of crystallisation above the glass transition temperature is related to the viscosity of the unfrozen serum phase and decreases as the temperature of storage approaches Tg` (Reid, 1990). Likewise, a direct relationship holds

for ice recrystallisation rates at temperatures between the storage temperature and Tg` (Hagiwara & Hartel, 1996).

3.5.3 Mix processing and optimisation of conditions

The conditions during which the mixes are processed affect the quality of frozen dairy desserts. For instance, while heat treatment increases the water-binding capacity of proteins, low temperatures increase the hydration of the proteins. The acidity of frozen yoghurt entering the freezer is yet another factor to be considered.

3.5.3.1 Pasteurisation

Pasteurisation denatures proteins and increases their water-binding capacity. The strong denatured protein film that subsequently surrounds the fat globules tends helps to stabilise the emulsion (Govin & Leeder, 1971). Pasteurisation also inactivates harmful bacteria and bacteriophages in milk and improves the viscosity of the finished product, e.g. denatured proteins have good water retention capability. According to Goff (1997), the temperatures used during pasteurisation are sufficient to cause melting of all the fat present.

3.5.3.2 Homogenisation

Homogenisation leads to the formation of a fat/protein complex (i.e. the formation of a new fat globule membrane) and to a broadening of the fat globule size distribution. There is thus a reduced risk of creaming and butter granule formation when the mix is properly homogenised (Abd El-Rahman *et al.*, 1997; Arbuckle, 1977; Berger, 1997). During homogenisation in the presence of amphiphilic molecules, a membrane quickly forms around the fat globule. This newly formed fat globule membrane acts to lower the interfacial tension (surface free energy) between oil and water (Goff & Jordan, 1989; Kanno *et al.*, 1991; Goff, 1997). Caseins (especially the α_s- caseins) are adsorbed to the fat globule in preference to the whey proteins (Gelin, Poyen, Courthaudon, Meste &

Lorient, 1994; Sharma, Singh & Taylor, 1996). The reduction in the interfacial tension is very important for emulsion stability in ice-creams. Homogenisation also affects the process of fat crystallisation during ageing since homogenised fat globules would require subsequent supercooling below the melting point of the highest melting triglycerides (Walstra & Jenness, 1984).

3.5.3.3 Ageing

The length of ageing depends primarily on the type of stabilisers used. The purpose of ageing the mix includes further fat crystallisation, adsorption of emulsifiers to the fat globules, which loosen the attachment of the protein, the hydration of proteins and stabilisers (Marshall & Arbuckle, 1996; Berger, 1997; Abd El-Rahmann *et al.*, 1997). One of the purposes of the ageing process is therefore to increase the amount of solidified fat (Abd El-Rahman, 1997). During ageing the high melting glycerides will form crystals while the lowest melting point glycerides will still be liquid (Marshall & Arbuckle, 1996). The fat globule is then characterised by the presence of an outer concentrical shell of crystallised fat (Berger, 1997). A second proposed theory is that the fat crystals can also be formed within the globule (Goff, 1997). Fat crystallisation is therefore incomplete during ageing. Most of the fat crystals would be in the β`-form and the transformation of the α-crystal to the β`-crystal is expected to distort the shape of the fat globule and hence making them more susceptible to shear stress (Berger, 1997). The presence of partly crystalline fat is essential to induce partial coalescence of the fat globules during whipping and ageing (Goff, 1997). It is hypothesised that the fat crystals distort the shape of the fat globules and hence help in aggregation (Dickinson, 1997; Goff, 1997). The membranes surrounding the fat globules continue to develop during the ageing step until the lowest possible energy state is achieved (Berger, 1997). The emulsifiers loosen the attachment of milk proteins to the fat globules (Berger, 1997) leading to a partial desorption of the proteins from the fat globule membrane (Gelin *et al.*, 1994). As proteins are displaced, the membrane becomes more susceptible to subsequent destabilisation because the protein molecules are much larger than the emulsifiers. Thus a membrane made of emulsifiers is thinner and has different properties and hence becomes more

susceptible to shear stress (Goff, 1997). The fat globule clusters formed during the process of partial coalescence are responsible for surrounding and stabilising the air cells and creating a semicontinuous network or matrix of fat throughout the product (Goff, 1997). If gelatin is used as stabiliser, it combines with water and swells (Marshall & Arbuckle, 1996). Hydration of other stabilisers is generally faster than that of gelatin. The overall effect is an increase in the viscosity of the mix.

3.5.3.4 Incubation

The fermentation process decreases the pH and increases the viscosity of the mix. Factors in a food system that modify the conformation of proteins, e.g. changes in pH have a considerable influence on the binding of volatile compounds (Fisher & Widdler, 1997). As the pH decreases, more protein is expected to adsorb to the fat globule (Dickinson, 1997). The extent of pH decrease is also expected to affect the type and strength of gel formed (Gastaldi, Lagaude & Tarodo de La Fuente, 1996).

3.5.3.5 Whipping and freezing

A knowledge of the events during whipping and freezing is crucial because they influence the texture and body of the frozen dairy desserts. The smoothness of ice-cream and other frozen dairy desserts is related to the degree of iciness in the product and small ice crystals give a sensation of smoothness compared to large ice crystals that yield a much coarser product (Modler *et al.*, 1990). According to Hartel (1996), the process by which ice crystals are formed in ice-cream and the factors that must be controlled to produce proper texture are not fully understood. The following events are known to happen during the whipping and freezing of an ice-cream mix:

1. Further desorption of proteins, increased collision between the fat globules, further crystallisation of fat (Gelin *et al.*, 1994), together with disruption of the fat globule membrane and clumping and partial coalescence of fat globules (Caldwell *et al.*, 1992a; Goff & Jordan, 1994; Marshall & Arbuckle, 1996). Temperature changes

during freezing may be responsible for fat globule distortion during fat crystallisation. This distortion of the fat globules causes them to fracture under stress. The disruption of the fat globule membrane causes liquid fat to flow from the globules and cause the aggregation of the fat globules (Goff & Jordan, 1989). Clumping of the fat globules is also enhanced by protein-protein interaction (Berger, 1997). The rate of agglomeration and coalescence is primarily a function of the degree of agitation, but is also affected by the melting point of fat, temperature of freezer and salt content (Marshall & Arbuckle, 1996).

2. Air is incorporated into the mix (Hartel, 1996); the overrun which reflects the amount of air incorporated also influences product quality (Arbuckle, 1977). The incorporation of air increases the risks of coalescence by reducing the interfacial surface- tension (Mulder & Walstra, 1974). When air is first introduced in the mix, the large difference in interfacial tension between the air-serum interface and fat-serum interface may cause some of the protein layer of the fat-serum interface to adsorb to the newly created air bubble. The disruption of the membrane together with the distortion of the fat globules then cause a spreading of liquid fat onto the surface of the air bubble (Anderson & Brooker, 1988; Goff & Jordan, 1989). The factors that contribute to adsorption of the fat globules to the air bubble are thus surface tension and fat crystallisation (Anderson & Brooker, 1988). If fat crystals are present at the oil-water interface, irreversible adsorption occurs when a fat crystal comes into contact with a clean air-water interface (Darling, 1982). Fat destabilisation is thus a consequence of the introduction of air and shear stress due to agitation and ice crystallisation (Goff & Jordan, 1989). However, the incorporation of too much air in the final product can result in a fluffy product (Baer *et al.*, 1997) and may induce shrinkage (Dubey & White, 1997) while too little air produces a soggy and heavy product (Baer *et al.*, 1997). On the other hand, smaller air cells enhance the perception of smoothness and forms the rationale behind pre- aeration.

3. The viscosity of the mix initially decreases due to the disruption of the gel structure of the aged mix and breaking of fat clusters (Arbuckle, 1977) and then increases

slightly as a result of the freeze-concentration process (Buck, Walker & Pierce, 1986; Goff *et al.*, 1993).

4. The temperature of the mix drops in the freezer and this happens before any ice crystals are formed (Marshall & Arbuckle, 1996). When the freezing point of the mix is reached, water crystallises out in the form of ice nuclei. Only a slight decrease in temperature is experienced while ice is formed. Many tiny ice crystal nuclei are then scraped from the walls of the freezing cylinder into the bulk of the mix by the rotating action of the dasher where crystal perfection and growth will occur (Hartel, 1996). As more ice is formed, the solution left behind becomes more concentrated.

The ice-cream mix usually enters the freezer at a temperature of about 4°C, which is above its freezing point (Hartel, 1996). A refrigerant, usually ammonia in the case of continuous freezers and a halocarbon in the case of batch freezers, applied on the outside of the freezer barrel serves to cool the mix to below its freezing point and hence to cause nucleation of ice (Hartel, 1996). Nucleation is required to initiate ice crystallisation. In a typical ice-cream freezer, there is a substantial temperature driving force for ice crystal nucleation at the barrel wall (Hartel, 1996). This extremely large supercooling causes rapid and massive nucleation in the region of the barrel wall (Caldwell *et al.*, 1992b; Hartel, 1996). The degree of supercooling determines the equilibrium ice phase volume of the product (Hartel, 1996). Nucleation can take place by various mechanisms comprising both primary and secondary processes. Under the practical terms of ice-cream manufacture, ice nucleation would also occur through heterogeneous mechanisms (Goff, 1992) on foreign particles present just beneath the barrel wall or on the pitted surface of the barrel wall (Hartel & Chung, 1993; Hartel, 1996). It can be postulated that a substantial amount of crystal nuclei form at the inner side of the barrel wall and because of the temperature driving force that exists at the barrel wall, the crystals grow in the shape of dendrites inwards (Hartel, 1996). These dendrites are then subsequently scraped off by the blades of the dasher into the mix, where they grow and ripen (Fig.3) (Hartel, 1996). Alternatively, nuclei may also be formed through secondary/contact nucleation at a much lower subcooling as a result of removal of some components of the crystals by physical means such as collisions by the ice crystals and the growth of dendritic

structures followed by shearing of those dendrites (Hartel & Chung, 1993; Hartel, 1996). However, secondary nucleation has not been conclusively proven in ice-cream (Hartel, 1996).

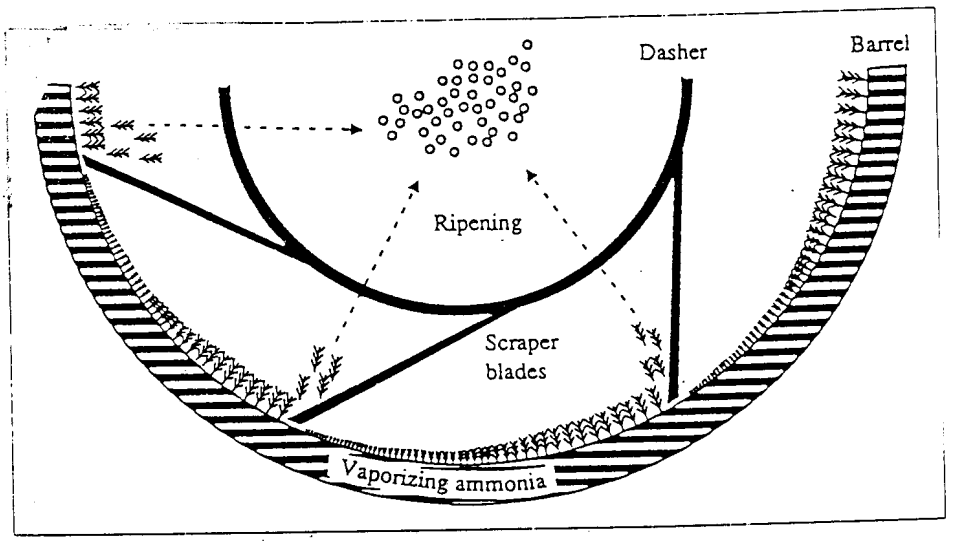


Figure 3: Shearing of dendrite crystals from the inside of the barrel of an ice-cream freezer (Hartel, 1996)

As freezing time and agitation increase and air is introduced, the fat globules become more crystalline (Marshall & Arbuckle, 1996; Goff, 1997), the membranes surrounding the fat globules rupture, releasing liquid fat which cements the globules together, forming globs and large clumps, both ranging in size from 3-5 μm (Buck *et al.*, 1986). This clumping or partial coalescence of the fat globules is responsible for stand-up properties and a dry melt-resistant ice-cream, i.e. there is a decreased rate of melting with increasing clumping (Keeney & Josephon, 1958; Kloser & Keeney, 1959; Knightly, 1959; Govin & Leeder, 1971; Goff & Jordan, 1989). The coalesced fat globules also surround and

stabilise the air cells thereby conferring a smooth texture to frozen dairy desserts (Berger, 1997; Goff, 1997). Too much agitation is, however, not good for the quality of ice-creams as the repulsive charges on the fat globules are lost and churning is highly enhanced (Marshall & Arbuckle, 1996). As water crystallises out as pure ice during the freeze concentration process, the solution left behind becomes more and more concentrated (the sugars and stabilisers are forced into smaller areas surrounding the ice phase) and as such further depresses the freezing point and therefore the temperature has to drop for further ice-crystallisation to occur (Goff, 1992). This freeze concentration process may eventually change properties such as pH, ionic strength, viscosity and lead to other unwanted reactions such as protein denaturation and lactose crystallisation (Kuntz, 1995). A point is however reached where the solution becomes so saturated that no further freezing can occur. This theoretical point leads to a maximally freeze-concentrated serum phase. During the freeze concentration process the stabilisers become more entangled, thereby further increasing the sub-zero viscosity of the unfrozen phase and hence impeding mass transfer (Goff *et al.*,1993). Likewise, the fat content of the freeze-concentrated phase also increases, making the fat globules more susceptible to flocculation and damage (Mulder & Walstra, 1974; Kokubo, Sakurai, Hakamata, Tomita & Yoshida, 1996). This is probably due to increased fat density that would increase the risks of flocculation. The freeze-concentration process also results in an increase in the glass transition temperature of the unfrozen matrix.

The main purpose of the freezing process is to produce a large number of small ice crystals (Hagiwara & Hartel, 1996). One of the conditions for a well-textured frozen dairy dessert is to freeze and draw the mix as quickly as possible since ice crystals that are formed quickly, are smaller than those formed slowly (Arbuckle, 1977; Goff, 1992). This illustrates the importance of the freezing rate. The type of freezer used to whip and freeze the mix is also very important as it has a direct impact on the freezing rate. Batch freezers tend to accentuate the development of larger and more irregular ice crystals compared to continuous freezers (Caldwell *et al.*,1992b; Marshall & Arbuckle, 1996). Rapid freezing rate enhances ice nucleation and thus the formation of many small ice crystals. According to Hartel (1996), the use of dull blades encourages the formation of

bigger ice crystals, most probably because they are not able to properly scrape the mix from the inner wall of the freezer. In general, the lower the draw temperature achieved, the smaller will be the size of the ice crystals (Hagiwara & Hartel, 1996). The draw temperature is a measure of heat removal and of the volume of ice phase achieved in the freezer (Hartel, 1996). The volume of ice phase attained at the freezing step is also a very important factor affecting the quality of frozen dairy desserts, since a higher amount of unfrozen water allows ice recrystallisation and ice crystal growth to occur at a faster rate (Carrington *et al.*, 1996).

The processing parameters of the freezer also affect the degree of fat de-emulsification and a higher degree of de-emulsification has been correlated with higher dasher speed (Kokubo *et al.*, 1996).

3.5.3.6 The hardening conditions

According to Caldwell *et al.* (1992a), the structure of ice-creams after drawing is in a non-equilibrium state. Studies carried out by the authors revealed that the fresh products at drawing consisted of a number of small air bubbles in a network structure of small ice crystals with poorly defined borders. Not all the freezable water is frozen at that stage. There is thus a need to harden ice-cream, as it leaves the freezer, as quickly as possible to a core temperature of -18°C (Arbuckle, 1977) since ice crystallisation continues during the hardening step but at a much slower rate (Hartel, 1996). This can lead to the formation of ice crystals of bigger size (Caldwell *et al.*, 1992b). During hardening, the amount of ice increases but no new ice nuclei are formed (Hagiwara & Hartel, 1996). Caldwell *et al.* (1992a) investigated the effects of hardening rate on the microstructure of frozen dairy desserts and concluded that freezing in liquid nitrogen yielded a structure characterised by a large number of small ice crystals as well as air bubbles. In contrast, plate hardening (for one hour) resulted in a structure with larger ice crystals (~25 μm) than the former process. Hardening in an ordinary cold room resulted in even bigger ice crystals (35 μm). They concluded that, as the hardening rate became slower, the average

size of ice crystals became larger and the average area of air bubbles decreased (smaller air bubbles collapsed to from larger ones).

3.5.3.7 Storage conditions

The temperature of storage has to be properly monitored, since even small ice crystals can grow, especially during heat shock (Carrington, Goff & Stanley, 1996). The mechanisms of ice recrystallisation that leads to the formation of bigger ice crystals in frozen dairy desserts can be grouped into migration (Otswald ripening) or the accretion theory.

Migration recrystallisation or Otswald ripening, is the process by which large ice crystals grow at the expense of smaller ones. Small ice crystals have a high surface area-to-volume ratio and consequently a high excess surface energy. They are thus thermodynamically unstable and tend to disappear with time and increase the size of bigger ice crystals. Accretion is the joining together of two crystals or more to form one larger one (Sutton, Evans & Crilly, 1994). The longer the storage time the higher the rate of ice recrystallisation (Hagiwara & Hartel, 1996).

Temperature fluctuations at the retail market is yet another factor affecting the quality of frozen dairy desserts since they have been shown to cause melting and growth of existing ice crystals (Goff *et al.*, 1993). Temperature fluctuations can also occur during the defrost cycles of the freezer used (Keeny, 1982). During temperature fluctuations ice crystals melt and when the temperature drops back no new nuclei are formed and ice recrystallisation takes place on existing crystals (Larsen, 1990). This temperature abuse phenomenon is referred to as heat shock and is influenced by a number of factors including fat replacement.

Melting of ice also decreases the viscosity of the unfrozen matrix and hence increase diffusion-limited reactions in food (Roos, 1995). It is thus acknowledged that constant subzero temperatures with minimal temperature fluctuations inhibit the process of ice

crystallisation (Goff, 1992). Moreover, freezing and subsequent thawing of the product may also enhance further clumping and coalescence of fat globules (Mulder & Walstra, 1974; Walstra & Jenness, 1984). Temperature fluctuations can also cause polymorphism in the crystallised fat (Mulder & Walstra, 1974) thus affecting the melting properties of the ice cream.

