THE USE OF ULTRAFILTRATION PROCESS FOR THE MANUFACTURE OF ICE CREAM AND CAJETA

by

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ABSTRACT

Retentate obtained from ultrafiltration was used as a substitute for skim milk powder, in the manufacture of ice cream and cajeta (Mexican dairy spread). The products were assessed by Chemical, physical, sensory and structural analysis. Ice creams made using ultrafiltered retentate had increased ash, protein, calcium, phosphorous and magnesium, but reduced lactose, potassium and sodium contents. Physical evaluation showed that UF-products were harder, more viscous and had better melting resistance, but had lower overrun and extrusion temperature than control ice cream. In Sensory analysis UF-products scored better for iciness, sandiness and fluffiness, and resisted heat shock treatment better. No consumer preference for UF-based ice cream or control ice cream was found. The UF-ice cream took longer to soften to eating consistency.

Structural examination of ice cream products by various microscopy techniques revealed air cell, ice crystal and fat droplet structures within a sugar and protein matrix.

Freeze substitution was applied to ice cream for Transmission Electron Microscopy to produce unique thin sectioned samples. This showed a more agglomerated casein structure with UF-based ice cream.

Heat shock changed ice cream structure. Ice crystal size increased and crystals fused into a network. Air cells could be distorted into a modified channel shape.

Chemical, physical, microbiological and sensorial analysis of cajeta were carried out. UF-cajeta had slightly higher protein calcium and phosphorous contents and lower lactose, potassium and sodium contents. UF-cajeta showed better sensory attributes after storage than the control, however as shelf life was extended yeast and mould growth was possible.

Structural examination of cajeta showed ultrafiltered retentate in cajeta manufacture prevented the formation of larger lactose crystals and prevented sandiness that developed in the control product.

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ABBREVIATIONS

Abs	Absent
AMF	Anhydrous milk fat
a_W	Water activity
BSI	British Standards Institution
CFU	Colony forming unit
cm	Centimetre
DM	Dry matter
HPLC	High performance liquid chromatography
IC	Ice cream
ICP	Inductively coupled plasma
IDF	International Dairy Federation
kPa	Kilo Pascal
LM	Light microscopy
MF	Microfiltration
MPN	Most probable number
MS	Membrane separation
MSNF	Milk solids non fat
MW	Molecular weight
mm	Millimetre
MPa	Mega Pascal
µm	Micrometer or micron
N	Newtons
NAFTA	North American Free Trade Agreement
PCA	Principal components analysis
pH	$-Log_{10}(H_3O^+)$
REML	Residual maximum likelihood
RO	Reverse osmosis
r	Correlation coefficient
SAC	Scottish Agricultural College
SCM	Sweetened condensed milk
SEDiff	Standard error of difference
SEM	Scanning electron microscopy
SM	Skim milk
SMP	Skim milk powder
SSM	Semi-skim milk

TEM	Transmission electron microscopy
TS	Total solids
UF	Ultrafiltration
UF-IC	Ultrafiltered ice cream
UF-P	Ultrafiltered permeate
UF-R	Ultrafiltered retentate
VR	Volume reduction
WM	Whole milk
WPC	Whey protein concentrate

CHAPTER ONE

LITERATURE

REVIEW

CHAPTER ONE: LITERATURE REVIEW

1.1 MEMBRANE FILTRATION

1.1.1 Introduction

In membrane technology, according to Ferguson (1989) there are three types of processes: Reverse osmosis (RO), Ultrafiltration (UF) and Microfiltration (MF). They may be distinguished by the size of particle or molecule they are capable of retaining. In Reverse Osmosis the membrane pore size is in the range of 0.0001μm to 0.001 μm. The process is used for dewatering purposes or for water purification duties. Desalination of sea and brackish water is one of the processes that illustrates the use of reverse osmosis, but also it has been used for other purposes such as the concentration of whey and in fruit juices for clarification and removal of pectin.

In ultrafiltration the membrane pore size is in the range of 0.001 to 0.1μm, and it has been used in the food industry for the separation and concentration of low and high molecular weight components. Among other things, ultrafiltration is commonly used for concentration and purification of whey proteins, for production of whey protein concentrate, for concentration of milk for cheese production, and for protein standardisation.

Microfiltration, (MF) involves an even more open membrane which will reject colloids, suspended particles, bacteria and some viruses. Among other things, microfiltration is used for sterile filtration and clarification processes as an alternative to precipitation by chemicals and centrifugal separation. In MF the pore size is in the range of 0.1 to 10 μm and in this case only very large

macromolecular groups and suspended particles are held back by the membrane, the remainder of the components in the solution are filtered across. The filtration spectrum shown in Figure (1.1) illustrates the size and character of a number of particles which can be separated by the filtration processes.

The word membrane in Latin is 'membrana' which means the skin of the body, and in all the process mentioned before, a membrane is used as the filter medium in which pressure is the driving force that achieves a certain throughput. A membrane is porous medium and, depending on the pore size and other separating characteristics of the membrane, the terms hyper, ultra and micro are applied. Membranes according to Kosikowski (1986) have a thin surface layer, or skin, where permeation occurs, and most have an open, porous interior or backing to support the surface skin. Initially, cellulose acetate was practically the only material used in fabricating membranes, but in recent years complex polymers, as thin film composites supported on polysulfone membranes or as polysulfones, have been replacing cellulose acetate for separations. Cellulose acetate membranes are sensitive to extremes in temperature, pH, and chlorine concentration. Polysulfone membranes are relatively insensitive to these influences and show a more satisfactory concentration polarisation and higher flux rates and oxidation. According to Renner and El Salam (1991), it is because the sulphur atom is in its highest oxidation state and the sulphone group tends to draw electrons from the adjacent benzene rings to stabilise them against oxidation. On the other hand, ceramic membranes are very resistant to pH and high temperatures, but they are very expensive.

In this study a hollow fibre module was used, this is produced by extrusion through annular dies. With a internal diameter of 0.5 or 1.0 mm and

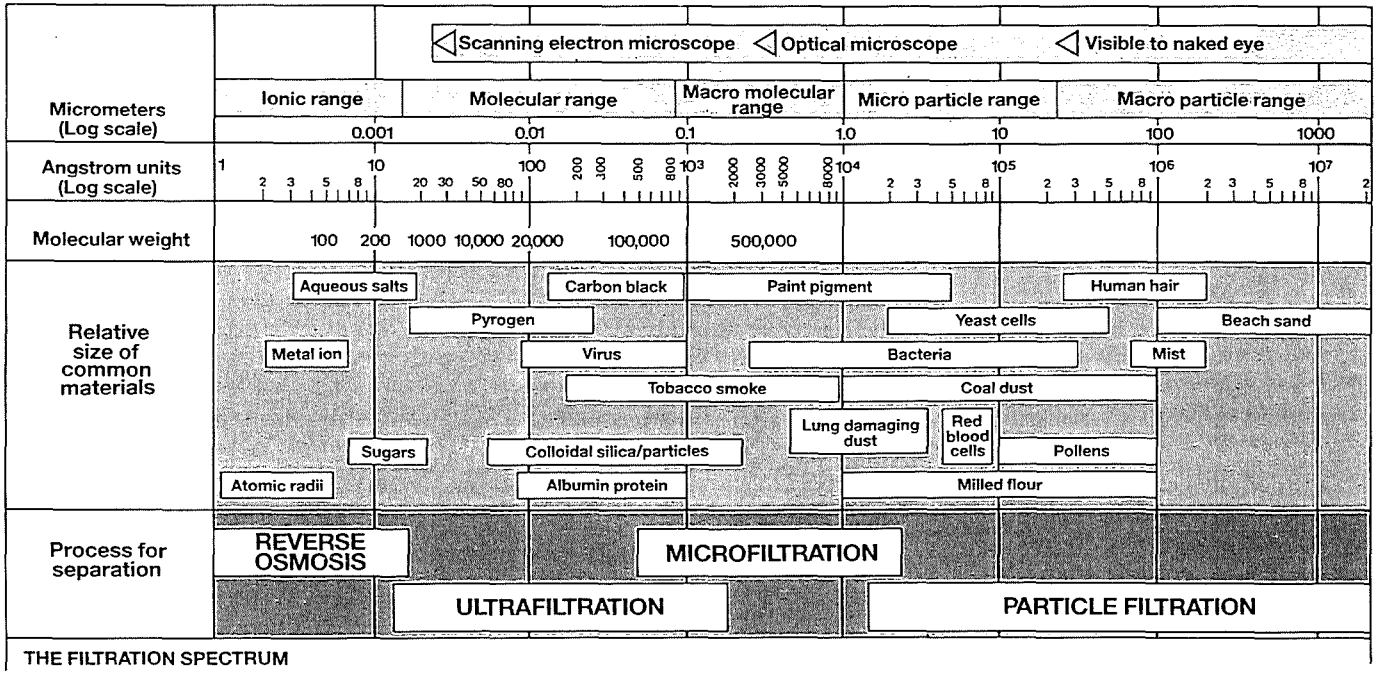


Figure No. 1.1 The filtration spectrum for size and character of a number of particles which can be separated by filtration process.

(Ottosen, 1990)

1 m long. The structure, according to Glover (1985) is dense on the inner surface, 0.1 to 1.5 μm thick and much looser towards the outer surface in a layer from 50 to 250 μm thick. These fibres are then gathered in a bundle of several thousands and sealed in a clear plastic cartridge. They can stand high internal or external pressures. Kosikowski (1986) mentions that membranes are designed in various configurations for specific functional space-saving making the equipment to most functional possible without taking large areas of space where they are installed. Those configurations include tubular, flat sheet or plate, spiral wound, and hollow fibre.

1.1.2 Ultrafiltration of milk

Ultrafiltration is a physico-chemical separation technique in which a pressurised solution flows over a porous membrane. The membrane allows the passage of only relatively small molecules, and the retentate flows over the membrane, while under the influence of pressure, water flows through the membrane together with the low molecular weight solutes. Protein is retained by the membrane and is concentrated relative to other solutes in the retentate. Ottosen (1990) describes how besides a flow over the membrane surface, the pumps in the membrane filtration plant create a pressure on one side of the membrane while the other is close to atmospheric pressure. This pressure difference across the membrane is the "driving force" which allows the separation to take place.

The fraction passing through the membrane is called permeate and consists normally of water, lactose and another small molecules, such as minerals. Meanwhile the fraction retained by the membrane is called retentate and contains water, large molecules and also part of the small molecules. In

practice, the retentate passes over the membrane surface many times and every time more water and small molecules are removed as permeate. As a result, the retentate becomes higher in total solids. See Table No (1.1) for the molecular weight of some milk components, and Table No (1.2) for some terms and expressions used in membrane separation process.

Ultrafiltration is now a well-established process for the separation and concentration of chemical molecules, due to the differences in their molecular weights (Rajagopalan and Cheryan, 1991, Rener and Abd El-Salam 1991, Ferguson 1989, and Glover *et al.* 1978).

During UF of milk, a dynamic layer, consisting primarily of fat and protein, forms on the membrane surface. The dynamic layer controls the flux and separation characteristics of the membrane system (Mohr *et al.* 1989). One of the most important parameters in evaluating the efficiency of membrane filtration systems is by checking the permeate flux during the ultrafiltration process.

In ultrafiltration processes the concentration polarisation begins after a few seconds of starting. Solids begin to collect near the membrane. They are then absorbed on to the membrane surface and invade the pores so that within minutes there is a rapid decline in permeate flux. As ultrafiltration process proceeds the flux continues to decline, though much more slowly, as a gel layer builds up on the membrane. Concentration polarisation in membrane transport has a profound effect on permeation rates. If concentration polarisation becomes too severe, membrane fouling follows. The process becomes controlled by fouling and the characteristics of the membrane become secondary. Concentration polarisation is inherent in the process; it can never be eliminated

Table No. 1.1 Characteristics Of Some Milk Constituents

SUBSTANCE	RELATIVE MOLECULAR MASS (Kg/Kmol)	DIAMETER (nm)
Water	18.0	0.3
Chloride ion	35.0	0.4
Calcium ion	40.0	0.4
Magnesium ion	24.0	0.2
Phosphorus ion	31.0	0.3
Sodium ion	21.0	0.2
Potassium ion	39.0	0.4
Lactose	342.0	0.8
α -Lactalbumin	14,500.0	3.0
β -Lactoglobulin	36,000.0	4.0
Blood serum albumin	69,000.0	5.0
Fat		130 - 1300
Casein micelles	10^7 - 10^9	25...130

Taken from Kessler, (1981)

Table No. 1.2 Terms and expressions in membrane separation process.

UF.- Ultrafiltration

Cut-off value or molecular weight cut-off.- Refers to the molecular weight of moleules rejected by an ultrafiltration membrane.

Flux.- The amount of permeate passing through the membrane with a given surface area. Normally expressed as litres/m^2/hour (l/m^2/h)

Fouling.- Accumulation of solids deposits on the membrane surface. Fouling reduces the flux substantially.

Permeability.- Expresses the fraction of a solute retained by the membrane

Permeate.- Means the filtrate passing through the membrane.

Rejection.- The rejection of a component means the fraction of a solute rejected by the membrane expressed in per cent.

Retention coefficient.- Expresses the fraction of a solute retained by the membrane

Retentate.- Means the concentrated solution coming out of a membrane filtration plant. Retentate and concentrate are synonyms.

Volume reduction or Concentration factor.- Is the ratio of the initial to the final volume of the concentrate.

Concentration polarization.- A higher concentration of retained solute species adjacent to the membrane surface than in the bulk stream.

Taken from Glover, (1985)

but only minimised (Glover, 1985; Kiviniemi, 1979); however Mohr *et al.* (1989) suggested that concentration polarisation can be controlled by use of crossflow filtration, use of turbulence promoters, high flowrates, and operation at the maximum temperature permitted by the membrane material and membrane module. Fouling can be minimised by the use of pre-treatments, such as filtration, precipitation, dissolution by acids, or foulant suspension as well as module design with crossflow configuration, where feed stream flows parallel to the membrane surface.

1.1.3 Chemical partition of the milk

In the dairy industry UF-membranes are made from polysulphone, polyvinylidene fluoride, regenerated cellulose or cellulose acetate, but polysulphone are the ones most used. Generally the usefulness of a membrane is determined by its selectivity, its flux and its chemical, mechanical and thermal stability

Ultrafiltration separates milk into two liquids, according to Wagner (1979) a corpuscular (fat globules, casein micelles) and high-molecular weight fraction (soluble caseins, whey proteins) which is retained as the retentate, and a low molecular weight fraction (lactose, minerals), as the permeate. This fractionation depends on different factors such as concentration polarisation, volume reduction of the liquid phase, molecular weight of the component, and membrane pore size. As a result of concentration polarisation and deposit formation on the membrane, retention usually increases as concentration proceeds.

Renner and Abd El Salam, (1991) stated that, depending on the degree of separation and concentration achieved by ultrafiltration, it is possible to obtain retentates and permeates with different compositions and properties which are different from the original fluid and which are suitable for processing into a new generation of diversified products.

Glover, (1985) mentions that for a single component system, concentration may be expressed simply in terms of the whole system. However milk contains many components, some of which are completely retained by the membrane and form a considerable proportion of the concentrate, some are partly retained, others pass freely through the membrane. The reduction in volume during UF is from the water phase only. Hence there is a greater loss of some water phase components than the overall concentration factor indicates, resulting in lower concentrations of the more diffusible components. Such concentrations are not a true representation of the behaviour of the membrane. For a component whose retention coefficient is zero or small the concentration in the permeate will appear to fall as UF proceeds which is why reports occur in the literature of negative retention coefficients, as for example for lactose in the following example given by Glover (1985).

100 kg MILK

Fat 3.8 %	Lactose 4.8%	Water 87.5 %
Protein 3.2 %	Salts 0.7 %	

CONCENTRATION FACTOR 5

UF-RETENTATE (20 kg)	**UF-PERMEATE (80 kg)**
Lactose 0.67 g	Lactose 4.13 g

$$\text{Concentration of lactose in water phase} = \frac{4.8}{93} \times 100 = 5.2\ \%$$

Concentration of lactose in the UF-Permeate = 4.1 %

Concentration of lactose in the UF-Retentate = 3.4 %

Rejection coefficients (See definition in Table No. 1.2) for the individual milk components are calculated from their concentration in the permeate related to the content in the base milk. The incomplete protein rejection coefficients of somewhat more than 90% may be due in part to the distribution of pore size in the membrane and in part to the distribution of molecular weights among the milk proteins. A rejection coefficient of approximately zero for lactose results from the fact that lactose content in the permeate is almost the same as in the base milk (Yan *et al.* 1979). Likewise Kessler *et al.* (1982), investigated the effects on UF of low molecular weight milk constituents and found that in the absence of protein all dissolved low molecular weight constituents passed the membrane without additional resistance.

On the other hand, Renner and Abd El Salam (1991), mention that the rejection coefficients are not constant but vary with the concentration factor or level of concentration and they cite that this is supported by (Bastian *et al.* 1991).

1.1.3.1 Nitrogen

According to Renner and Abd El Salam, (1991), during UF of milk, a great change occurs in the distribution of the individual nitrogen fractions

related to total nitrogen; the proportions of casein as well as of whey proteins increase in the retentate with elevated concentrations factors due to the corresponding decreases of all the other chemical fractions. It seems that the protease-peptone components are partly retained by the membrane. As virtually all milk proteins are concentrated, then, no significant change occurs in the composition of essential amino acids and hence no change in its biological protein value.

Glover (1985) points out that milk proteins subjected to ultrafiltration have been examined for damage. The whey proteins by their solubilities and the casein through the electron microscope. No denaturation of the whey proteins or damage of the casein micelles was detected such as reduction in size. Retention coefficients of non protein nitrogen are generally 20 - 40 %, increasing with concentration factor. Losses through the membrane are mainly urea and some free aminoacids. This is supported by Barbano *et al.* (1988)

1.1.3.2 Lactose

Retention coefficients for lactose are generally reported around 10%. In the aqueous phase of the feed the concentration of lactose then rises as ultrafiltration proceeds, as it does also in the permeate, though to a lesser extent (Glover 1985).

1.1.3.3 Fat

Fat normally is expected to be retained in the retentate due to its high molecular weight (See Table No. 1.1). Some ultrafiltration plants damage the fat globules in milk, causing a degree of homogenisation and churning of the fat.

The damage occurs as the milk passes through the pressure retaining valve. The damage is the result of mechanical action, not a consequence of the concentration. The effect is particularly marked in batch processing (Glover 1985). So, it is recommended to use skimmed milk for ultrafiltration processes.

1.1.3.4 Minerals

Minerals in milk exist in two forms according to Renner and Abd El Salam (1991). Some are completely free in solution and some, namely calcium, magnesium, phosphate and citrate, are partly bound to protein. The retention of the free minerals is apparently zero for all membrane types and geometries. The concentrations of minerals in the permeate are therefore equal to the concentrations of these minerals in the aqueous phase of the milk. Because the concentrations of minerals in this water phase remains constant, no transfer of minerals to or from the casein micelles occurs during ultrafiltration. Concentration factors of minerals bound to the protein are therefore identical with concentration factors of the protein. The ratio of the soluble calcium to total amount of calcium present varies with concentration factor. The distribution of calcium between the aqueous and micellar phases in milk is highly pH-dependent. Calcium content in retentate increases as the concentration factor increases (Glover 1985).

1.1.4 Applications of Ultrafiltration in the Dairy Industry

The use of ultrafiltration by the Dairy Industry has already led to the creation of new products with high nutritional value. Maubois (1989) estimates, that there are more than 150 000 m^2 of membrane for ultrafiltration of dairy products world-wide and this is growing at a rate of 20% yearly. Table No. 1.3

Table No. 1.3 Ultrafiltration plants sold by APV Pasilac[1] within the dairy industry.*

APPLICATION	NUMBER OF PLANTS	MEMBRANE AREA (m^2)
Trial purposes	98	1,390
Sweet whey	60	20,600
Acid whey	7	5,300
Whole milk	58	8,500
Skimmed milk	33	2,960
Fermented milk and cream	32	944

* Data taken from Ottosen (1990)
1 From 1972 to 1990

Table No. 1.4 Ultrafiltration applications within the dairy industry.

MILK (Normal pH)

Protein standarisation

Cheese:

-Consistent milk composition all year independent of seasonal variations.

-Better utilisation of existing equipment (lower milk volume)

Powder:

-Powder with same standarised protein content all year

-Powder with more or less protein in dry matter than normally possible

-Surplus protein can be used for cheese, retentate powder, etc.

Market Milk:

-Same milk composition all year

-Surplus protein can be utilised for cheese, etc.

-Protein-enriched milk products

UF-Cheeses

-Increase yield/better process economy than by conventional methods for existing cheese types.

-New cheese types with considerably better process economy than existing cheese types.

Yoghurt, Ymer

-Increase of protein content (higher viscosity) without addition of powder or evaporation.

FERMENTED MILK AND CREAM

Quarg, Cream cheese, Mascarpone and other fresh soft cheeses

-Considerably better yield and process economy than by conventional technology.

-Very flexible process as the same UF-equipment can be used for both skimmed milk quarg, cream cheese and any product types in between.

Table No. 1.4 Ultrafiltration applications within the dairy industry. (continued)

WHEY (SWEET OR ACID)

WPC

-Utilisation of valuable whey proteins in WPC with a standarised protein content of up to more than 80% protein in dry matter.

-Preconcentration to save transport costs before transport to whey protein manufactures.

WPC to Cheese

-Concentration of whey protein before redosing of denaturated whey protein into cheese milk (better yield).

Special products

-Products with a special protein composition for health food, baby food, pharmaceutical purposes, etc.

-Permeate with a special composition for health food, baby food, pharmaceutical purposes, etc.

SWEET BUTTERMILK

Retentate

-Retentate used as addition to yoghurt, butter and other products as protein sources.

Powder

-Preconcentration before powder manufacture.

ACID BUTTERMILK

Quarg, other fresh cheeses

-Buttermilk quarg, etc. or partial substitute for milk in these products

Taken from Ottosen (1990)

shows the number of ultrafiltration-plants that have been sold by one company from 1972 to 1990 to the dairy industry.

Milk and particularly whey proteins have the advantage of offering dual benefit, that is, the nutritional value, and the physico-chemical characteristics (gelling, foaming, emulsification, water holding capacity), which have wide functional applications in the food industry (Maubois and Ollivier, 1991) (See Table No. 1.4). Reimerdes and Mehrens (1991) mention that milk proteins offer tremendous scope as functional ingredients in food systems, because of the various possibilities that exist to exploit their structural features and physical behaviour. Likewise, Glover (1985) suggests that the application of this new process must be seen in relation to changing patterns in the use of milk, in the development of new products from milk and in the quest for improvements in efficiency of processes. Wilbey (1990), mentions that the adoption of alternative technology in the processing of dairy products can have a profound effect on the quality of the product. The author gave an example of membrane technology in the processing of dairy products (e.g. yogurt).

In the dairy industry the removal of water from milk using reverse osmosis during the production of milk powder accounts for a significant proportion of reducing the cost of the powder (Abbot *et al.* 1979), and ultrafiltration process can be used to supply a new source of milk solids non fat (MSNF), where the heat treatment is relatively mild giving energy savings by avoiding prolonged heating as in the manufacture of skim milk powder.

Rothwell (1992a), (1993a), suggests that one method to supply MSNF in ice cream formulations is to concentrate the milk by membrane processing, where, by the use of special membranes, water can be removed from skim milk

without such complicated equipment as for heat concentration. Ice cream was produced by Kosikowski and Masters (1983) by mixing a 3.3X-retentate with cream (containing 55 wt/% fat), sucrose, corn syrup solids and stabiliser. The mix was homogenised, pasteurised and cooled. After two days of ageing, vanilla ice cream was made from the mix. The resulting ice creams, containing 10 to 12 wt% fat and 35 to 37 wt% total solids, were given excellent ratings for flavour appearance, and body. These ice creams maintained good overrun and had low lactose content (1.8 wt% as compared to 5.6 wt% for the control ice creams. Nielsen (1992) suggests that membrane filtration may be used in the food and dairy industry as a means to improve food processing through better process economy, higher yields, improved quality of products, new products, utilisation of by-products and solution to some environmental problems. There is a report, Chavez (1995), about an ultrafiltration plant in California, USA, which has been used for whey processing. It confirms that the membrane system has a pay-back of two years. This is achieved by the combination of eliminating disposal of the whey and selling the whey protein concentrate.

It is difficult to present an accurate picture on the cost savings that occur in food processing when membrane separation (MS) technology either wholly or partially replaces traditional practices, because the factors contributing to costs are complex and vary widely with time and the given situation. However, Mullikin (1993); Abbot *et al.* (1979); Muir and Banks (1985); Jensen (1994) and Chavez (1995), report some benefits in using MS in the dairy industry such as making milk powder, the treatment of cheese whey, and cheese manufacture.

In another report Boer and Koenraads (1991) state that the application of liquid whey protein concentrate (WPC) for partial skim milk replacement in

dairy desserts and yogurts appears feasible and that the main reason for using WPC is to reduce the cost of the ingredients by replacing milk constituents. The economical advantage varies from country to country due to differences in the value of skim milk powder and the costs of electricity. On the other hand, the process costs of WPC can be reduced by reducing energy to concentrate the product.

The perspectives and expectations of ultrafiltration technology since commercialisation within the dairy industry have been many. The most important are a higher yield, in line systems, continuous processes, higher flexibility and new technologies and products. All of these have two things in common - economics and commercial viability. There is no point in changing a process or technology, if not to achieve a higher quality benefit or profit, Jensen (1994). Ostergaard (1986) mentions that ultrafiltration technology opens up new prospects for better utilisation of milk, providing for considerable reductions in milk consumption for the manufacture of products.

On the other hand, cajeta is a typical Mexican sweet spread, normally made from whole milk and similar to sweetened condensed milk, which is concentrated by heat. The ultrafiltration process may have a good advantages in reducing the processing time and preventing sandiness by lowering the final lactose content which is the most significant technological problem in cajeta production, reducing product acceptability (Sabioni *et al.* 1984a).

The major advantage of the UF-process is that it yields a higher protein and lower lactose milk ingredient with excellent nutritional and functional properties, (Lee and White 1991). In the concentration of milk by ultrafiltration, proteins are the ones providing good benefits to the new dairy products. In one

report Burgess (1987) mentions that the structure of casein makes it one of the most effective emulsifying and foaming agents of all the major food proteins and that its structure also gives it excellent water binding properties. Hofi (1989) reports that ultrafiltration can be used to vary the protein content in dairy products such as ice cream within a wide range, without adverse effect on their organoleptic properties.