One of the major consideration during storage is the difference in temperature between storage (T_s) is from the glass transition temperature ($T_g`$) of the food system, i.e. ($T_s$-$T_g`$), (Roos & Karel, 1991a). According to Reid (1990), reactions during storage change as an exponential function of the difference between the storage temperature and $T_g`$ and not as the absolute value of $T_s$. At temperatures near the $T_g`$ water molecules can no longer migrate to the ice crystals.

The type of container used will also affect the quality of frozen dairy desserts. The use of paperboard containers allows the diffusion of gases and off-flavours in and out of the packaging systems (Dubey & White, 1997). Moreover, the shipment of frozen dairy desserts from different altitudes may induce shrinkage (Dubey & White, 1997).

3.6 Microparticulated proteins

3.6.1 The basis for fat replacement

Simplesse®, a microparticulated fat mimetics, has been affirmed as a GRAS ingredient for use in frozen desserts in 1990 (Cheftel & Dumay, 1993). Singer, Latella & Yamamoto (1990) claimed that Simplesse® has been formulated to produce a rich creamy taste and texture similar to fat because of the technology (microparticulation) used to produce the fat mimetics. According to the same authors, particle size distribution plays an important role in determining the perceived sensation of creaminess and grittiness (Fig. 4).

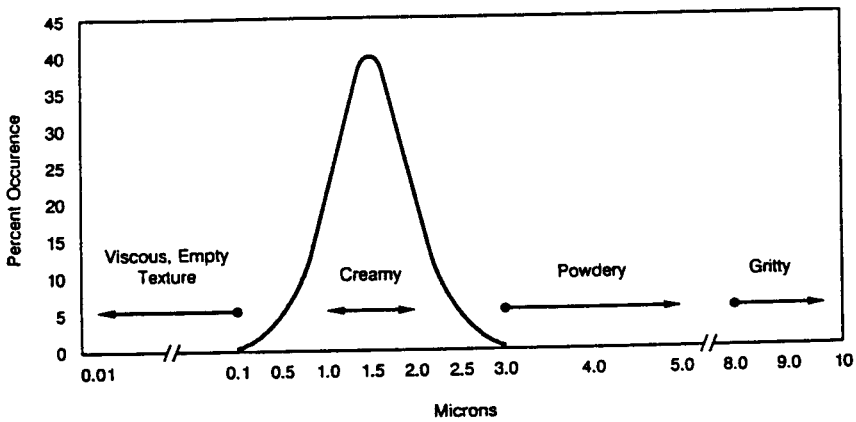


Figure 4: Particle size and perceived texture characteristics (Singer, 1990)

The size of the fat globules and clumps in ice-cream falls within the range for creaminess. In addition to the particle size, a creamy texture would also require the particles to be uniformly round and to roll freely over one another in response to shear (Singer *et al.*, 1990; Cheftel & Dumay, 1993). The microparticulated proteins have thus been formulated to have particle sizes ranging from 0.1-3.0 μm. It is the size range, shape and hydration of the microparticulated proteins that allow these particles to flow and create the creamy feel in the mouth (Singer & Dunn, 1990; Singer *et al.*, 1990). The particularity of protein-based fat replacer is that they do not replace fat on a one gram to one-gram basis but three parts of fat are replaced by one part of the protein and two parts of hydration (Anon, 1990 a).

3.6.2 The technology of microparticulated proteins

The whole process of making microparticulated proteins involves a series of denaturing treatments like thermocoagulation under moderate acidic conditions followed by microparticulation (Singer *et al.*, 1989; Singer *et al.*, 1990; Cheftel & Dumay, 1993;). Various substances may be added such as lecithin, xanthan gum and maltodextrins in the process to increase resistance to thermal aggregation (Fang & Snook, 1991; Cheftel & Dumay, 1993). The protein source can also be treated to remove traces of fat and other impurities (Singer *et al.*, 1989, 1990). The most preferred protein source includes globular

non-fibrous proteins that have not been previously subjected to protein denaturing processing e.g. whey proteins, caseins and egg albumin.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

- Fresh cream of 36-38% (m/m) fat, skim milk powder (96% (m/m) TS) and UHT-skim-milk (Ultramel) were all provided by Clover S.A.
- Whole milk (3.8-4% (m/m) fat) was obtained from the experimental farm, University of Pretoria and used to standardise the cream to 30% (m/m) fat.
- Starch (Mapps 949), one of the stabilisers used, was supplied by African Products (Pty) Ltd, Isando, SA.
- Gelatin grade 45, with a Bloom strength of 200, was supplied by Leiner Davis Gelatin SA, Krugersdorp, SA.
- Sucrose and Corn Syrup Solids (47% SE) were used as the sweeteners. Corn syrup solids was provided by African Products (Pty) Ltd, Isando, SA.
- Simplesse®500, a microparticulated whey protein concentrate (WPC), used as fat substitute, was manufactured by The NutraSweet Kelco Co., San Diego, California, USA and supplied by Tranarc Holdings, Ltd, Benmore, SA. It was available as a powder.
- The culture used was an ABT culture (ABT 5) for stirred yoghurt available in the freeze-dried form (Chr. Hansens, Denmark). The cultures were stored according to the manufacturer's recommendations at -18°C.
- The artificial strawberry flavour, Strawberry French D.3694, was supplied by Bush Boake Allen (Pty) Ltd, Isando, SA.
- The colouring, pontceau red 4R, was obtained from Haarmann & Reimer SA, Isando, SA.

4.2 Experimental design

4.2.1 The 'treatments'

Four different frozen yoghurt mixes were formulated as follows:

'Treatment' 1: A frozen yoghurt mix containing 10% (m/m) milk fat, more typical of ice-cream products on the market (Tharp & Gottenmoller, 1990), acted as a 'control'.

'Treatment' 2: A frozen yoghurt mix containing 3.4% (m/m) of microparticulated WPC as fat substitute to replace 10% (m/m) milk fat since one gram of fat would be replaced by one gram of protein which would hydrate with two grams of water (Anon, 1990a).

'Treatment' 3: A frozen yoghurt mix containing 5% (m/m) milk fat was formulated because the frozen yoghurt on the market and that used in some studies (Holocomb *et al.*, 1991; Laroia & Martin, 1991; Thompson & Mistry, 1994; Westerbeek, 1995; Anon, 1996) contain 5% (m/m) fat.

'Treatment' 4: A frozen yoghurt mix containing 5% (m/m) of microparticulated WPC, which is the upper limit for substitution. This frozen yoghurt mix would almost have the same total solids content as the mix containing 5% (m/m) milk fat and thus would substitute 15% (m/m) milk fat.

4.2.2 Sampling protocol

The time at which the various samples were taken for analysis is shown in Fig.5. A sterilised spatula was used to remove the surface layer of the frozen yoghurt to a depth of about 10 mm (IDF, 1995) before taking any sample. Samples of 100 g each were taken from three randomly selected cups to constitute the sample for the tests (IDF, 1995). All the tests were carried out on triplicate samples and the whole experiment repeated three times.

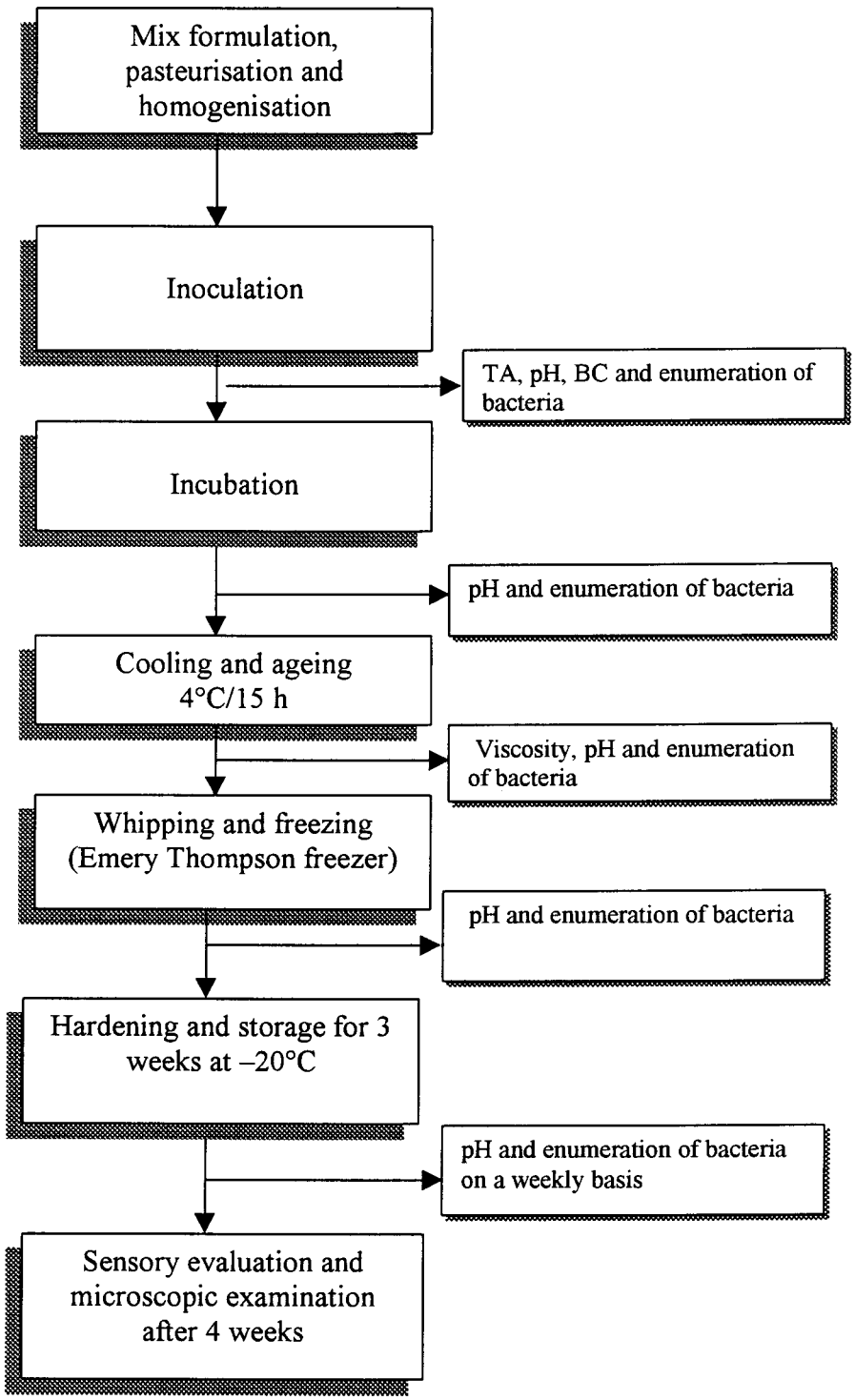


Figure 5: Protocol for taking samples for the indicated tests (BC= buffering capacity, TA= titratable acidity)

4.3 Methods

4.3.1 Yoghurt Manufacture

4.3.1.1 Mix formulation

Frozen yoghurt mixes of the ice-cream type were formulated as discussed in section 4.2.1 and manufactured by fermenting the entire mix after pasteurisation and homogenisation (Fig. 6). The composition of the four mixes was as shown in Table 2 and the ingredients used as listed in appendix A.

Table 2: Formulated composition of the four frozen yoghurt mixes

Composition % (m/m)	10% milk fat mix	5% milk fat mix	5% fat substitute mix	3.4% fat substitute mix
TS*	38.36	33.92	33.72	32.18
Fat [φ]	10.00	5.00	0.00	0.00
Fat substitute[○]	0.00	0.00	5.00	3.40
Solids-not-fat[γ]	10.50	11.06	11.06	11.06
Sugar[Φ]	14.00	14.00	14.00	14.00
Stabiliser[κ]	1.00	1.00	1.00	1.00

* Total solids
[φ] Include fat contributed to by the cream (standardised to 30% (m/m) fat) only
[○] Simplesse® 500
[γ] Does not take into account milk solids-not-fat contributed by the fat substitute
[Φ] 20% of sucrose was replaced by corn syrup solids (47% sucrose equivalent, 96.5% (m/m) total solids)
[κ] Consisting of starch and gelatin (used at 0.5% (m/m) each)

Fresh cream was used since it is the most desirable concentrated source of butterfat for use in the mix (Arbuckle, 1977). Starch and gelatin were used as stabilisers (Inoue *et al.*, 1998). Corn syrup solids, 47% sucrose equivalent (SE), were used to replace 25% of the sucrose. The microparticulated WPC fat substitute, Simplesse® 500, was available in the dry form and added to the pasteuriser vat at 45°C, before addition of the stabilisers.

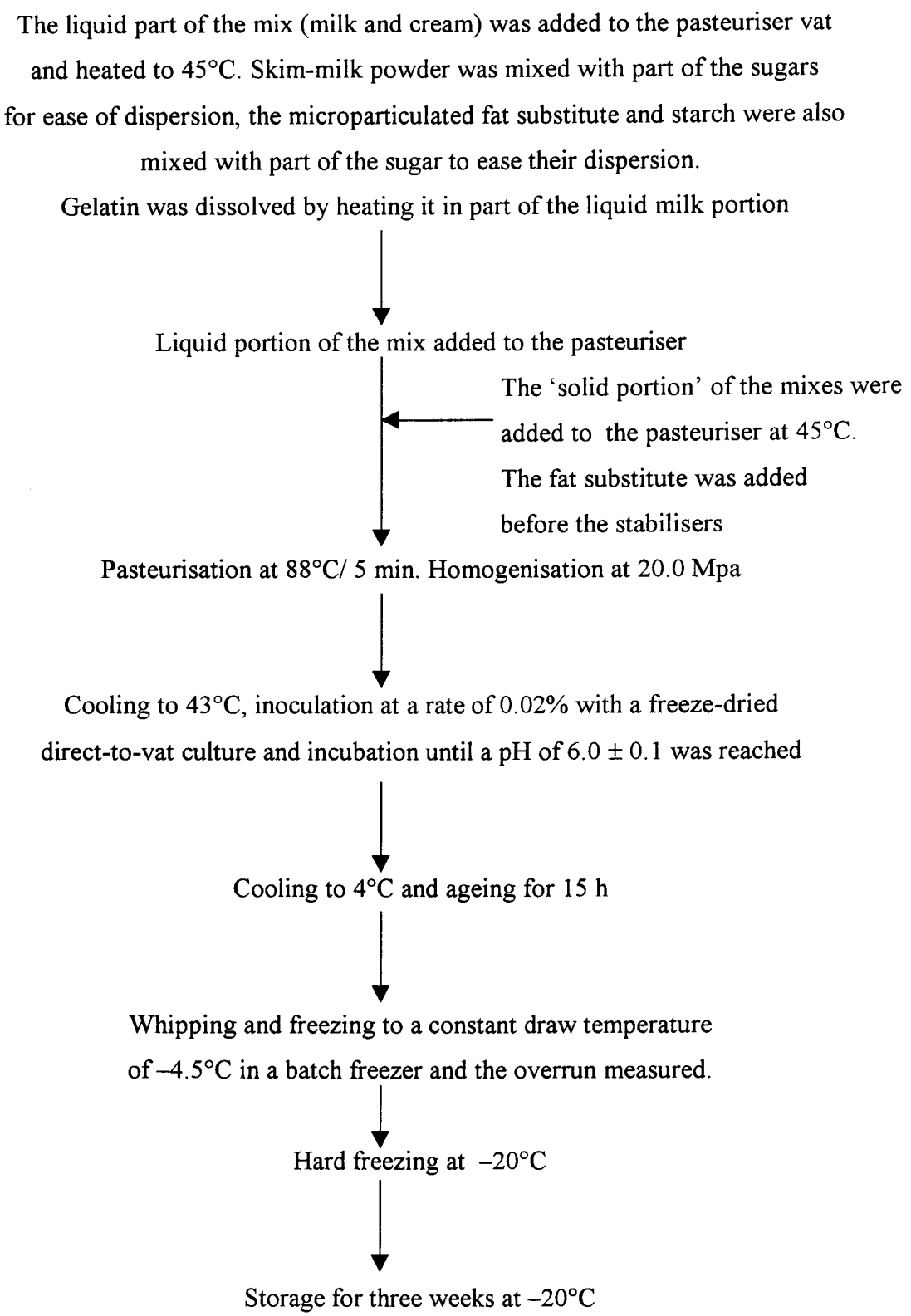


Figure 6: Flow chart of the frozen yoghurt manufacture

The milk solids-not-fat (MSNF) content of the 10% fat mix was calculated to be 10.5% (m/m) and that of the 5% fat mix to be 11.06% (m/m) by making use of the serum point method described by Arbuckle (1977) and Marshall & Arbuckle (1996). The mix containing 5% of Simplesse® 500 had the same milk solids-not-fat content as the mix containing 5% fat to avoid any significant differences in total solids content between the treatments. The level of sugar for all the four different frozen yoghurt mixes was maintained at 14%. (m/m).

4.3.1.2 Mixing of ingredients

All liquid ingredients were placed in the pasteuriser vat and agitation and heating started at once (Marshall & Arbuckle, 1996). The sugars were mixed with the skim-milk powder, the starch and the microparticulated WPC (in the case of the fat substitute samples) for proper dispersion. According to the supplier of Simplesse® 500, the microparticulated whey protein concentrate could be either hydrated before use or used in the dry form. Therefore, in the case of the fat substituted mixes, the microparticulated WPC was added directly to the pasteuriser vat along with the blend of sugar, starch and skim-milk powder when the liquid ingredients reached a temperature of 45°C (Marshall & Arbuckle, 1996). Gelatin was soaked and heated with part of the liquid skim milk to dissolve it and was added to rest of the mix in the vat at 45°C (Arbuckle, 1977).

4.3.1.3 Pasteurisation

The pasteurisation step is very important as it inactivates bacteria and bacteriophages in addition to the advantages listed in section 3.6.3.1. A heat treatment of 88°C for at least 2 min was used during the experiment.

4.3.1.4 Homogenisation

Following pasteurisation, the mix was homogenised at 20.0 Mpa and a temperature of 66°C using a Rannie homogeniser, KP 2, (National Dairy Equipment (Pty) Ltd, Johannesburg, SA). The homogenisation pressure used during the experiment is in accordance with values suggested by Marshall & Arbuckle (1996) for ice cream with a fat content of 8-12 % (m/m).

4.3.1.5 Cooling

The mix was cooled to 43°C, which was the temperature at which the yoghurt was inoculated and incubated.

4.3.1.6 Inoculation

The mix was inoculated at a 0.02% (v/v) inoculation rate. The inoculated milk was stirred for 2 min to mix the culture completely into the yoghurt mix.

4.3.1.7 Incubation

The yoghurt mixes were incubated at 43°C until a pH of 6.0 ± 0.1 was reached. A high pH frozen yoghurt would allow better survival of the probiotic bacteria (Laroia & Martin, 1991). Moreover, residual fermentation during cooling to 4°C would decrease the pH by 0.2-0.3 units (Dave & Shah, 1997a, 1997b).

4.3.1.8 Ageing

The mixes were aged at 4°C for 15 h to allow for the complete action of gelatin. When gelatin is used with a batch freezer, a long ageing period is essential and recommended (Marshall & Arbuckle, 1996).

4.3.1.9 Flavouring

Following ageing, the mixes were flavoured with an artificial strawberry flavour, added at a rate of 0.08% (v/v) according to the supplier's recommendation. The mixes were also coloured with ponceau red 4R (Haarmann & Reimer SA, Isando, SA) and the flavourings properly mixed with the aged mix.

4.3.1.10 Whipping and freezing

When ice-cream is drawn it must be firm enough to form a ribbon yet soft enough to slowly settle into the container and lose its shape (Marshall & Arbuckle, 1996). Following preliminary trials a draw temperature of -4.5°C was found to provide a good firmness for all the treatments involved. Consequently, the aged mix was frozen in a batch freezer (Emery Thompson Freezer) to a constant draw temperature of -4.5°C and packaged in cups of 500 ml and 125 ml.

4.3.1.11 Hardening and storage

Following whipping and freezing, the samples of frozen yoghurt were stored at a temperature of -20°C for 6 weeks during which time several tests were performed on them at specified times (Fig. 5).

4.3.2 Physical analyses

4.3.2.1 Apparent viscosity

Apparent viscosity of the aged mix was measured using a Haake Rotovisco Viscometer VT 24 (Haake-Buchler Instruments Inc., Karlsruhe, Germany) equipped with a MVII sensor. An ice water bath was used to maintain the temperature of the sample at 4°C throughout the measurements. The apparent viscosity of the mixes was compared at a shear rate of 20.4 s^{-1}. The precautions prescribed by the manufacturer were carefully followed for setting up the apparatus before taking any measurements.

4.3.2.2 Overrun

The overrun of the frozen yoghurt, which represents the amount of air incorporated into the product (Marshall & Arbuckle, 1996) was determined using the following formula (Buck *et al.*, 1986):

Overrun = [(mass of mix - mass of frozen yoghurt from freezer) / mass of frozen yoghurt from freezer] x 100

4.3.3 Chemical analyses

4.3.3.1 pH

The pH of the mix and the frozen yoghurt was measured using a pH meter (Mettler DL 25 Titrator, Mettler Co. Greinfensee, Switzerland) equipped with a combined electrode (pH electrode, Mettler Toledo, DG 111-SG). The temperature of the samples was adjusted to 25°C in a water bath (Inoue *et al.*, 1998). Before taking any measurement the instrument was calibrated using buffer solutions at pH 4.0 and 7.0 respectively. The electrode was directly immersed into the sample so that the pH sensitive bulb and the

reference junction were in good contact with the sample (Case, Bradley & Williams, 1985).

4.3.3.2 Titratable acidity (TA)

Titratable acidity (TA) was determined following the method of Arbuckle (1977). The temperature of the samples was adjusted to 25°C and the titration carried out as quickly as possible so that the samples remained at that temperature (Case *et al.*, 1985). A 9 g sample was thoroughly mixed with 9 ml of distilled water and titrated against 0.1 N NaOH to an end point of pH 8.3. The end point was determined using a Mettler DL 25 Titrator (Mettler Co. Greinfensee, Switzerland). The percent titratable acidity was calculated using the following equation (Arbuckle, 1977):

% TA = ml 0.1N NaOH / 10.

4.3.3.3 Buffering capacity

The buffering capacity of the mix was determined by the method described by Kailasapathy, Supriadi & Hourigan (1996). Samples (10 g) were thoroughly mixed with 10 ml of distilled water and titrated against 0.05 ml increments of either 1.0 N NaOH or 1.0 N HCl to an end point of pH 8.3 and pH 2.0 respectively. Each of the increments of the acid or alkali was thoroughly mixed with the sample and the pH change measured with a Mettler DL 25 Titrator (Mettler Co. Greinfensee, Switzerland). The final volume of either acid or alkali added was used as a measure of the buffering capacity (Martini, Bollweg, Levitt & Savaiano, 1987; Kailasapathy & Supriadi, 1996; Kailasapathy, Supriadi & Hourigan, 1996)

4.3.3.4 Fat content

The percentage (m/m) of fat of the frozen yoghurt was determined by the ether-extraction gravimetric method (IDF, 1983), based on the Rose-Gottlieb procedure.

4.3.3.5 Protein Content

The protein content was determined by the semi-micro Kjedahl method using the Buchi 430 Digester and the Buchi 332 Distillation Unit (Buchi Laboratoriums-Technik AG, Switzerland). The percentage (m/m) crude protein was obtained by multiplying the nitrogen content by a factor of 6.38 (IDF, 1993).

4.3.3.6 Total solids content

The total solids (TS) were determined by the official method described by Arbuckle (1977). Approximately 2.0 g of the sample was weighed accurately into a flat-bottom metal dish. The sample was heated on a steam bath for 30 min and then dried in an air oven for 3.5 h at 105°C. The dish with the dried sample was then cooled in a desiccator and weighed.

Percentage (m/m) TS = (mass left after drying/ mass of sample) × 100

4.3.4 Microbiological analyses

Unless otherwise stated the media, diluents and instruments (e.g., spatula, knives, membrane filters and the membrane filter set-up) were sterilised by autoclaving at 121.1°C for 15 minutes (IDF, 1984). All heat sensitive ingredients such as glucose, maltose and L-cysteine hydrogen chloride were filter-sterilised through a Whatman membrane of 0.45µm pore size (Martin & Chou, 1992).

The pour plate method was used for enumerating *Streptococcus salivarius* subsp. *thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Lankaputhra & Shah, 1995; Dave & Shah 1997a, 1997b, 1997c) and only plates with 25-250 colonies were used for calculating the numbers of 'colony forming units' (cfu) per gram of sample.

4.3.4.1 Dilutions

Peptone/saline solution was used as the diluent (IDF, 1984; Klaver *et al.*, 1993). The 10^{-1} dilution was constituted by aseptically transferring 10 g sample to 90 ml of sterile peptone/saline solution and mixed thoroughly (IDF, 1987). One millilitre of the 10^{-1} dilution was then transferred to 99 ml sterilised peptone/saline solution and mixed to make the 10^{-3} dilution. Subsequent uneven dilutions (10^{-5} and 10^{-7}) were prepared in the same way. Whenever an even dilution was needed, 0.1 ml of the lower uneven dilution was plated e.g. a 10^{-4} dilution was obtained by plating 0.1 ml of the 10^{-3} dilution. Dilutions 10^{-3} to 10^{-8} were plated.

4.3.4.2 Enumeration of *Bifidobacterium bifidum*

The enumeration of *B. bifidum* was carried out on MRS-NPNL (Naladixic acid, .Paramomycin sulphate, Neomycin sulphate and Lithium Chloride) medium (Rasic, 1990; Laroia & Martin, 1991; Dave & Shah, 1996). L-cysteine hydrogen chloride was added to lower the redox potential of the medium and hence enhance the growth of bifidobacteria (Rasic, 1990; Dave & Shah, 1996).

The required amount of MRS agar (de Man, Rogosa & Sharpe, 1960) was suspended in 1000 ml of distilled water and boiled to dissolve the ingredients completely. The pH of the medium was adjusted to be at pH 6.9 ±0.1 after sterilisation at 121.1°C/ 15 min.

The NPNL solution (Laroia & Martin, 1991; Lourens, 1998) consisted of 0.2 g Neomycin sulphate (Sigma N-1876), 0.03 g Naladixic acid (Sigma N-8878), 6 g Lithium Chloride (univAR 394 5320) and 0.25 g Paramomycin Sulphate (Sigma P-9297). The ingredients were suspended in 100 ml of distilled water, the pH adjusted to 7.2 -7.5 and the solution was filter-sterilised.

A 20% glucose solution (m/v) was prepared by dissolving 20 g of dextrose (Oxoid L71) in 100 ml of distilled water. The solution was sterilised by filtration.

A 10% (m/v) solution of L-cysteine hydrogen chloride (Merck No.2839) was prepared by dissolving 10 g of L-cysteine HCl in 100 ml of distilled water and then filter-sterilised.

To prepare the MRS-NPNL medium 100 ml of 20% (m/v) glucose, 50 ml of NPNL solution and 5 ml of L-cysteine HCl was added to 1000 ml of MRS medium at 50°C.

The pour plates were incubated anaerobically at 37°C for 72 h (Rasic, 1990; Laroia & Martin, 1991; Dave & Shah, 1996). The anaerobic atmosphere was generated by using Anaeorcult® A blocks (Merck).

4.3.4.3 Enumeration of *Lactobacillus acidophilus*

Lactobacillus acidophilus was enumerated on MRS-maltose agar (IDF, 1991; Hull & Roberts, 1993; Dave & Shah, 1996). The MRS- Maltose medium consisted of MRS agar to which a filter-sterilised 20% (m/v) maltose solution was added. On such medium *L. acidophilus* forms large convex colonies approximately 2 mm in diameter (Hull & Roberts, 1984).

The plates were incubated aerobically at 37°C for 72 h (Dave & Shah, 1996).

4.3.4.4 Enumeration of *Streptococcus salivarius* subsp. *thermophilus*

Streptococcus salivarius subsp. .*thermophilus* was enumerated on M17 agar (Terzaghi & Sandine, 1975; Roberts & Maust, 1995).

The complete M17 agar was prepared by adding 50 ml of a filter-sterilised 10% (m/v) lactose solution to 950 ml of the M17 agar at 45°C.

The plates were incubated aerobically at 37°C for 48 h. *Streptococcus salivarius* subsp. *thermophilus* forms lenticular-shaped colonies.

4.3.5 Sensory evaluation

The sensory evaluation tests were carried out at the sensory evaluation area of the Department of Food Science, University of Pretoria. The IDF standard 50B (IDF, 1981) spells out the conditions required for sensory evaluation.

Generic descriptive analysis was used to characterise and quantify the differences between the four treatments. Generic sensory evaluation is the technique whereby the panel is able to identify all the perceptions and then describe and measure them. Descriptive analysis is a sensory evaluation technique that allows the quantification of complex changes that occur in a food formulation.

4.3.5.1 Recruitment and training of panel

A sensory panel was recruited amongst the students and personnel of the University of Pretoria and screened for their sensitivity to the four basic tastes (Jellinek, 1985) as well as their motivation and interest in participating in the sensory evaluation. After the screening process, the selected panelists were exposed to the basic principles of sensory evaluation and given the usual instructions, i.e. not to smoke and not to eat at least 30 min before each session and not to wear any perfume. They were then trained on samples of the four treatments and were asked to generate terms that best described the differences between the samples. The panel had also to note the order in which those attributes appeared. Both the final list of attributes and the order in which they appeared were agreed on by the panelists through consensus. The panelists were asked to evaluate the texture first and then the flavour. Texture was analysed by manipulating a sample of frozen yoghurt between the tongue and the palate. After the generation of terms, a further screening was carried out to constitute the final panel on their ability to generate consistent and reliable data. The training session lasted for a week with three sessions a day.

4.3.5.2 The sensory evaluation test

Samples of approximately 30 g each were scooped out into styrofoam cups and covered. The trained panelists were randomly served three-digit coded samples to avoid any bias during the evaluation. Testing of the samples was conducted in separate booths at about 22°C and under red illumination to mask any colour differences. The panelists were provided with a 10-cm long line scale marked at each end by standards generated by the panelists.

4.3.6 Electron microscopy

The microscopic analysis of the frozen yoghurt was carried out at the laboratory for Microscopy and Micro-analysis, University of Pretoria. Scanning electron microscopy (SEM) was used to examine the differences in the microstructure of the samples of the different treatments in terms of the behaviour of the fat and the fat substitute and the presence or absence of lactose crystals.

By using a spatula, which was dipped in liquid nitrogen at -196°C to avoid distributing heat while taking a sample of the frozen yoghurt, a sample was transferred to the sample holder of the sample stand and immersed in slushed nitrogen when it becomes liquid again at -210°C (Caldwell *et al.*, 1992b). After fixing the sample, it was transferred to the sample preparation area of the microscope where it was fractured in vacuo by a blade to reveal the surface of the frozen yoghurt sample (Inoue *et al.*, 1998). The samples were then sputter-coated with gold for 5 min and viewed with a cryo-scanning electron microscope (Jeol Scanning Microscope- 840) at approximately -186°C. The temperature of the microscope was constantly monitored and maintained by the use of liquid nitrogen.

4.3.7 Statistical analysis

The statistical analysis of the results were done using Statistica software package tool, Version 5.0 (StatSoft, Inc., Tulsa OK, USA). The numbers of microbiological counts were converted to $\log_{10}$ cfu/g. Means and standard deviations of the values were calculated. The least significant difference between the means was determined and compared. Analysis of Variance and correlations were also determined to fit the data.

Only one sample from each repetition was used for the microscopic examination.

CHAPTER 5

RESULTS

5.1 Composition of the yoghurt mixes

The composition of the various mixes for the experimental yoghurt is shown in Table 3. The values obtained after analysis did not differ much from the composition aimed at during formulation. Substituting fat by a microparticulated whey protein concentrate resulted in a significant increase ($p < 0.05$) in the protein content of the mixes compared to those containing 5% or 10% milk fat. The protein content of the mix containing 5% milk fat was also significantly higher than that of the mix containing 10% milk fat. The protein content of the yoghurt mix containing 5% microparticulated whey protein concentrate also differed significantly from that containing 3.4% whey protein concentrate. The yoghurt mixes containing 3.4% and 5% of the fat substitute did not differ significantly from each other in terms of their fat content. The mix containing 10% fat differed significantly from the other mixes as far as total solids content was concerned.

Since the fat-substituted mixes were high in protein and low in fat compared to those containing fat, they will at times be referred to as 'high-protein' or 'low-fat' mixes, depending upon the circumstances.

Table 3: Composition of the yoghurt mixes containing either milk fat or fat substitute in the form of a microparticulated whey protein concentrates (WPC)

Yoghurt mixes	Fat content[1] % (m/m)	Protein content[1] % (m/m)	Total solids content[1] % (m/m)
10% milk fat	9.92[a]	4.28[a]	37.69[a]
	(±0.34)[2]	(±0.15)	(±0.392)
5% milk fat	5.34[b]	4.40[b]	33.92[b]
	(±0.54)	(±0.09)	(±0.634)
3,4% WPC	0.62[c]	5.47[c]	32.72[c]
	(±0.08)	(±0.13)	(±0.735)
5.0% WPC	0.69[c]	6.09[d]	33.86[b]
	(±0.10)	(±0.08)	(±0.435)

1. Means with different letters in the same column are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

5.2 pH and titratable acidity (TA) of the yoghurt mixes before incubation

Fat substitution by a microparticulated WPC resulted in yoghurt mixes with higher TA and lower pH (Table 4). The mixes with either 10% fat or 5% fat did not differ significantly from each other in pH and titratable acidity. The titratable acidity was positively correlated to the protein content ($r = 0.909$, $p < 0.05$).

Table 4: The pH, titratable acidity (TA) and buffering capacity of the yoghurt mixes containing either milk fat or fat substitute in the form of a microparticulated whey protein concentrates (WPC)

Yoghurt mixes	pH[1]	TA (%)[1]	Volume of 1.0 N NaOH[1] (ml /10 g)	Volume of 1.0 N HCl[1] (ml / 10 g)
10% milk fat	6.55[a]	0.181[a]	0.175[a]	1.72[a]
	(±0.05)[2]	(±0.009)	(±0.004)	(±0.09)
5% milk fat	6.53[a]	0.190[a]	0.184[a]	1.78[a]
	(±0.04)	(±0.011)	(±0.003)	(±0.08)
3.4% WPC	6.47[b]	0.218[b]	0.211[b]	2.01[b]
	(±0.03)	(±0.012)	(±0.008)	(±0.09)
5% WPC	6.45[b]	0.223[b]	0.218[b]	2.08[b]
	(±0.04)	(±0.011)	(±0.007)	(±0.10)

1. Means with different letters in the same column are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

5.3 Buffering capacity of the yoghurt mixes

The addition of a microparticulated WPC in replacement of milk fat resulted in a significant increase in the buffering capacity of the fat-substituted yoghurt mixes (Table 4). Although increased buffering capacity in alkaline conditions was positively correlated to the protein content ($r = 0.93$, $p < 0.05$), the samples containing milk fat required the addition of slightly more alkali per gram of protein (0.041 ml/g and 0.042 ml/g respectively) than those containing the fat subtitute (0.038 ml/g and 0.036 ml/g respectively). Likewise, although the buffering capacity in acidic conditions was also positively correlated to the protein content ($r = 0.85$, $p < 0.05$), the samples containing milk fat required the addition of more acid per gram of protein (0.40 ml/g and 0.41ml/g) compared to the mixes containing the fat substitute (0.36 ml/g and 0.34 ml/g).

5.4 The decrease in pH of the yoghurt mixes during various processing stages

The pH of all the yoghurt mixes decreased during fermentation and ageing (Fig. 7). However, during ageing, the decrease in the pH of the 'low-buffering capacity' mixes (containing fat) was significantly higher than that of the two mixes containing the microparticulated WPC. Freezing at –4.5° C and storage of the mixes at –20°C did not result in a significant decrease in the pH.