On the other hand, Nijpels (1981) mentions that lactose in the human intestine has to be hydrolysed by lactase into glucose and galactose, otherwise it would not be digested causing gastrointestinal discomfort, i.e. abdominal pains, diarrhoea, flatulence etc. Renner and Abd El Salam, (1991) mention that in areas with a high prevalence of lactose malabsorption, there is a need for low-lactose milk, which could help in the treatment of protein-calorie malnutrition and which could also serve as the main food in such areas. By applying the UF-technique, up to more than 80% of the lactose can be removed from milk. The ingestion of 500 ml of this low-lactose milk gave rise to significantly fewer gastro-intestinal disorders than regular skim milk. Such a low-lactose milk may be of potential usefulness in the treatment of protein energy malnutrition in developing countries, where lactose malabsorption is highly prevalent. Nijpels (1978) mentions that lactose intolerance affects the following groups: some people from the moment of birth lack lactase activity, premature birth people, and people during the weaning period. Anonymous (1992) from Nestle states that adult type lactose intolerance is considered the world's most widespread genetic disorder.

In the production of dairy desserts, the UF-process has not been widely used. There are few reports of UF-Retentate replacing skim milk powder to supply MSNF in the production of ice cream (Kosikowski and Masters 1983;

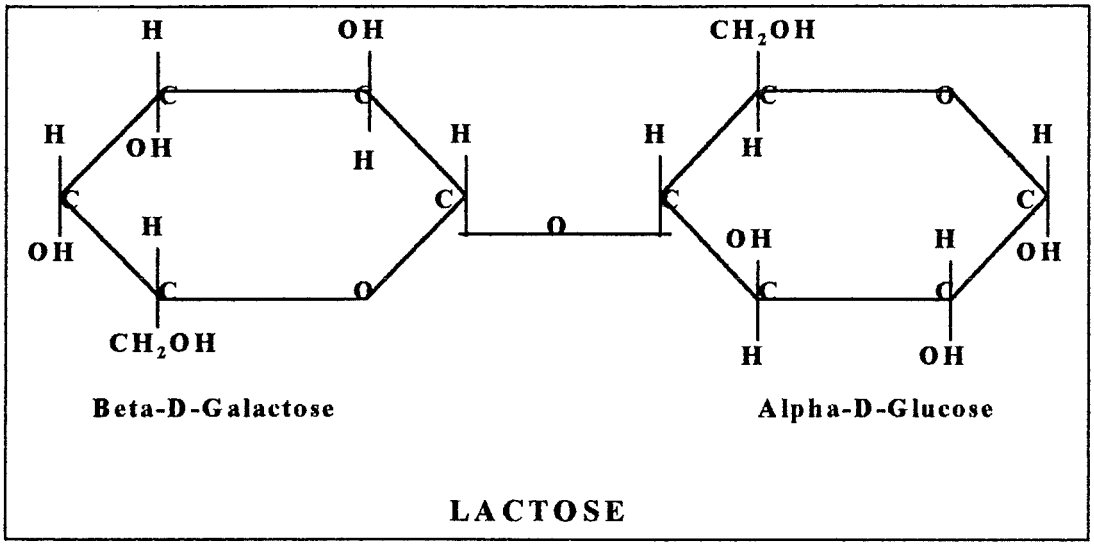
Jensen *et al.* 1989; Tong *et al.* 1989; Bundgaard, 1974 and Hofi, 1989), but not enough information is given to characterise the product. Consequently, there is a lack of scientific information, about the possibilities in obtaining frozen products where some nutrients such as protein and some minerals have been increased, and others such as lactose and sodium decreased.

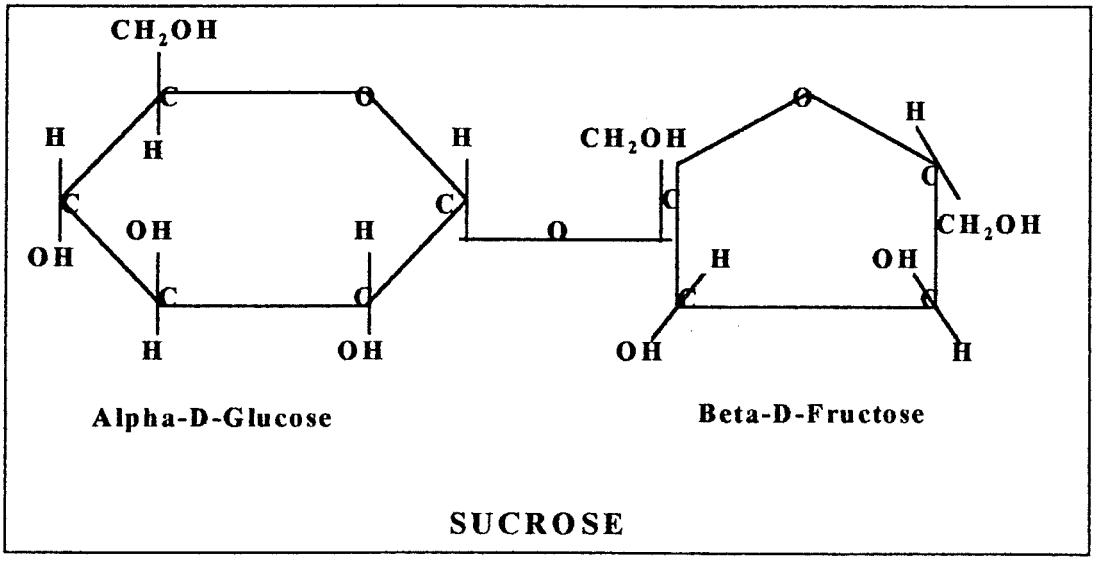
1.2 LACTOSE CRYSTALLISATION

Lactose is refered to as milk sugar. It is a disaccharide comprising glucose and galactose, and it occurs as two optical isomers, α-lactose and β-lactose (See Figure No 1.2 for lactose representation). The two forms have different physical properties, for specific rotation, melting point, hygroscopicity and, sensorially in intensity of sweetness. α-Lactose crystallises out of aqueous solutions at temperatures below 93.5° C with one molecule of water of crystallisation. β-Lactose is formed by crystallisation above 93.5° C. The β- form exhibits a specific rotation of $[\alpha]^{20}_{D}$ = +35.0° whereas the α-isomer shows a specific rotation of $[\alpha]^{20}_{D}$ = +89.4°, both on the anhydrous weight basis. All of these forms of lactose undergo mutarotation in aqueous solution, yielding a specific rotation of $[\alpha]^{20}_{D}$ = +55.4° (anhydrous basis) at equilibrium which requires 24 hrs at 20° C.

In crystallisation not only the properties of individual atoms and molecules but also the interactions between particles must be considered. A number of different bonds may be active in holding a substance in the ordered arrangement of a crystal. Covalently bonded molecules are held in the crystal lattice primarily by the relatively weak van der Waals forces. Another type of force that may be involved in the maintenance of crystal structure is by hydrogen bonds.

Figure No. 1.2 Representation of ring forms of lactose and sucrose *





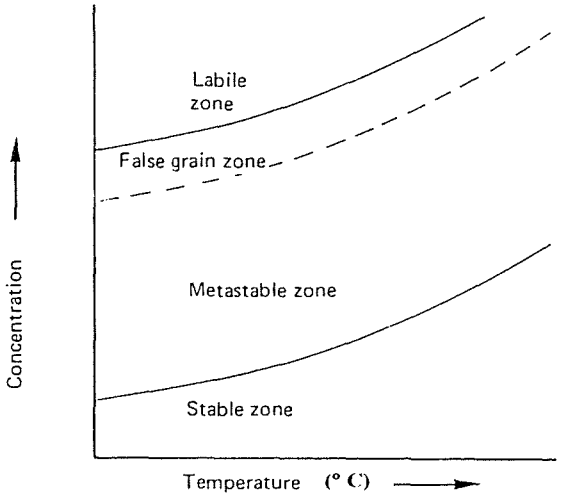
* Sucrose from Meade and Chen (1977), and Lactose from Muir (1990).

In crystallisation, the development of crystals requires the formation of nuclei and the continued deposition of molecules on these nuclei to form perceptible crystals. The formation and growth of crystals are influenced by the nature of the crystallising substance, the concentration, the temperature, rate of cooling, degree of agitation, impurities in the solution, nature of the container walls, and the size and characteristics of the sample.

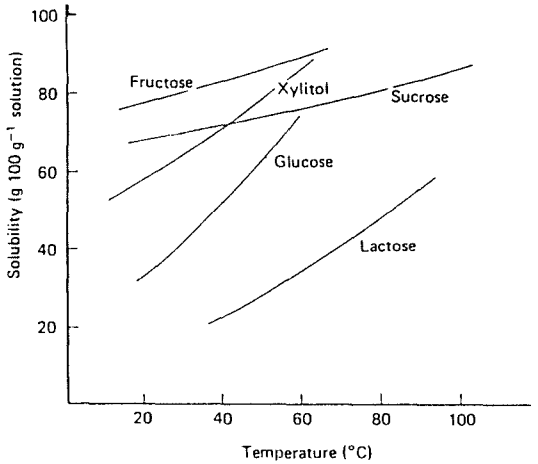
Some degree of supersaturation of a solution, or supercooling of a liquid, is required before crystal formation can proceed. Heat is generally given out on crystallisation so in the end the crystals must have a lower energy level. However, as molecules come together there will be an energy hump to be overcome. As the first few molecules come together to form the nuclei, there is an increase in free energy until the critical size is reached. At this point, further increase in size leads to a decrease in free energy, so the nucleus is stable and will continue to grow. A system that is supersaturated, but not sufficiently to generate nuclei, is called metastable (See Figure No. 1.3). Tutton (1924) mentions that within the metastable range, treatments such as seeding or agitation will lead to instant crystallisation. Seeding may be deliberate, as by the addition of crystals of the compound to be crystallised, or accidental from dust in the air.

Van Hook (1961) lists five steps involved in the crystal growth by addition of particles properly oriented to fit into the crystal lattice. One is the transport from the medium to the growing environment; two is the adsorption on the crystal surface; three is the orientation in the surface; four is the desorption of the products; and five is the dissipation of the products. The product of crystallisation is the heat of transition from liquid to solid, so steps four and five

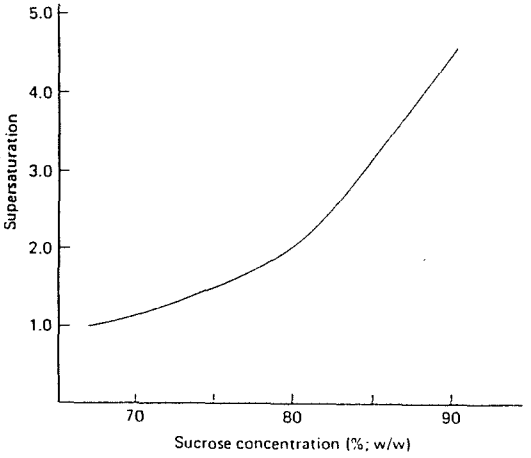
Figure No. 1.3 Crystallisation zones and solubilities of some carbohydrate
(Lindley, 1988)



A: Crystallisation zones for supersaturated solutions



B: Solubility of some carbohydrates



C: Relationship between supersaturation and concentration of sucrose

are concerned with removal of heat energy. The amount of heat generated may be considerable if crystallisation is rapid.

Supersaturation favours the development of small crystals. Nuclei form more readily in more concentrated solutions. The viscosity of a very supersaturated product delays crystal growth, since the thickness of the system hinders the transport of the solute particles from the medium to the surface of the growing crystal.

Bancroft (1920) mentions that the higher the temperature at which crystal formation is initiated, the coarser the crystals, and that the most favourable temperature for crystal growth in a saturated sucrose solution boiled to 112° C is between 70 and 90° C.

Stirring a solution favours the formation of nuclei and hinders the depositing of the material of the solution on the nuclei already formed producing more individual crystals. Stirring also helps to prevent formation of aggregates of crystal since in a system crystallising without agitation, neighbouring crystals may touch and grow together, forming perceptible masses, while stirring keeps the crystals in motion through the solution, producing more individual crystals. Reversible stirring is frequently used to keep lactose crystals in suspension because they have a density greater than water (e.g. whey crystallisation).

According to Paul and Palmer (1972), the shapes of crystals seem endlessly variable, but the forms follow certain principles dealing with axes, angles, and symmetry. The external form of crystals of the same material may vary depending on the conditions under which the crystals are grown. For example, one axis or face may grow more rapidly than another. Crystals may

grow together, or extend variously in different directions. This author mentions that polymorphism is another source of variation in crystal form. For example, crystallisation from supersaturated solutions of lactose below 93.5° C, yields α-lactose monohydrate and above 93.5° C, β-lactose is obtained.

The final solubility of lactose in water at 25° C is approximately 18% by weight. The initial solubility is that of the α-form. The increasing solubility with time is due to mutarotation (Nickerson, 1980). As some of the α is converted to β, the solution becomes unsaturated with respect to α, and more α-hydrate dissolves. When crystallisation is carried out above 93.5 ° C the crystals formed are of the β-anhydrous type. Under normal conditions the α-hydrate form is the stable one and other crystal forms will change to that form dictated by thermodynamic equilibrium requirement. At equilibrium and at room temperature the β-form is much more soluble and the amount of α-form is small. However, because of its lower solubility the α-hydrate will crystallise out and the equilibrium will shift to convert β into α-hydrate. This process continues until equilibrium is established between α and β in solution and no more α-hydrate can dissolve, giving the final solubility (See Figure No. 1.4).

Shear forces can cause local concentration increases, and hence induce growth. Hence shear can induce crystallisation in solutions that would not support crystal growth ordinarily. Crystal coarseness depends on the rate of crystallisation. Fast crystallisation produces fine crystals, slow crystallisation large crystals.

The solubility of lactose is less than that of most other sugars (See Figure No. 1.3) and this may present problems in a number of foods containing lactose. When milk is concentrated 3:1 the concentration of lactose approaches

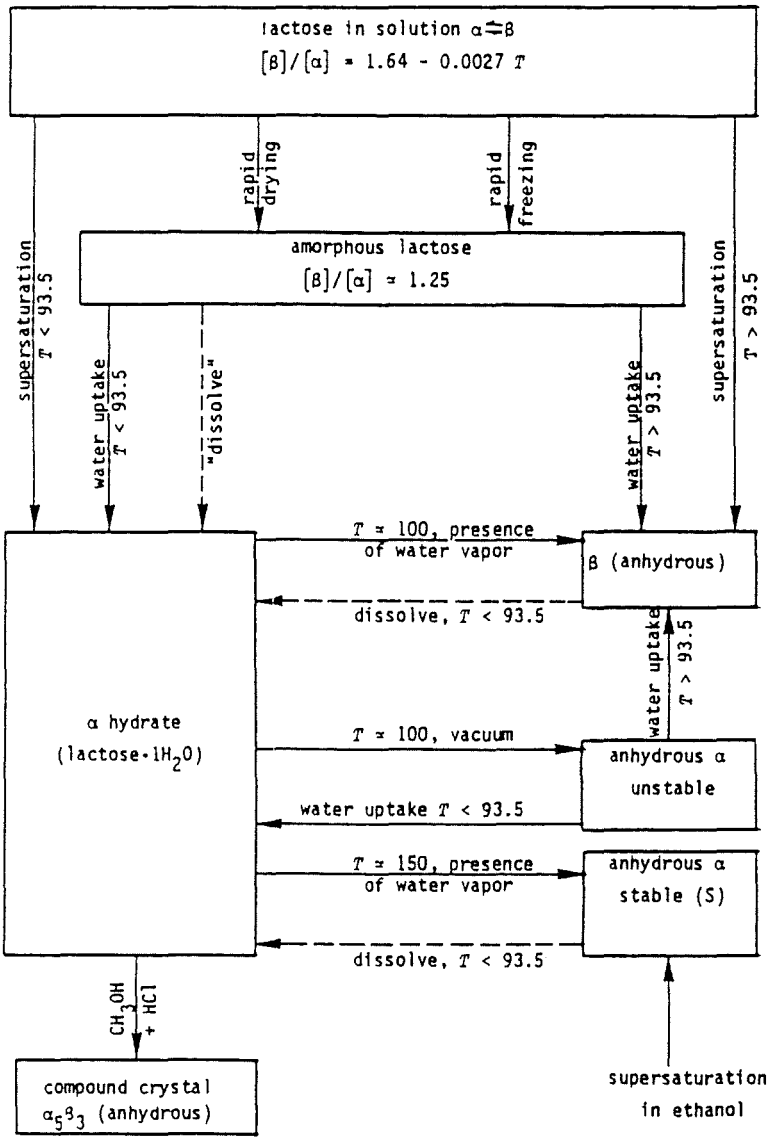


Figure No. 1.4 Modifications of Lactose (T = ° C)
Taken from Walstra and Jenness (1984)

its saturation solubility. When this product is cooled or sucrose is added, crystals of α-hydrate may develop. Such crystals are very hard and sharp and when left undisturbed may develop to a size at which they appear as a sensation of grittiness or sandiness. However, in products like whey powder, α-hydrate crystals are desirable because they are less hygroscopic than the β-form. As a consequence whey powder with a high proportion of the α-hydrate form of lactose does not cake very rapidly.

The crystals of α-lactose monohydrate usually occur in prism or tomahawk shape. Amorphous or glassy lactose is formed when lactose-containing solutions are dried quickly. The dry lactose is non crystalline and contains the same ratio of α/β as in the original product (De Man, 1980). On storage amorphous lactose may pick up moisture , depending on the packaging material used.

An important factor in the manufacture of confectionery products according to Dodson (1975) is the effect of one sugar on the solubility of another sugar.

In general it has been found that one ingredient tends to depress the solubility of another. However this author in the same article discussed the effect of lactose in solutions containing sucrose and concluded that the reduction in sucrose solubility is never more than 5% and also appears to be independent of temperature, but the effect of sucrose on the lactose solubility is positive at lower temperature.

Lactose differs from other commonly occurring sugars (e.g. Sucrose, fructose, glucose and galactose) by its reduced sweetness, extremely low

hygroscopicity of the α-lactose monohydrate and low solubility. According to Muir (1990) as a reducing sugar, lactose can react with free amino groups in milk proteins (Maillard reaction) under appropriate conditions of pH, temperature (above 100° C) and water activity (Maximum velocity of reaction at A_w= 0.3 - 0.7).

A number of food manufacturing processes involve a crystallisation operation in which no separation of the crystals is desired. Such operations occur in the production of frozen foods (including ice cream), sweetened condensed milk and certain sugar confectionery. A common requirement for such processes is that the crystals produced should be below a certain size (e.g. less than 15 μm for sweetened condensed milk). Doan (1958) mentions that lactose crystallisation often occurs during the processing of some milk products. Usually it occurs in the manufacture of condensed and dried wheys, and may take place in such products as ice cream, condensed, dry and frozen products.

The reasons for lactose to crystallise in all cases are either an insufficiency of water to hold it in solution under the prevailing conditions, or sufficient water to furnish a labile concentration when lactose is in the amorphous or glass state. Doan (1958) affirms that in the freezing of ice cream, the lactose solution apparently passes through the labile zone so rapidly, and at so low a temperature, that no opportunity exists for the molecules to diffuse and orient into crystal structures. However, the high viscosity of the unfrozen liquid is a crucial factor in this connection. The same author cites that when ice cream is warmed to, and held at, dipping temperatures or dispensing-cabinet temperatures, some ice melts, and there must be produced an infinite variety of lactose concentrations, over a period of time, as molecules diffuse slowly into

these water droplets from the amorphous glass. Some of the concentrations doubtless will be in the labile zone for the temperature, and permit spontaneous crystallisation; others will be in the metastable zone, where crystallisation can occur if a stimulus in the form of lactose crystal nuclei, or fine particles of extraneous matter exists.

In ice cream, the growth of objectionably sized lactose crystals will be promoted by storage, particularly at fluctuating temperatures, another factor is the amount of MSNF in the formulation. Zuczkowa (1970), mentions that in ice cream formulations MSNF should not exceed 12% and lactose concentration in the liquid phase of ice cream should not exceed 9%. If so then ice crystal growth is more likely to result and cause coarseness.

In the manufacture of sweetened condensed milk, which is similar to cajeta, according to Evenhuis and De Vries (1957); Doan (1958), water is removed by evaporation in the vacuum pan from the mixture of milk, sucrose and lactose. When cooled to room temperature, the remaining water in the condensed milk becomes heavily supersaturated in respect of lactose. This results in lactose crystallisation (See Figure No. 1.3).

During the processing of sweetened condensed milk and cajeta the temperature is normally above 93.5° C. Lactose crystals are not present due to the fact that at that temperature lactose concentration is below the saturation point. Doan (1958) mentions that when sweetened condensed milk (SCM) is cooled to 60 or 65 ° C, between two-fifths and two-thirds of the lactose present will emerge as crystalline α-lactose hydrate, this is because lactose is soluble to the extent of only about 15 parts to 100 parts of the water as found in the product. Choi (1958) mentions that the best temperature to crystallise lactose is

30° C. In SCM, there are 40 to 47 parts of lactose per 100 of water, and a composition made up of an equilibrium mixture of about 40% α form and 60% β form, as a result of mutarotation.

1.3 ICE CREAM

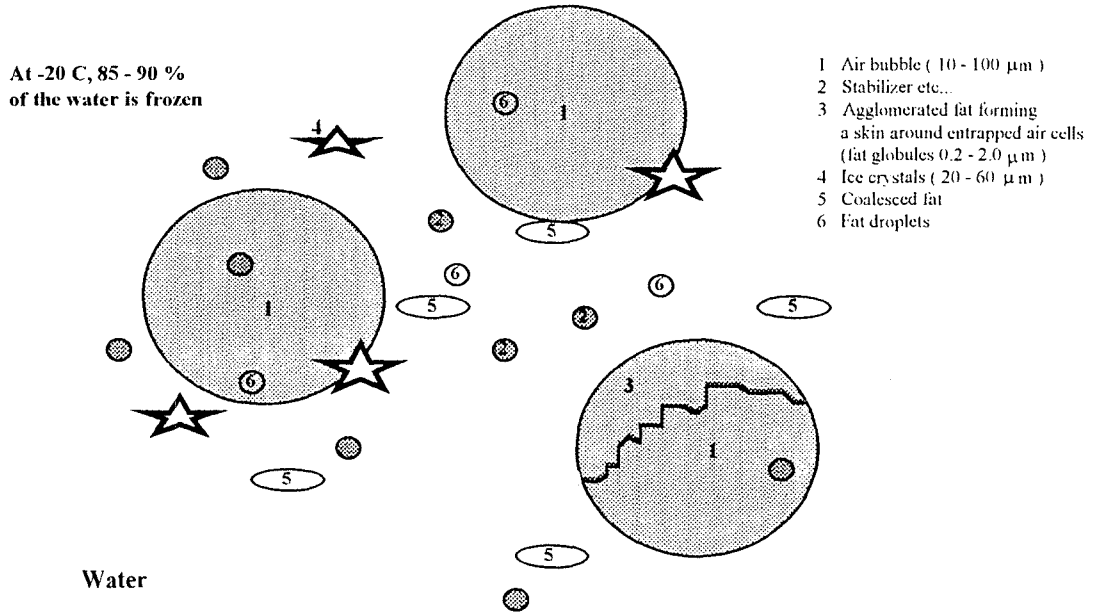
1.3.1 Introduction

Ice cream is a foam based product in which incorporated air is distributed in the solution-mixture as bubbles within the partly-frozen continuous phase of an oil-in-water emulsion. This serum phase also includes dissolved added solids such as sugars and salts in genuine solution, and colloidal elements such as proteins and stabilisers which, together with the fat globule agglomerates help to stabilise the air cells (Rothwell, 1991c). See Figure No. (1.5) for a drawn representation of ice cream microstructure.

The structure of the ice cream is developed in the freezing barrel. The mix is frozen to between -4° C and -6° C while the air is incorporated and distributed by means of dasher and scraper blades. The ice cream structure is completed by the hardening process, in which most of the water becomes frozen.

The size of the air cells depends on many factors such as, the composition of the mix, drawing temperature of the freezer, and the freezer design. The average size of the air cell is 60 μm although it can vary from 5 to 300 μm (Berger *et al* 1972), Rothwell (1991c) reports air cell size in the range of 10 to 150 μm, (See Table No. 1.5). According to Rothwell (1991b) if too much air is incorporated for the solids the ice cream may probably be weak and watery with

FIGURE No. 1.5 REPRESENTATION OF ICE CREAM MICROSTRUCTURE



poor body, while if the overrun is too low, there will not be enough ice cream for a given amount of mix.

Table No. 1.5 Dimensions of ice cream components

COMPONENT	SIZE RANGE	SIZE AVERAGE
Fat globules	0.2 - 2.0 μm	0.6 μm
Air cells	10.1 - 150.0 μm	60.0 μm
Ice crystals	10.0 - 75.0 μm	40.0 μm
Casein micelles	40.0 - 400 nm	100 nm
Casein sub-units		- 10 nm

Taken from Nielsen (1984b)

Ice cream mix composition, quality of mix components, production method, freezing operation, hardening and storage conditions are some factors that affect the final quality of the ice cream. The quality of ice cream is mainly determined by its flavour, body, texture and meltdown. These properties depend upon the dimensions of the ice crystals, the size, distribution and stability of the incorporated air cells, and the amount of frozen water.

1.3.2 Raw material

Ingredients used in ice cream can vary depending of the raw material available and the type of product to be made. Table No. 1.6 shows a standard ice cream target.

Table No. 1.6 Typical target for ice cream formulation

CONSTITUENTS	(%)
Fat	10.00
M.S.N.F	10.92
Sucrose	13.00
Stabiliser and emulsifiers	0.50
Total solids	34.32

a) Milk Solids Non Fat (MSNF).

They may be found in liquid whole milk, concentrated skim milk, skim milk powder, skim milk, evaporated milk, sweetened condensed milk, butter milk powder, whey powder, whey protein concentrate or other types of dried solids.

MSNF are composed of casein, albumin, globulin, lactose, salts and traces of other non fatty constituents such as inmunoglobulins and vitamins. Proteins bind water and make the ice cream more compact and smooth and thus tend to prevent a weak body and coarse texture; and minerals tend to carry a slightly salty taste which enhances the flavour of the finished ice cream. Blenford (1992), mentions that bound water is attached water to other substances creating a compact system which significantly affects both the eating and keeping qualities of food.