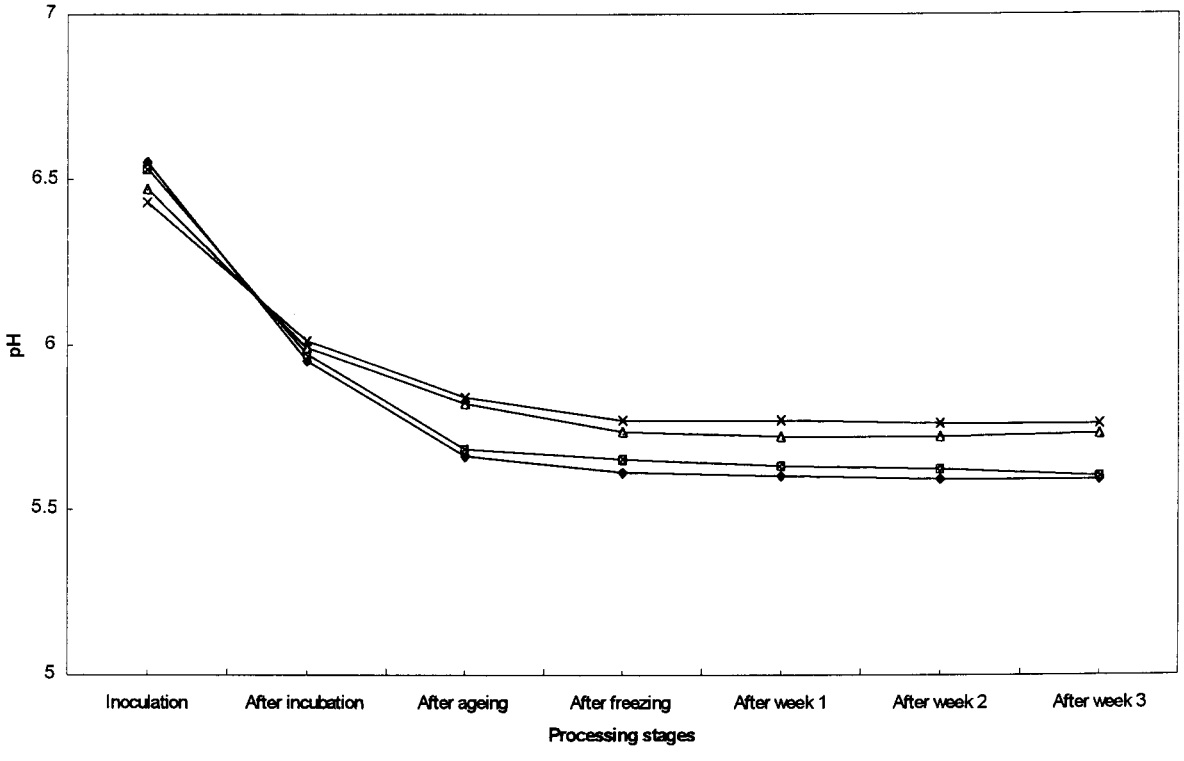


Figure 7: The pH at various stages during the manufacture of frozen yoghurt containing milk fat or fat substitute in the form of a whey protein concentrate (WPC)
(◆ 10%Fat ■ 5%Fat △ 3.4%WPC × 5%WPC)

5.5 Microbiological analysis

The numbers of *S. salivarius* subsp. *thermophilus* increased significantly ($p < 0.05$) in all four mixes until the end of ageing of the mixes (Table 5; Fig. 8). During freezing, there was a significant ($p < 0.05$) decrease in numbers of *S. salivarius* subsp. *thermophilus* in all four yoghurt mixes. During storage of the frozen yoghurt there was a further slight, but insignificant ($p > 0.05$), decrease in numbers of *S. salivarius* subsp. *thermophilus.* At no stage during the manufacturing process were there any significant differences in the numbers of *S. salivarius* subsp. *thermophilus* between the four batches of frozen yoghurt (Table 5).

Table 5: Numbers (log_{10} cfu/g) of *S. salivarius* subsp. *thermophilus* during the manufacture and storage of frozen yoghurt containing either milk fat or fat substitute in the form of a whey protein concentrate (WPC)

Stages	10% milk fat[1]	5% milk fat[1]	3.4% WPC[1]	5% WPC[1]
Inoculation	7.12^{a}	7.08^{a}	7.04^{a}	7.07^{a}
	(±0.17)[2]	(±0.23)	(±0.19)	(±0.17)
After incubation	8.03^{bd}	8.05^{b}	7.97^{bd}	8.00^{bd}
	(±0.17)	(±0.21)	(±0.14)	(±0.11)
After ageing	8.29^{c}	8.27^{c}	8.21^{c}	8.20^{c}
	(±0.17)	(±0.17)	(±0.16)	(±0.15)
After freezing	8.01^{bd}	8.02^{b}	7.97^{bd}	7.99^{bd}
	(±0.12)	(±0.13)	(±0.12)	(±0.17)
After week 1	8.01^{bd}	7.99^{bd}	7.92^{bd}	7.95^{bd}
	(±0.14)	(±0.17)	(±0.10)	(±0.17)
After week 2	8.00^{bd}	7.96^{bd}	7.89^{bd}	7.90^{bd}
	(±0.16)	(±0.17)	(±0.18)	(±0.19)
After week 3	7.99^{bd}	7.91^{bd}	7.85^{d}	7.88^{bd}
	(±0.18)	(±0.19)	(±0.20)	(±0.17)

1. Means with different letters in the same column and row are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

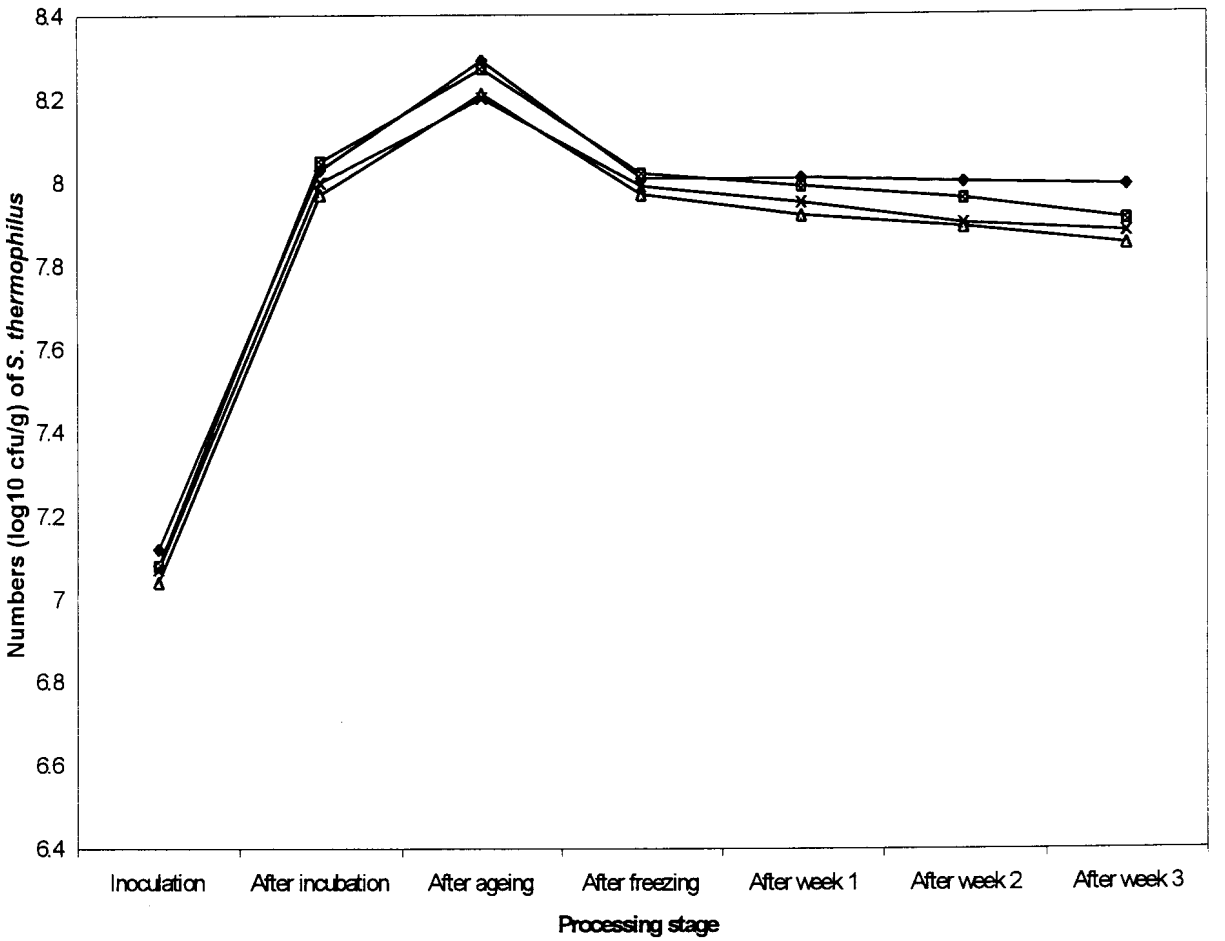


Figure 8: The numbers of *S. salivarius* subsp. *thermophilus* at various stages of the manufacture of frozen yoghurt containing milk fat or fat substitute in the form of a whey protein concentrate (WPC) (10%Fat 5%Fat 3.4%WPC 5%WPC)

The same trend was observed in the numbers of *L. acidophilus*. There was a significant increase in the counts of *L. acidophilus* during incubation in all the yoghurt mixes (Table 6 and Fig. 9.). However, in this case the increase in numbers during ageing was not significant. During freezing there was a significant decrease in numbers of *L. acidophilus*, regardless of whether fat or the fat substitute was present in the mix.

Table 6: Numbers (log_{10} cfu/g) of *L. acidophilus* during the manufacture and storage of frozen yoghurt containing either milk fat or fat substitute in the form of a whey protein concentrate (WPC)

Stages	10% milk fat[1]	5% milk fat[1]	3.4% WPC[1]	5% WPC[1]
Inoculation	6.88^{a}	7.01^{a}	7.01^{a}	6.96^{a}
	(±0.25)[2]	(±0.22)	(±0.21)	(±0.15)
After incubation	7.50^{bc}	7.56^{bc}	7.48^{bc}	7.46^{bc}
	(±0.19)	(±0.19)	(±0.13)	(±0.12)
After ageing	7.65^{b}	7.71^{b}	7.59^{b}	7.58^{b}
	(±0.20)	(±0.22)	(±0.13)	(±0.10)
After freezing	7.34^{cd}	7.39^{cd}	7.33^{cd}	7.33^{cd}
	(±0.22)	(±0.24)	(±0.16)	(±0.19)
After week 1	7.27^{d}	7.28^{d}	7.27^{d}	7.25^{d}
	(±0.18)	(±0.21)	(±0.14)	(±0.18)
After week 2	7.19^{ad}	7.19^{ad}	7.15^{ad}	7.16^{ad}
	(±0.20)	(±0.22)	(±0.18)	(±0.11)
After week 3	7.17^{ad}	7.18^{ad}	7.15^{ad}	7.15^{ad}
	(±0.18)	(±0.16)	(±0.22)	(±0.18)

1. Means with different letters in the same column and row are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

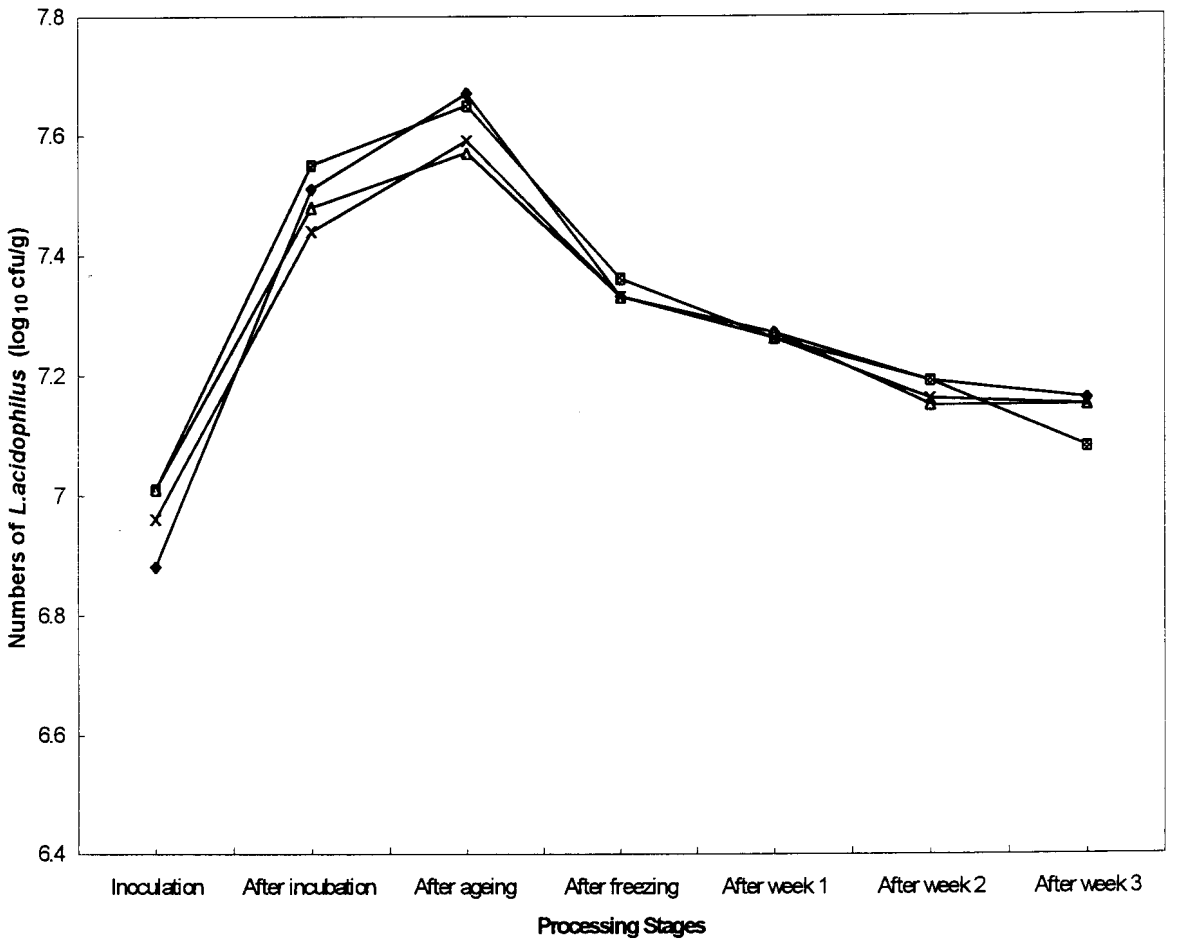


Figure 9: The numbers of *L. acidophilus* at various stages of the manufacture of frozen yoghurt containing either milk fat or fat substitute in the form of a whey protein concentrate (WPC) (10%Fat 5%Fat 3.4%WPC 5%WPC)

There was no growth of *B. bifidum* in any of the frozen yoghurt mixes (Table 7 and Fig. 10), but rather a decrease in their numbers from the onset of incubation until the final storage time of three weeks.

Table 7: Numbers (log cfu/g) of *B. bifidum* during the manufacture and storage of frozen yoghurt containing either milk fat or fat substitute in the form of a whey protein concentrate (WPC)

Stages	10% milk fat	5%milk fat	3.4% WPC	5% WPC
Inoculation	[1] 6.76[a]	6.74[a]	6.75[a]	6.73[a]
	(±0.21)[2]	(±0.20)	(±0.25)	(±0.21)
After incubation	6.34[b]	6.37[b]	6.45[b]	6.41[b]
	(±0.14)	(±0.21)	(±0.17)	(±0.15)
After ageing	6.21[b]	6.21[b]	6.27[b]	6.29[b]
	(±0.17)	(±0.21)	(±0.18)	(±0.18)
After freezing	6.02[c]	6.01[c]	6.09[c]	6.10[c]
	(±0.15)	(±0.12)	(±0.15)	(±0.22)
After week 1	5.98[cd]	5.97[cd]	6.04[cd]	6.02[cd]
	(±0.20)	(±0.19)	(±0.19)	(±0.18)
After week 2	5.81[cd]	5.85[cd]	5.93[cd]	5.94[cd]
	(±0.24)	(±0.26)	(±0.14)	(±0.17)
After week 3	5.78[cd]	5.82[cd]	5.90[cd]	5.93[cd]
	(±0.19)	(±0.24)	(±0.14)	(±0.21)

1. Means with different letters in the same column are significantly different ($p<0.05$) from each other
2. Numbers in parentheses are standard deviations

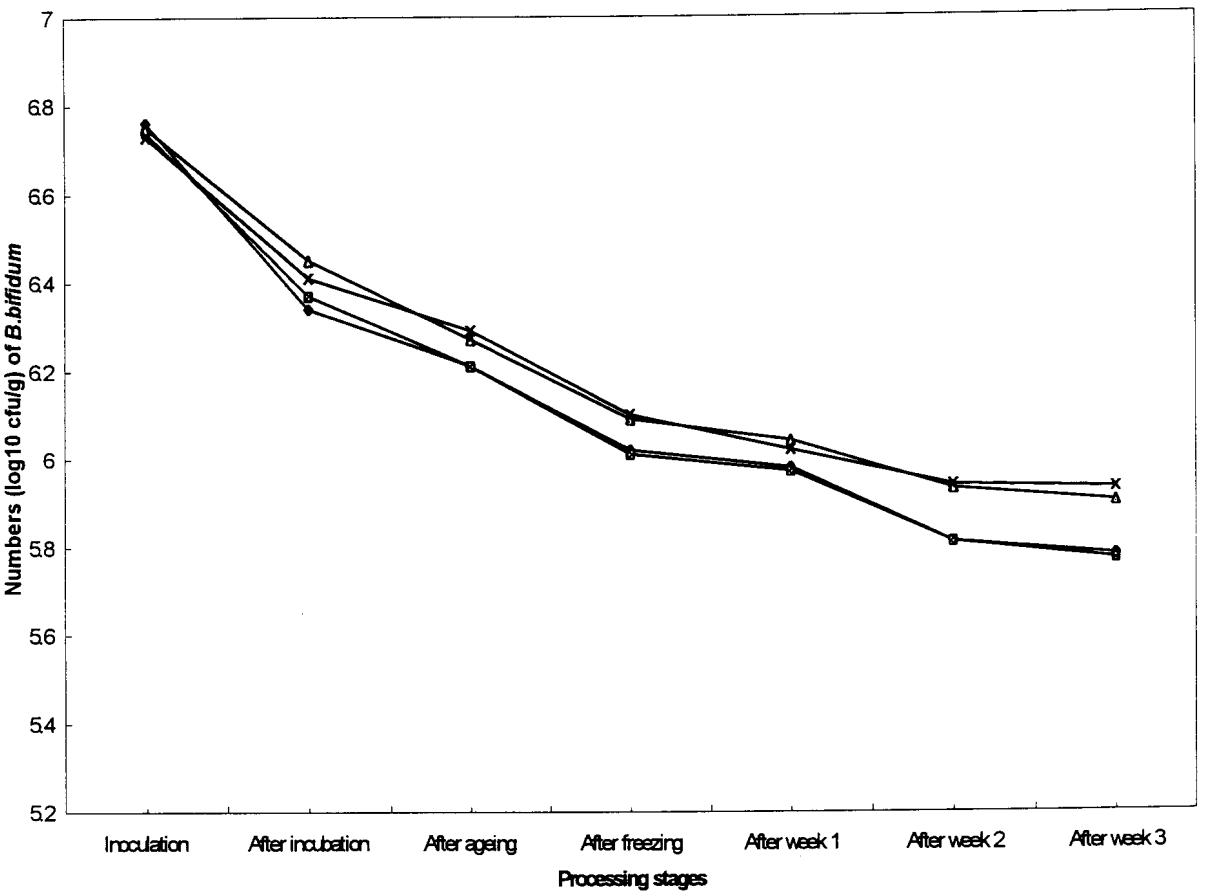


Figure 10: The numbers of *B. bifidum* at various stages of the manufacture of frozen yoghurt containing milk fat of fat substitute in the form of a microparticulated whey protein concentrate (WPC) (10%Fat 5%Fat 3.4%WPC 5%WPC)

5.6 Physical analyses of the yoghurt mixes and frozen yoghurts

5.6.1 Apparent viscosity

Table 8 shows the apparent viscosity of the various yoghurt mixes after 15 h of ageing. The yoghurt mixes containing fat had a very high apparent viscosity and there was a strong correlation between the fat content and the viscosity ($r = 0.988$, $p < 0.05$) (Fig. 11). The apparent viscosity was also positively, although weakly, correlated to the pH achieved after ageing ($r = 0.71$, $p < 0.05$). The apparent viscosity of the two samples containing the fat substitute did not differ significantly ($p > 0.05$) from one another.