MSNF according to Rothwell (1992a) is necessary in a normal level (e.g. 10 to 11%) for a good ice cream. However, in excess it may make the ice cream sandy, as lactose may crystallise, however on the other hand,

Berger *et al.* (1972) mention that increasing MSNF lowers the freezing point of the mix and the amount of water present as ice at a given temperature, and this results in smaller ice crystals and larger intercrystal distances. Wilbey (1986) mentions that the optimum level of MSNF occurs at the ratio of 1 part of milk solids non fat : 6 parts of water. In another report (Wilbey, 1990) cites that higher water ratios give a watery ice cream, while lower water ratios will increase product costs and may increase the risk of sandiness defects as a result of lactose crystallisation.

Liquid milk and skim milk are very useful sources of this, but MSNF present in milk is not enough to supply the right amount, so another source of MSNF must be used, such as skim milk powder, (Rothwell 1992a). The amount of MSNF which would normally be present in a good ice cream will be of the order of 10 to 11 %. In a study carried out by Zuczkowa (1970) concluded that to prevent lactose crystallisation in ice cream, the MSNF content should not exceed 12 %. However, in recent years studies have been carried out (Jensen *et al.* 1989, Tong *et al.* 1989, Hofi, 1989, Lee and White, 1991 and Geilman and Schmidt 1992) using the ultrafiltration process to obtain ultrafiltered retentate to supply partial or full MSNF in ice cream formulations, and in none of the cases was sandiness analysed.

According to Hamilton (1990) MSNF increase the viscosity and the melting resistance of the ice cream. Higher MSNF give a smoother ice cream due to the proteins absorbing free moisture and holding it as water of hydration. This prevents the growth of large ice crystals which would give a coarse texture. Too high amounts of MSNF lead to sandiness due to the formation of lactose crystals which are extremely hard and feel rough on the palate. The milk

proteins mainly caseins help to emulsify the fat since they constitute part of the fat globule membranes.

b) Fat.

Fat is essential to give richness and mellowness to the flavour of the ice cream since it and other ingredients such as vanilla contribute with aroma compounds i.e. the fatty acids in fat and the natural flavour in the flavoring. According to Watts (1992) the flavour in food is primarily the result of natural organic compounds such as the fatty acids present in the lipid fraction. The more fat present the smoother is the ice cream: too much, however, will reduce its palatability. The normal range to be used in ice cream is from 8% minimum to 12 % maximum (Hamilton 1990). The fat is present in the ice cream mix as a fine emulsion, produced by homogenisation. The size of the fat globule in a properly homogenised mix ranges from 0.5 to 4.0 μm Berger *et al.* (1972).

The fat content and composition, as well as its distribution greatly influence the texture by restricting the growth of ice crystals through mechanical obstruction.

The best source of milk fat is evidently cream but butter can be used, however it must be unsalted. Butter oil or anhydrous milk fat (AMF) is a very good source of fat too.

c) Sugar.

Sucrose may be present in ice cream up to 16%. According to Kessler (1981), and Hamilton (1990) it increases the viscosity and the total solids

content thus, lowering the freezing point of the ice cream mix. It enhances the effect of aroma substances and improves the body and texture of the ice cream. The major source of sucrose is from cane or beet sugar and it is used because of its solubility and its high sweetening power. Other sugars are used, notably, glucose syrups, produced from corn. Dextrose is also used to a limited extent.

d) Stabilisers and Emulsifiers. (ST/EM)

Because ice cream is a very complex system (Jones, 1989), the use of stabilisers and emulsifiers is essential. To obtain the best texture it is essential that all ingredients are very well blended together and that when the product is frozen and stored, changes in properties will not occur. To prevent this and to ensure that the product is smooth, it is necessary to use a well balanced mix, to keep the ice cream frozen at a constant temperature of about -20° C, and to use a stabiliser and an emulsifier. They can be used separated or together and will vary according to the fat content of the mix.

The emulsifier according to Nielsen (1984a; Penny (1992; and Blenford (1993) is a product which, due to their hydrophilic-lipophilic properties, orientates to the interfacial layer between fat/protein and water. The primary effect of emulsifiers is related to their properties to de-emulsify the fat globule membrane formed during homogenisation. This de-emulsification is necessary in order that agglomeration and coalescence of the fat globules may take place during the processing in the ice cream freezer.

Emulsifiers are incorporated in the fat-protein complex formed on the fat globule surface during the homogenisation process where the number and thereby the surface of the fat globules is increased, according to Banks (1993) by

the order of 10 times. The quantity of natural phospholipoid membrane material will not be sufficient to cover the newly created surfaces, which have a far larger energy potential than previously, for which the system will try to compensate by attracting interfacial tension reducing substances, as in this case the emulsifier. With the addition of a small percent of emulsifier there is more than sufficient emulsifier present to cover the newly created fat globule surfaces with a mono-molecular layer (Nielsen, 1984b).

There are three types of emulsifiers: cation active, anion active and non-ionic substances. The ones used in ice cream are mainly non-ionic derivatives of natural fats.

The main functions of emulsifier on ice cream are:

a) Improve fat dispersability in mix
b) Promote fat-protein interactions
c) Control fat agglomerations and coalescence
d) Facilitate air incorporation
e) Impart smoother texture and consistency
f) Improve resistance against shrinkage
g) Delay melting

Stabilisers are hydrocolloids, long carbon chain polymer substances which, when dispersed in water, gradually hydrate, whereby a large number of water molecules are bound primarily by means of hydrogen bonds. A three dimensional network is formed due to intra-and inter-molecular links between several stabiliser molecules in combination with protein, so that the mobility of the residual aqueous phase is limited. Flack (1991) mentions that stabilisers are used due to their influence on the mobility of water, partly through their ability

to form hydrogen bonds and partly because they form a three dimensional network throughout the liquid. The water binding effect improves the storage stability of the ice cream and retards the development of an icy texture if temperatures fluctuates during storage.

The functions of stabilisers are to:

a) Increase mix viscosity (Interact with proteins)

b) Increase air incorporation

c) Consolidate body and texture

d) Retard ice crystal formation and growth

e) Inhibit syneresis during melting

f) Increase water binding properties

1.3.3 Mix preparation and ageing

a) Formulation and preparation of the mix.

Ingredients of good quality are calculated and then weighed and additives must be dissolved in water or emulsified in the fat phase and then all the ingredients are blended together. In this study a basic formulation was created using a computer spreadsheet software program Excel™ version 5. It was based in the principle of the serum point method recommended by Hyde and Rothwell (1993).

b) Heat treatment

The mix is heat treated to comply with the legislation and to prevent bacterial growth. According to Hamilton (1990) the pasteurisation of the ice

cream mix should be carried out at not less than 71.1° C for at least 10 minutes, or not less than 65.5° C for at least 30 minutes or not less than 79.4° C for at least 15 seconds. The mix has to be cooled preferably to below 4° C to prevent bacterial growth until it is frozen.

c) Homogenisation

Homogenisation can be done before final heat treatment. It is carried out to break down the fat into smaller globules and disperse it more uniformly in the mix. After homogenisation the mix must be cooled to prevent bacterial growth and for fat crystallisation to begin.

The fat in the ice cream is divided in the form of very small fat globules by using mechanical means. This produces an emulsion which is stabilised by the new formed small globules and to ensure a good stable emulsion an emulsifier is needed to be added to get a mix which is well mixed and homogeneous. Caseins play an important role as an emulsifying agent (Rothwell 1993b).

During homogenisation the fat is stabilised in an emulsion, but it should not be too strong as some de-emulsification (fat agglomeration) is desired during freezing. The emulsifier, which is found on the fat globule interface, will destabilise the fat emulsion so that fat agglomeration can take place.

d) Ageing

After pasteurisation and homogenisation, the mix is cooled in a plate heat exchanger to 3 - 4 °C and left for ageing during no less than three hours (Rothwell, 1991a). During ageing, the following processes take place:

1. Hydration of milk proteins
2. Complete hydration of stabilisers
3. Crystallisation of liquid fat
4. Protein desorption

When the ice cream mix is stored for ageing, the physical structure of the casein micelles changes gradually, leading to the creation of more hydrophilic molecular structure. This change in the casein micelles continues during the ageing period, and full hydration of the casein micelles may be achieved in two or three hours. During pasteurisation of the mix some denaturation of the whey proteins takes place and the partly denaturated whey protein will have a water binding effect which will reach a level similar to that casein, i.e. 3 g water/g protein. At this stage milk proteins are dispersed in the aqueous phase and are absorbed on the fat surface globules. Many fat globules are disrupted and have coalesced to partially support the structure and to segregate water droplets in the interstices between them (Morely, 1989). The membrane should be strong enough to stabilise the fat, but weak enough to undergo disruption subsequently. The fat globule and its membrane are believed to consist of a core of liquid fat surrounded by the crystallised triglycerides. Emulsifiers and proteins are then layered around this core.

1.3.4 Freezing and hardening

According to Rothwell (1992c) this involves the change of state of the water from a liquid to a solid, also a partial churning of the fat emulsion, and also air is incorporated to increase the ice cream volume (overrun).

According to Rothwell (1991b) during the freezing process the temperature of the mix is reduced at the same time as the air is whipped into the ice cream. The speed of freezing depends on the amount of soluble sugars, protein and milk salts in the mix, and these depress the point at which the mix begins to freeze to about -2° C or -3° C. Pure ice crystals only begin to be formed when the temperature gets lower than this, then as the water is removed as ice, the remaining liquid becomes more concentrated and the freezing point is further, and progressively lowered. The average ice crystal size normally is about 40 μm in a satisfactory product.

The temperature of the mix has to be reduced continuously until the freezing process is completed. During freezing of the mix, the fat globule membranes are disrupted due to the combination of aeration, cooling and agitation, thus free fat will flow out of the core of the globules and will coat and therefore stabilise the air bubbles (Diamond *et al.* 1988). The air bubbles will be further stabilised by unagglomerated fat globules surrounding the coating of free fat. Coalescence of these fat globules does not occur because of the crystallised fat. The movement of air bubbles is obstructed by the presence of ice crystals and the very viscous nature of the unfrozen aqueous phase. Nielsen (1984a) mentions that the degree of the disruption or the de-emulsification of the membranes is mainly influenced by the type of emulsifying agent, the dosage, and to a minor extent by the relation between fat/msnf and drawing temperature.

An emulsifying agent where the lipophilic part dominates the hydrophilic characteristics, will have far more affinity for fat than for water and thus cause a more moderated de-emulsification. This partial disruption or de-emulsification of the membranes is essential for the texture and consistency of ice cream, because disruption facilitates the agglomeration and coalescence of the fat globules. This agglomeration and coalescence is considered to be the first step in an actual churning out of the fat phase. All these three stages of fat dispersions - agglomerated, coalesced and churned out fat globules - are present in ice cream, and they are considered important as these intermediate stages allow for squeezing out of the liquid fraction of the fat which envelops the fat clusters, and stabilises the air/serum interface of ice cream.

The freezer may be vertical or horizontal; batch or continuous and every one has its own limitations. Horizontal freezers normally give high overruns and have greater holding capacities than vertical freezers. Once the ice cream is made, it has to be stored in a cold room (-20°C to -30°C) until it is dispatched. During this process the proportion of frozen water increases.

1.4 CAJETA

1.4.1 Introduction

Cajeta is a typical Mexican sweet, (Dulce de Leche in some Latin American countries) similar to sweetened condensed milk. This product has been produced mainly in Argentina, Brazil and Uruguay (Sabioni *et al.* 1984a). However Mexico is probably the only country in Central and North America producing this product, which is marketed in Mexico and USA.

The manufacture of cajeta in Mexico is done mainly using goats milk, however cow's milk is also used. According to Anon. (1994) from The Cheese reporter milk production is estimated at 10.7 million tons in 1993. Mercado (1982) mentions that the production of goat milk accounts for a very important income for goats dairy farmers, in 1980 it totalled 279.7 million litres and from this figure 25% was consumed as liquid milk, and the rest for cheese and cajeta manufacture. The Cheese reporter of USA, Anonymous (1993b), reviewed some aspects about the North American Free Trade Agreement (NAFTA) mentioning that Mexican goat's milk cajeta has an immediate tariff-free access to USA. It is therefore, one of the few products which contribute external income for the Mexican dairy industry.

Cajeta is made by concentrating milk by evaporation at atmospheric pressure in the presence of added sucrose and some glucose, Table No. 1.7, shows a typical standard recipe for cajeta manufacture. It is used as a dessert or as a confectionery ingredient. Sucrose is normally partially replaced by glucose to prevent crystallisation. Sodium bicarbonate is added to increase the pH in order to prevent coagulation of proteins and to increase the browning reaction. The high solute concentration of cajeta results in a water activity (a_w) usually ranging from 0.80 to 0.85.

Due to the prevailing conditions during preparation nonenzymatic browning reactions occur extensively, leading to a brown-coloured product which has a characteristic and pleasant flavour (Ferramondo *et al.* 1984). Flavouring compounds can consist of over 50 different chemical substances representing a number of reactive groups, and therefore it should be expected that chemical reactions take place between the components.

Table No. 1.7 Typical formulation for cajeta manufacture

INGREDIENT	(%)
Milk	100
Sucrose	20
Glucose Syrup *	2
Vanilla	Flavouring
Sodium Bicarbonate	To get pH 7.0

* Added at 10% on sucrose base

Holmes (1970) tried to explain odour and flavour formation in a foodstuff and reports that, chemical reactions between sugars and amino acids occurs most readily in concentrated aqueous solutions and is favoured by high pH and high temperatures. The aroma produced by heating model amino acid-sugar mixture are composed mainly of reactions between glucose, fructose, maltose and sucrose with some amino-acids. The chemical structure of toffee, which is similar to dulce de leche consists mainly of sugars, milk proteins, and fat. The fat contributes to the texture, and in some extent to the flavour. The sugars in this case which are sucrose and lactose do not contribute to the caramel flavour or to darkening. Levulose darkens the product and gives an acid flavour. Dextrose is the one producing the brown colour and the toffee flavour (Holmes, 1970; Hunziker, 1934), but brown coloration becomes more pronounced in the presence of an alkali, such as carbonates. Casein and maybe albumin are capable of reacting with dextrose to give rise the toffee flavour. Holmes believes that the compounds responsible for toffee flavour are produced initially by a reaction between casein and corn syrup and that this complex is then thermally degraded to yield some highly-nonpolar and volatile compounds which probably has moderately low molecular weight. It is possible that effect of the casein

depends on the amino acid composition of the protein. Lewis (1990a) mentions that the basic structure of toffee is fat droplets and proteins dispersed in a glassy sugar matrix, which may be similar in cajeta but the matrix will be less viscous since the moisture content is higher in cajeta.

1.4.2 Raw Material

a) Milk solids

In cajeta manufacture the main ingredient supplying the solids normally are either whole milk, or skim milk, however concentrated milk may be used to reduce the boiling processing time. The presence of milk solids according to Lees and Jackson (1992) in caramelised products cause the product to be different in its properties to other type of confectionery mainly on texture, flavour and colour. The higher level of milk solids present in caramel, the harder will be the caramel, casein being the component which contributes hardness.

The function of milk protein in cajeta, a toffee like product is complex according to Stansell (1990). Apart from the reaction with reducing sugars to provide the characteristic flavour and colour, which is apparently specific to milk protein, it also stabilises the emulsion of fat in the sugar phase possible binding some of the water.

The function of the fat is to provide chewing characteristics on the product good texture, colour and flavour. Low fat levels tend to produce products which are sticky and difficult to chew and when high fat is used without the addition of emulsifier it leads to oiling on the surface of the confection (Lees and Jackson, 1992).

b) Sucrose

According to James (1990) sucrose is one of the basic ingredients for classical sugar confectionery. It is a disaccharide, can be broken down into a mixture of two mono-saccharides, known as dextrose (glucose) and laevulose (fructose) (See Figure No. 1.2 for sucrose representation), by inversion which is promoted by the action of acid, heat and mineral matter. Sugar is readily soluble in water, and at room temperature one part of water will dissolve two parts of sugar (67%). The solubility rises to 83% at 100°C. When sugar is present in a solution, together with invert sugar and/or glucose syrup, a higher total concentration of the mixed sugars can be achieved than may be obtained with the individual sugars alone (Fabry 1990). In sugar/invert sugar mixtures, above the range 76 - 78% total sugars there is the likelihood of dextrose crystallisation. However, stable solutions at much higher concentrations can be achieved when using regular glucose.

Sucrose inversion in sweetened condensed milk under normal processing conditions might occur, but it is highly improbable (Hunziker, 1934). Thus, dulce de leche being similar to it has the same probability of sucrose inversion

c) Glucose

Alternative sugars such as glucose, are generally used to replace a proportion of the sucrose in confectionery product in order to modify the sweetness and/or textural properties (Pepper 1990). The mono-saccharide glucose (dextrose) occurs widely in nature where it is found, together with fructose, in most fruits and in honey. It can be obtained from starch by enzymatic hydrolysis or alternatively

may be produced from sucrose by hydrolysis (inversion) to its constituents glucose plus fructose, followed by separation.

Glucose is commercially available in either monohydrate or anhydrous form. The monohydrate form, containing about 9% water, is most commonly used in the confectionery industry and the anhydrous form in the chocolate manufacture.

Glucose has a lower sweetness, lower solubility and lower viscosity than sucrose (See Figure No. 1.3). It is a better humectant and provides better preservative properties owing to its lower water activity. Since it is a reducing sugar, glucose is more reactive than sucrose. Glucose solutions have a greater tendency to browning on boiling (particularly between pH 5 and 6) and participate more readily in the Maillard reaction with proteins. The use of glucose and other sugars in sweetened condensed milk according to Hunziker (1934) has a positive effect in preserving the product because osmotic effects inhibit microbial growth.

In cajeta manufacture replacement of 5-15% of the sucrose with glucose will have the effect of lowering the overall crystal size and/or smoothing the confection. It will also increase the tendency to crystallisation during manufacture (Pepper, 1990).

On the other hand, glucose syrup has long been used to supply glucose, to replace part of the sucrose in the formulations due to the fact that sucrose solubility in formulations can only give 67.1% w/w at 20°C, so if the product is intended to be concentrated to above 70% w/w it has to be used. Another reason is that in cajeta manufacture, lactose is already present in the milk and if sucrose

is added the supersaturation point is then reached causing the crystallisation of the lactose giving grains to the product so with the presence of glucose syrup much higher solids can be obtained before saturation is achieved (Howling and Jackson 1990). Additionally glucose syrup has an influence on the plasticity of the product.

1.4.3 Cajeta manufacture

A standard initial formulation according to Hough *et al.* (1990) is 10 parts of milk and 2 parts of sucrose. A typical Mexican recipe would be a quantity of milk, and 20% of sucrose and if glucose is added it should be at 10 % of the sucrose weight, but it should be subtracted from the original sugar weight (See Table No 1.7). The sucrose and the glucose in sweetened condensed milk according to Hunziker (1934) should be added to the milk when it is hot (40 to 50° C) in order to dissolve the sucrose in the solution, and this applies in cajeta. This is concentrated to about 70% total solids by boiling at atmospheric pressure, then the cooling process should be done very quickly in order to promote a very uniform crystallisation down to be packed at 50 ° C. Caric´(1994) mentions that sometimes $NaHCO_3$ is added for acidity correction. However the use of neutralizants in cajeta manufacture are essential based on the fact of that in milk the acidity is in the range of 0.14 to 0.18% lactic acid equivalent, so when the mixture is evaporated, lactic acid is concentrated and may cause protein coagulation, if not neutralised.

Glucose syrup is used as a 'doctor ' to replace some of the sucrose used in order to diminish the development of sandiness (Pepper, 1990).

Chemical compositional standard for traditional "dulce de leche" is: moisture, maximum 28.0%; milk solids 26 % (Lactose 10%; Proteins 7 %; Fat 7.5 %; ash, maximum 2.0%) and sucrose 44%.

The high solute concentration of dulce de leche, results in a water activity (a_W) usually below 0.85 (Ferramondo *et al.*, 1984), which constitutes the main preservation factor in this product. The stability of dulce de leche to bacterial spoilage at room temperature is well known even under household conditions. However yeast and mould growth may occur when the product is stored at room temperature for long periods of time.

One of the major problems facing the cajeta industry is that there is no accurate technique to measure the endpoint of the cooking process. Refractometry has been use for this purpose, but it has some disadvantages because it is affected mainly by temperature and bulk temperatures during cajeta processing are in the range of 94 to 98° C, with temperatures higher at the heating surface. The corrections recommended by Kirk and Sawyer (1991), and the manufacturers leaflet do not reach that point. Hough *et al.* (1988), have tried to develop a technique to solve this problem, however, Moro and Hough (1985) studied the relationship between solids by oven drying and refractometric solids at 20° C. They found that those variables are correlated, however no easy technique to be used in the cajeta industry was developed.

1.4.3.1 Evaporation

The evaporation of the mixture is carried out checking the temperature of the product in order to add the sucrose and the glucose syrup, and to monitor the

holding temperature which is in the range of 94 to 98° C. This is because if the temperature exceeds 100° C, foaming will occur.

1.4.3.2 Sandiness

The most relevant technological problem in dulce de leche production concerns its physical stability as related to prevention of lactose crystallisation. Crystallisation causes a sandy texture and lowers product acceptability (Sabioni *et al.* 1984a); Caric' (1994).

According to Hough et al (1990) lactose crystallisation in dulce de leche is inevitable due to the fact that in a milk with 12% total solids and 4.5% lactose, lactose concentration in dulce de leche is 9.85 g/100 g, and considering the water phase, the lactose concentration is 33 g/100 g water. Solubility of lactose at 15 and 30° C is 16.9 and 24.8 g/100 g water, respectively. Thus, even without interference, lactose in dulce de leche is initially in a supersaturated solution, and this is compounded by the simultaneous presence of sucrose (146 g/ 100 g water), which substantially reduces lactose solubility.

Sandiness in dulce de leche is caused by high concentration of lactose. Lactose crystals in concentrated dairy products such as condensed milk and dulce de leche may cause a sandy texture and reduce consumer acceptability. Crystals tend to aggregate and alter the physical character of the product. Under normal conditions for dairy products, alpha-lactose monohydrate is the major determinant of the nature and degree of crystallisation (Nickerson and Moore, 1973).

According to Hough et al (1990) sandiness can be prevented, by reducing the lactose content in dulce de leche. With crystal size below 6μm sandiness is not detected, even if all lactose in dulce de leche is crystallised. Above this size, the detection threshold depends on number of crystals. In Sweetened condensed milk according to Buyze (1952) the acceptable size of the lactose crystal is 10 to 20 μm.

Some efforts in Argentina and Brazil have been made to control sandiness problem using different methods, such as seeding the product with lactose and by enzymatic means but the latter seems to be costly (Martinez *et al.* 1990, Sabioni *et al*, 1984a, and Sabioni 1984b). Seeding apparently is a good technique to force crystallisation in condensed milk (Buyze, 1952). But according to Sabioni *et al.* (1984a), Martinez *et al.* (1990) dulce de leche industries face certain technical difficulties in the application of this technique, such as controlled cooling and proper seeding techniques, in addition, it increases total operation time and induces air bubble formation in the product due to agitation and product contamination. There are two brief reports, Christiansen *et al.* (1987) and Edelsten *et al.* (1987) using UF-process for the production of Dulce de Leche where sandiness was prevented, however, no more information is given. Caric' (1994) mentions that ultrafiltration can be used to prevent lactose crystallisation. In another report Martinez *et al.* (1990), mention that in sweetened condensed milk, this defect is prevented by seeding with lactose microcrystals. Seeding has not been used in dulce de leche due to its high viscosity and due to contamination problems at the recommended seeding temperature (30°C), and also they mention that UF-Technology is not economically feasible in Argentina.

1.5 Research objectives.

It has been established that UF-processes may be adopted as a new technology to be used in the food industry, and has current applications in the dairy industry.

Nevertheless, little data are available on the application of UF-process in ice cream and far less in cajeta manufacture, where it may offer good possibilities in improving the general characteristics of the products. With this in mind, this study was undertaken in order to:

1) Study the applicability of UF-process to provide UF-Retentate to substitute for skim milk powder in the manufacture of ice cream.

a) Obtaining a product concentrated in protein and low in lactose.

2) Study the applicability of UF-process to provide UF-Retentate to substitute for whole milk in the manufacture of cajeta.

a) Decrease in the processing time in cajeta manufacture

b) Reduction of sandiness problem in cajeta.

CHAPTER TWO

MATERIALS

AND

METHODS

CHAPTER TWO: MATERIALS AND METHODS

2.1 Raw Material

2.1.1 Ice Cream

2.1.1.1 Skim milk (SM)

Whole milk from The SAC-Auchincruive farm, was separated in a fat separator No. 27914 (L' Electro Ecremeuse. France) to provide skim milk (Fat content 0.1%) for the ultrafiltration process.

2.1.1.2 Skim milk Powder (SMP)

Skimmed milk powder (Medium heat, heat number 81) with moisture content of 3.0%, protein 36.0%, fat 0.7% and lactose 52.3%, ash 8.0%, total solids 97.0 and a solubility index of 0.2 ml. It was used for the production of the ice cream control. The skim milk powder was obtained from A. N. Garrett & Co. Ltd. Bristol, U.K. in 25 kg bags and stored in a cold place.

2.1.1.3 Ultrafiltered Retentate (UF-R)

Ultrafiltered retentate from the ultrafiltration of skim milk was used in this section to supply MSNF for ice cream manufacture (For chemical composition see Table No. 3.9 of Ice Cream Chapter).

2.1.1.4 Ultrafiltered Permeate (UF-P)

Ultrafiltered permeate from the ultrafiltration of skim milk was used to standardize the UF-Retentate in ice cream manufacture (For chemical composition see Table No. 3.9 of Ice Cream Chapter).

2.1.1.5 Butter

Butter (Fat content 82% (min.), and moisture 16% (max.) and salt plus curd (2.5 to 3.0 %)) was used to supply the fat in the ice cream formulation. It was supplied by Food Science and Technology Department at SAC-Auchincruive, Ayr, Scotland, U.K.

2.1.1.6 Sucrose

The sucrose in this study was obtained from Tate & Lyle Thames Refinery, London, U.K.