5.6.2 Overrun

There was a significant ($p < 0.05$) decrease in the overrun as the fat content of the frozen yoghurt increased (Table 8). The overrun thus showed the opposite trend to the viscosity of the various frozen yoghurt samples. The 'high-protein' frozen yoghurts (containing the fat substitute) did not differ significantly ($p > 0.05$) from each other in terms of their overrun.

Table 8: Apparent viscosity and overrun of yoghurt mixes and frozen yoghurts containing either milk fat or fat substitute in the form of a whey protein concentrate (WPC)

Frozen yoghurt mixes	Apparent viscosity (cP) [1]	Overrun (%) [1]
10% milkfat	1831[a]	64[a]
	(±156)[2]	(±5.10)
5% milk fat	1076[b]	76[b]
	(±120)	(±7.43)
3.4% WPC	405[c]	131[c]
	(±20)	(±16.32)
5% WPC	416[c]	129[c]
	(±12)	(±12.77)

1. Means with different letters in the same column are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

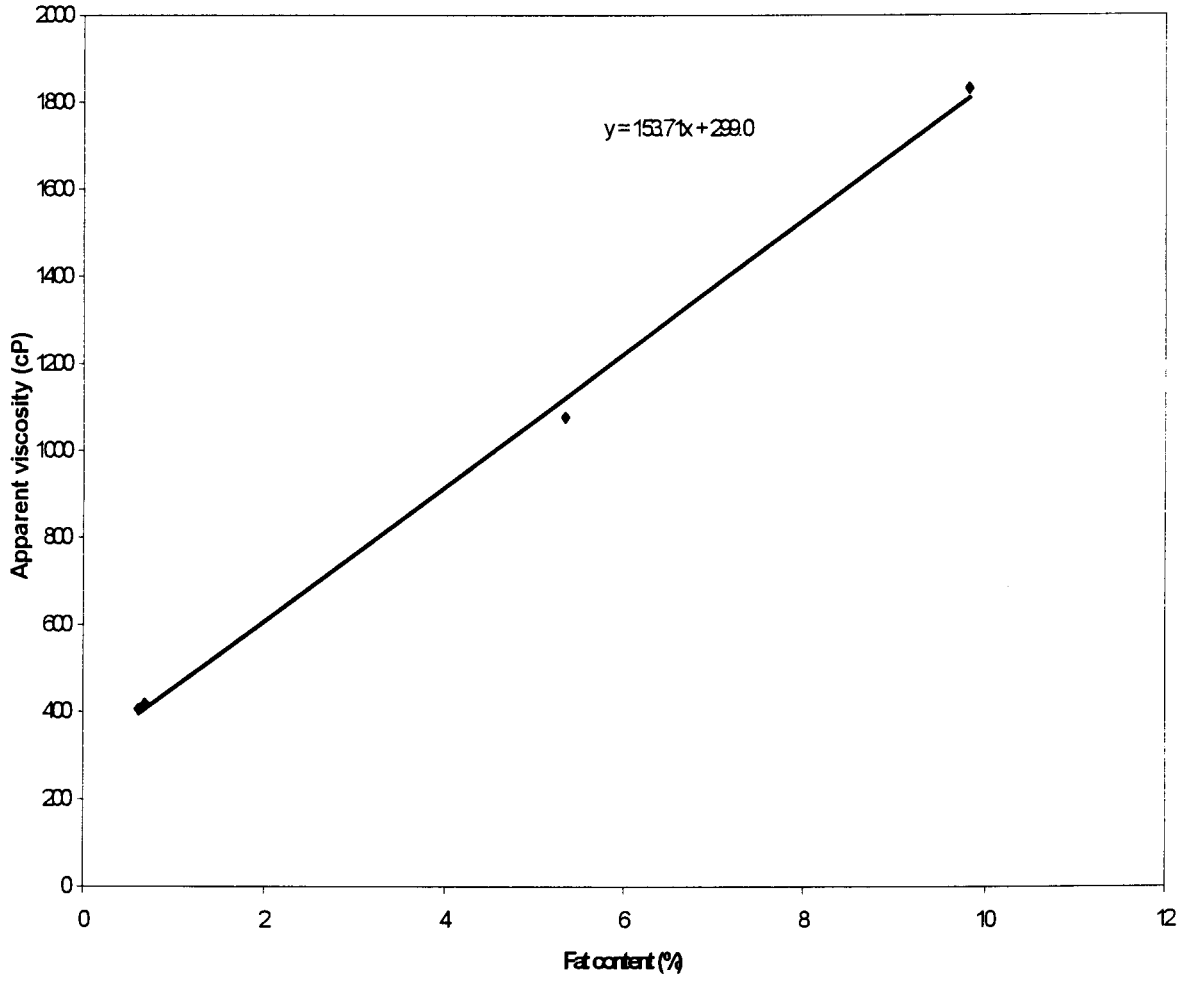


Figure 11: Correlation between apparent viscosity (cP) and fat content of frozen yoghurt samples

5.7 Sensory evaluation

Three terms that best described the differences in flavour and three terms that best described the differences in texture between the four frozen yoghurts (Appendix B for a copy of the sheet used during the sensory evaluation) were generated during the initial analysis of the frozen yoghurts during training. The generated terms as well as their definitions were agreed on by consensus between the panelists and used for the sensory evaluation. There was an obvious difference between the panelists' evaluation of the samples as shown by the high standard deviations for all the parameters involved.

5.7.1 Flavour

Decreasing the fat content from 10% milk fat to 5% fat, led to a statistically insignificant ($p > 0.05$) increase in the intensity of the strawberry flavour. On the other hand, replacing the fat entirely resulted in a significant increase ($p < 0.05$) in the perception of the intensity of strawberry flavour (Table 9). The two frozen yoghurts containing the fat substitute did not differ significantly from each other in the intensity of strawberry flavour. A decrease in the fat content due to fat substitution, resulted in an obvious and significant increase in the perception of an aftertaste. The latter characteristic was often referred to as being bitter and astringent. Decreasing the fat content of the frozen yoghurts (from 10% milk fat to 5% milk fat) led to a significant decrease in sweetness (Table 9). On the other hand, the addition of the fat substitute caused an increase in the perception of sweetness compared to the frozen yoghurt containing 5% fat. The two frozen yoghurts containing the fat substitute did not differ significantly from the frozen yoghurt containing 10% milk fat, while only the frozen yoghurt containing 5% of the microparticulated WPC differed significantly from the frozen yoghurt containing 5% milk fat. The latter was perceived as being more sweet than the frozen yoghurt containing 5% fat.

Table 9: Flavour characteristics of frozen yoghurts containing either milk fat or fat substitute in the form of a microparticulated whey protein concentrate (WPC)

Frozen yoghurt	Sweetness[1]	Strawberry intensity[1]	Aftertaste[1]
10% milk fat	6.50[ac]	4.66[a]	2.30[a]
	(±1.75)[2]	(±2.14)	(±1.75)
5% milk fat	5.90[b]	4.79[a]	2.31[a]
	(±1.78)	(±2.36)	(±1.95)
3.4% WPC	6.23[ab]	8.87[b]	4.97[b]
	(±2.14)	(±2.26)	(±2.16)
5% WPC	7.03[c]	7.63[b]	4.58[b]
	(±1.92)	(±2.15)	(±2.06)

1. Means with different letters in the same column are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

5.7.2 Texture

Decreasing the fat content of the frozen yoghurt from 10% to 5% milk fat, led to a significant increase in coarseness of the product as perceived by sensory evaluation (Table 10). The frozen yoghurt mixes containing the fat substitute also differed significantly in terms of coarseness from the frozen yoghurt containing milk fat. The two frozen yoghurts containing the fat substitute did not differ significantly from each other in terms of coarseness.

The two frozen yoghurts containing the fat substitute and with high overruns had a higher foamy meltdown than the frozen yoghurts containing milk fat. The two frozen yoghurts with the fat substitute did not differ from one another in terms of foamy meltdown.

Increasing fat content led to a significant increase in thickness of the frozen yoghurt perceived in the mouth. Fat subtitution led to a significant decrease in thickness of the frozen yoghurts.

Table 10: Texture of frozen yoghurt containing milk fat or fat substitute in the form of a microparticulated whey protein concentrate (WPC)

Frozen yoghurt	Coarseness[1]	Thickness[1]	Foamy meltdown[1]
10% milk fat	1.03^{a}	7.59^{a}	1.39^{a}
	(±0.82)[2]	(±1.96)	(±1.11)
5% milk fat	3.12^{b}	6.74^{b}	2.12^{a}
	(±1.19)	(±2.23)	(±1.17)
3.4% WPC	5.14^{c}	3.43^{c}	5.46^{b}
	(±1.47)	(±2.17)	(±2.29)
5% WPC	4.40^{c}	4.08^{c}	5.75^{b}
	(±1.24)	(±2.37)	(±2.17)

1. Means with different letters in the same column are significantly different ($p < 0.05$) from each other
2. Numbers in parentheses are standard deviations

5.8 Microstructure of frozen yoghurt

More or less the same basic microstructure to that of ice cream was observed in the frozen yoghurts containing fat (Fig. 12). The fat globules coated the air bubbles to some extent (Fig. 13) and a serum phase separated the air bubbles from the ice crystals. The microstructure of the frozen yoghurts, containing milk fat also revealed a certain degree of fat clustering throughout the serum phase and to a lesser extent on the air bubbles. The samples containing the microparticulated WPC shared more or less the same microstructure (Fig. 14), with the exception that the air bubble was not coated by fat globules, neither were they coated by the microparticulated WPC (Fig. 14). The microparticles could not be properly observed within the serum phase. However, all the samples (fat and fat substituted) were very unstable during their examination and yielded spoilt images (Fig. 15) which made more qualitative and quantitative analyses very difficult, if not impossible. An attempt to examine the microstructure after 6 weeks of manufacture also failed.

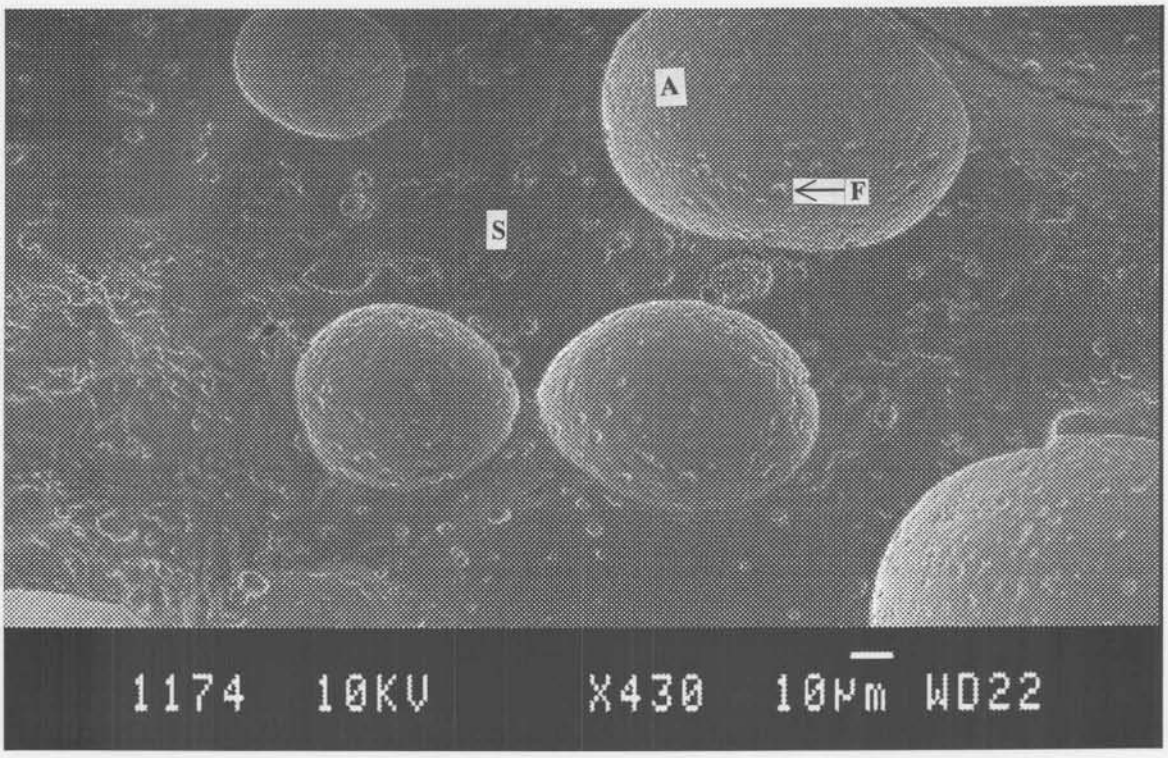


Figure 12: The microsture of a frozen yoghurt containing 10% fat (A=air bubble, F= fat globule, S=serum phase)

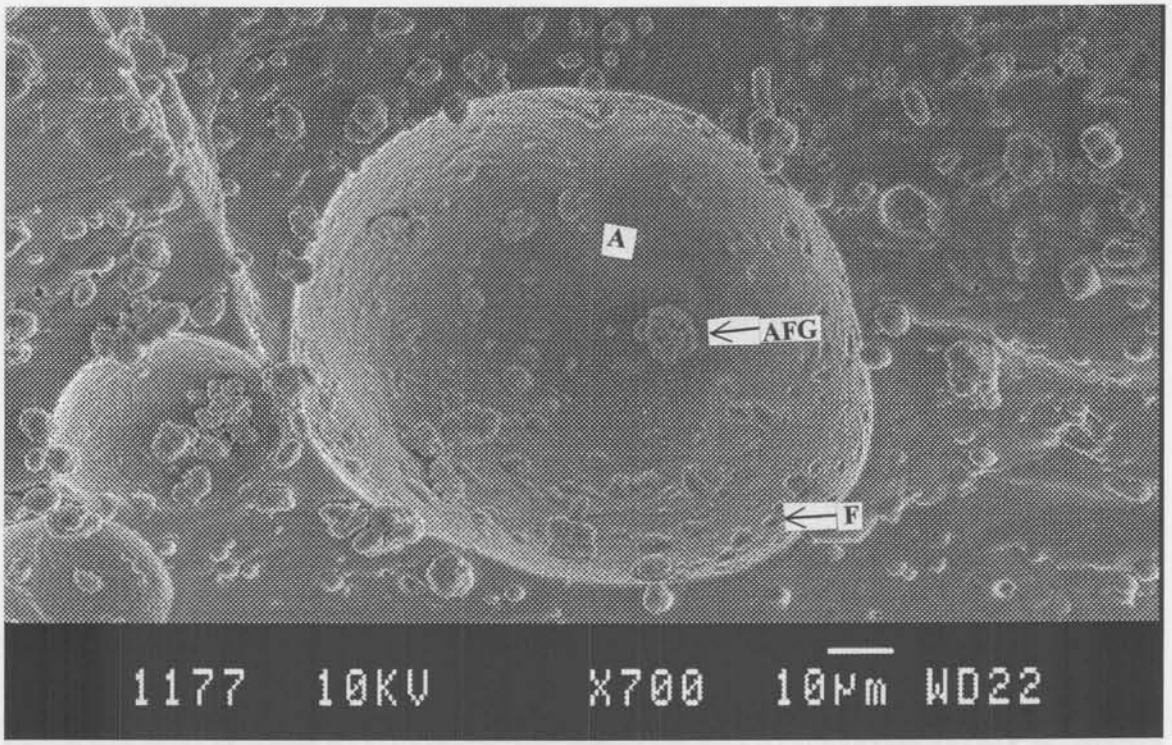


Figure 13: An air bubble coated by fat globules in a frozen yoghurt containing 10% milk fat (A= air bubble, AFG= Agglomerated fat globules, F= fat globule)

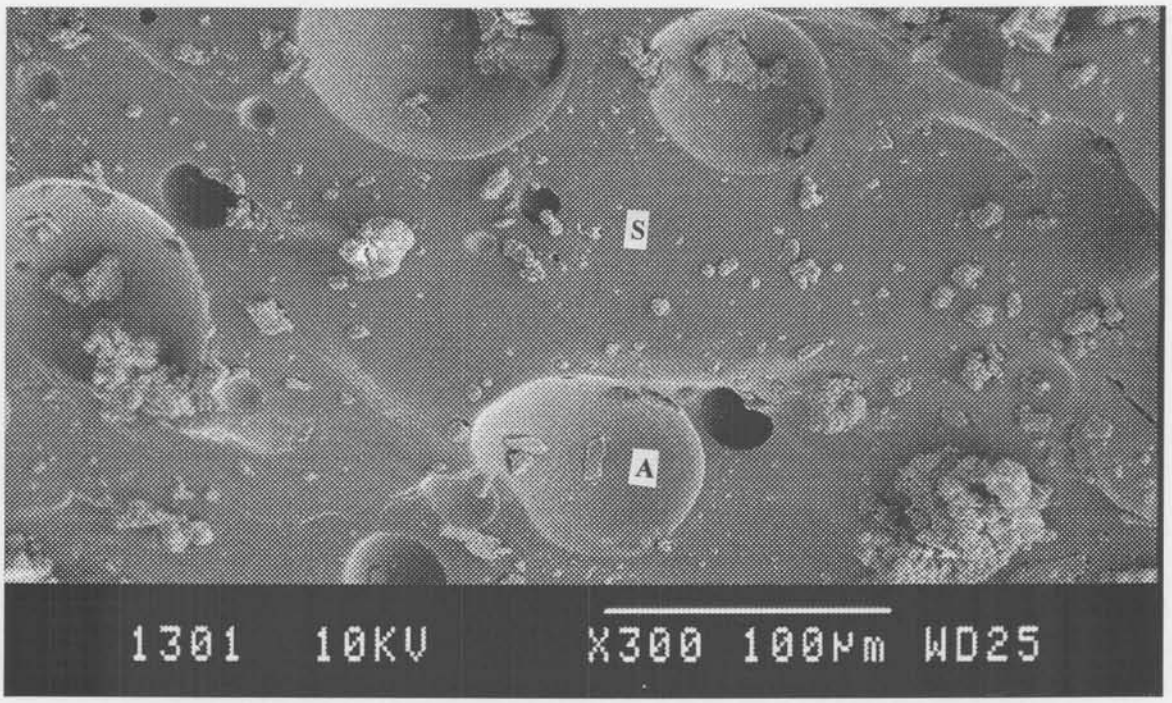


Figure 14: The general microstructure of a frozen yoghurt containing the microparticulated whey protein concentrate (A= air bubble; S=serum phase)

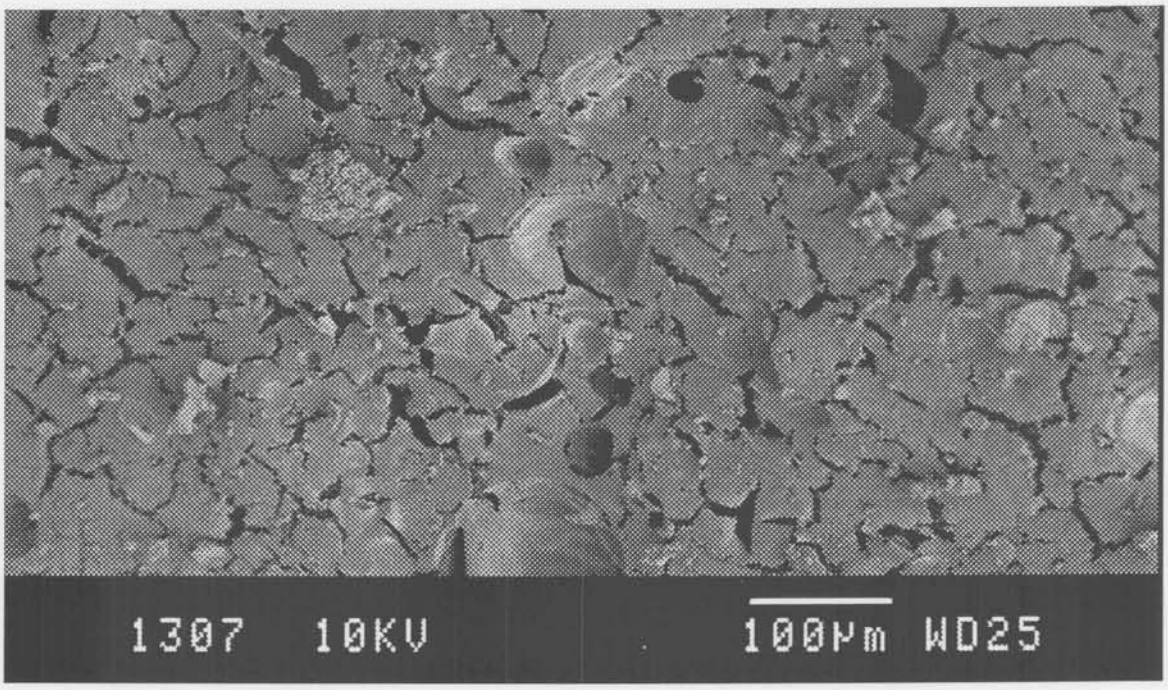


Figure 15: The various samples (here a frozen yoghurt sample containing 3.4% microparticulated whey protein concentrate) were very unstable after a few minutes of analysis

CHAPTER 6

DISCUSSION

Substituting fat by the microparticulated whey protein concentrate, Simplesse® 500, decreased the pH and increased the titratable acidity (TA) of the yoghurt mix. These results support those of Lee & White (1991) who also reported a decrease in the pH of ice-cream mixes when a WPC substituted milk solids-not-fat. The pH of the microparticulated whey protein concentrate is often given in the range of pH 6.0-6.5 (Cheftel & Dumay, 1993), which thus could have influenced the acidity of the frozen yoghurt mixes. Various components of the milk solids-not-fat are capable of splitting protons (Rogers, 1935; Walstra & Jenness, 1984; Rosenthal, 1991) and thus make them available to the pH electrode during pH measurements. Only a few independent studies on fat substitution by Simplesse® are available in the literature. In one such study, Schmidt, Lundy, Reynolds & Yee (1993) also observed a decrease in the pH of ice-milk mixes following substitution of milk fat by Simplesse® 100. The decrease in pH in the present study was less than that observed by Lee & White (1991) and Schmidt *et al.* (1993). Two factors could have explained this phenomenon. Firstly, pH measurements depend on availability of protons to the pH electrode. Secondly wide variations are expected in the composition of WPC depending on the source of the whey proteins and the processing conditions (Kailasapathy *et al.*, 1996).

Although the yoghurt mix containing 5% fat had a higher protein content and milk solids-not-fat content, its acidity did not differ significantly from that containing 10% fat. This could be attributed to the fact that the acidity of the mixes are in the plasma portion of the mix (Atherton & Newlander, 1977). The acidity of the frozen yoghurt mix, and hence the acid-base equilibria, depends on the dissociation constants of weak acids and salts in the aqueous phase. The % TA usually represents the amount of alkali necessary to shift the proteins and salt buffer systems from their initial state of labile equilibrium to a pH of 8.3 (Rogers, 1935). The yoghurt mix containing 10% fat had a significantly lower water content compared to the mix containing 5% fat. The higher water content of the yoghurt

mix contaning 5% fat therefore tended to 'dilute' the effect of the weak acids, such as proteins and phosphoric acid. The proportion of MSNF to water in the mix containing 5% fat was only slightly higher (0.167 kg/l) compared to the mixes containing 10% fat (0.166 kg/l). The water content of the mix containing 5% fat therefore diluted and counteracted the effect of the increased MSNF. The addition of more MSNF in the case of the fat-substituted yoghurt mixes resulted in a significantly higher acidity compared to the mixes containing fat. These mixes did not differ much in terms of their water content compared to those containing 5% fat. Hence, the increased MSNF content resulted in an increase in the acidity of the mixes through the contribution of the weak acids. Arbuckle (1977) and Marshall & Arbuckle (1996) also stated that an increase in the acidity of ice-cream mixes would result from an increase in the MSNF content.

According to Simpkins (1993), cellular processes must take place in a medium of which the pH is carefully regulated since they are sensitive to pH changes. Milk acts as a buffer since it is to a large extent a mixture of partly neutralised weak acids (Rosenthal, 1991) and it contains both acidic and basic groups (Walstra & Jenness, 1984). The buffer components of milk include the phosphate, carbonate and citrate systems, which occur in both the acid form as well as their derivative salts, casein and whey proteins (Rogers, 1935; Davies, 1939; Jenness, Shipe & Sherbon, 1974; Rosenthal, 1991). The addition of more solids-not-fat, in the form of a microparticulated WPC, resulted in a significant increase in the buffer capacity, in both acidic and alkaline conditions, as was also reported by Davies (1939). This would most probably be through the contribution of the various components of the buffer system of milk. Moreover, the acid-base equilibria of dairy products is affected by a number of factors, including the source and concentration of milk solids-not-fat, the type of proteins, any treatments affecting the configuration of proteins, the concentration of phosphate, citrate and carbonate salts as well as the analyses used to determine buffer capacity (Rogers, 1935; Davies, 1939; Jenness *et al.*, 1974; Rosenthal, 1991). For example, heat treatment leads to the precipitation of the colloidal phosphate (the solid phase), with the release of protons (Rosenthal, 1991). The two yoghurt mixes containing the microparticulated WPC did not, however, differ significantly in their buffer capacity. The relatively small increment of 1.6% in the WPC

(which also contain some impurities e.g. sugars) only resulted in an increase of 0.56% in the protein content and a small increment in the other buffer components. Moreover, the base or acid binding capacity of proteins depends on the type of proteins. Whey proteins have weaker buffer capacity than caseins (Kailasapathy & Supriadi, 1996) and show some degree of anomalous dissociation (Swaisgood, 1982). For example, β-lactoglobulin C variant, in addition to $-NH_2$ and –COOH groups, also contains histidyl residues, possibly two per dimer, which become removed from the titration as the pH is lowered from 6.0 to 4.5 (Swaisgood, 1982). This behaviour is suggested to arise from a conformational change which buries the group as an ion-pair presumably with a carboxylate ion that remains unprotonated at the acid end point (Swaisgood, 1982).

Although the titratable acidity and buffer capacity were only weakly correlated to the protein contents, the mixes containing fat needed addition of a higher amount of acid or alkali per protein content than the fat-substituted mixes. Caseins are less compact than whey proteins and as such their ionisable groups are more readily available for reactions. Whey proteins on the other hand are often characterised by an anomalous dissociation constant (Swaisgood, 1982). These proteins have some 'buried' or 'abnormal' ionisable groups, which would only become available through treatments that expose them. Thus, comparison with compositional data indicated that two β, γ-carboxyls per dimer do not normally ionise with the normal pKa value but rather these two protons dissociate following a conformational change of the protein (Swaisgood, 1982). Other components of the buffer systems of the yoghurt mixes therefore also determined the amount of acid and alkali required to shift the pH to the respective end-points.

The pH of the various mixes continued to decrease during the ageing period. Despite the use of an ABT culture, *S. salivarius* subsp. *thermophilus* remain active even at refrigeration temperatures and produce small amounts of lactic acid (Shah *et al.*, 1995). A decrease in pH of 0.07-0.42 units was observed during a 5 weeks storage period. Kneifel *et al.* (1993) observed a 22.3% increase in the titratable acidity of yoghurt and 14.9% in yoghurt-related products during storage of various yoghurt and yoghurt-related products. Dave & Shah (1997a) argued that the decrease in pH of 0.17 units from the set point of

4.5 was obviously due to continued fermentation during overnight cooling until the temperature of the product reached 4°C. According to Rasic & Kurman (1978), the pH of yoghurt decreases during cooling to 5°C and below, the extent of which depends on the cooling regime. This is a reflection of continued bacterial activity. The pH of the frozen yoghurt mixes showed a very slight and non-significant decrease after freezing and during storage. The same phenomenon was observed by Modler *et al.* (1990), Laroia & Martin (1991) and Hekmat & McMahon (1993). Although freezing of milk leads to a decrease in pH the reaction is reversible (Fox & McSweeney, 1998).

The larger decrease in pH of the yoghurt mixes containing milk fat compared to the fat-substituted mixes, attained at the ageing step, can potentially be explained in terms of the peak of buffer capacity and the pK_a values. In general, weak acids are most effective in buffering against pH changes in the vicinity of their pKa value (Styrer, 1988). Jenness, Sharpe & Sherbon (1974) argued that, while it is relatively easy to determine the buffer components, a quantitative assignment of the buffer capacity of each component is much more difficult. One can thus expect a difference in the buffering capacity of several different dairy products such as milk, yoghurt and ice cream. The latter two products often contains more milk solids-not-fat because of the addition of milk powders, whey protein concentrate and other protein concentrates and isolates. According to Rogers (1935), caseins have a maximum buffering capacity at a pH of 5.2, while whey proteins buffer more at pH 6.0. Mann & Malik (1996) determined the maximum buffering capacity of a whey protein-carboxymethyl cellulose (WP-CMC) complex and of an ultrafiltered whey protein concentrate (UF-WPC). They concluded that the WP-CMC complex had a high buffering capacity below pH 3.0 and above pH 6.0, while UF-WPC also had a peak around pH 6.0. There is a general agreement in the literature on the pH region of maximum buffer capacity of the carbonates, phosphates and citrates. The citrates have three pK_a values at pH 3.08, 4.74 and 5.4, the phosphates have three peaks at pH 1.96, 6.83 and 12.32, while the carbonates have two pK_a values at pH 6.37 and 10.25 (Walstra & Jenness, 1984; Fox & McSweeney, 1998). The addition of the microparticulated WPC with the concomitant increase in whey proteins and milk salts (especially the carbonates), therefore resulted in a higher buffering action at a pH below

and above 6.0 - enough to affect the decrease in pH. This would certainly have been the result of the buffering action of whey proteins as well as the bicarbonates. According to Davies (1939), the $-NH_2$ group is most effective as a base in acidic conditions. Ventling & Mistry (1993) argued that, because of increased buffering capacity of ultrafiltered milks, pH 5.5 or higher was maintained for a longer period of time during fermentation.

Moreover, the processing of the microparticulated whey protein concentrate involves a number of pH adjustment steps and heat treatments, which could have contributed to more inonisable groups. For example, the first generation of Simplesse® required the protein source to be adjusted, by the addition of citric acid, to a pH of 1.0 units below the isoelectric point of the composite curve. This pH adjustment step would ensure that the proteins do not form large aggregates that would be detected as being gritty instead of creamy (Singer *et al.*, 1989, 1990; Cheftel & Dumay, 1993). For example, β-lactoglobulin exhibits a high degree of tetramerisation at their isoelectric region (Swaisgood, 1982; Fox & McSweeney, 1998). This acid treatment is often followed by processing at a pH of 6.0 (i.e. neutralisation of the acid solution) (Singer *et al.*, 1990; Cheftel & Dumay, 1993). Moreover, according to Fang & Snook (1991) and Cheftel & Dumay (1993) the present technology for the production of Simplesse might involve a pH adjustment above the isoelectric point. This would involve the addition of certain bases, since according to Marshall (1982), even sweet whey has a maximum pH of 5.6. The additional bases could thus have bound more protons and hinder a decrease in pH during ageing of the fat-substituted mixes.

Other factors that could have explained the lesser decrease in pH could have been a lower activity of the bacteria. Dave & Shah (1998) observed that increased levels of cysteine and whey proteins (WP) adversely affected *S. salivarius* subsp. *thermophilus* due to a very low redox potential. *Streptococcus salivarius* subsp. *thermophilus* is micro-aerophilic to aerobic, *L. acidophilus* is anaerobic to micro-aerophilic and bifidobacteria are strictly anaerobic (Dave & Shah, 1998). The mixes used by Dave & Shah (1998) were fortified with skim-milk powder, whey proteins or WPC and they were also subdivided into several lots and cystein added at different levels. The mixes containing the WPC in

their study did not affect *S. salivarius* subsp. *thermophilus*. However, the levels of WPC used in the present study were higher than that used by Dave & Shah (1998). Whey proteins are known to release –SH group after heat treatment and thus reduce the redox potential (Marshall, 1982; Fox & McSweeney, 1998). This could have caused a decrease in the redox potential in the present study that would have adversely affected *S. salivarius* subsp. *thermophilus*. *Lactobacillus acidophilus* would not have been much affected, while *B. bifidum* could have benefited from the low redox potential. However, the latter was not the case. This would have led to a longer fermentation process for the fat-substituted frozen yoghurt mixes and less acidification during the ageing process. This is however less likely to be the case since *S. salivarius* subsp. *thermophilus* did increase in numbers during the ageing period, though to a lesser extent. The sulphydryl group of β-lactoglobulin in its native form is usually buried and only become available for reaction following denaturation processes such as heat treatment (Fox & McSweeney, 1998). So far, the stability of microparticulated whey proteins has not been systematically investigated and proven (Cheftel & Dumay, 1993). On the other hand, the whey protein concentrate source is subjected to a heat treatment during its preparation, which could thus have exposed the –SH group before its incorporation into the food system. However, fermentation often results in a decrease in the dissoloved oxygen of the yoghurt base (Driessen, 1992; Shah *et al.*, 1995; Dave & Shah, 1997a). The redox potential could thus have increased with fermentation and became slightly more favourable to the *S. salivarius* subsp. *thermophilus* during ageing and less favourable to *B. bifidum* during incubation.

The specific heat capacity of fat, acting as an insulator, could have resulted in the mixes containing fat taking more time to reach cooling temperature. However, the two mixes containing fat did not differ significantly from each other in terms of their numbers of *S. salivarius* subsp. *thermophilus* after ageing. Moreover, the numbers of *S. salivarius* subsp. *thermophilus* after ageing in the fat-substituted mixes did not differ significantly from those containing fat, making the effect of specific heat capacity of fat less likely. This can be explained on the ground that even though cooling might have been slower in the case of the yoghurt mixes containing fat, the bacteria would not have been exposed to

a higher temperature for a considerable amount of time. Therefore, bacterial activity would not differ between the various yoghurt mixes

Both the numbers of *S. salivarius* subsp. *thermophilus* and *L. acidophilus* increased during incubation and ageing. *Streptococcus salivarius* subsp. *thermophilus* appears to be the organism that grew the best and especially so in the yoghurt mixes containing fat. *Streptococcus salivarius* subsp. *thermophilus* is the main fermenting organisms in an ABT culture (Dave & Shah, 1998), which is confirmed by the better growth of the species in the present study. The continued bacterial activity during ageing resulted in a decrease in the pH of the various yoghurt mixes.