2.1.1.7 Stabiliser and Emulsifier

The combined stabiliser and emulsifier type Velpeco 164. It is composed by emulsifier E471 (Mono- and di-glycerides of fatty acids), stabilisers E466 (Carboxymethylcellulose, sodium salt), E407 (Carrageenan), acidity regulator E450-a (trisodium diphosphate) and fat content of 66.5%. It was obtained from Pritchitt Foods. Kent, U.K.

2.1.1.8 Vanilla

The vanilla flavoring P6A used in ice cream manufacture was supplied by The Rayner Essence Group Ltd. London

2.2 Cajeta

2.2.1 Whole milk

Whole milk in this section was used for cajeta manufacture and for the ultrafiltration process to obtain UF-Retentate to be use in UF-cajeta manufacture. It was obtained as described in 2.1.1.1

2.2.2 Ultrafiltered Retentate

Ultrafiltered retentate was obtained from the ultrafiltration of whole milk and the equipment is described in section 2.3.1 of this chapter.

2.2.3 Ultrafiltered Permeate

Ultrafiltered permeate was obtained from the ultrafiltration of whole milk and it was used to standardize the UF-Retentate in the cajeta manufacture

2.2.4 Sucrose

Sucrose was obtained (as described in 2.1.1.6)

2.2.5 Glucose Syrup

The glucose syrup used in this section was GL-01132 type, recommended for use in the sugar confectionery. It has dextrose equivalent of 40, dry matter 80.0%. With a carbohydrate composition of dextrose 18.0%, Maltose 15.0%, Maltotriose 13.0%, and High carbon sugars 54.0%. It was obtained from Cerestar UK Ltd., Manchester, U.K.

2.2.6 Sodium hydrogen carbonate

The sodium hydrogen carbonate, with purity of 99.5% was supplied by BDH Chemicals Ltd. Poole, England.

2.2.7 Vanilla

Vanilla for cajeta manufacture was obtained as described in section 2.1.1.7.

2.3 Equipment and Utensils

2.3.1 Ultrafiltration Plants

In the preliminary ultrafiltration of milk trial, a Pellicon Millipore ultrafiltration unit supplied by Millipore Corporation, Bedford, U.K. with a regenerated cellulose membrane, (molecular weight cut-off of 30,000 Daltons) supplied by the same company was used to ultrafilter the milk.

In the ultrafiltration of skim milk for ice cream and whole milk for cajeta manufacture a pilot-scale ultrafiltration unit type UFP No. 2979625 was used. It was supplied by Alfa-Laval A/B, Lund, Sweden. The membrane was a hollow fibre, PM 50 type. The fibre internal diameter was 1.5 mm and effective surface area 1.3 m^2, with a nominal molecular weight cut-off of 50,000 Daltons. It was supplied by Romicon Inc. Massachusetts, USA. The inlet and outlet pressure were 0.15 and 0.12 MPa (gauge) respectively

2.3.2 Batch Pasteuriser

A large scale steam-heated water bath was used to pasteurise the 5 l batches of ice cream mix in buckets at 72° C for 10 min. Pasteurised mixes were homogenised and cooled to 4° C with cold water in a sink with stirring of the mix.

2.3.3 Homogeniser

An homogeniser model Lab 4746/72 (Rannie Machine Works Ltd., DK-2620 Albertslund, Denmark) was used for the homogenisation of the ice cream mixes at 14 MPa at 72° C.

2.3.4 Freezer

A vertical freezer with capacity of two litres mix was used to make the ice cream (T. Giusti & Son Ltd., London U.K.)

2.3.5 Boiling open pan

A steam-jacketed open boiling pan with capacity of 10 litres was used for the evaporation and concentration process in cajeta manufacture. (Brierley Collier & Hartley Equipment Ltd., Rochdale, England.)

2.3.6 Measuring Instruments

2.3.6.1 Thermometer

In all the trials in this study a portable digital thermometer Testo 900 (Testoterm Ltd., Hampshire, U.K.) was used for temperature monitoring. With a resolution of 0.1° C (up to +199.9° C) and 1° C (above +200° C) and accuracy of ± 0.5 ° C (up to + 100° C).

2.3.6.2 Scale

Two scales were used for the weighing of ingredients in ice cream and cajeta manufacture (Type 3901 AAG., W. & T. Avery, Birmingham, England) for large quantities with accuracy of ± 2g. An electronic digital (OHAUS 1-10, serial 13118. London, U.K.) with accuracy/linearity of 0.001% of full scale capacity, for small quantities, and another special scale No 5-50387 with a chart marked by 0.01 lb. divisions (W. & T. Avery Ltd., Birmingham, England) for overrun determination in ice cream manufacture.

2.3.6.3 pH-meter

A portable pH stick meter model PHK-120-B (Gallenkamp Express, Leicestershire, U.K.) with automatic temperature correction, was used to measure the pH value in milk and UF-R in cajeta manufacture. Calibration buffer was used for pH 7 and 4, with a level of accuracy of 0.2 pH.

2.3.6.4 Sugar refractometer

A refractometer with a range of 50 to 80% of sugar was used in order to check the sucrose concentration level of cajeta. It was supplied by Bellingham & Stanley, Ltd., London, England.

2.4 Analysis of Raw Material

2.4.1 Skim Milk Powder (SMP)

2.4.1.1 Fat Content

Fat content of the skim milk powder was determinated by using the method (IDF, 1987a) which is based on the Rose-Gottlieb method. It is based in the principle of extraction of an ammoniacal ethanolic solution of a test portion with diethyl ether and light petroleum, removal of the solvents by vacuum evaporation (vacuum oven supplied by Gallenkamp, U.K.) at 90° C for one hour, and determination of the mass of the substances extracted which are soluble in light petroleum. The sample weight was 1.5 g for skim milk powder.

2.4.1.2 Total Nitrogen Content

The total nitrogen content of skim milk powder, (expressed as percentage of protein) were determined according the method recommended by the IDF:1993, which is based on the wet combustion of the sample by heating at approximately 350 ° C with a mixture of concentrated sulfuric acid, and copper tablets (BDH Chemicals Ltd., Poole, England) instead of mercuric oxide as catalyst, to effect the reduction of organic nitrogen in the sample to ammonia in the form of ammonium sulphate. The digest is distilled to release the ammonia which is trapped and titrated in the Micro-Kjeldahl unit.

1 ml, 0.5 ml and 3 ml of milk, ultrafiltered retentate and ultrafiltered permeate respectively with 10 ml of sulfuric acid and 2 tablets of copper catalyst

were digested in a Kjeldahl digestion tube placed in a block-digestion apparatus for one and half hours. Distillation and titration was completed approximately within one minute in the micro-Kjeldahl unit. The digital reading given by the micro-Kjeldahl apparatus was used in the following formula:

$$\% \text{ PROTEIN} = \frac{(\text{Reading sample} - \text{Blank reading})\ 178.7}{\text{Sample weight (mg)}}$$

2.4.1.3 Total Solids Content

Total solids in skim milk powder was determinated using the method of (BSI :1968b) by weighing 3g (± 1 mg) of sample on a AE 166 balance (Mettler Instruments Ltd., Buckinghamshire, U.K.) and dried at 102° C for 2 hour in a hot air oven to a constant weight.

2.4.1.4 Ash Content

Ash content of skim milk powder was determinated according to the method of (BSI:1988) by drying 2 g (± 1 mg) of sample, charred and ashed at 550°C using a muffle furnace (Baird & Tatlock, London, U.K.) to constant weight.

2.4.1.5 Determination of the heat number

The heat number method is preferred to the traditional whey protein index, because the latter is influenced by factors such as the cow nutrition, breed and state of lactation, as well as the degree of heat treatment.

The heat number of skim milk powder was determined according to the method (IDF, 1982). The principle is based on the casein plus heat-denaturated milk-serum protein in a certain volume of

reconstituted dried milk precipitated at a final pH of 4.8 by adding acetic acid solution (10%) and then sodium acetate solution (13.60%). The precipitate is collected and washed, and its nitrogen content is determinated by the Kjeldahal method. The total nitrogen content of the same volume of the reconstituted dried milk is similarly determined using the following formula.

$$H = \frac{V_0 - V_1}{V_2 - V_3}$$

Where:

H is the heat number

V_0 Is the volume, in milliliters, of the standard volumetric solution used in the Kjeldahl determination with the precipitate from 10 ml of the reconstituted milk.

V_1 Is the volume, in milliliters, of the standard volumetric solution used in the blank Kjeldahl determination with a filter paper.

V_2 Is the volume, in milliliters, of the standard volumetric solution used in the Kjeldahl determination with 10 ml of the reconstituted milk.

V_3 Is the volume, in milliliters, of the standard volumetric solution used in the blank Kjeldahl determination with 0.1 g of sucrose.

The heat number of the dried milk is calculated directly from the two volumes of standard volumetric solution, each being corrected by a blank Kjeldahl determination. The heat class of the dried milk is derived from the heat

number according to a proposed heat-classification scheme consisting of four heat classes, namely Low Heat (80 or less), Medium Heat (80.1 to 83.0), Medium-High Heat (83.1 to 88.0) and High Heat (88.1 or more).

2.4.1.6 Determination of lactose content.

Lactose content in skim milk powder was determinated using High Performance Liquid Chromatography (See Appendix A1). The specifications of the HPLC used as follow:

2.4.1.6.1 Apparatus

The HPLC instrument used was a SP87000 gradient pump, with a loop injection system Varian 9090 (20μml). The integrator model SP4270 was supplied by Spectra Physics.

The chromatography column was Spherisorb 5μm, aminobonded. The mass detector was the Model 750/14, supplied by Burke Electrics Ltd. Glasgow.

2.4.1.6.2 Reagents

All the chemical reagents were of analytical grade. The mobil phase, consisted of a mixture of acetonitrile and water (CH_3CN:H_2O = 90:10 v/v initially, followed by 80:20, after 20 mins, then stabilised to 90:10 at 37 mins up to 45 mins.), which was degassed under vacuum before starting the analysis.

Calibration standards were prepared by weighing accurately 2 g of sucrose, 2 g of xylose, 2 g of glucose, 2 g of fructose and 2 g of lactose. (all from Sigma Chemical Company, Poole, UK), dissolving in distilled water and making up to 100 ml in a volumetric flask in order to get the response factor using the following formula (Lindsay, 1992).

$$r = \frac{C/A}{C_s/A_s}$$

Where:

C = Concentration of the component of interest

A = Peak area for this component

C_s = Concentration of internal standard

A_s = Peak area of internal standard

2.4.1.6.3 Sample preparation

A sample of 8.011 g of skim milk powder was dissolved in 50 ml of distilled water to make a skim milk solution. 12.005 g of the prepared solution and 0.5 g of xylose (as internal standard) were weighed into a 50 ml volumetric flask. Acetonitrile was added to make up to 50 ml. The sample plus reagents was mixed by repeated inversions for three minutes and followed by filtration through a fluted filter paper (Whatman No 1, 12.5 cm diameter) and filtering again through a Millipore filter, of 0.45 μm pore size, 13 mm diameter (Millipore Ltd., Harrow, Middlesex, UK) to ensure complete removal of suspended matter (such as protein and fat) before injection into the HPLC system.

2.4.1.6.4 Operation of the HPLC

The system was gradient operated at room temperature and the flow rate was adjusted to 1 ml/min. The injection valve was fitted with fixed volume loop (20μml) and the samples were loaded onto the column while the pump was in operation. A computer software (Chrome Perfect Program® supplied by Justice

Innovations, Inc. U.S.A.) was used to analyze the responses and to obtain the chromatograms for every sample.

The concentration of the component was calculated using the following formula (Lindsay, 1992).

$$C_u = A_u \times r \times \frac{C_s}{A_s}$$

Where:

C_u = Concentration of the component

A_u = Peak area

C_s = Concentration of internal standard

A_s = Peak area of internal standard

r = Response factor

2.4.2 Skim milk, Whole milk, Ultrafiltered Retentate and Permeate

2.4.2.1 Fat content

The fat content of the Skim milk, UF-Retentate and UF-Permeate were determinated by using the methods of the International Dairy Federation. For skim milk and UF-permeate (IDF:1987d) weighing 10 g (± 1 mg), and the (IDF:1987b) for the UF-Retentate weighing 5 g (± 1 mg) of sample.

2.4.2.2 Total Nitrogen Content

The total nitrogen content of Skim milk, UF-Retentate and UF-Permeate (weight of 1 ml, 0.5 and 3 ml of sample [± 1mg] respectively) were determined according the method recommended by the International Dairy Federation as described in section 2.4.1.2.

2.4.2.3 Ash Content

Skim milk, UF-Retentate and UF-Permeate were analyzed using the method recommended by British Standards (BSI:1988). Weighing 10 g (± 1 mg) of skim milk and UF-permeate, and 5 g (± 1 mg) of UF-Retentate.

2.4.2.4 Lactose Content

Lactose content in skim milk, UF-Retentate and UF-Permeate was analysed using different methods. (See Appendix No. A1).

2.4.2.4.1 Enzymatic Method

Lactose content in skim milk, ultrafiltered retentate, and ultrafiltered permeate samples were determined using the enzymatic method recommended by IDF (1991b). This method was developed by Boehringer (Anon., 1989). It is based on the principle of lactose is hydrolyzed to glucose and β-galactose in the presence of β-galactosidase and water. β-Galactose is then oxidised by nicotinamide-adenine dinuclotide to galactonic acid in the presence of β-galactose dehydrogenase. The amount of reduced nicotinamide-adenine dinuclotide formed is stoichiometric with the amount of lactose and is measured at 340 nm in a spectrophotometer possessing a slit width of ≤ 10 nm.

The samples were prepared weighing approximately 2g (± 1mg) of sample (Milk, UF-Retentate or UF-Permeate) into a volumetric flask. They were diluted with 20 ml of distillated water. One ml of Trichloroacetic acid was added (3mol/l) for protein precipitation. After 10 min. incubation at room temperature, the samples were neutralized with NaOH (1 mol/l) and made up to 100 ml with distillated water, and filtered. Then the test-kit (Lactose/D-Galactose, UV-method supplied by Boehringer-Mannheim) was used, following

the supplier instructions manual (Anon. 1989). An UV spectrophotometer model SP 1800 (PYE Unicam Ltd.) was used at wavelength of 340 nm.

2.4.2.4.2 Polarimetric Method

The polarimetric method described by Biggs and Szijreto (1963) was used for the determination of lactose of skim milk, ultrafiltered retentate and ultrafiltered permeate. It consisted in a digital polarimeter model AA-100 (Digital activity Ltd., Cornwall, U.K.). A pump (i.e. serial No. 9138), which was obtained from Watson-Marlow Ltd., Cambridge, U.K., was attached to the polarimeter. Sodium light was used as a source of light and the tube length was 17 cm. The reagent solution was prepared by mixing 12.5% (w/v) of zinc acetate ($(CH_3\ COO)_2\ Zn\ 2H_2O$), dodeca-Tungstophosphoric acid ($H_3PO_4\ 12WO_3\ XH_2O$) 6.25% (w/v) and 10% (w/v) of glacial acetic acid ($C_2H_4O_2$). All reagents were Analar grade obtained from BDH Chemicals Ltd. Filter paper No. 42 (Whatman Ltd., Maidstone, U.K.) was used to clarify the test solution. A standard sucrose solution (BDH Chemicals.) was prepared to give an optical rotation of 3.460.

10 ml of the reagent was added to 40 ml of sample (skim milk, ultrafiltered retentate, ultrafiltered permeate). The mixture was filtered using the filter paper No. 42 (Whatman Ltd., Maidstone, U.K.) for 15 mins. The filtrate was analyzed at 20° C in the polarimeter for lactose determination.

2.4.2.4.3 High Performance Liquid Chromatography Method (HPLC)

Lactose content was determined by using the HPLC technique as described in section 2.4.1.6. using 12g (±1 mg) of sample in each case, and using 0.5g (±1 mg) of xylose as internal standard.

2.4.2.5 Mineral Content

Calcium, Phosphorus, Magnesium, Potassium and Sodium were analysed using an Inductively Coupled Plasma Spectrometer (I.C.P.) (Model IL Plasma-100 supplied by Thermo Electron Ltd., Birchwood, Warrington, U.K.) following the technique proposed by Alexander *et al.* (1985). To 5 ml of digest solution (obtained from protein analysis) was added 1 ml of nitric acid/Triton solution. Standards of known concentrations of the minerals to be determined were prepared. The solutions were analyzed on the I.C.P. The output of the I.C.P. is adjusted to give the result of the appropriate mineral in g/Kg.

The accuracy of the method is reported by the same authors, with low coefficients of variation between the analyses for Ca (± 0.1 g/Kg), Mg (± 0.2 g/Kg), P (± 0.02 g/Kg), K (± 0.04 g/Kg), Cu (± 0.1 ppm), Zn (± 0.5 ppm), Fe (± 8 ppm) and Mn (± 1 ppm).

2.4.2.6 Total Solids Content

Total solids in skim milk, UF-Retentate, UF-Permeate were determined according the method recommended by IDF:1987c. The principle is based in the predrying of a mixed with sand sample on a steam bath and to completely evaporation of the water at temperature of 102° C in a drying oven to constant temperature. A samples of 3 g (± 1 mg) of UF-permeate, 1 g (± 1 mg) of skim milk and 0.5 g (± 1 mg) of UF-retentate were weighed for total solids analysis.

2.4.2.7 Milk Solids Non Fat Content (MSNF)

The MSNF value for every case, was obtained subtracting the fat from the total solids values.

2.4.2.8 Titratable Acidity Determination

The titratable acidity in the skim milk was determined by using the method of British Standards Institution (BS) 1741, Section 10.1:1989. Using 10 ml of milk and 1 ml of 0.5 per cent (w/v) solution of alcoholic phenolphthalein as indicator. Titrant used was N/9 NaOH solution. The volume of NaOH solution used divided by 10 gives the acidity as percentage lactic acid.

2.4.2.9 Determination of pH

The hydrogen ion concentration in the milk was measured using the pH-meter described in 2.3.7.3, at temperature of 20° C. Buffer solutions of pH 4 and pH 7 were used for calibration.

2.4.2.10 Total Viable Count

The total viable count was determined by using the method recommended by (IDF:1991c). The method is based on the preparation of poured plates using a Milk Plate Count Agar CM 21 (Oxoid Ltd.) medium and 1ml of solution sample incubated at 30° C for 3 days. A modified preparation of solution sample used Ringer solution BR 52 (Oxoid Ltd.) (1g of milk sample in 9ml of diluent) to get 10^{-1}, 10^{-2} and 10^{-3} .

2.4.2.11 Coliform Count

The method recommended by IDF (1985), and modified in sample preparation was used to enumerate the coliforms count by using the techniques of colony count. The principle is based on mixing a 1 ml test portion or a series of decimal dilutions (10^{-1}, 10^{-2} and 10^{-3}) of the sample with the culture medium (Violet Red Bile CM 107) in Petri dishes and incubation at 30° C for 24 h. Preparation of solution sample(1ml of milk sample in 9ml of diluent) used Ringer solution BR 52 (Oxoid Ltd.) to get 10^{-1} dilution.

2.4.2.12 Microscopy

2..4.2.12.1 Transmission Microscopy

Milk and UF-Retentate were examined by using an AEI Corinth Transmission Electron Microscope (TEM) type 275. The samples were warmed to 45° C and some drops were placed in warm agar CM 463 supplied by Oxoid™ which was allowed to cool and solidify. The sample was cut into ~ 2 mm cubes and placed in glutaraldehyde solution (2.0 %) for two hours, in water overnight; then they were placed in ethanol at 70%, 80%, and 90% for one hour in each case. Finally they were held overnight in absolute ethanol. Next day the samples were transferred to fresh absolute ethanol for one hour, placed in LR White resin for five hours and finally encapsulated with LR White resin and left overnight at 60° C. Once the samples were embedded with resin they were sectioned using an UB ultra-tome®, type 8801A. The sections were collected on copper grids (type G215 of 3.05 mm diameter). The sections were stained with uranyl acetate for one minute and were then washed twice by aqueous immersion in distilled water. The uranyl acetate was filtered using a 0.45 μm membrane filter (Sartorius™).

2.4.3 Ice Cream

2.4.3.1 Fat Content

The fat content in 2 g (± 1 mg) of ice cream mixes was determined by using the method recommended by International Dairy Federation (IDF:1987e). The principle is discussed in 2.4.1.1.

2.4.3.2 Total Nitrogen Content

The total nitrogen content in ice cream mixes was determined by using the method described in 2.4.1.2 weighing 2 g (± 1 mg) of ice cream mix sample.

2.4.3.3 Ash Content

The ash content in ice cream mixes was determined using the method recommended by (BSI :1966), by weighing 8 g (± 1 mg) of ice cream mix sample.

2.4.3.4 Carbohydrates Content

The lactose content of ice cream were analyzed by using the enzymatic method described previously in section 2.4.2.4, and by the HPLC method (See Appendix No. A1.

2.4.3.4.1 Enzymatic Method

The ice cream samples were prepared weighing 1 g (± 1 mg) into 100 ml volumetric flask and adding 60 ml of distillated water and incubated for 15 min at 70° C. After cooling trichloroacetic acid (3 mol/l) was added for protein precipitation, followed by filtration and neutralization by using NaOH (1 mol/l) to pH 7 and made up to 100ml with distilled water. Then a test-kit and a procedure as described 2.4.2.4. was used.

The sucrose content in the ice cream samples was obtained by difference of the sum of all the chemical components including the lactose value.

2.4.3.4.2 High Performance Liquid Chromatography Method (HPLC)

The HPLC technique was used to analyze sucrose and lactose content in ice cream samples (See Appendix No. A1). The samples were prepared weighing 4g (± 1 mg) of ice cream mix and 0.5 g of xylose (as an internal standard) into 50 ml volumetric flask and 10 ml of water (as a solvent). Trichloroacetic acid (20%) was used to precipitate the proteins, followed by filtration through a fluted filter paper (Whatman No 1, 12.5 cm diameter) Then a neutralization of the solution was carried out with sodium hydroxide (20%) to get a pH of 6.8.

Water was added to make up to 20 ml. Acetonitrile was added to make up 50 ml. The samples were stored a 4° C overnight, then the samples were centrifuged for 3 minutes at 6000 rev/minute followed by filtration using a Millipore filter, 0.45 μm pore size, 13 mm diameter (Millipore Ltd., Harrow, Middlesex, UK) to ensure complete removal of suspended matter before injection into the HPLC system. (See section 2.4.1.6 for operation conditions).

2.4.3.5 Total Solids Content

Total solids in an ice cream sample mixed with sand were analyzed using the method described by the International Dairy Federation (IDF:1972).

2.4.3.6 Milk Solids Non Fat

The MSNF were obtained as described in 2.4.2.7

2.4.3.7 Methylene Blue

The methylene blue test was used to evaluate the bacteriological quality of ice cream It was done by using the method proposed by British Standards Supplement No. 1, 1970 (BS:1968a). The method is based in the principle of the discoloration of the ice cream with methylene blue caused by the use of oxygen by microbial growth. 1 ml of methylene blue solution, 7 ml of strength Ringer solution, and 2 ml of melted sample are placed in a 10 ml tube. Two controls tubes were prepared: a) *Ice cream colour.*- 8 ml of Ringer solution and 2 ml of ice cream to make up to 10 ml. b) *Methylene blue colour.*- 2 ml of sterile ice cream and 8 ml methylene blue solution to make up 10 ml.

Incubation of the test and the control tubes for 17 h in a water bath at 20° C followed by incubation in a water bath at 37°C, inverting the tubes once every half hour until complete decolorisation.

The time for complete decolorisation of the methylene blue should be interpreted as follows:

Provisional grade	*Time taken to reduce methylene blue*
1	Fails to reduce in 4 h
2	2½ - 4 h
3	½ - 2 h
4	0

This test is proposed for routine grading and it is used essentially to indicate if further investigations in manufacturing are advisable. It is recommended, that samples falling in higher grades should be analyzed for plate counts and coliforms (Kirk and Sawyer, 1991).

2.4.3.8 Viscosity

The apparent viscosities of the ice cream mixes were evaluated using a Brookfield Synchro-lectric Viscometer Model LVT (Brookfield Engineering Laboratories, Massachusetts) with a spindle Type 2, at 12 revolutions per minute at 20° C and three readings were recorded, averaged and converted to Newton second per meter squared (N s/m^2). The ice cream mix viscosity was determined as 100 ml sample in a 200 ml beaker.

2.4.3.9 Hardness

A Steven's-LFRA Texture Analyser (C. Steven's & Son Ltd, Hertfordshire, U.K.) was used to measure the hardness of the ice cream in terms of compression forces (Newtons) resulting from the penetration of a probe. The determinations were carried out at -5, -16 and -18° C using a needle type TA-16, and T8 (at -5°). The penetration distance was 15 mm, and the speed of penetration was 1.0 mm/sec. The data was recorded as a direct digital reading and fed to a chart recorder (Model BS 271. supplied by C. Steven's & Son Ltd,

Hertfordshire, U.K.) with a single channel. 250 mm chart width. Pen response 0.333 seconds for full scale deflection.

2.4.3.10 Overrun

The overrun was determined according to the method proposed by Rothwell (1991b) by weighing a container filled exactly to the brim with the ice cream mix to be frozen. When the freezing operation was done, the same container, was filled with ice cream and weighed. The overrun is given by the following formula.

$$\text{Overrun \%} = \frac{\text{Wt. of mix} - \text{Wt of Ice cream}}{\text{Wt. of the Ice cream}} \times 100$$

2.4.3.11 Extrusion Temperature

The extrusion temperature was recorded by monitoring the ice cream temperature (using a thermometer described in 2.3.7.1) during the freezing process. The extrusion temperature was measured in each batch after 10 minutes from starting the freezing operation and before removal from the freezer.

2.4.3.12 Microscopy

2.4.3.12.1 Transmission Electron Microscopy (TEM)

The microstructure of ice cream was examined and photographed by using an AEI Corinth TEM, type 275. Small pieces of ice creams sample were cut at -40° C and placed in a solution prepared with ethanol (95%) and glutaraldehyde at 1.25 % for one week at -40° C. The solution was changed to absolute ethanol for one week at -20° C, the samples were warmed to -10° C for two days and then to 0° C before placing in LR White resin overnight. Next day the samples were encapsulated with fresh LR White resin and placed at

60° C overnight. Once the samples were embedded with resin they were sectioned using an UB ultra-tome, type 8801A. The sections were collected on copper grids (type G215 of 3.05 mm of diameter. The sections were stained for one minute in uranyl acetate and were then washed twice by immersion in distilled water. The uranyl acetate was filtered using a 0.45 μm membrane filter (Sartorius).

2.4.3.12.2 Scanning Electron Microscopy

The microstructure of ice cream was also examined using a scanning electron microscopy (SEM) Cambridge Model S250. The ice cream samples were cut under liquid nitrogen (-180° C) and were placed in a sample holder. Silver DAG (Acheson Colloids) was used as an adhesive; the frozen ice cream was placed on the silver DAG and the holder immediately plunged into liquid nitrogen to avoid melting. The holder was transferred to a cryo unit (Emscope SP2000) under vacuum. The samples were fractured in the cryo unit and 'etched' in the microscope to enhance the ice crystals. Etching was achieved at 80° C whilst observing the microscope. The sample was then transferred back to the cryo unit and spatter coated using gold. They were then examined in the microscope and photographs were taken on a Kodak HC110 film.

2.4.3.12.3 Light Microscopy

The microstructure of ice cream was also analyzed using light microscopy techniques to investigate each ice cream phase separately.

Ice crystals and air cells identification were carried out using a section obtained from a specimen previously prepared for TEM, which is described in section 2.4.3.12.1. The sections were placed on a slide and left to dry, then were stained using eosin (Supplied by Raymond A. Lamb, UK) and immersion oil.

The analysis of the sections were done using an Olympus CH2 Microscope (Bright field and magnification X 10 and X 40) loaded with Ektachrome (160T) film.

Oil droplets in melted ice cream were analyzed, by mixing 0.1g of ice cream mix with 2.5 ml. of Lauryl Sulfate (Supplied by Sigma Chemicals Co. England) and 2.5 ml. of Oil Red O® and 1 ml. of Glycerol (Supplied by BDH Chemicals Ltd. Poole, England) for staining using an Olympus Vanox microscope with differential interface contrast (DIC) with magnification X 100 and loaded with an Ektachrome film (160T). An Optimas software program was used to count the fat droplets present in ten different fields for each sample.

2.4.3.13 Sensory Analysis

The samples of ice cream were stored at -22° C and evaluated by ten judges, after one, four and twelve weeks. Familiarization of the judges with various attributes was carried out in one session before the evaluation. Ice cream attributes were described and discussed with the judges. When a test involves more than one sample the order in which the samples are tested is very important. People may respond differently to the samples simply because of the order of presentation. Presentation order was fixed according to Halliday *et al* (1989), to allow for estimation of any effects of order of presentation. A sensory vocabulary, comprising eight ice cream attributes (iciness, sandiness, gummines, watery, fluffiness, flavour strength, colour and overall acceptability) was used and the judges scored each attribute on a 150 mm scale with anchor points (Lang and Shepherd, 1988). Each panellist scored an attribute by placing a mark on a 150 mm scale (See Figure No. 3.5 in Chapter 3 of Ice Cream). Water was given to the judges to be used after every sample tasting.

2.4.3.14 Heat Shock Properties

Samples of each ice cream were subjected to heat shock to simulate abuse of the product during handling. Samples were removed from the hardening room and stored for two hours at -4° C, then for a further hour at room temperature (20° C). The samples were then returned to the hardening room. The sensory panel then evaluated the ice creams for the three characteristics; **iciness, flavour and acceptability.** Each panellist scored an attribute by placing a mark on a 150 mm scale (Lang and Shepherd, 1988). Each end of the scale represented a response as shown in Figure No. 3.15 of Chapter 3 of Ice Cream. Presentation order was fixed according to Halliday *et al* (1989), to allow for estimation of any effects of order of presentation.

2.4.3.15 Consumer Acceptance

After one year, a consumer acceptance evaluation for ultrafiltered ice cream-1 and the control was carried out by 61 randomly selected students from SAC-Auchincruive using freshly prepared ice cream samples. The students were asked to score both products for overall preference from a seven-point Hedonic scale (Lang and Shepherd, 1988). Presentation order was fixed according to Halliday *et al* (1989), to allow for estimation of any effects of order of presentation. (See Figure No. 3.16, of Chapter 3 of Ice Cream, for the score card).

2.4.3.16 Melting Properties

The melting determination of ice cream samples was carried out by tempering 150 g of sample at -14° C for 48 hrs. The samples were placed in a funnel with a plastic mesh integrated in a graduated cylinder to record the liquid collected. The mesh (9.6 inch) had 81 perforations of 1/16 in each square inch.

Measurements were made from the first drop, continuing every five minutes for up to ninety minutes at temperatures ranging from 20.1 to 20.6° C.

2.4.4 Cajeta

2.4.4.1 Fat Determination

The fat content was determined by using the method recommended by the IDF as described in section 2.4.2.1. weighing 2g (± 1mg) of cajeta sample.

2.4.4.2 Total Nitrogen

The total nitrogen content was determined by using the method described in 2.4.1.2, weighing 2g (± 1mg) of cajeta sample.

2.4.4.3 Ash Content

The ash content in cajeta was determined as described in 2.4.1.4, weighing 3 g (± 1mg) of sample.

2.4.4.4 Carbohydrates Content

The carbohydrate content in 4g (± 1mg) of cajeta was analyzed by using High Performance Liquid Chromatographic instrument as described in section 2.4.3.4.

The sample preparation involved different trials (See Appendix No. A1). However a standardised technique for carbohydrate analysis in cajeta was obtained as a follows:

1.- Weigh 4 g of cajeta sample in a volumetric flask
2.- Weigh 0.5 g of Xylose as an internal standard
3.- Add 5 ml of distilled water to dissolve the mixture
4.- Add Trichloroacetic acid (TCA, 20%) to protein precipitation

5.- Add 10 ml of distilled water and shake the mixture for three minutes to precipitate proteins

6.- Filter the solution using a Whatman filter No 1 of 9 cm diameter

7.- Wash the cake formed on the filter with a little distilled water.

8.- Neutralize the solution using aqueous sodium hydroxide (NaOH, 20%) to pH of 6.8

9.- Add distilled water to make up 20 ml

10.- Add acetronitrile to make up 50 ml and shake well

11.- Store the solution overnight at 4° C, to allow time for carbohydrates to dissolve.

12.- The solution divided into two layers overnight. Each layer was sampled and HPLC analysis showed that carbohydrate was only present in the acetonitrile layer which was isolated for analysis by using a separating funnel

13.- Pass the solution through a cellulose acetate filter of 0.45 μm pore size, 13 mm. diameter

14.- Inject the solution into the HPLC (20μml)

2.4.4.5 Minerals Content

The mineral content was carried out as described in 2.4.2.5, weighing 0.250 g of dried sample.

2.4.4.6 Total Solids Content

Total solids were determined by using a method recommended by IDF :1991a, for sweetened condensed milk which is similar to cajeta. Two grams (± 1mg) of sample was mixed with sand and weighed on a AE 166 balance (Mettler Instruments Ltd., Buckinghamshire, U.K.) and dried at 102° C for two hours in a hot air oven to a constant weight.

2.4.4.7 Milk Solids Non Fat

The MSNF were obtained by difference of total solids subtracting the fat content in the sample.

2.4.4.8 Refractive Index

A refractometer was used to determine the end point in concentrating cajeta as described in 2.3.7.4.

2.4.4.9 Consistency

The consistency of cajeta was evaluated using 200 g of sample in a food grade plastic container. The texture analyser as described in section 2.4.3.9 was used to measure the consistency in terms of penetration resistance (Newtons) resulting from the penetration of a probe. The determinations were carried out at 10° C, 20° C, 30° C and 40° C, using the probe type TA-16 at 15 mm penetration distance with speed penetration of 1.0 mm/sec.

2.4.4.10 Total Viable Count

Total viable count was determined using the method recommended in (IDF:1991c). This method was partially modified after personal communication with Dr. James Bruce, by preparing a Ringer solution BR 52 (Oxoid Ltd.) with and without sucrose at 20% in order to give different osmotic conditions for bacteria growth. 10 g of sample was mixed with 90 ml of diluent. 1 ml of sample solution was mixed with 15 ml of Plate Count Agar CM 325 (Oxoid Ltd.) and incubated at 30°C for 3 days.

2.4.4.11 Coliform Count

The coliforms count in cajeta were determined by using the method recommended by the (IDF:1985) based in the colony count and most probable number (MPN) techniques. This method was partially modified by using

McConkey Broth and the recommended Violet Red Bile Agar CM 107 (Oxoid Ltd.) for the plate technique and for the MPN. The sample preparation and incubation is described in section 2.4.4.10. The MPN technique used 3 tubes with 1 ml of sample solution at 30° C for 3 days.

2.4.4.12 Yeast and Mould Count

The method was suggested by Dr. James Bruce (personal communication) to determine the presence or absence of yeasts and moulds in cajeta product after manufacturing was used. The analysis was carried out using 5 x 10g of cajeta sample mixed with 5 x 90 ml of Malt Extract Broth CM57 (dilution 7% sugar) supplied by Oxoid Ltd, in 100 ml bottles with Durham tubes. The procedure was repeated with 5 x 1g portions of cajeta added to 5 x 9 ml of medium. The samples were incubated at 25° C for 5 days and 10 days and examined for gas production. If any sample was positive, the samples would be tested following the method recommended by IDF, as described below.

The method recommended by the IDF (1990) was used to determine the presence of yeast and moulds in cajeta samples after eight months storage. It was modified by using Malt Agar (supplied by Oxoid Ltd) as recommended by personal communication of Dr. James Bruce. One ml of solution sample was mixed with 15 ml of agar and incubated at 25° C for 5 days. Sample preparation was as described in section 2.4.4.9

2.4.4.13 Microscopy

2.4.4.13.1 Transmission Electron Microscopy

The microstructure of cajeta samples were examined by using a TEM as described in section 2..4.2.12.1.

2.4.4.14 Light Microscopy

A representative (~ 0.005 g) sample of each cajeta product was placed on a slide covered by a cover slip and sealed with a special seal-mountant. In every slide six circles of 30 mm were drawn to be used as a reference point for the analysis of lactose crystal number. A Nikon microscopy Model 128753 with a calibrated scale to allow sectorizing an observed field and measurement of crystal size was used to keep track of the size and quantity of lactose crystals in each field. Photographs of the lactose crystals were taken using an Olympus CH2 microscope (magnification X 10) loaded with Ektachrome (160T) film.

2.4.4.14.1 Crystal Size

The size of the lactose crystals in each slide was measured using an integrated scale in the eyepiece and using a X10 objective in the microscope as described in section 2.4.4.13. The lactose crystals were selected at random and the measurement was made on the longest side. In general the crystals were triangular.

2.4.4.14.2 Crystal Number

The number of lactose crystals present in each circle in every slide were counted using the microscope as described in 2.4.4.13, using an objective with magnification X10.

2.4.4.15 Sensory Evaluation Analysis

The samples of cajeta were evaluated by twelve judges, after one, five and nine weeks from processing. Familiarisation of the judges was carried out in one session before the evaluation and cajeta attributes were described and discussed with the judges. Presentation order was fixed to allow for estimation of any effects of order of presentation. A sensory vocabulary, comprising five attributes (e.g. sandiness, stickiness, smoothness, flavour and acceptability), was

used and the judges scored each attribute on a 150 mm scale with anchor points (Lang and Shepherd , 1988). (See Figure No. 4.13 of Chapter 4). In addition, the judges were asked to score each product in terms of overall acceptability. Water was given to the judges to be used after every sample tasting. Presentation order was fixed according to Halliday *et al.* (1989), to allow for estimation of any effects of order of presentation.

2.4.4.15.1 Visual evaluation of sandiness

Cajeta samples were evaluated by twelve judges for visual appearance in order to detect sandiness on the product after eight months in storage at 4 C using a four-point Hedonic scale (from undetectable to very sandy) (Lang and Shepherd, 1988). Panelist were asked to decide to score sandiness in the samples (See Figure No. 4.20 of Chapter 4).

2.4.5 Butter

2.4.5.1 Fat Content

The fat content in 2 g of butter was determined according to the method described in 2.4.1.1.

2.4.5.2 Moisture Content

The moisture content was determinated by method as described by (IDF:1986). It is based in the principle of a known mass of butter (10 g ± 1mg of butter) is heated under controlled conditions (102° C ± 2°C) in an open beaker to evaporate the volatile constituents. The mass is calculated using the followed formula:

$$E = \frac{m_1 - m_2}{m_1 - m_o} \times 100$$

Where:

m_0 = Is the mass, in grams of the empty beaker

m_1 = Is the mass, in grams, of the test portion and beaker

m_2 = Is the mass, in grams, of the test portion and beaker after heating.

2.5 Statistical Analysis

The data were analyzed by univariate [Analysis of variance (REML), regression and correlation] and multivariate analysis [Principal components analysis (PCA)], by the Genstat ™ computer programme, version 5 (Copyright 1993) Agricultural Trust, Rothamsted Experimental Station and the Minitab™ Release 8 (1991) computer programme (Minitab Inc. Pennsylvania State College, PA 16801, USA, respectively).

The method of Residual Maximum Likelihood (REML) was used in the statistical analysis of sensory evaluation data. The REML estimates the treatment effects and variance components in a linear mixed model (linear model with both fixed and random effects). This technique is used to analyze unbalanced data sets, and can also account for more than one source of variation in the data, providing an estimate of the variance components associated with the random terms in the model. It can also be used to combine information over similar experiments conducted at different times or in different places. Fixed effects are used to describe treatments imposed in a experiment where it is the effect of those specific choices of treatment that are of interest. Random effects are generally used to describe the effects of factors where the values present in the experiment represent a random selection of the values in some larger homogeneous population (Anonymous, 1993a).

The multivariate analysis gives mathematical relations between some characteristics arising from some sensorial analysis carried out on the product. These analyses are not conventional statistical methods using one hypothesis or the estimation of one probability. However they are used to simplify a great quantity of data from a group of variables or characteristics and show the interrelation between them to facilitate their interpretation (Pedrero and Pangborn, 1989). According to Fry (1993), the axes or components are successively extracted from a matrix of similarities, typically correlations or covariances between the variables. PCA is a particular form of the more general principal coordinates analysis which can utilize either similarities or a distance matrix.

To give a better idea of the overall tendencies of the samples, a principal components analysis was carried out taking account of all of the attributes which characterised the ice cream samples in every period of time. From the Residual Maximum Likelihood (REML) analysis, each ice cream has a mean score for each of eight characteristics, with the effects of judge and time within judge removed. To show all these characteristics completely would require an 8-dimensional plot. A principal components analysis projects this hypothetical plot to a 2-dimensional scatterplot in the way which maximises the observed variation.

CHAPTER THREE

ULTRAFILTRATION IN ICE CREAM MANUFACTURE

CHAPTER THREE: ICE CREAM MANUFACTURE

3.1 PRELIMINARY ULTRAFILTRATION TRIALS

3.1.1 Introduction

It is well known that the chemical composition of the ultrafiltered retentate (UF-R) can vary as the volume reduction (VR) of the permeate changes. Depending on the degree of separation and concentration achieved by ultrafiltration process (UF), it is possible to obtain retentates and permeates with different composition and properties which are different from the original fluid, and which are suitable for processing into a new generation of diversified products (Renner and Abd El-Salam, 1991). For this reason, a good level of volume reduction of the permeate in the ultrafiltration process is required to get an ultrafiltered retentate with enough milk solids non fat (MSNF) to be used as the sole ingredient in ice cream formulations

The objective of this preliminary section was to find the level of permeate volume reduction to get the required level of milk solids non fat (MSNF) in the ultrafiltered retentate to be used in an experimental ice cream formulation.

3.1.2 Materials and Methods

Seven trials were undertaken using different volume reductions of permeate (20, 30, 40, 50, 60, 70, and 75%) (See Table No. 3.1). In every trial approximately two litres of skim milk obtained by local purchase, were used as a raw material for the ultrafiltration process at 50° C. The volume reduction varied in every trial as a result of the time applied.

A Pelican Millipore ultrafiltration unit supplied by Millipore Corporation, Bedford, U.K. with a regenerated cellulose membrane, having a molecular weight cut-off of 30,000 Daltons supplied by the same company was used to ultrafilter the milk in each trial. The ultrafiltration process was carried out at inlet and outlet pressures of 290 and 276 kPa gauge respectively.

3.1.3 Results and Discussion

The chemical composition of products obtained using the ultrafiltration process at different levels of volume reduction, showed considerable differences, reflecting the behaviour of all components during UF processes (See Table No. 3.1). In particular the total solids content of the original milk was increased in the ultrafiltered retentate in each trial, showing a non linear relationship at different levels of volume reduction (See Figure No. 3.1). The MSNF values gave a similar non linear relationship response as they were determined by subtracting the fat content from the total solids values. In a similar way, proteins were increased as expected as shown in (See Figure No. 3.2).

The fat was not allowed to pass into the ultrafiltered permeate and the recovery in the ultrafiltered retentate in every trial was always almost 100%.

The mass recovery for every component in each trial varied from 94 to 99 % (See Table No. 3.2).

3.1.4 Conclusions

The technical feasibility of concentrating and fractionating skim milk by ultrafiltration was established. Protein, fat, and other solids were maintained in

the retentate providing the opportunity of using the ultrafiltered retentate in the manufacture of dairy products with a higher concentration of protein. If the fat causes fouling problems in ultrafiltration, it is possible to use high efficiency centrifugation to start with skim milk of fat content around 0.01%.

The main advantage of the ultrafiltration process is that the protein content in the ultrafiltered retentate is totally concentrated apart from a small process loss, and total solids are increased. According to Lee and White (1991), lactose within the retentate is reduced.

Volume reduction may be used as a variable, to adjust the total solids and MSNF content in the ultrafiltered retentate to be used for the manufacture of dairy products.

Ice creams made from milk or skim milk powder, normally have a maximum MSNF content of about 11%. Although the proportion of protein, fat, lactose, minerals and other trace constituents is not the same as in UF-Retentate, the MSNF in UF-Retentate can be used as a guideline for preliminary trials with ice cream. On this basis the volume reduction has to be approximately 70%. The value in any given situation will depend upon the composition of the milk supply with the protein content possibly having the most effect on the ice cream properties.

TABLE No. 3.1 CHEMICAL COMPOSITION OF SEMI-SKIM (SSM) AND SKIMMED MILK (SM) USED IN PRELIMINARY TRIALS *

SAMPLE	VOLUMEN (Lts)	VOL.RED.[3] (%)	PROTEIN (%)	FAT (%)	MSNF (%)	TOTAL SOLIDS (%)
SSM	2.090	20	3.17	1.70	6.90	8.60
UF-R[1]	1.650		3.90	2.10	7.59	9.69
UF-P[2]	0.418		0.18		4.20	4.20
SM	1.580	30	3.91	0.80	7.90	8.70
UF-R	1.050		5.71	1.17	9.30	10.47
UF-P	0.474		0.16		4.35	4.35
SSM	2.180	40	3.48	1.50	7.10	8.60
UF-R	1.300		5.75	2.50	8.88	11.38
UF-P	0.872		0.10		4.10	4.10
SM	1.010	50	4.05	0.70	8.00	8.70
UF-R	0.500		7.80	1.40	11.30	12.70
UF-P	0.505		0.24		4.45	4.45
SSM	2.090	60	3.36	1.60	6.90	8.50
UF-R	0.820		8.15	4.02	10.18	14.20
UF-P	1.254		0.24		4.46	4.46
SSM	2.070	70	3.30	1.60	7.00	8.60
UF-R	0.610		10.54	5.20	13.32	18.52
UF-P	1.449		0.25		4.48	4.48
SM	2.045	75	3.26	0.20	8.50	8.70
UF-R	0.500		12.38	0.80	21.20	22.00
UF-P	1.534		0.27		4.42	4.42

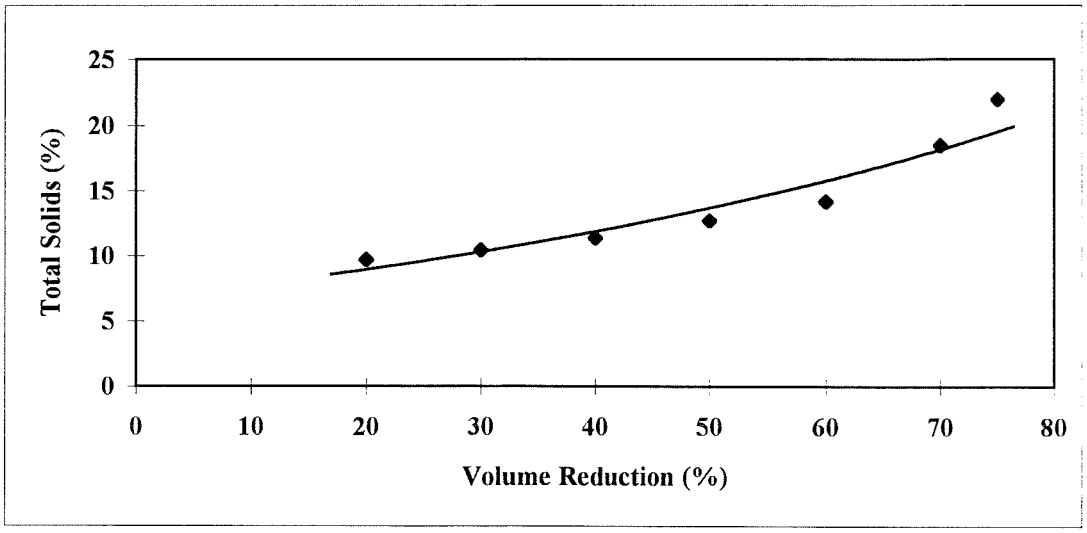
* Trials were carried out using a Pelican ultrafiltration unit with a membrane of 30,000 Nominal Molecular Weight Cut-off with inlet and outlet pressure of 290 and 276 kPa (gauge) respectively at 50 ° C

[1] Ultrafiltered retentate

[2] Ultrafiltered permeate

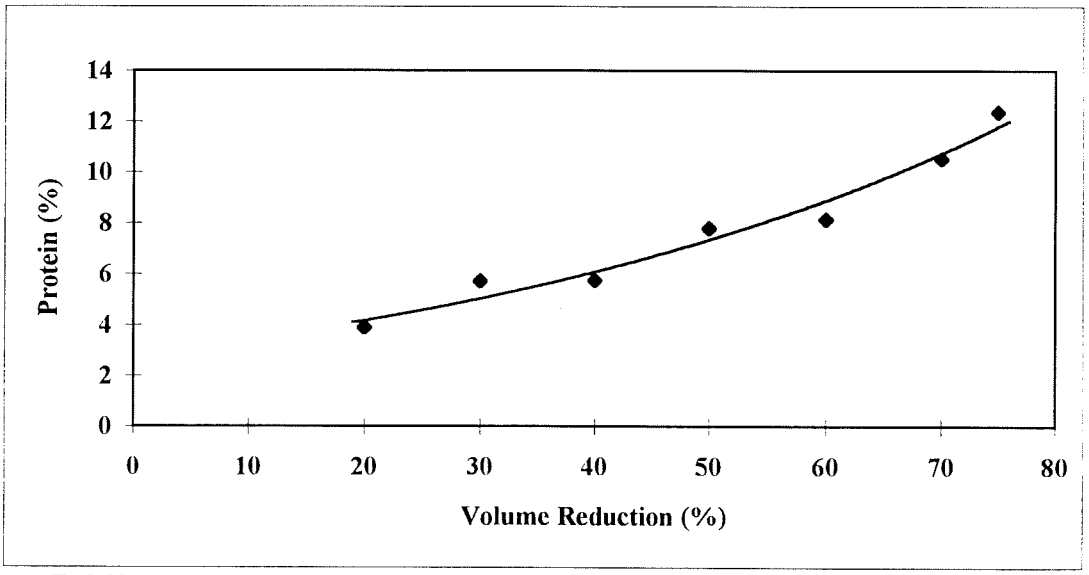
[3] Volume reduction

FIGURE No. 3.1 RELATIONSHIP BETWEEN VOLUME REDUCTION AND TOTAL SOLIDS CONTENT OF THE UF-RETENTATE IN PRELIMINARY TRIALS



* (P<0.003)

FIGURE No. 3.2 RELATIONSHIP BETWEEN VOLUME REDUCTION AND PROTEIN CONTENT OF THE UF-RETENTATE IN PRELIMINARY TRIALS



* (P<0.001)

TABLE No. 3.2 MASS BALANCE OF CHEMICAL COMPONETS IN PRELIMINARY TRIALS *

SAMPLE	Volume (l)	Vol.Red.[1] (%)	Fat (%)	Mass (kg)	Protein (%)	Mass (kg)	T. Solids (%)	Mass (kg)
SSM[2]	2.09	20	1.7	0.036	3.2	0.066	8.6	0.180
UF-R[3]	1.65		2.1	0.035	3.9	0.064	9.7	0.160
UF-P[4]	0.42			0.000	0.2	0.001	4.2	0.018
RECOVERY *	99			98		98		99
SM[5]	1.58	30	0.8	0.013	3.9	0.062	8.7	0.137
UF-R	1.05		1.2	0.012	5.7	0.060	10.5	0.110
UF-P	0.47			0.000	0.2	0.001	4.4	0.021
RECOVERY *	96			97		98		95
SSM	2.18	40	1.5	0.033	3.5	0.076	8.6	0.187
UF-R	1.30		2.5	0.032	5.8	0.075	11.4	0.148
UF-P	0.87			0.000	0.1	0.001	4.1	0.036
RECOVERY *	100			98		100		98
SM	1.01	50	0.7	0.007	4.1	0.041	8.7	0.088
UF-R	0.50		1.4	0.006	7.8	0.039	12.7	0.064
UF-P	0.51			0.000	0.2	0.001	4.5	0.022
RECOVERY *	100			85		98		98
SMM	2.09	60	1.6	0.033	3.4	0.070	8.5	0.178
UF-R	0.82		4.0	0.032	8.2	0.067	14.2	0.116
UF-P	1.25			0.000	0.2	0.003	4.5	0.056
RECOVERY *	99			96		99		97
SSM	2.07	70	1.6	0.033	3.3	0.068	8.6	0.178
UF-R	0.61		5.2	0.032	10.5	0.064	18.5	0.113
UF-P	1.45			0.000	0.3	0.004	4.5	0.065
RECOVERY *	99			96		99		100
SM	2.05	75	0.2	0.004	3.3	0.067	8.7	0.178
UF-R	0.50		0.8	0.003	12.4	0.062	22.0	0.110
UF-P	1.53			0.000	0.3	0.004	4.4	0.068
RECOVERY *	99			73		99		100

* Trials were carried out using a Pelican Millipore Ultrafiltration unit, with a membrane of 30.000 Nominal Molecular Weight Cut-off, with inlet and outlet pressure of 290 and 258 KPa (gauge) respectively at 50° C

[1] Permeate volume reduction

[2] Semi skim milk

[3] Ultrafiltered retentate

[4] Ultrafiltered permeate

[5] Skimmed milk

3.2 ULTRAFILTRATION OF MILK FOR ICE CREAM MANUFACTURE

3.2.1 Introduction

The major advantage claimed for the ultrafiltered process is that it yields a higher protein and lower lactose milk ingredient with excellent nutritional and functional properties, (Lee and White 1991). Hofi (1989) states that UF can be used to vary the protein content in dairy products within a wide range, without adverse effect on their organoleptic properties. So with this in mind ultrafiltered retentate should have a good effect in increasing the protein content and lowering the lactose content in ice cream products. Directly this will have a positive effect in preventing sandiness. It would be useful if lactose-intolerant people could consume the product without adverse effect.