Although the incorporation of microparticulated WPC increased the protein content of the fat-substituted yoghurt mixes, *B. bifidum* did not grow in them nor in the mixes containing fat. Two other factors must be mentioned here. Firstly, caseins are less compact than whey proteins and hence more susceptible to proteolysis (Swaisgood, 1982). In fact the high proline content of caseins results in a very low content of α-helix or β-sheet structures in the casein. The caseins are, therefore, readily susceptible to proteolysis without prior denaturation by, for example, acid or heat (Fox & McSweeney, 1998). On the other hand, whey proteins have well-developed secondary, tertiary and quaternary structures (Jelen & Rattray, 1995). Although the microparticulated WPC could have provided more free amino acids and small peptides, the amino acid requirements of bifidobacteria seems to be highly species as well as strain dependent (Dave & Shah, 1997a). Dave & Shah (1997 a, b, c) observed a decrease in the numbers of *B. bifidum* of about 3-log cycles during fermentation of a yoghurt base using a certain ABT culture. Bacteria have a certain generation time, by the end of which they become more susceptible to end-product inhibition and other limiting factors. Desjardin *et al.* (1990) observed a marked difference in growth of various strains of *B. bifidum.* Ageing of a low-acid frozen yoghurt mix also resulted in a decrease in the numbers of *Bifidobacterium longum* (Modler & Villa-Garcia, 1993). Dave & Shah (1997a,b,c) also observed no growth of *B. bifidum* in a yoghurt fermented by a certain ABT culture. The same problems could thus have been the reasons for the lack of growth of this species

during the present study. Champagne, S^{t} Gelais & Audet (1996) concluded that WPC could be used for the production of starter cultures of lactobacilli, but supplementation with milk protein hydrolysate proved essential. The milk hydrolysates, due to a certain degree of hydrolysis, provided the bifidobacteria with smaller peptides and more amino acids that would have improved the growth of bifidobacteria (Gomes *et al.*, 1993). Poch & Bezkorovainy (1988) observed that the addition of bovine casein digest and yeast extract yielded best performance of bifidobacteria. The low viability of bifidobacteria in this study can thus be explained in terms of a lack of appropriate proteolytic activity. Gomes *et al.* (1993) and Klaver *et al.* (1993) stated that, because of the lack of appropriate proteolytic activity, bifidobacteria cannot grow to high density in milk unless co-cultured or being provided with additional growth factors.

The first step in the degradation of milk proteins by lactobacilli is mediated by cell-wall located proteinases (exopeptidases) that cleave casein into oligopeptides. Further degradation of the oligopeptides into peptides is then mediated by a large variety of peptidases comprising a set of at least 11-12 different types of aminopeptidases and endopeptidases. The final step involves the amino acid and peptide transport system in the cell membrane which delivers the products of extracellular hydrolysis to the intracellular systems involved in bacterial protein synthesis (Pritchard & Coolbear, 1993; Sasaki *et al.*, 1995). A point of interest raised by Pritchard & Coolbear (1993) is that, while the products of proteinase action are mostly peptides of seven or more amino acid residues, the upper limit for peptide transport systems is currently accepted to be around 6-7 residues. Extracellular-oriented peptidases might act as a bridge to this effect, although this has not been systematically proved (Pritchard & Coolbear, 1993). The strain of bifidobacteria used in the present study might have lacked a component of the proteolytic activity or the hypothetical exocellular peptidase bridge. Martin & Chou (1992) observed that the viability of *Bifidobacterium* spp was species and strain dependent and that the viability greatly varied among them.

All the bacteria decreased in numbers at the freezing step. Modler *et al.* (1990), by hardening frozen yoghurt and subsequent storage at –17°C, observed a decrease of less

than one log unit in the counts of *B. longum*, *B. brevis* and *B. infantis*. Mashayek & Brown (1992) reported a decrease of 1 log cycle in the counts of yogurt bacteria while data by Laroia & Martin (1991) suggested a decrease of slightly less than one log cycle, as far as *B. bifidum* and *L. acidophilus* are concerned at a 100% overrun. Moreover, Modler & Villa-Garcia (1993) observed that encapsulating *Bifidobacterium longum* in milk fat prior to incorporation in the yoghurt did not improve their survival. Bielecka *et al.* (1988) suggested that protective agents such as carbohydrates, proteins, emulsifiers and stabilisers might help in protecting the bacteria against freezing injury. Glycerol and mannitol (Sheu *et al.*, 1993) are also expected to act as good cryoprotectants. The cryoprotectants either have high water binding capacity or prevent the formation of larger ice crystals and hence help protect against freezing injury of the bacteria.

A number of factors affect ice cream quality such as the composition of the mix and production method. It depends to a large extent of the size and distribution of de-emulsified fat globules, ice crystals, air cells and an unfrozen phase (Kokobu *et al.*, 1996). Fat is very important since it contributes to creaminess, appearance, palatability, texture and lubricity in foods (Akoh, 1998).

Increasing fat content led to a higher apparent viscosity of the aged mixes containing fat, measured at 4°C. A number of factors can potentially explain the higher apparent viscosity of the aged mixes containing milk fat relative to those containing the fat substitute. First of all, ageing leads to an increase in the amount of solidified fat (Abd El-Rahman *et al.*, 1997). Cooling to a sufficiently low temperature leads to nucleation of fat crystals. It is generally argued that in the case of milk fat triglygerides and surfactants act as impurities for the formation of fat crystals. During ageing fat crystallisation proceeds even further together with adsorption of proteins and emulsifiers to the fat globule (Marshall & Arbuckle, 1996; Goff, 1997; Dickinson, 1997). Starch and gelatin also hydrate and thereby increase the viscosity even further (Marshall & Arbuckle, 1996). A number of studies in ice-cream sustain the findings of the present study. For example, Li *et al.* (1997) also observed a marked decrease in apparent viscosity as the fat content of ice-cream mixes decreased, i.e. from 0.074 Pa.s for an aged ice-cream mix containing

10% milk fat to 0.02 Pa.s for an ice-cream mix containing 0.53 % milk fat. The decrease in apparent viscosity, measured at 4°C, of the aged mix containing 10% milk fat to that containing 0.53% milk fat was more than by a third. The viscosity of ice-cream mixes decreased when a whey protein concentrate replaced skim milk powder (Lee & White, 1991). Abd El-Rahman *et al.* (1997) observed that the use of cream resulted in a much higher viscosity after the ageing period compared to other sources of fat. The apparent viscosity in their study ranged from 754.7 cP to 1143.7 cP.

Moreover, the mixes containing fat achieved a much lower pH after ageing. The decrease in pH of the mixes containing milk fat could thus have caused a conformational change in the milk proteins, thereby exposing some of the water binding groups, with a concomitant increase in viscosity.

The formation of a well-defined network structure involving milk fat as fillers inside a matrix consisting of casein micelles is less likely to have happened in the present study due to a relatively high pH achieved after ageing. Recent studies by Xiong, Aguilera & Kinsella (1991) and Lucey, Monro & Singh (1998) showed that increasing the fat content increased the pH of gelation in yoghurt. However, the pH achieved after ageing in our study was much higher than the pH at which gelation occurred for yoghurt in the studies of Xiong *et al.* (1991) and Lucey *et al.* (1998). They noted that, in the absence of milk fat, gelation occurred at a pH of 5.0 while in the presence of fat, gelation took place at a pH of 5.2. Increase in fat content is very often to the detriment of water rather than other components. The proteins thus have less water to bind. However, the frozen yoghurts formulated with the fat substitute contained more proteins than the frozen yoghurt containing 5% milk fat but yet did not differ much in terms of water content. Increasing the fat content in the present study, could thus have increased the water binding capacity of the proteins by decreasing the pH at a much faster rate. On the other hand, the increased use of milk solids-not-fat hindered the decrease in pH after the ageing period and thus prevented the exposure of more water binding groups in the case of the fat-substituted mixes. On the other hand, a study by Tamime, Kalab, Muir & Barrantes (1995), where anhydrous milk fat was substituted by the microparticulated whey protein

concentrate, revealed that the microstructure of the yoghurt differed depending upon the presence of milk fat or the fat substitute. Casein micelle aggregation was less evident when the microparticulated whey protein concentrate substituted anhydrous milk fat. Moreover, the yoghurt containing the fat substitute was also less firm. Gastaldi, Lagaude, Marchesseau & Tarodo de la Fuente (1997) observed that the effect of pH decrease on casein micelle interactions occurred essentially between pH 6.0 and 5.3. Depending upon the total solids content, casein micelles started to fuse at a pH of 5.5 in the case of 20% total solids, and 5.8 in the case of 10% total solids. The increase in total solids was a result of an increase in milk solids-not-fat. The caseins thus started to lose their individuality and became aggregated into clusters of particles. Moreover, denatured whey proteins have a much higher iso-electric point than caseins and therefore aggregation of whey proteins is expected to occur earlier (Lucey *et al.*, 1998). They are known to tetramerise at pH near their iso-electric point.

It is therefore most likely that the increased viscosity would have been due to the formation of fused casein micelles, solidified fat, increased water binding capacity of the proteins, due to some conformational changes of the proteins as a result of reduced pH, early aggregation of whey proteins and gel matrix formation of gelatin. This would thus increase the shear stress required to measure the viscosity. It is unfortunate that no analysis was done to reveal the nature of the microstructure of the aged yoghurt mixes containing the milk fat.

The overrun showed an opposite trend to the apparent viscosity. Increasing fat content and viscosity lead to impaired incorporation of air. This is quite expected, since increasing fat content is known to decrease the amount of air incorporated in an ice-cream mix. Marshall & Arbuckle (1996) argued that a certain level of viscosity is essential for the incorporation of air, but that too much of a high viscosity is detrimental to the overrun. This is confirmed by the present study. A batch freezer was used in the present study and the draw temperature was maintained constant for all the four mixes. According to Mulvihill (1992), the presence of lipids affects the foaming abilities of milk proteins. This is expected since the ease of formation of milk foams is reduced as the fat

content increases (Anderson & Brooker, 1988). Surface-active lipids present in dairy products will adhere to the air-serum interface thereby leading to a decrease in foaming abilities (Anderson & Brooker, 1988). Cream does contain phospholipids and according to Dickinson *et al.* (1988), oil molecules preferentially solvate the hydrophobic side-chains of proteins. On the other hand, caseins adsorb readily at air-water interfaces due to their open structure, the relatively high content of apolar amino acids and the uneven distribution of amino acids (Fox & McSweeney, 1998). This will give caseins higher foaming properties, which will be fully available in the absence of fat.

Studies by Schmidt *et al.* (1993) also showed that fat substitution led to an increase in the amount of air incorporated. Moreover, data by Christiansen, Edelsten, Kristiansen & Nielsen (1996) suggested that decreasing pH also resulted in a decrease in the overrun of frozen yoghurts.

The sensory evaluation was characterised by large standard deviations, resulting from differences between panelists. This is not surprising since the interpretation of flavour, for example, depends on the taster and the context. For instance, bitterness can be detected by some of the people but not by others (Leland, 1997).

Increasing fat content led to a significant decrease in coarseness of the frozen yoghurts. The frozen yoghurt containing 10% milk fat was scored lowest in terms of coarseness followed by the frozen yoghurt containing 5% milk fat. The two frozen yoghurts containing the fat substitute were significantly coarser than the two other frozen yoghurts. For the purpose of the present study, coarseness was defined as being the result of detection of bigger ice crystals. The frozen yoghurts containing milk fat also contained less solutes in the aqueous phase compared to the two samples containing the fat substitute. Even though the fat substitute was in the form of a microparticulated whey protein concentrate, it also contained a number of other impurities such some polyhydroxy compounds. The frozen yoghurt containing the microparticulated WPC at a 5% level did not differ significantly from that containing 5% milk fat in terms of water content. The increased amount of solutes in the water phase therefore resulted in a lower

freezing point of the mix. Ohmes *et al.* (1998) also observed that ice-cream mixes containing the fat substitute had a lower freezing point. Likewise, Smith, Bakshi & Lomauro (1984) observed that substituting MSNF with whey solids resulted in a decrease in the freezing point of ice-creams. All the frozen yoghurts were drawn at the same temperature. The lower freezing point will therefore result in a lower amount of ice nuclei formed, as a result of freezing, due to the difference between the temperature of the system and the draw temperature. This would result in a larger amount of unfrozen water after drawing (Hagiwara & Hartel, 1996). These frozen yoghurts would therefore be very soft after extrusion. This would in turn lead to a higher amount of unfrozen water available to crystallise during the hardening stage (Hagiwara & Hartel, 1996). Ice crystallisation during hardening is a quiescent process and does not involve further ice nucleation (Hartel, 1996). The larger amount of unfrozen water therefore contributes to the growth of the already formed ice crystals. These therefore could have grown to such an extent that they would be detected as being coarser in the present study. The mixes containing fat would on the other hand exhibit a higher degree of subcooling that would result in more nucleation. The frozen yoghurt containing 10 % milk fat had a higher amount of solutes (0.27 kg/ l H_2O) in the aqueous phase than the frozen yoghurt containing 5% milk fat (0.26 kg/l H_2O). The latter frozen yoghurt also contained a slightly higher amount of milk solids-not-fat. The frozen yoghurt containing 10% milk fat might have had a slightly lower freezing point compared to the frozen yoghurt containing 5% milk fat.

According to Goff *et al.* (1993), nucleation rate is largely a function of the freezing rate, which is related to the temperature differential within the freezer barrel and to the conductive and convective heat transfer coefficients of the freezing system. It can thus be argued that the increased amount of fat could have resulted in a higher viscosity in the freezer barrel and fat acting as an insulator could also have decreased the nucleation rate. The mixes containing milk fat did have a higher viscosity at the time of entry into the freezer barrel. However, the initial whipping and freezing would break the gel matrix, thereby resulting in a decrease in the viscosity. Moreover, these frozen yoghurts also had a higher freezing point and therefore a larger subcooling, which would thus lead to

subsequent nucleation. A system nucleates spontaneously once a crtitical mass of nuclei is formed. It is thus likely that the increased amount of fat would have resulted in an increase in the time taken for a certain number of ice nuclei to be formed rather than leading to a decrease in the total volume of ice nuclei formed.

However, a higher amount of fat might also increase the sub-zero viscosity of the unfrozen phase, compared to a fat-free system and therefore impede movement of water molecules to the growing ice crystals. On the other hand, fat decreases ice crystal growth by obstruction (Donhowe *et al.*, 1993). Increasing fat content therefore resulted in a higher subzero viscosity as well as prevented growth of ice crystals by obstruction. Data by Hagiwara & Hartel (1996) suggested that increasing the amount of small molecules also result in a decrease in the glass transition temperature of ice-creams. The difference between storage temperature and the glass transition temperature also determines the extent to which recrystallisation is possible during storage. Too low a freezing point depression can also result in a product that is easily heat-shocked and hence become coarser (Smith & Bradley, 1983). Shirai *et al.* (1985) argued that a large freezing point depression would result in an increase in growth rate of the ice crystals. This would certainly be due to the larger amount of unfrozen water available for crystallisation during hardening. Recrystallisation is a thermodynamic process whereby small ice crystals dissolve and are incorporated into larger crystals that grow. The result is an increase in the mean size of ice crystals and a broadening of the size distribution (Sutton, Cooke & Russell, 1997). A crystal size distribution with a large mean size and a wide variation results in a coarse product (Donhowe *et al.*, 1991).

Increasing the fat content is also known to increase smoothness in frozen dairy desserts other than through the size of ice crystals. This would certainly be a result of the fat globules coating the air cells. Therefore, the increasing fat content significantly influenced the panelists to score those samples as being smoother.

Although whey proteins decreases the rate of ice crystals growth (Hartel & Chung, 1993), this did not have a significant effect in this present study. Budiaman & Fennema (1987)

argued that the resistance to crystallisation might depend on molecular associations (intrapolymer, water polymer) within the hydration sphere of a single hydrocolloid molecule. It thus means that the presence of microparticulated whey protein concentrate was not sufficient in the present study. It should be mentioned that the frozen yoghurts containing milk fat also had a lower pH, which thus could have increased the water binding capacity of the proteins.

Increasing fat content also results in an increase in the thickness of the various samples in the mouth. This is quite obvious since fat led to a considerable increase in the viscosity of the mixes. The temperature in the mouth would not allow for the melting of all fat crystals during eating. So it can be argued that the increasing fat content together with more solidified fat led to an increase in the viscosity of the frozen yoghurt in the mouth.

Increasing the protein content of the various yoghurt mixes resulted in a significant increase in the foamy meltdown. This would certainly be the result of a higher overrun and the low freezing point of the frozen yoghurts containing the fat substitute. Although caseins have higher foaming abilities it also leads to the formation of less stable foams (Mulvihill, 1992). Moreover, high overruns can also lead to a phenomenon referred to as foam shrinkage (Dubey & White, 1997).

Increasing milk fat content from 5% to 10% milk fat resulted in an increase in the perception of sweetness. Weit, Ketelsen, Davis & Beyts (1993) also observed that an increase in fat content led to an increase in sweetness perception. They concluded that fat content affected perceived sweetness. Likewise, Roland, Philips & Boor (1999 a) also observed a significant difference in the perception of sweetness as the fat content decreased from 10% fat to 7% fat. Although the mixes were formulated to have the same sweetness values of 14 % sucrose, the amount of sugars in the aqueous phase was higher in the frozen yoghurt containing 10% milk fat than that containing 5% milk fat. This is because the increase in fat content replaced some of the water. Conforti (1994) also observed that increasing fat content, from 10%-16%, increased the perception of sweetness when sucrose was used as the only source of sweetener. On the other hand,

although fat substitution led to a decrease in fat content, it also led to a significant increase in sweetness, especially when used at 5% substitution level. In the present study, a microparticulated whey protein concentrate replaced milk fat. This particular fat substitute is processed under high temperature and shear stress. According to Singer *et al.* (1990), the protein solutions used for the manufacture of the microparticulated proteins may also contain one or more polyhydroxy compounds, such as glucose, fructose and lactose. These may either be present as a component in the source of the material used to provide the soluble proteins, e.g. lactose and the products of its enzymatic hydrolysis, i.e. glucose and galactose, present in whey protein concentrate or may be added as an additive (Singer *et al.*, 1990). Other proteinaceous starting materials, such as dairy whey purified by chromatography contains essentially no sugars and would benefit from the addition of polyhydroxy compounds. According to the same authors, sucrose and lactitol are among the most desired non-reducing sugars to be added. The addition of such polyhydroxy compounds would most certainly help against thermal instability of the proteins. Therefore, the addition of the fat substitute at a 5% substitution level resulted in an increase in perceived sweetness when compared to the frozen yoghurt with 5% fat. These frozen yoghurts did not differ in terms of water content and therefore the increased use of the fat substitute led to an increase in sweetness. Ohmes *et al.* (1998) also found that when used at 5% substitution level, ice-creams containing Simplesse® 100 were sweeter than a corresponding control ice-cream with 5% milk fat. It is not quite clear from their data whether these samples differed significantly or not. Decreasing the level of the fat substitute from 5% to 3.4%, resulted in an insignificant decrease in sweetness due to decreased level of sugar and increased amount of water.