In the production of dairy desserts, the UF-process has not been widely used, and there is a lack of scientific information. Therefore the objective of this study is to use the ultrafiltration process to provide ultrafiltered retentate as a source of MSNF to replace skim milk powder in the production of ice cream.

3.2.2 Ultrafiltration Process

Whole milk from SAC-Auchincruive farm was separated using a centrifugal separator (supplied by L' Electro Ecremeuse, Boulogne, France). The milk was divided into two parts for processing with a target of 70% of ultra filtered permeate volume reduction , and around 20% of total solids in the ultrafiltered retentate . The ultrafiltered retentates were mixed and used as one of the sources of MSNF in formulations for ice cream.

The permeability of the membrane was checked before the process by comparing its flux rate, at different temperatures with water at 50°C. (See Figure No. 3.3). Appendix A.2, shows the values for the flux rate of water.

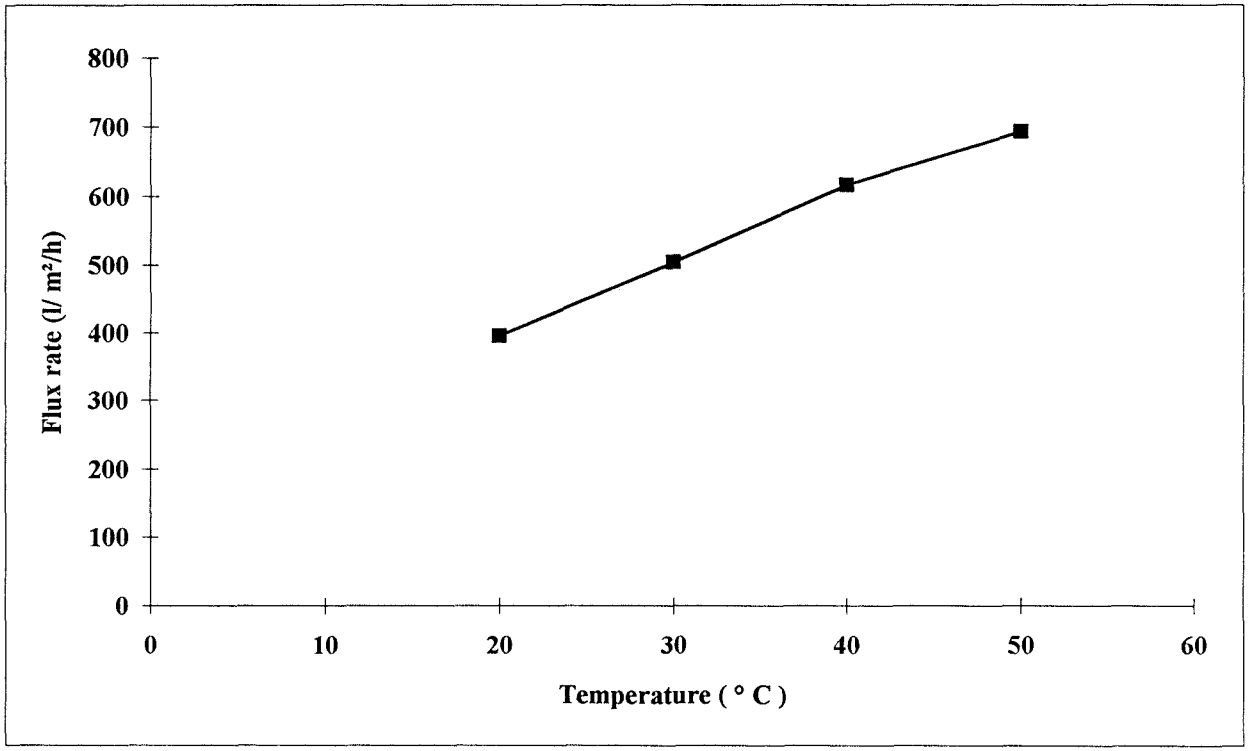
During the Ultrafiltration processes of skim milk for ice cream manufacture, the flux rate of the ultrafiltered permeate was checked at 10 minute intervals giving a total average of 726 ml/min (Figure No. 3.4). The values of trials are shown in Appendix A.3.

3.3 ICE CREAM MANUFACTURE

3.3.1 Methodology

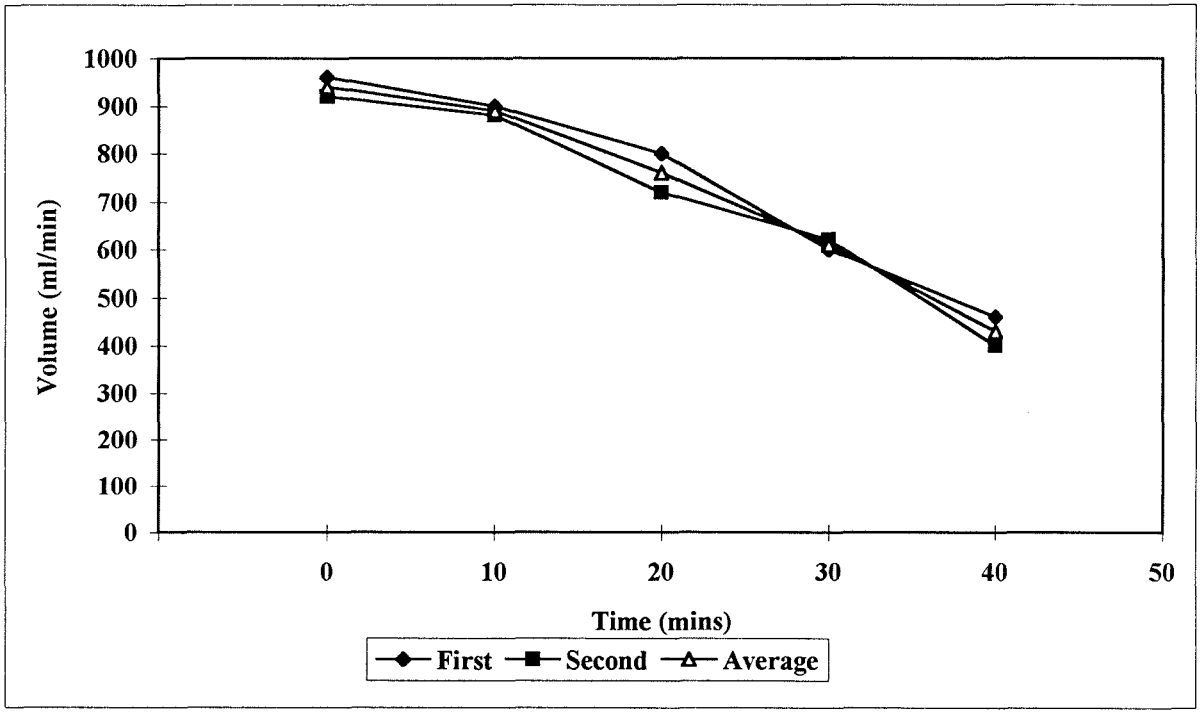
A traditional ice cream formula was used for both the control using skim milk powder and for ultrafiltered ice cream with an initial target of 10% Fat, 10.92% MSNF, 13% Sugar, 0.5% Stabiliser/Emulsifier (S/E), giving 34.42% of Total Solids (See Table No. 3.4 for the target and Tables No. 3.5 and 3.6 for formulations). Another formula for ultrafiltered retentate, having a initial target of 10% fat, 13% MSNF, 13% sugar and 0.5 S/E giving 36.5% of Total Solids (See Tables No. 3.3 for the target and Table No. 3.7 for formulation). MSNF were increased in order to make evident that increasing the MSNF in ice cream formulation by using ultrafiltered retentate reduces the possibility of sandiness, while increasing the protein content of the final product. Wilbey (1990), suggested to break away the norms by increasing the protein content using low lactose content milk in order to increase the acceptability of the product. Vanilla was used as a flavouring at a rate of 1 ml per litre of mix. Water was used to balance the formula in both cases. The mixes were pasteurised at 72° C for 10 minutes and homogenised at 17 MPa (gauge) at 71.5 ° C, and cooled to 4° C,

FIGURE No. 3.3 FLUX RATE OF UF-ROMICON MEMBRANE USING WATER AT DIFFERENT TEMPERATURES *



* Membrane of 50,000 Daltons of Nominal Molecular Weight Cutoff with Inlet and outlet were 0.15 and 0.12 mPa (gauge) respectively

FIGURE No. 3.4 FLUX RATES OF SKIM MILK IN TWO ULTRAFILTRATION PROCESSES AT 50° C *



* Membrane of 50 000 Daltons of Nominal Molecular Weight Cutoff and Inlet and Outlet pressures of 0.15 and 0.12 mPa (gauge) respectively

TABLE No. 3.3 ICE CREAM TARGET No 1 [1]

CONSTITUENTS	(%)
FAT	10.00
M.S.N.F. *	13.00
SUCROSE	13.00
ST/EM **	0.50
TOTAL SOLIDS	36.50

[1] Formulation used for ultrafiltered ice cream-1
* Milk solids non fat
** Stabiliser and emulsifier

TABLE No. 3.4 ICE CREAM TARGET No 2 [2]

CONSTITUENTS	(%)
FAT	10.00
M.S.N.F. *	10.92
SUCROSE	13.00
ST/EM **	0.50
TOTAL SOLIDS	34.42

[2] Formulation used for control and ultrafiltered ice cream-2
* Milk solids non fat
** Stabiliser and emulsifier

TABLE No. 3.5 CONTROL ICE CREAM FORMULATION USING SMP (10.9% MSNF [3])

VOLUMEN (Kg)	5	UF-R*	0	SMP[4]	100	
INGREDIENTS	**WEIGHT (Kg)**	**FAT**	**MSNF[3]**	**SUGAR**	**ST/EM[1]**	**T.SOLIDS[2]**
ST/EM[1]	0.025				0.025	0.025
SUCROSE	0.650			0.650		0.650
BUTTER	0.591	0.496				0.496
UF-RETENTATE*						
SKIM MILK						
SMP[4]	0.567	0.004	0.546			0.550
WATER	3.168					
FORMULA (Kg)	5.000	0.500	0.546	0.650	0.025	1.721

* Ultrafiltered retentate
1 Stabiliser and emulsifier
2 Total solids
3 Milk solids non fat
4 Skim milk powder

TABLE No. 3.6 ICE CREAM FORMULATION USING UF-R (10.9% MSNF[3])

VOLUMEN (Kg)	5	UF-R*	100	SMP[4]	0	
INGREDIENTS	WEIGHT (Kg	FAT	MSNF[3]	SUGAR	ST/EM[1]	T.SOLIDS[2]
ST/EM[1]	0.025				0.025	0.025
SUCROSE	0.650			0.650		0.650
BUTTER	0.579	0.486				0.486
UF-RETENTATE*	2.807	0.013	0.546			0.559
SKIM MILK						
SMP[4]						
WATER	0.938					
FORMULA (Kg)	5.000	0.500	0.546	0.650	0.025	1.720

TABLE No. 3.7 ICE CREAM FORMULATION USING UF-R (13% MSNF[3])

VOLUMEN (Kg)	5	UF-R*	100	SMP[4]	0	
INGREDIENTS	WEIGHT (Kg	FAT	MSNF[3]	SUGAR	ST/EM[1]	T.SOLIDS[2]
ST/EM[1]	0.025				0.025	0.025
SUCROSE	0.650			0.650		0.650
BUTTER	0.576	0.484				0.484
UF-RETENTATE*	3.342	0.016	0.650			0.666
SKIM MILK						
SMP[4]						
WATER	0.407					
FORMULA (Kg)	5.000	0.500	0.650	0.650	0.025	1.825

* Ultrafiltered retentate

[1] Stabiliser and emulsifier

[2] Total solids

[3] Milk solids non fat

[4] Skim milk powder

followed by storage overnight at 4° C. Next day a vertical freezer (T. Giusti & Son Ltd., London, U.K.) was used for the ice cream manufacture of a 5 l batch. During the process a sample of ice cream mix and ice cream were taken in order to determine the overrun percentage of ice cream.

The ice creams were filled into 100 g plastic containers sufficient for the range of analytical and organoleptic testing that were carried out (See sections 2.4.3 and 2.4.3.13 of Materials and Methods chapter 2). Samples for testing at 1, 4 and 12 weeks storage, were kept in a hardening room at -22° C.

3.3.2 Chemical Composition of UF-Ingredients

The skim milk used for ultrafiltration process was 80.5 kg (7.11 kg Dry matter). After the ultrafiltration process 16.7 kg (3.33 kg Dry matter) and 61.0 kg (3.39 kg Dry matter) of ultrafiltered retentate and ultrafiltered permeate respectively were obtained, giving a total recovery of 94.51 %. A mass balance was carried out in order to verify the partition of milk components (See Table No. 3.8). The recovery of the chemical components after ultrafiltration process ranged from 84.56 % to 99.6 %. The difference is explained in terms of residual loses of milk inside the ultrafiltration plant, including the fouling layer formed on the ultrafiltration membrane. As a result of this loss, differences may be found in the recovery of the chemical components of milk after the ultrafiltration process Glover (1971). Kessler *et al.* (1982) found in one study that in ultrafiltration of milk, protein was the major cause of blocking of the UF-membrane during the ultrafiltration process.

Skim milk, ultrafiltered retentate and ultrafiltered permeate were analysed for chemical composition (See Table 3.9). Total solids content of the original milk was increased in the ultrafiltered retentate from 8.83 to 19.93 %.

TABLE No. 3.8 MASS BALANCE FOR ICE CREAM UF-INGREDIENTS

	SM*	UF-R[1]	UF-P[2]	RECOVERY (%)
VOLUME (kg)	80.5	16.7	61	
MASS (kg)	7.1	3.3	3.4	94.5
Ash %	0.8	1.7	0.5	
Mass (kg)	0.61	0.28	0.30	93.8
Protein%	3.2	12.9	0.4	
Mass (kg)	2.58	2.16	0.25	93.3
Fat%	0.1	0.5	0.0	
Mass (kg)	0.08	0.08	0.00	99.6
Lactose%	4.8	4.9	4.7	
Mass (kg)	3.84	0.82	2.84	95.3
T.Solids%	8.8	19.9	5.6	
Mass (kg)	7.11	3.33	3.39	94.5
Ca (mg/100g)	1359.8	1906.7	538.6	
Mass (g)	96.68	63.49	18.26	84.6
P (mg/100g)	1019.8	999.6	978.2	
Mass (g)	72.51	33.29	33.16	91.6
Mg (mg/100g)	113.3	180.3	46.2	
Mass (g)	8.06	6.00	1.56	94.0
K (mg/100g)	1660.1	820.4	2513.6	
Mass (g)	118.03	27.32	85.21	95.3
Na (mg/100g)	555.2	265.9	825.9	
Mass (g)	39.48	8.86	28.00	93.4

* Skim milk

[1] Ultrafiltered retentate

[2] Ultrafiltered permeate

TABLE No. 3.9 CHEMICAL COMPOSITION OF RAW MATERIAL FOR ICE CREAM MANUFACTURE

SAMPLE	ASH (%)	PROTEIN (%)	LACTOSE [1] (%)	FAT (%)	TOTAL SOLIDS (%)	M.S.N.F (%)
SKIM MILK	0.76 a	3.20 a	4.72 a	0.10 a	8.83 a	8.73 a
UF-RETENTATE	1.65 b	12.90 b	4.82 b	0.48 b	19.93 b	19.45 b
UF-PERMEATE	0.49 c	0.41 c	4.58 c	0.00 c	5.57 c	5.57 c
SEDifference	0.141	0.029	0.055	0.002	0.035	0.033
RECOVERY (%)[2]	93.8	93.3	95.3	99.6	94.5	

a,b,c Means within the same column followed by the same letter are not significantly different ($P<0.05$)

[1] Determined by enzymatic method (Anon. 1989)

[2] Recovery in dry matter basis

MSNF had a similar response as they were determined by subtracting the fat content from the total solids values. However, protein was increased from 3.20 to 12.9 %, representing a 4-fold increase of concentration. In the ultrafiltered permeate the protein content was 0.41 %. Possibly some whey proteins were in the permeate as reported by Bastian *et al.* (1991); and urea, amino acids and NH_3 reported by Green *et al.* (1984).

The non-protein nitrogen content of skim milk, ultrafiltered retentate and ultrafiltered permeate in one of the trials was measured yielding values of 0.21 %, 0.23 and 0.19% respectively.

Renner and Abd El Salam, (1991) mention that lactose is fractionated between the retentate and the permeate, and mention that the extent of this fractionation will depend on the degree of concentration of protein in the final retentate. In another report Yan *et al.* (1979) stated that large differences in lactose rejection coefficients were found and suggests that it may be due to variability in membrane fouling, and error in measurement.

The lactose content of skim milk (4.72%), ultrafiltered retentate (4.82%) and ultrafiltered permeate (4.58%) are similar as would be expected for a compound in solution with a molecular weight of 342 Daltons. Clearly most of the lactose is in the permeate which has a greater volumetric flow rate than the retentate.

The mineral content showed some changes in the ultrafiltration process (See Table 3.10). During the ultrafiltration process removal of minerals may be expected since their molecular weights are less than 1000 Daltons. However the

TABLE No. 3.10 MINERAL CONTENT IN RAW MATERIAL (Dry Basis) FOR ICE CREAM MANUFACTURE

SAMPLE	CALCIUM (mg/100 g)	PHOSPHORUS (mg/100 g)	MAGNESIUM (mg/100 g)	POTASSIUM (mg/100 g)	SODIUM (mg/100 g)
SKIM MILK	1360 a	1020 a	113 a	1660 a	555 a
UF- RETENTATE*	1907 b	999 b	180 b	820 b	266 b
UF-PERMEATE ****	539 c	978 c	46 c	2514 c	826 c
SEDifference	3.76	4.46	0.19	15.00	4.91
RECOVERY (%)[1]	84.6	91.6	94.0	95.3	93.4

a,b,c Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

* Ultrafiltered retentate

** Ultrafiltered permeate

1 Recovery in dry matter basis

incomplete removal of some minerals from ultrafiltered retentate, (mainly Calcium, Magnesium and Phosphorus) can be due to their association with proteins. Concentration by Ultrafiltration usually involves only a mild heat treatment, and the pressure perturbs the equilibrium only slightly, so the small changes in diffusible salt concentrations observed can be explained as a result of concentrating the proteins, mainly casein micelles. The calcium content in milk, has two thirds in colloidal form associated with the casein micelles and the remaining one third is soluble. Calcium showed an increase from 1359 mg/100g in milk to 1906 mg/100g in ultrafiltered retentate, but in the ultrafiltered permeate it was reduced to 538 mg/100g.

The decrease in calcium phosphate solubility with temperature increase and concentration polarisation effects at the membrane surface may also have contributed to low calcium recovery. The total mass of Phosphorus divides almost equally between ultrafiltered permeate and ultrafiltered retentate (See Table No. 3.8). However, on a dry matter basis it was slightly higher in the ultrafiltered retentate (See Table No. 3.10). Almost 35% of the magnesium remained in the ultrafiltered retentate (180 g/100g), from 113 mg/100g in milk. This is expected since it has been determined that 0.8% is bound in the casein micelle and 44% is associated with the whey proteins (Flynn and Power, 1985). Bastian *et al.* (1991), report a range of calcium retention from 82% to 99% during ultrafiltration, and diafiltration of unacidified and acidified whole milk.

Sodium and potassium in milk are believed to be present almost entirely as free ions (Flynn and Power, 1985). The potassium and sodium content of the skim milk were 1660 mg/100g and 555 mg/100g respectively. The proportion of these elements reduced in the ultrafiltered retentate to 820 mg/100g and 265 mg/100g respectively, but were increased in ultrafiltered permeate to 2513

TABLE No. 3.11 CHEMICAL COMPOSITION OF ICE CREAM MIXES

SAMPLE DESCRIPTION	ASH (%)	PROTEIN (%)	LACTOSE[2] (%)	SUCROSE[1] (%)	FAT (%)	TOTAL SOLIDS (%)
UF-MIX 1[3]	1.04 a	8.83a	3.29 a	13.65	9.8 a	36.61 a
UF-MIX 2[4]	1.02 b	7.13b	2.91 b	13.24	9.9 b	34.25 b
CONTROL	0.96 c	4.39c	5.06 c	13.62	10 c	34.03 c
SEDifference	0.001	0.116	0.182		0.037	0.077

a,b,c Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

1 Sucrose was determined by difference

2 Lactose was determined by the enzymatic method (Anonymous, 1989)

3 Ultrafiltered ice cream mix 1

4 Ultrafiltered ice cream mix 2

mg/100g and 825 mg/100g. The fact, that these minerals increased in the ultrafiltered permeate was as a result of their low molecular weights and because they are not linked to any chemical network in the milk. This agrees with results shown by Eckner and Zottola (1992), about partition of skim milk components during ultrafiltration processes.

3.3.3 Chemical Characteristics of Ice Cream Mixes

The chemical composition in ice creams were statistically different ($P< 0.05$) in all cases. The use of ultrafiltered retentate as a source of MSNF in ice cream formulations, slightly increases the content of ash (e.g. 1.04 %/1.02% against 0.96 %) and protein and reduces the lactose content in the final product (See Table No. 3.11).

The protein content in ultrafiltered ice cream mixes (Mix-1 8.83% and Mix-2 7.13%) were almost double that in the control (4.39%). The lactose content in Mix-1 (3.29 %) and Mix-2 (2.91 %), were lower than the control with 5.06 % . This agrees with Renner and Abd El-Salam (1991) who mention that by means of the ultrafiltration process, high protein and low lactose products can be obtained. In this case, ultrafiltered mix-1 obtained more protein also as a result of increasing the MSNF (13.16 %) in its formulation compared with ultrafiltered mix-2 (11.11%) and the control with 10.41 % of MSNF.

The mineral content of the ice cream mixes showed that calcium, phosphorus, potassium and sodium were all significantly different ($P< 0.05$) between the two ultrafiltration mixes and the control (See Table No. 3.12). There was no difference between the magnesium content of ultrafiltered mix-2 and the control at 29.4 mg/100g. This is probably due to the fact that they had

similar MSNF content, whereas the ultrafiltered mix-1 had a higher MSNF content (13.16%).

The calcium content of ultrafiltered mixes 1 and 2 were 73% and 55% higher than the calcium in the control mix. Phosphorus was 47% higher in ultrafiltered mix-1 and 36% higher in ultrafiltered mix-2 than the control. These properties might be useful in the diet of older people who suffer from osteoporosis.

The potassium content of ultrafiltered mix-1 was 59% less than the control and 76% less for the ultrafiltered mix-2. Sodium levels were both 40% less than the control for ultrafiltered mixes 1 and 2. Decreased sodium levels could be beneficial in the diet.

TABLE No. 3.12 MINERAL CONTENT IN ICE CREAM MIXES (Dry Basis)

SAMPLE	CALCIUM (mg/100 g)	PHOSPHORUS (mg/100 g)	MAGNESIUM (mg/100 g)	POTASSIUM (mg/100 g)	SODIUM (mg/100 g)
UF- MIX 1[1]	765 a	519 a	55 a	348 a	154 a
UF-MIX 2[2]	682 b	482 b	29 b	314 b	153 a
CONTROL	441 c	353 c	29 b	553 c	216 b
SEDifference	11.88	11.89	0.26	3.89	3.51

a,b,c Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

[1] Ultrafiltered ice cream mix 1

[2] Ultrafiltered ice cream mix 2

3.4 Physical Properties of Ice Cream

The physical properties of any product are largely affected by the type of ingredients used and the manufacturing process. The use of concentrated protein ingredients, from ultrafiltered retentate, affects the majority of the product physical characteristics, due principally to the chemical properties of the protein, such as binding of water.

3.4.1 Hardness

Hardness in ice cream is measured by the resistance of the product to the penetration by a probe at a certain temperature.

Olsen (1992), mentions, that when cooling mixes from pasteurisation to ageing temperature, the physical structure of the casein micelles change gradually, leading to the creation of a more hydrophilic molecular structure. He also states, that, during pasteurisation of the mix, partial denaturation of some of the whey proteins will take place, causing some coiled whey protein molecules to unwind. During ageing the partly denatured whey protein could have a water-binding effect, which may reach a level similar to that of casein, (i.e. around 3 g water/g protein.). The hydration process, that takes place during ageing, results in an increase in viscosity of the mix and subsequent hardness of ice creams , confirmed by the results (See Table No. 3.14).

Hardness values were statistically different ($P<0.05$) in some cases (See Table No. 3.13). Using probe TA16 at -18° C, -16° C and -5° C, the UF-ice creams were found to be about four times harder than the control. Ultrafiltered ice cream-1, was slightly harder than ultrafiltered ice cream-2. The increase in hardness may be explained in terms of protein concentration in the mixes as well as the temperature of the sample (See Table No. 3.11). Ultrafiltered ice

cream-1 is slightly harder than ultrafiltered ice cream-2, probably due to a higher total solids content (e.g. 36.61% / 34.25%).

3.4.2 Viscosity

Viscosity normally is affected by the level of solids used in formulae, type and amount of stabiliser, and pH. Sufficient viscosity in an ice cream mix, according to Diamond *et al.* (1988), is important in order to avoid serum separation during ageing and storage, and to ensure optimum incorporation and distribution of air cells during freezing. But according to Tanis (1988), too high a viscosity makes it difficult for the mix to entrap air. With too low a viscosity the mix will have difficulty in keeping the air entrapped. In this study the viscosities of all mixes, showed significant differences ($P > 0.05$) (See Table No. 3.14). The viscosity of mixes containing ultrafiltered retentate were higher than the control. Ultrafiltered mix-1 recorded the highest viscosity with 1.27 Ns/m^2 followed by ultrafiltered mix-2 with 0.87 Ns/m^2 versus 0.31 Ns/m^2 in the control. These results show how ultrafiltered retentate, which has a raised level of proteins, and milk solids, significantly increases the viscosity of the ultrafiltered mixes.

Higher viscosities can improve the perceived qualities of the frozen products and can minimise ice crystal growth during frozen storage. Hence, using ultrafiltered retentate in frozen dessert formulations could possibly reduce or eliminate the need for other viscosity building agents such as stabilisers.

3.4.3 Overrun

The overrun is the amount of air incorporated into the mix during the freezing process. The functions of air in ice cream according to Berger *et al.* (1972), are to provide lightness of body and smooth texture. Too little air gives

the ice cream a heavy, soggy body, while too much air gives a fluffy body. Large air cells give a snowy or flaky texture, while smaller air cells are associated with smooth texture. The overrun according to Tanis (1988), also depends on the percentage of whipping agent, the mixing temperature at which whipping occurs, and processing equipment. In UF-mixes 1 and 2, the overruns were 54 and 63 % respectively, which were lower than the control with 66 % (See Table No. 3.14). This finding agrees with other reports where ultrafiltered retentate was a partial or total source of MSNF in the formulation. There are no reports explaining the cause for this decrease in overrun, but it is thought that increasing the quantity of protein in the mixture will create a stronger matrix. It would be difficult for air bubbles to form because of the concentration of total solids in the mix. When the mix flows over the beater blades air is incorporated in the turbulent zone downstream from the blades. An increase in the viscosity of the mix will make it more difficult for air to be incorporated. In this study a vertical freezer was used and the overall overrun was expected to be under 70 %. However, the differences between the control and ultrafiltration mixes may be explained in terms of the amount of ultrafiltered retentate used in the formulation, and increasing the MSNF content present in the mix. It has been explained by Arbuckle (1986), that increasing total solids in the formulation will result in less water being frozen. The increased concentration of mixes, causes mechanical obstruction to crystal growth and air incorporation during the freezing process which is also hindered by the increase of viscosity caused by the MSNF as cited by Crowhurst (1993)

3.4.4 Extrusion Temperature

Extrusion temperature is the temperature of the ice cream by the time it has to be withdrawn from the freezer. According to Rothwell (1991a), (1992c), it is typically in the range of - 4° C to - 6° C, when approximately 50 % of the

TABLE No. 3.13 HARDNESS OF ICE CREAM SAMPLES (Newtons) *

SAMPLE	PROBE TA 16 -18° C	-16° C	- 5° C	PROBE TA 8 - 5° C
UF-MIX1	6.11 a	5.53 a	0.113 a	0.24 a
UF-MIX2	6.01 a	5.07 b	0.113 a	0.23 b
CONTROL	1.46 b	1.29 c	0.024 c	0.06 c
SEDifference	0.117	0.026	0.006	0.026

a,b,c Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

* Room Temperature of 13° C

TABLE No. 3.14 PHYSICAL CHARACTERISTICS OF ICE CREAM MIXES

TRIALS	VISCOSITY ($N\ s/m^2$)	OVERRUN (%)	EXTRUSION * TEMPERATURE (° C)
UF-MIX1	1.27 a	54 b	- 3.6 a
UF-MIX2	0.87 b	63 a	- 3.4 b
CONTROL	0.31 c	67 c	- 4.6c
SEDifference	0.0285	1.31	0.08

a,b,c Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

* 10 Minutes freezing in all cases

water is frozen. It is affected by the amount of solids but mainly by the amount of minerals and sugars present in the mix. The extrusion temperature of the ice creams was lower in the control with -4.6° C against -3.6 and -3.4 ° C in ultrafiltered mix-1 and ultrafiltered mix-2 respectively (See Table No. 3.14). This response is due to the lower amounts of lactose in the UF-Mixes.

3.4.5 Melting Properties

According to Bradley (1985) and Flack (1988), any frozen dessert when tested should melt to a consistency similar to that of the mix.

The melting characteristics of ice creams are shown in Table No. 3.15, and all the samples were statistically different ($P < 0.05$) at all stages of melting as confirmed by values of the standard error of difference.

The liquid collected was recorded from the first drop released and then recorded in duplicate every five minutes at a temperature of 20.5° C. Ultrafiltered ice cream-1 and ultrafiltered ice cream-2 released the first drop at 74 and 62 minutes respectively, compared with 27 mins for the control.

It took 16 minutes from the first melting to get the first 10 ml of ultrafiltered ice cream-1; 18.5 minutes for the ultrafiltered ice cream-2 and 22.5 minutes for the control. However after the tenth ml the rate of melting was similar for all ice creams at 0.8 ml/min for ultrafiltered ice cream, ultrafiltered ice cream-2 with 0.81 ml/min and 0.83 ml/min for the control. The final time required to collect 90 ml was highest in ultrafiltered ice cream-1 with 154 mins and then ultrafiltered ice cream-2 with 145.5 mins against 116 mins of the control. Slow melting in UF-mixes may be attributed to the higher protein content in the mixes which have a water binding effect, also the freezing point

TABLE No. 3.15 MELTING CHARACTERISTICS OF ICE CREAMS [1]

LIQUID COLLECTED *(ml)*	TRIALS UF-IC1* *(min)*	UF-IC2** *(min)*	CONTROL *(min)*	*SEDifference*
FIRST DROP	74	62	27	5.20
5	84	75	41	3.50
10	90	81	50	2.20
15	96	86	56	1.90
20	101	92	61	2.00
25	106	97	66	1.90
30	109	102	70	1.90
35	113	106	75	1.20
40	118	111	79	0.70
45	122	115	83	0.70
50	127	119	87	0.70
55	131	123	90	0.60
60	134	126	93	0.60
65	139	130	96	1.00
70	143	133	101	1.20
75	147	136	105	1.50
80	149	139	109	1.10
85	152	143	113	1.00
90	154	146	116	1.20

[1] Melting temperature ranged from 20.1 ° C to 20.6 ° C
* Ice cream 1 using ultrafiltered retentate
** Ice cream 2 using ultrafiltered retentate

of the control was approximately 1° C lower than the UF-ice creams, and hence the control mix should start melting first. Once a layer of melted ice cream is formed, heat transfer through the unfrozen ice cream is likely to be the rate-limiting step. This would account for the similar melting rates after first melting has occurred. Arbuckle (1986) suggests that increasing the level of MSNF in formulations increases the viscosity of mixes and resistance to melting.

3.5 Sensory Characteristics of Ice Cream

Sensory analysis according to Lyon *et al.* (1992) is used to establish difference and to characterise and measure sensory attributes of products, or to establish whether product differences are acceptable or unacceptable, and noticeable to the consumer. In product development and quality control, understanding, determining and evaluating the sensory characteristics of products are important in many applications such as shelf-life studies, product matching, product mapping, product reformulation and product acceptability.

Residual Maximum Likelihood (REML) was used to fit a mixed model to the data. Random effects of judge and time within judge were estimated. Fixed effects by time on ice cream were estimated along with the effects of order of presentation. These presentation effects must be taken into account in a properly designed sensory trial.

3.5.1 General Sensory Properties

The methylene blue test was carried out before each sensory evaluation session in order to evaluate the general microbiological conditions of the ice creams. In all cases the results were satisfactory. The average values for the sensory attributes, are presented in Table No. 3.16. Figure No. 3.5, shows the

TABLE No. 3.16 MEAN SCORES IN SENSORY EVALUATION OF ICE CREAMS *

ATTRIBUTE	UF-IC1[1]	UF-IC2[2]	CONTROL	SEDiff
ICINESS	13.8a	16.7b	16.4cb	2.27
SANDINESS	9.3a	10.9ab	12.7b	1.81
GUMMINESS	38.9a	31.4b	22.4c	3.68
WATERY	36.5a	49.0b	39.3a	4.24
FLUFFINESS	18.6a	19.8a	33.3b	3.19
FLAVOUR STRENGTH	55.1a	49.7b	74.2c	3.36
COLOUR	57.4a	53.2b	54.7b	2.06
ACCEPTABILITY	55.8a	67.6b	46.3c	4.32

a,b,c Means within the same row followed by the same letter are not significantly different ($P<0.05$)

* Means are the average of three sessions

[1] Ice cream 1 using ultrafiltered retentate

[2] Ice cream 2 using ultrafiltered retentate

FIGURE No. 3.5 SCORE CARD FOR SENSORY ANALYSIS OF ICE CREAMS

NAME_____

SAMPLE No_____

ICINESS

None Extremely

SANDINESS

None Extremely

GUMMINESS

None Extremely

WATERY

Not Extremely

FLUFFY

Not Extremely

FLAVOUR STRENGTH

Weak Strong

COLOUR

Pale Intense

OVERALL ACCEPTABILITY

Like Dislike

COMMENTS_____

* Attributes lines are 150 mm long

score card used in this study. Ultrafiltered ice cream-1 and ultrafiltered ice cream-2 scored as the preferred products being significantly different ($P<0.05$) from the control made using skim milk powder. Iciness recorded lower scores in ultrafiltered ice cream-1 than the control, but the iciness scores for ultrafiltered ice cream 2 and the control were similar. This behaviour may be attributed to the amount of MSNF, and hence protein, present in ultrafiltered ice cream-1 formula (13.16 %), which tends to retain more water, thus having a stabiliser effect on the product. (Table No. 3.11, gave the chemical composition of ice cream mixes).

Although sandiness was lower in ultrafiltered ice cream-1 than ultrafiltered ice cream-2, it was not significantly different. This also applied to sandiness in ultrafiltered ice cream-2 and the control. Sandiness is normally caused by the presence of large lactose crystals, and according to Hyde and Rothwell (1973) it is caused by high MSNF in relation to the water in the ice cream formulation. However in this study two formulae were used; one for ultrafiltered ice cream-1 using 13.16 % of MSNF (3.29% lactose) and the other for ultrafiltered ice cream-2 with 11.11% of MSNF (2.91% lactose) and the control with 10.41 % MSNF (5.06% lactose). Hence, it is expected that ultrafiltered ice cream-2 would show the least sandiness.

Gumminess in ultrafiltered ice cream-1 and ultrafiltered ice cream-2 were markedly higher than the control at 38.9 and 31.4 respectively, but were significantly different at $P<0.05$. This may be explained by the fact that UF-mixes had more stabiliser effect from the increased proteins. No significant differences in the watery response were found between ultrafiltered ice cream-1 and the control at ($P<0.05$). Ultrafiltered ice cream-2 had a much higher watery response (49.0) than either ultrafiltered ice cream-1 or the control. This result

was unexpected because of the higher level of protein in ultrafiltered ice cream-2 compared with the control. The control ice cream was more fluffy than the ultrafiltered ice cream which were similar to one another.

With regard to flavour strength, significant differences ($P<0.05$) were found between all samples. The control had the strongest flavour against the UF-ice creams, with added ultrafiltered retentate reducing the strength of the flavour. Colour responses were similar for ultrafiltered ice cream-2 and the control. Even though ultrafiltered ice cream-1 had a colour score of 57.4, it was only a few points higher than the other two ice cream samples.

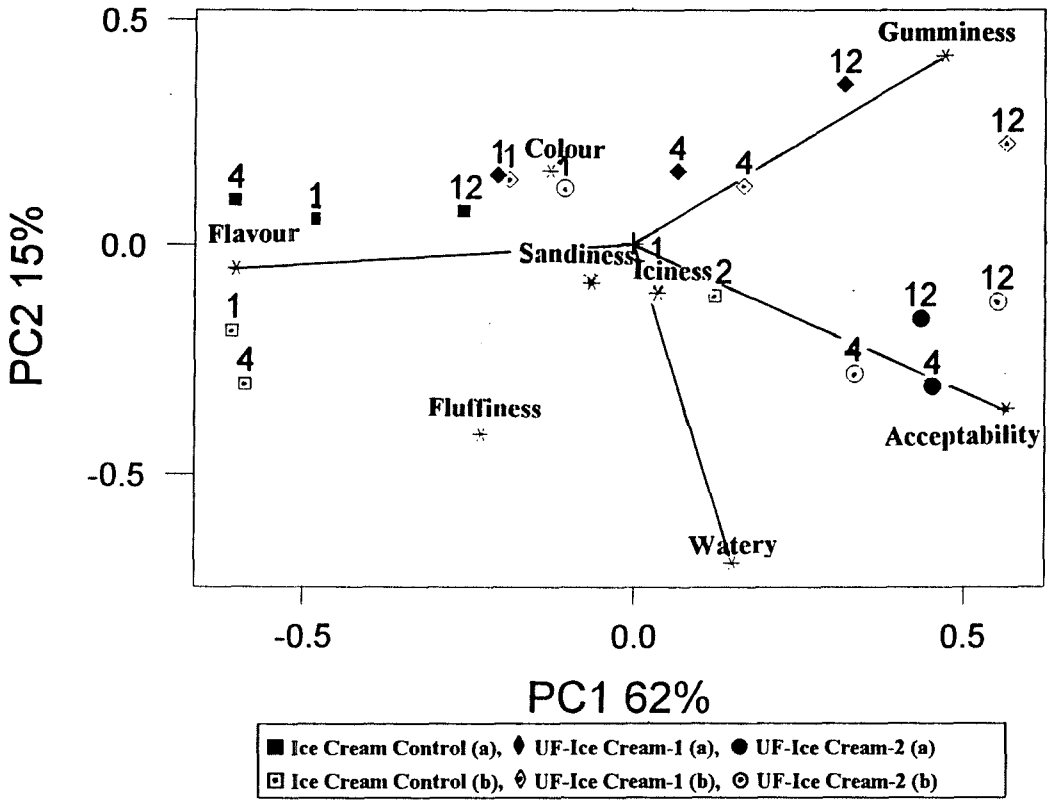
Overall acceptability between ultrafiltered ice cream-1, ultrafiltered ice cream-2 and control were significantly different ($P<0.05$) from each other, with the control preferred.

3.5.2 Principal components analysis (PCA)

In this study the results from sensory analysis were used for principal components analysis. The first principal component accounts for 62 % of the total variation, and the second another 15 % to get an accumulated representation of 77% of the total variation of the data. The rest of the variation does not contribute and can be eliminated to visualise the data in two dimensions. The principal components are an arbitrary linear combination of the sensory scores and cannot be interpreted to get measured values directly; stars showing where the actual characteristics appear on the scatterplot have been added to the figure to produce a biplot (See Figure No. 3.6).

The important vectors are acceptability, gummy, flavour, fluffiness and watery. The rest are less important in describing the products (e.g. colour).

FIGURE No. 3.6 PRINCIPAL COMPONENTS ANALYSIS OF ORGANOLEPTIC EVALUATION OF ICE CREAM AT 1, 4 AND 12 WEEKS



According to the PC-diagram the first principal component basically separates the control from the UF-ice creams, and the second principal component separates the UF-ice creams showing a general relationship between them. The controls are more fluffy and somewhat more sandy than the ultrafiltered ice creams. The colour of all ice creams are fairly similar. Ultrafiltered ice cream-1, was the most gummy of the samples. Ultrafiltered ice cream-2 was eventually the most watery. The control had the strongest flavour. Higher scores for overall acceptability indicate increasing dislike for the ice cream and on this basis the control is preferred and is shown on the PC-biplot as the cluster of points furthest away from overall acceptability.

3.5.3 Storage periods

Scores for organoleptic evaluation after one, four and twelve weeks are shown in Appendix A.4. In all cases referred to, the levels of significance are at $P<0.05$.

The texture of ice cream should be smooth. This requires that the ice crystals and any other solid particle present must be small. The most common defect is that the texture is coarse and icy due to the presence of large ice crystals and coarse structure in general.

There are some factors affecting the iciness defect. The incorporation of air as small air cells in the ice cream should be helpful in producing a smooth texture (See Section No. 3.6.2 for air cell sizes). The composition of the mix has a strong effect on the final texture, because if total solids of the mix are increased there will be less water to be frozen into ice crystals, and because the ice crystals that are formed will be interspersed with solids more generously and crystal growth will be hindered by mechanical obstruction.

In this study, increases in iciness in all samples occurred as expected with the growth of water crystals through storage. Iciness was significantly different, between ultrafiltered ice cream-1 and ultrafiltered ice cream-2 on the first week, but ultrafiltered ice cream-1 and the control had similar iciness scores. However after the fourth week all samples were significantly different. After twelve weeks storage all samples were similar in iciness (See Figure No. 3.7).

Ultrafiltered ice cream-1 had lower iciness scores, and this may be due to the higher total solids and protein content, which helps in reducing the formation of ice crystals. It had 9% less carbohydrate content than the control, and 4.6% more than ultrafiltered ice cream-2. It is known that the sugar content is important in determining the freezing point of the mixes; increasing the sugar content will lower the freezing point of the mix. A lower freezing point means that at any given temperature the ice cream will be less completely frozen; there will be fewer ice crystals and the product will appear smoother. However as the ice cream samples were hardened to the same temperature (- 22° C), most of the ice will have been frozen in all samples only if the solute concentration is similar.

Sandiness in ice cream is caused by the presence of lactose crystals. Those crystals are result of the crystallisation of lactose when it reaches the saturation point. The lactose solubility is only 11.9 parts per 100 parts of water at 0° C. The average lactose content of UF-ice creams was 4.8 parts of lactose to 100 parts of water, but when about 70% of the water is frozen, there would be only 28.6 parts of water remaining to hold the lactose in solution. This would be equivalent to 15.9 parts of lactose to 100 parts of water, which is above to the saturation point of lactose. But when this point has been reached, the unfrozen

portion is then so viscous that it is in the glass state or at least in conditions where crystallisation will be exceedingly slow. With a higher solids content the point of spontaneous crystallisation is reached earlier, and crystallisation will be more rapid.

The lactose crystals in ice cream are hard and do not dissolve at once in the mouth, and if they increase above approximately 30 μm, the ice cream becomes progressively more gritty.

Initially sandiness in all samples was not significantly different, but after four weeks all samples were significantly different in the control scored the highest sandiness. After twelve weeks storage ultrafiltered ice cream-2, sandiness was significantly different from ultrafiltered ice cream-1 but similar to the control (See Figure No. 3.8). It seems to indicate that the source of MSNF affected the presence of sandiness in the ice creams. Ice cream-1 made using UF-retentate with low lactose content had lower sandiness scores compared with the ice cream control (See Table No. 3.11).

Gummines is largely affected by the presence of high quantities of stabiliser in the ice cream formula. Ultrafiltered ice cream-1 and ultrafiltered ice cream-2 were not significantly different from each other, but were significantly higher than the control after one week's storage (See Appendix III and Figure No. 3.9). The gummines of all ultrafiltered samples increased by the fourth week storage period, but control decreased from 17.3 to 13.5. Ultrafiltered ice cream-1 was significantly more gummy than the other ice creams after twelve weeks storage but ultrafiltered ice cream-2 and the control were similar.

Ultrafiltered products were expected to be gummy due to the viscosity increase caused by the protein. The results agrees with the melting resistance responses (See Table No. 3.15).

Watery defect causes the ice cream to melt rapidly. It is caused by both low total solids and low protein content in the formulation, especially if the stabiliser content is low. Watery scores after one week storage were not significantly different in all samples. However, after four and twelve weeks of storage ultrafiltered ice cream-1 and control were not significantly different , but both were significantly less watery than ultrafiltered ice cream-2 (See Figure No. 3.10). However, this does not correlate with the high melting rate results (See Table No. 3.15).

Fluffiness or snowy fault, results when a large amount of air is incorporated as large air cells. There are some causes which affect the size of the cell. For instance, when there is low total solids content, the mix does not offer enough resistance to the whipping mechanism in the freezer to cause the fine subdivisions (air-cell walls or lamellae) of the incorporated air. The results are large air cells and if the overrun is high the lamellae will be thin, hence a flaky texture.

After twelve weeks ultrafiltered ice cream-1 and ultrafiltered ice cream-2 had both decreased in fluffiness and there was no significant difference between them throughout storage. On the other hand, the fluffiness of the control was greater than the ultrafiltered samples after one week and increased throughout storage. This may be explained in terms of higher overrun which cause the ice cream to be lighter in weight and is probably due to the much lower protein content of the control (See Figure No. 3.11). In this study, the cause for higher

fluffiness scores for the ice cream control may be associated with the relatively high overrun (67 %) and low total solids (34.03%). The air cells for the ice cream control ranged from 20 to 200 μm (See section 3.6.2.1.1)

Flavour strength at one and four weeks of storage for all samples were significantly different with the control having the strongest flavour. After twelve weeks ultrafiltered ice cream-1 and ultrafiltered ice cream-2 continued to have significantly less flavour strength scores than the control (See Appendix III, and Figure No. 3.13). The flavour strength of both ultrafiltered ice cream samples was reduced during storage and the control, flavour strength also clearly decreased, being 36% of the value after the first week. By comparison the ultrafiltered ice cream samples 1 and 2 were 27% and 28%, respectively, of their first week flavour strength values. Flavour responses may have been influenced by the viscosity of the mix with higher viscosities dampening the flavour release and hence causing lower scores. The time of the year may also have influenced scores. It was noted that the scores levels were generally lower at the winter tasting session.

Colour scores, after one week of storage, in ultrafiltered ice cream-1 and ultrafiltered ice cream-2 were similar, but significantly different from the control which had a slightly lower colour score. However, statistical differences were found between all samples after four weeks storage with a very noticeable drop in the colour score of ultrafiltered ice cream-2. After twelve weeks storage the scores for all the samples were very similar and no significant differences were found at the $P<0.05$ level (See Figure No. 3.12), though the control had the greater percent change (-14%) in colour score.