Increasing the fat content led to a decrease in the perceived intensity of strawberry flavour. Increasing fat content from 5% fat to 10% fat did not result in a statistically significant decrease in the perception of the intensity of strawberry flavour while fat substitution by a microparticulated whey protein concentrate resulted in increased intensity of strawberry flavour. The same trend was observed for an aftertaste. Strawberry flavour consists of a complex mixture of volatiles comprising compounds of different chemical classes and molecular weights. The complex mixture of strawberry flavour

includes acids, alcohol, aldehydes, esters, lactones, acetals, furans, terpenes and glucosides (Zebetakis & Holden, 1997). Synthetic strawberry flavour emulating the typical strawberry flavour would also consist of a variety of compounds. Recent research indicate that 2,5-Dimethyl-4-hydroxy-2H furan-3-one (DMHF), also referred to as furaneol®, is one of the important character compounds found in strawberry flavour (Zebetakis & Holden, 1997; Whitehead, 1998). This compound is only slightly soluble in water but highly soluble in alcohol and oils (Furia & Bellanca, 1975). Likewise, other compounds found in strawberry flavour have varying solubility in water and oil.

Milk fat functions as a carrier of important flavour notes, the perception of which can be expected to differ when the fat quantity is varied around the concentration of fat marginally sufficient to carry the amount of flavourant to the olfactory senses (Ohmes *et al.*, 1997). As the fat content increases, more time is needed for a lipophilic flavour compound to be released into the headspace. In the absence of fat, lipophilic flavour compounds become poorly bound to the food matrix, hence resulting in their headspace concentration being higher, thus leading to an increased flavour perception (Schirle-Keller *et al.*, 1994; Li *et al.*, 1997; Ohmes *et al.*, 1998). For example, the headspace of compounds such as, ethyl heptanoate, ethyl hexanoate and ethyl methyl-phenyl-glycidate, which are more soluble in oil than in water, will be lower at higher fat content. On the other hand, a fat-free product will run the risk of an early release of those compounds and would be perceived as highly intense. This is because the initial rate of flavour release is faster for emulsions of lower oil content (Harisson & Hills, 1997). The types of molecules released as well as their quantities are thus the key elements to the perceived flavour (Leland, 1997). Fat content therefore affects the headspace concentration of flavour compounds by influencing their vapour pressure (Schirle-Keller *et al.*, 1994; Li *et al.*, 1997). It follows that the time needed to reach maximum intensity decreases in fat-free systems. It is thus a common practice to adjust the flavour components when formulating low fat / fat-free systems to get a proper flavour balancing (Honer, 1994). The perception of the intensity of strawberry flavour was not statistically different in the frozen yoghurts containing 10% and 5% milk fat. This suggests that the marginal or 'threshold' fat content is below those levels. The two frozen yoghurts containing the fat

substitute did not differ from each other in terms of the strawberry intensity as they did not differ in terms of their fat content. There is unfortunately no existing data in the literature regarding perception of strawberry flavour in fat systems versus fat-free systems.

A recent set of studies by Schirle-Keller *et al.* (1992) and Schirle-Keller *et al.* (1994) suggested that the microparticulated whey protein concentrate Simplesse® 100 does bind to certain lipophilic flavour compounds more than any other fat substitutes tested. They argued that it might be due to some fat present in the fat substitute. However, although Simplesse® 100 did react with some flavour compounds, this was not to the same extent as fat itself. The authors concluded that all formulations, based on the various fat substitutes tested, would require changes to the flavour component to maintain the flavour profile.

Fat content will have very little direct effect on the vapour pressure of water-soluble compounds, such as hexanal, but will affect the perception of such compounds mainly through its influence on the melting rate of the product and mass transfer. The latter two phenomena will also influence the perception of fat-soluble flavour compounds. The time for certain compounds to reach maximum intensity increased with increasing oil fraction irrespective of whether the flavour compounds were fat-soluble or water-soluble (Harisson & Harris, 1997).

Flavour release in the mouth is a dynamic process involving essentially the food, saliva and air (Hills & Harission, 1995). During eating a food is broken down by mastication, mixed with saliva, is partially dissolved and subjected to an air flow (Bakker, Brown, Hills, Boudaud, Wilson & Harisson, 1996). In the case of ice-creams, the food is sucked rather than masticated and the release of flavour compounds is even slower. It is highly probable that interfacial mass transfer between food/saliva and saliva/gas interfaces is rate limiting during the release of flavour compounds to the headspace (Hills & Harission, 1995; Harisson & Hills, 1997). It is generally accepted that food components that affect the rate and extent of transfer of a flavour compound from the food to the

saliva influence perceived flavour (O'Neil & Kinsella, 1987). The frozen yoghurts containing milk fat were characterised as being higher in thickness compared to those containing the fat substitute. Thickness, in that case, was defined as the viscosity of the product from the time it was first chewed in the mouth until complete melting. This would most certainly prevent access of saliva to the product and the release of flavour compounds. According to Li *et al.* (1997), ice-creams with higher fat content melted into a fluid with high viscosity compared to lower fat products, which decreased the rate of diffusion of vanilla flavour compounds. Therefore, the rate of melting in the mouth will also influence the rate at which flavour compounds are released. The initial rate of release of flavour compounds depends on the interfacial surface area between the emulsion and air, initial flavour concentration and mass transfer coefficient as well as gas flow rate in the mouth (Harisson & Hills, 1997). The breathing pattern of an individual therefore also influences the flavour characteristics of a particular food. Breathing will result in a turbulent flow in the mouth, with the result that the flavour-enriched gas is driven into the back of the throat, leading to a decrease in the headspace concentration (Harisson & Hills, 1997). It can then be argued that a sample with a low melting rate will tend to result in more flavour being released per unit time, especially so if the flavour compound is soluble in fat and the system is fat-free. This would obviously result in an increased flavour perception. Linforth, Ingham & Taylor (1996) reported that, in one of their studies on flavour in strawberry fruits, the amount of esters released increased rapidly during the early stages of mastication, then reached a maximum after swallowing and then fell rapidly after the bolus was swallowed.

The fat-substituted frozen yoghurts contained small soluble molecules that would influence the freezing point of the frozen yoghurt. According to Bakker *et al.* (1996), the driving force for flavour release depends on the bulk melting temperature of the gel. In gels with melting points lower than the mouth temperature, flavour release is determined by the rate heat can diffuse into the gel matrix and initiate melting. Fat acts as an insulator in ice-creams and increasing fat content thus decreases the melting rate. Ohmes *et al.* (1998) observed that ice-cream mixes containing a fat substitute had lower freezing point and they argued that as such those ice-cream mixes melted at a much lower

temperature and therefore had a higher melting rate. Roland *et al.* (1999 b) also observed that all the fat-free ice-creams melted faster than a 10% fat-containing ice-cream. Moreover, fat globules at the interface of the serum phase and of the air bubbles act as an insulator to heat transfer. The rate of heat loss and heat gained by the frozen yoghurts containing fat is thus lower compared to those containing the fat substitute. The faster melting rate would ensure that flavour compounds are released much quicker in the headspace (Bakker *et al.*, 1996) and as such they are perceived as being 'stronger'. On the other hand, Guinard & Marty (1995) reported that increased gel firmness led to a maximum flavour intensity of compounds such as ethyl butyrate and benzaldehyde.

On the other hand, the omission of milk fat in the present study was accompanied by an addition of a microparticulated whey protein concentrate. Both β-lactoglobulin and α-lactalbumin have been found to have binding sites for a number of volatile compounds. For example, β-lactoglobulin has binding sites for certain alkalones, such as 2-octanone and 2-nonanone (O'Neil & Kinsella, 1987). Vanillin was perceived as being less intense in the presence of whey protein concentrate, due to cysteine-aldehyde condensation or shift base formation (Hansen & Heinis, 1991). However, the interaction was pH dependent and was more significant at alkaline pH. The binding abilities of proteins therefore depend on the conformational state of the proteins. Any chemical and physical changes that alter the conformational status of the proteins thus produce marked changes in the flavour binding characteristics of the proteins (O'Neil & Kinsella, 1987). However, a taste sensation will result when competition for flavour compounds between the food proteins and protein of the sensory receptors is won by the latter (Stevenson *et al.*, 1996). Moreover, other factors affect flavour perception such as viscosity and melting rate and according to Roberts & Acree (1996), the effects of proteins on aroma binding is usually overweighed by the effect of oil in the system.

Fat substitution by the microparticulated whey protein concentrate resulted in a higher and strong aftertaste. This could have been the result of the use of the fat substitute itself or increased vapour pressure of some of the constituents of the strawberry flavour. Strawberry flavour consists of a number of volatile compounds that have solubility in

both oil and water. Some of them are completely insoluble in water, while others prefer fat as a solvent. Decreasing the fat content would therefore result in an increased perception of oil-soluble compounds. Ethyl cinnamate, which is insoluble in water, has a cinnamon-like aroma (Furia & Bellanca, 1975) and 2,5-Dimethyl-4-hydroxy-2H furan-3-one (DMHF), which is slightly soluble in water but soluble in oils (Furia & Bellanca, 1975), has a caramel-like odour (Schieberle & Hofmann, 1997). Decreasing the fat content could thus have resulted in an increase in the perception of these compounds as a result of the various mechanisms outlined above.

On the other hand, Ohmes *et al.* (1998) observed that in general the whey-based fat substitute imparted more of the flavours considered as undesirable than did non-fat milk solids. The inclusion of WPC as fat substitute also resulted in higher bitter aftertaste (Ohmes *et al.*, 1998). The perception of a strong astringent and bitter aftertaste could also have influenced the panelists to score the strawberry intensity as being higher.

To conclude on the perception of strawberry flavour in frozen yoghurts as a result of fat substitute, it can be said that fat influences flavour perception of both water-soluble as well as fat-soluble flavour compounds. The mechanisms involved are:

- a reduction of the vapour pressure of fat soluble volatile compounds and thus a decrease in the headspace concentration,
- by preventing access of water soluble flavour compounds to some receptors
- by influencing the rate of melting and mass transfer and
- the increased perception of an aftertaste, either as a result of an increase in the vapour pressure of some of the constituting flavour molecules or as result of the whey protein concentrate.

The results of the sensory evaluation thus revealed that fat substitution resulted in products that do not compare favourably with those containing milk fat. According to Akoh (1998), several foods formulated with fat replacers do not compare favourably with fat containing counterparts. A preference study by Soliah & Dorsett (1995) revealed that some dairy desserts based on fat substitutes were not liked as much as premium ice-

creams. According to Roland *et al.* (1999 b) fat subtitutes do improve the quality of fat-free systems, e.g. an ice cream mix containing 0.1% fat, but they rarely do so to the same extent as ice-creams containing 10% fat.

The rapid deterioration of the samples during microscopic examination, made it difficult to reach clear and unambiguous conclusions. According to Botha, Technician at the Laboratory for Microscopy and Micro-analysis, University of Pretoria (1999 - personal communication) the high moisture content of the frozen yoghurts could have been the reason for such a behaviour. However, in a recent study on the microstructure of frozen yoghurt of the ice-cream type, Inoue *et al.* (1998) found that the microstructure of frozen yoghurt was similar to that of ice-cream. They also concluded that the microstructure of frozen yoghurt did not depend on pH. In the present study the surface structure revealed the presence of air bubbles coated by fat globules, a serum phase separating the ice-crystals from the air bubbles and fat globules in the serum phase. The microstructure also revealed some fat clusters on the air bubbles and also in the serum matrix. The amount of proteins adsorbed onto the fat globules is expected to increase with increasing acidity (Kanno *et al.*, 1991). This would result in fat globule-fat globule interaction.

According to Dickinson *et al.*(1988), the vast majority of food emulsions and foams are stabilised by a layer of adsorbed protein at the oil-water or air-water interfaces. The results of the microstructure tend to suggest that the microparticulated whey proteins did not coat the air bubbles, as did the fat globules. The microstructure showed some particles adhering to the air bubble at times. Those might actually be some fat globules contained in either the fat subtitute or skim milk powder. According to Dickinson *et al.* (1988), casein tends to prevent the unfolding of whey proteins at the air-water interface. This would certainly prevent the whey proteins from adhering to the interface. Moreover, caseins have high surface hydrophobicity, resulting in a high surface activity, in contrast to more globular whey proteins (Fox & McSweeney, 1998). In globular proteins, the hydrophobic residues are buried, as far as possible, within the molecule. Therefore, it appears that the microparticulated whey proteins will structure the aqueous phase by binding water and as a result of their size would provide the desirable creamy texture.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Fat substitution by the microparticulated whey protein concentrate, Simplesse®500, did not adversely affect the activity of probiotic bacteria at any stages. The microparticulated whey protein concentrate can thus be used in the formulation of fat-free frozen yoghurts as far as the viability of *S. salivarius* subsp. *thermophilus, L. acidophilus* and *B. bifidum* is concerned. It caused a decrease in the pH of the mixes before incubation but also increased the buffer capacity of the mixes, resulting possibly in a slightly longer fermentation. The longer fermentation process favoured the growth of *S. salivarius* subsp. *thermophilus* and *L. acidophilus* to the same extent as in the mixes containing milk fat. The viability of *B. bifidum* was very low in the frozen yoghurts containing fat. The bacteria did not grow in these frozen yoghurts. The addition of the microparticulated WPC into the mixes did not result in an increase in the number of *B. bifidum.* The viability of bifidobacteria had been known to be highly species and strain dependent. The numbers of *B. bifidum* remained below the recommended level for therapeutic effects (10^7 cfu/g) in frozen yoghurts containing either 10 % or 5 % milk fat as well as in frozen yoghurts containing the 3.4% and 5% of the microparticulated WPC. Different species and strains of bifidobacteria have different requirements as far as amino acids, vitamins and other growth factors are concerned. Since the addition of more whey proteins could have contributed to a slightly lower redox potential that could be beneficial to the bacterium as well as increasing the protein content, it can thus be concluded that their low viability is most likely to be related to a lack of an appropriate proteolytic system and / or the requirements of certain specific amino acids or vitamins on the part of the species / strains used in the present study. An increase in the inoculation rate might prove necessary to ensure that the bacteria be delivered at the right level at the time of consumption. On the other hand, it would also be very interesting from a research perspective to determine the strain of *B. bifidum* used in the present study. Moreover, a determination of the required essential amino acid (s), vitamins and/or any other growth factors would also be interesting. Although a lot has been done to determine the

proteolytic activity of lactobacilli, much still remains to be done. Those two aspects were beyond the scope of the present study.

Frozen dairy desserts are very complex systems and fat has long been known to play an important role in developing their structure. Very little information is at present available on fat-free frozen yoghurts. The present study clearly and unambiguously showed that fats play a key role in frozen yoghurt of the ice-cream type. The frozen yoghurts containing 5% milk fat and those containing 5% of the microparticulated WPC did not differ significantly in terms of their water content but yet the latter frozen yoghurt was characterised by being coarser and having a higher strawberry intensity and aftertaste. Removal of fat thus resulted in a void that had to be properly filled. This could not be done by adding more milk solids-not-fat or increasing the water content.

The most important change resulting from a reduction in fat content is the addition of other more soluble compounds to the aqueous portion of the mix. Fat is suspended rather than dissolved, while carbohydrates and other impurities present in whey protein concentrate, such as salts, are dissolved in the water portion of the mix. The amount of solutes present in the aqueous phase affects some important properties of the mix such as the texture of the frozen yoghurt. A coarser texture caused by an increased amount of solutes was most probably the result of less water being frozen during initial whipping and freezing due to an excessive lowering of the freezing point. More water was thus available to freeze during the hardening stage.

The solution to fat replacement might involve a properly engineered mix containing polysaccharides for high viscosity, microparticulated WPC for enhancing creaminess and the inclusion of compounds that would not lead to an excessive lowering of the freezing point of the mix. Low DE maltodextrin have less small molecules compared to a high conversion corn syrup and will therefore not depress the freezing point too much and would increase the glass transition temperature. The inclusion of such compounds would help to decrease the amount of unfrozen water at whipping and freezing and also hinder considerable ice crystal growth during hardening and storage.

The strategy could also be fat reduction rather than fat replacement. This would involve the formulation of a mix containing the microparticulated WPC, low DE maltodextrin, stabilisers, fat and emulsifiers. The microparticulated WPC would most certainly contribute to a sensation of creaminess and would also bind some water. The inclusion of fat at 2-2.5% levels would most probably ensure a build-up in the structure of the frozen desserts. Fat is also known for its lubricating properties, resulting from the sliding action of fat chains. Yoghurt cultures containing bacteria that produce polysaccharides during fermentation and hence increase the viscosity and enhance mouthfeel could also find a special use in low-fat frozen yoghurt.

APPENDIX A

The relative amounts of ingredients in the yoghurt mixes

Composition %(m/m)	10% fat	5% fat	5% fat substitute	3.4% fat substitute
Skim milk	43.71	60.22	71.87	73.63
Cream	33.33	16.67	0.00	0.00
Simplesse	0.00	0.00	5.00	3.40
SMP$^{\gamma}$	4.76	4.91	4.93	4.77
Sucrose	11.20	11.20	11.20	11.20
CSS$^{\alpha}$	6.00	6.00	6.00	6.00
Gelatin	0.50	0.50	0.50	0.50
Starch	0.50	0.50	0.50	0.50

γ Skim-milk powder

α Corn syrup solids (47% SE)

APPENDIX B

The Frozen yoghurt sensory evaluation

Name:

Seat No..... **Date** **Sample code.....** **Session No.**

Please read the following instructions carefully before starting the evaluation!!!!.

You are provided with four coded strawberry frozen yoghurt samples. Evaluate them(from left to right) and indicate (by drawing a vertical line),on the line scale provided, where you think the samples stand as far as each of the specified characteristic is concerned.

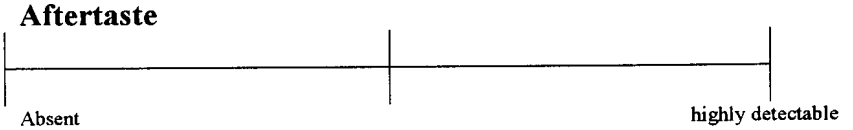
Remember to rinse your mouth thoroughly in between each sample and to use new spoons for each one of them.

Flavour

Sweetness

Strawberry intensity

Aftertaste



Texture and body

Coarseness

Thickness

Foamy meltdown

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