FIGURE No. 3.7 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

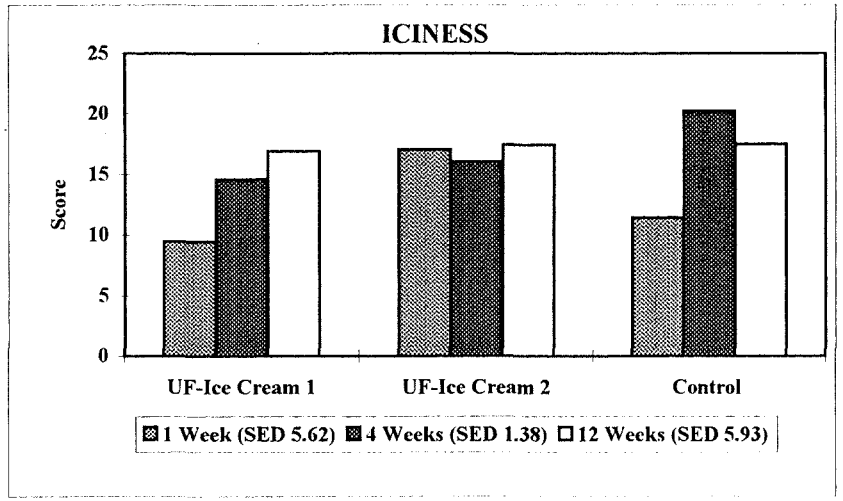


FIGURE No. 3.8 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

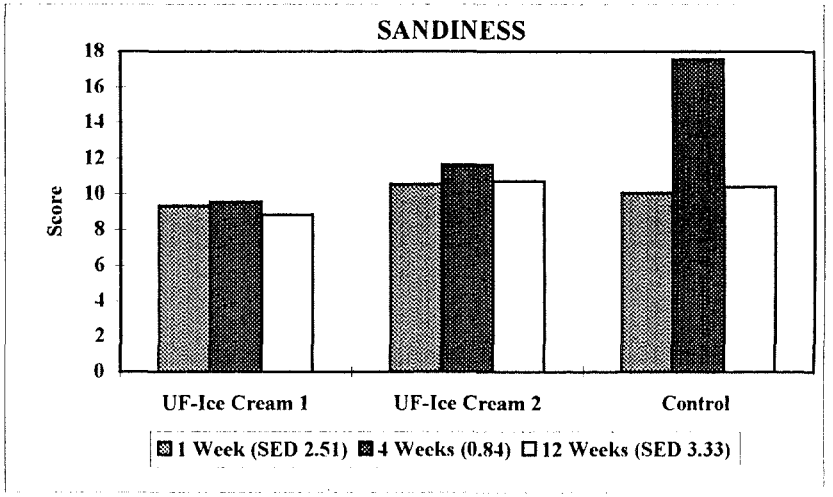


FIGURE No. 3.9 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

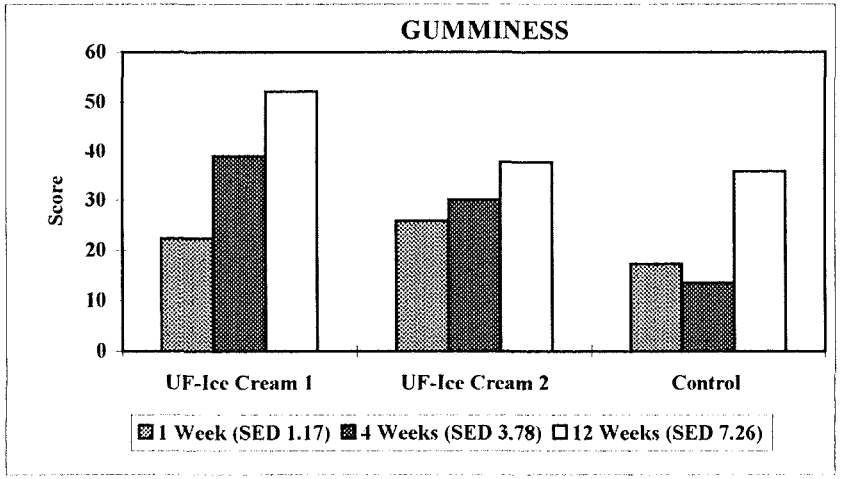


FIGURE No. 3.10 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

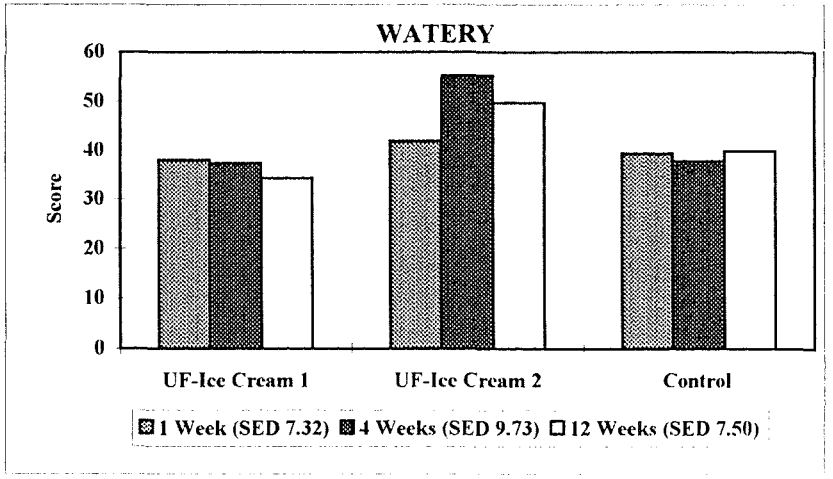


FIGURE No. 3.11 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

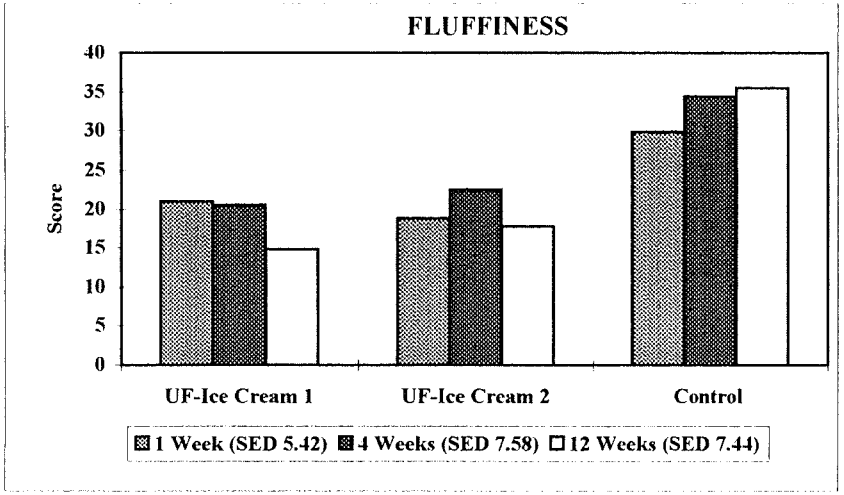


FIGURE No. 3.12 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

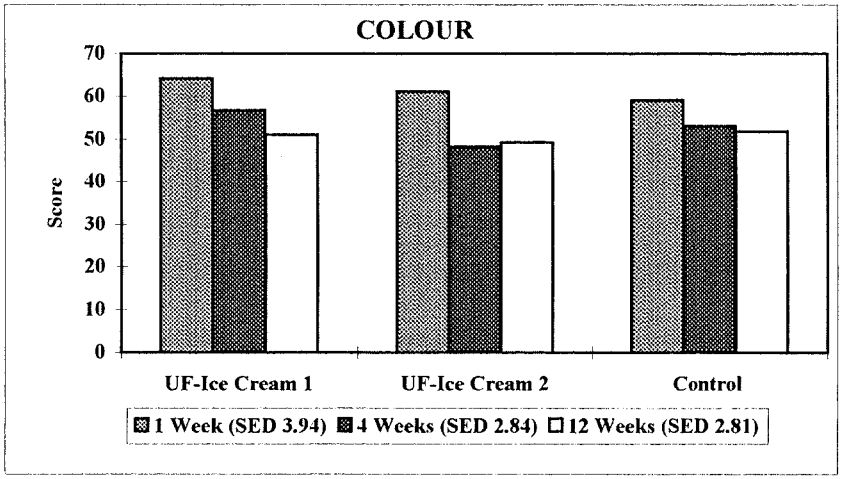


FIGURE No. 3.13 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME

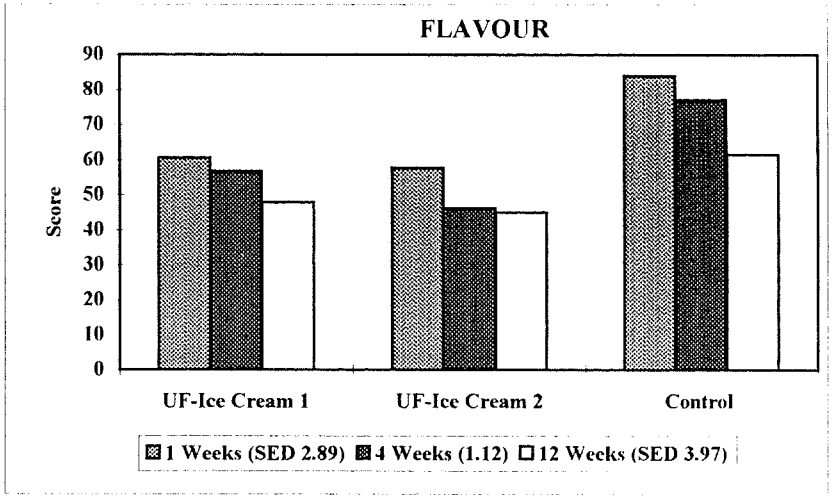
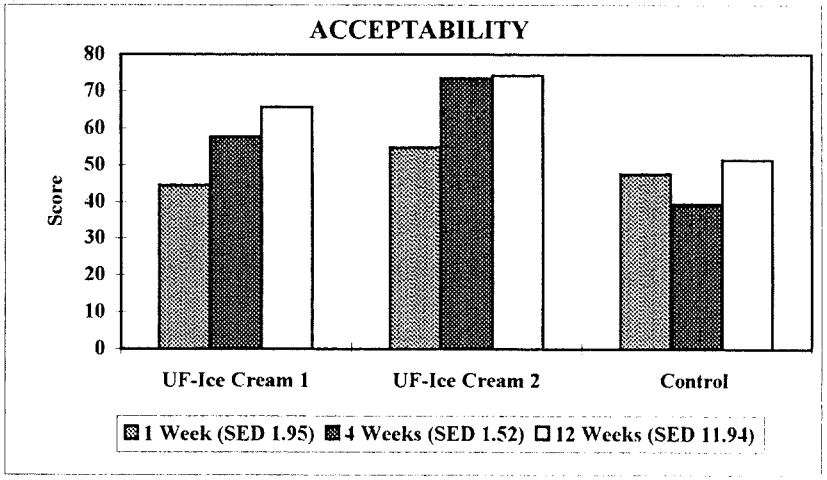


FIGURE No. 3.14 ICE CREAM SCORES OF SENSORY EVALUATION FOR TIME



For overall acceptability, statistical differences were found after the first week between all samples with ultrafiltered ice cream-1, having the lowest score and being the most preferred. However after twelve weeks storage ultrafiltered ice cream-1 and ultrafiltered ice cream-2 were not significantly different from one another. They were significantly different in acceptability from the control at 12 weeks and both had noticeable increases (48% and 35% respectively) in their scores. The control by contrast, only increased its acceptability score by 8% between the first and twelfth week of storage at which stage it was the most preferred ice cream (See Appendix III, and Figure No. 3.14). No strong linear correlation was found between overall acceptability and any of the individual attributes.

3.5.4 Heat Shock Stability

Heat shock stability in ice cream refers to the effect in which large ice crystals form in ice cream, usually as the result of uncontrolled temperature fluctuations. According to Hegenbart (1990) heat shock causes moisture to migrate, which results in large ice crystal formation. As the moisture forms ice crystals, excess milk sugar will begin to form lactose crystals eventually giving a grainy or coarse texture. Ice cream products can be vulnerable to heat shock during distribution and supermarket display, when changes in transport and storage procedures can lead to potentially harmful variations in temperature. The changes can reduce the quality of ice cream by changing texture and appearance, due to the melting of ice crystals during improper handling. If the temperature increase is followed by a temperature drop, the water which refreezes does not necessarily recrystallise as the original small crystals but tends to deposit on existing crystals, making them larger.

Sensory evaluation of the samples was carried out by ten panellists previously familiarised with the effects of heat shock. The methylene blue test was carried out before the evaluation session in order to evaluate the general microbiological conditions of the ice creams. In all cases the results were satisfactory. Figure No. 3.15, shows the score card used for this study. The mean scores for each product before heat shock treatment are shown in Table No. 3.17. In all cases referred to the levels of significance are at $P<0.05$.

Flavour strength was significantly different in all samples, with the ice cream control having the higher score. However the ultrafiltered ice creams presented similar scores around the middle of the score line (54.5 for ultrafiltered ice cream-1 and ultrafiltered ice cream-2 with 50.7). Body and iciness were not significantly different in all samples.

Table No. 3.18, shows the mean scores for ice creams after being heat shocked. Ultrafiltered ice cream-1 (13.16% MSNF) had the highest flavour strength response (90.6) compared with ultrafiltered ice cream-2 (70.9) having 11.11% MSNF and the ice cream control (76.5) using 10.41% of MSNF from skim milk powder.

Ultrafiltered ice cream-1 showed better body (84.5) and icy (21.0) response to heat shock conditions compared with ultrafiltered ice cream-2 (66.9/35.8) and the ice cream control (56.1/32.0).

According to the results ultrafiltered retentate used in ice cream making tends to intensify the flavour of the ice cream under heat shock conditions.

FIGURE No. 3.15 SCORE CARD FOR SENSORY EVALUATION OF HEAT SHOCKED ICE CREAM

NAME_____

DATE_____

SAMPLE No_____

FLAVOUR STRENGTH

_____

Unacceptable Excellent

BODY

_____

Poor Very good

ICINESS

_____

None Extremely

COMMENTS

_____

_____

_____

* Lines are 150 mm long

TABLE No. 3.17 ORGANOLEPTIC MEAN SCORES BEFORE HEAT SHOCK OF ICE CREAMS

ATTRIBUTE	UF-IC1[1]	UF-IC2[2]	CONTROL	SEDiff
FLAVOUR STRENGTH	54 a	51 b	72 c	3.38
BODY	26 a	28 a	27 a	3.23
ICINESS	15 a	17 a	17 a	2.67

a,b,c Means within the same row followed by the same letter are not significantly different ($P<0.05$)

1 Ice cream-1 using ultrafiltered retentate

2 Ice cream-2 using ultrafiltered retentate

TABLE No. 3.18 ORGANOLEPTIC MEAN SCORES AFTER HEAT SHOCK ON ICE CREAMS *

ATTRIBUTE	UF-IC1[1]	UF-IC2[2]	CONTROL	SEDiff
FLAVOUR STRENGTH	91a	71 b	76 b	9.90
BODY	85 a	67 b	56 c	9.09
ICINESS	21a	36 b	32 b	6.66

a,b,c Means within the same row followed by the same letter are not significantly different ($P<0.05$)

* Removed from hardening room and stored at - 4° C for 2 hrs and then 20° C for one hour. Results for two trials for each product using random presentation order

1 Ice cream-1 using ultrafiltered retentate

2 Ice cream-2 using ultrafiltered retentate

Ultrafiltered ice cream-1 was more stable to the heat shock treatment as a result of the high protein content which improves the stabilising effect of the ice cream components. On the other hand, the ice cream control may be affected by the heat shock treatment with some destabilisation of the fat, forming clusters and coalesced units, as shown in Figure No. 3.29 and Figure No. 3.32 of the ultrafiltered heat shock ice cream (See Section No. 3.6.2.3.3 for microstructure interpretation).

The ultrafiltered ice-1 cream presented better response to heat shock treatment for iciness and body than ultrafiltered ice cream-2 and the ice cream control. This may be due to the higher protein content (8.83%) of the product.

Iciness was increased in all samples as a result of the ice crystals refreezing forming large ice crystal networks (See Sections 3.6.2.3.1 and 3.6.2.4.1), but to the greatest extent with ultrafiltered ice cream-2.

3.5.5 Consumer Acceptance

From the organoleptic evaluation of ice cream products, ultrafiltered ice cream-1 and the control were selected to be evaluated against each other in a student consumer trial. The methylene blue test was carried out before the evaluation to evaluate the general microbiological conditions of the ice creams. In all cases the results were satisfactory. Figure No. 3.16, shows the score card used for this study. Fresh samples were prepared following the previous formulations and they were kept in a insulated box during the testing period

The results from a random 61 students in a scored evaluation, showed that UF-ice cream and the control were not statistically different ($P<0.05$) (See Table No. 3.19).

FIGURE No. 3.16 SCORE CARD FOR CONSUMER ACCEPTANCE OF ICE CREAMS

NAME_____

DATE_____

Circle one score for every sample of ice cream, using the table below to describe every number.

1	2	3	4	5	6	7
Dislike Extremely	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Extremely

SAMPLE No 1 **1 2 3 4 5 6 7**

SAMPLE No 2 **1 2 3 4 5 6 7**

COMMENTS

_____

_____

_____

TABLE No. 3.19 MEANS OF CONSUMER PREFERENCE EVALUATION OF ICE CREAMS

SAMPLE	MEANS	SE Diff
UF-ICE CREAM-1	5.56 a	
CONTROL	5.69 a	1.21

a Means within the same column followed by the same letter are not significantly different ($P < 0.05$)

TABLE No. 3.20 CROSS CLASSIFICATION OF CONSUMER PREFERENCE AT DIFFERENT STAGES OF TRIAL FOR UF-ICE CREAM AND ICE CREAM CONTROL

STAGES	NUMBER OF CONSUMERS				PROBABILITY * (%)
	EQUAL	UF-SAMPLE	CONTROL	TOTAL	
Early (< 30 mins)	3	2	11	16	0.022
Mid	7	9	19	35	0.084
Late (> 90 mins)	0	10	0	10	0.002
TOTAL	**10**	**21**	**30**	**61**	

* Probability that UF-Sample and Control are equally good given the distribution of preference at each stage (early, mid and late of trial)

However, during the evaluation of the ice cream samples a tasters preference of the control was observed during the first 30 minutes of the trial, due to the softness of the product, since ultrafiltered ice cream products are harder (See Table No. 3.13). A cross classification of consumer preference at different stages of the trial was made to evaluate the preferences at the early, mid and late stages of the trial (See Table No. 3.20).

However, as the evaluation passed to the next stage the proportion of scores for the UF-ice cream sample tended to increase. However at the last stage, all the preferences were for the UF-sample which tended to be soft and at the optimum point to be tasted, compared with the control which was very soft.

In summary, increasing the protein content in ice cream products by using ultrafiltered retentate with not compensating change in the sugar, can make the products harder. This would be an advantage in warmer countries where in refrigeration systems are not so readily available, and more use is made of insulated service units.

3.6 Microscopy Analysis of Raw Material and Ice Cream

Microscopy has been used to investigate a wide range of food stuffs and many food properties have been shown to be related to the structures found by microscopy (Aguilera and Stanley, 1990). Lewis (1990b) mentions that in food systems, at the finest structural level changes in molecular structure can alter the behaviour of an ingredient and the whole quality of the product, such as rheology, flavour, appearance and stability on processing.

Lewis (1990b) reviewed different microscopy techniques for the analysis of the food microstructure. In this study, the following techniques were used.

Light Microscopy (LM), offers the advantage of working at normal conditions of temperature and pressure and allows the use of filters and stains to give contrast in a specimen. There is a considerable literature on the light microscopy observations of a wide range of food related materials cited by White and Shenton (1976), (1977a), (1977b), (1980), (1981) and (1982). However, it lacks resolution and 3-D imaging. Sztehlo (1994), concluded from a series of investigations about light microscopy techniques of ice cream structure, that light microscopy can be used to investigate the structure of components in ice cream. This technique is cheaper in comparison with other techniques. Confocal laser scanning microscopy is a recent development in microscopy which improves the resolution of ligh microscopy, giving instant images, and allows computer 3D reconstructions. It scans the specimen with a laser beam, measuring the intensity of the re-emitted light. The confocal element means that only light from a very small depth of field is used to form the image (Lewis, 1990b).

Scanning Electron Microscopy (SEM), according to Sargent (1991), is a technique offering high resolution images and a great depth of field, hence giving better 3-D images. Special specimen preparation is needed since the microscope operates under high vacuum, and application of gold is required to avoid a build up of charge from the electron beam. It examines only surfaces but specimen preparation is easier than TEM. The development of cryo-SEM and more recently the environmental SEM have reduced the specimen preparation needs for SEM. In this technique the specimen is placed at low vacuum pressure whilst the rest of the microscope is maintained at a much higher vacuum. This allows the sample to be directly in the microscope without coating

with a conductive layer as a result of the high pressure around the sample. A bibliography of food related applications of SEM is given by Holcomb (1990).

Transmission Electron Microscopy (TEM) offers the best resolution of normal microscopy (0.2 μm), although 3-D images are not obtained. Specimen preparation requires more time and care, since the specimen has to be treated with chemicals for fixation and dehydration. TEM may be used for: sizing of small droplets in emulsions; casein micelle distribution; function of stabilisers; gelling and thickening agents; crystal form in fat systems; and recognition of small foreign body particles. Many applications of TEM to dairy products have been presented, notably reviews by Kalab (1993) and Brooker (1979).

According to Berger and White (1979), one reason for studying the structure of ice cream was to understand how to prevent the sandy texture which is caused by the presence of large lactose crystals. Likewise, they mention that light microscopes, and electron microscopes, have proved to be powerful tools for the investigation of ice cream, in terms of emulsification and subsequently stability of the fat phase.

3.6.1 Microscopy of skim milk and ultrafiltered retentate

In this study, Transmission Electron Microscopy (TEM) was the most appropriate technique to examine the microstructure (proteins) of skim milk and ultrafiltered retentate.

Skim milk and ultrafiltered retentate were examined using the TEM at x 7,500, x 20,000 and x 50,000. (See Figures No. 3.17 and 3.18).

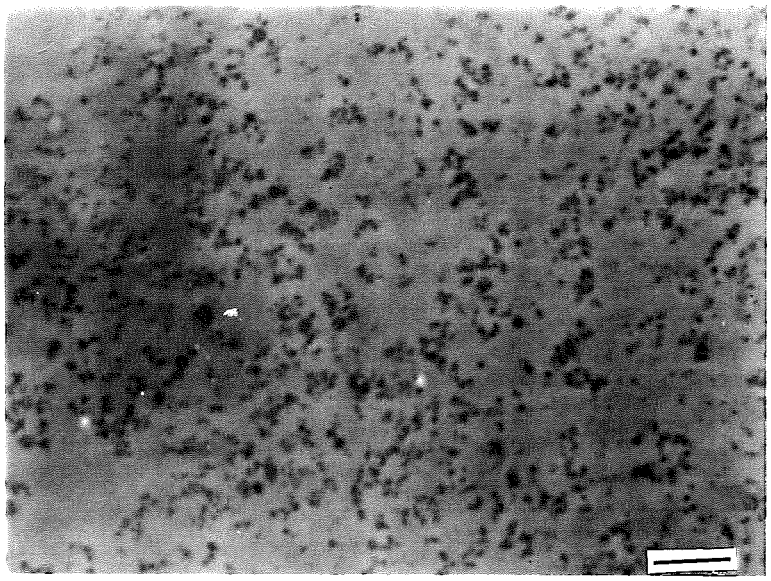
Skim milk (3.2 % protein) examination using TEM at x 7,500, x 20,000, and x 50,000 magnification shows a general overview of the protein distribution. The proteins are in most cases seen as small aggregates, distributed randomly (See arrow in Figure No. 3.17-C). The protein aggregates and fat droplets have clear spaces between them; only limited evidence of contact between proteins and fat is observed.

Ultrafiltered retentate (12.90 % protein) when examined using TEM at x 7,500, x 20,000 and x 50,000 magnification present a sharper definition of protein aggregates (See Figure No. 3.18-B), and some indication of order. In general the skim milk and ultrafiltered retentate were, similar in structure. There are some indications that casein micelle size has been increased (See arrows 'C' in Figure 3.18-B and C) in the ultrafiltered retentate. Srilaorkul *et al.* (1991), suggested that changes in size distribution, average diameter , and volume distribution of casein micelles in ultrafiltered skim milk may be due to the change in milk composition as a result of the ultrafiltration process. Macromolecules like casein, whey proteins and fat are retained by the membrane, and increase in concentration. In this study the concentration factor was 4-fold. However the real concentration of protein cannot be interpreted directly from the photographs.

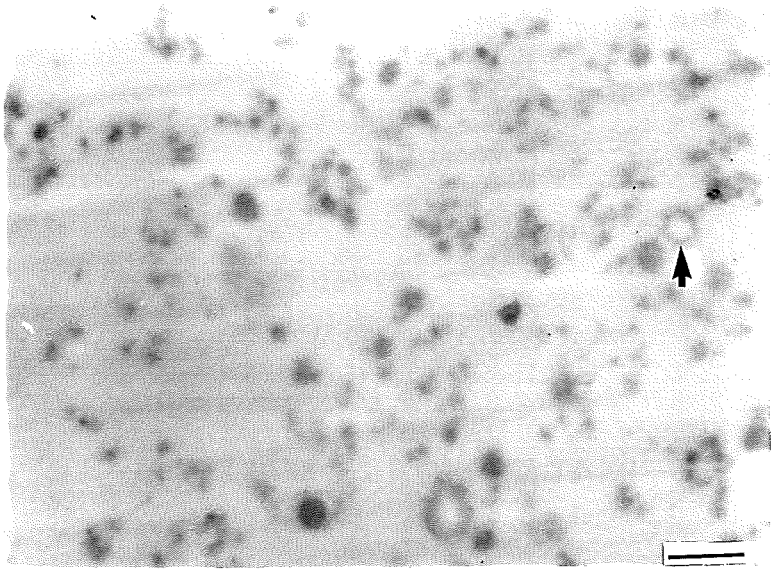
Some areas are interpreted as fat droplets (See arrow in Figure No. 3.17-B). These are clear areas surrounded by dark rings. The preparation technique was not designed to retain fat and so only "ghosts" are present. Free fat with no protein membrane will not be seen.

The casein in milk is present in roughly spherical particles (casein micelles). These micelles contain approximately 94% protein such as α_{s1}-, α_{s2}-,

A: Overall view of protein distribution
BAR = 1.3μm



B: Arrow is pointing fat "ghost" droplet
BAR = 0.5 μm



C: Arrow is pointing to casein micelle clusters
BAR = 0.2μm

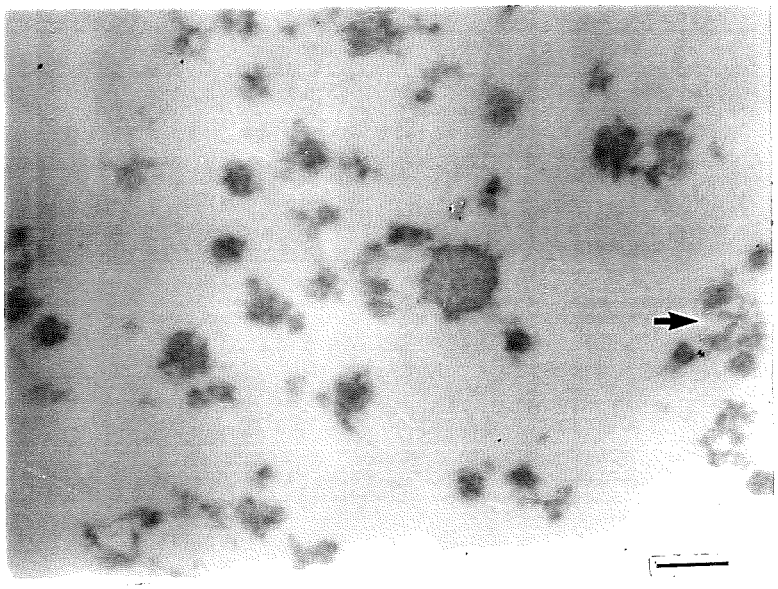
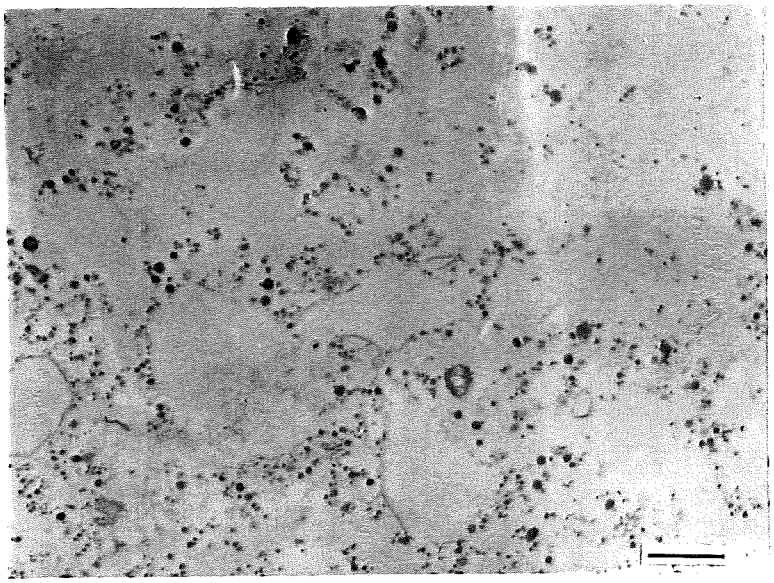
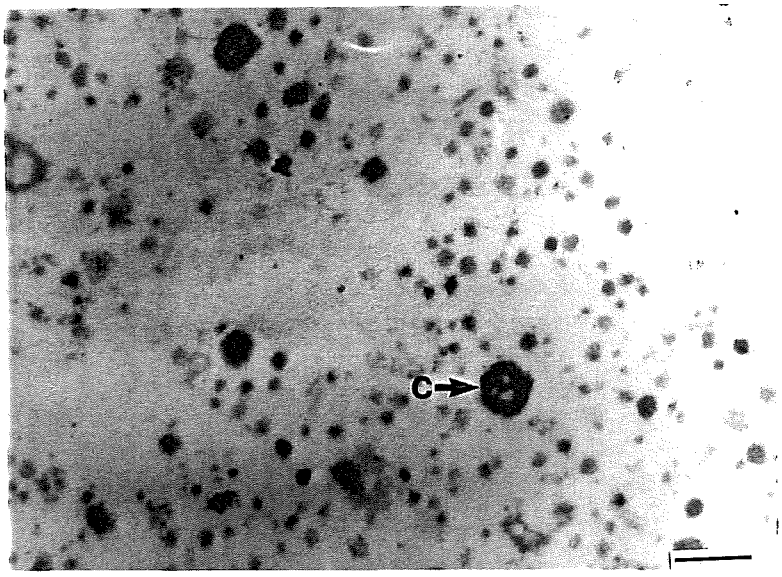


Figure No. 3.17 Transmission Electron Micrographs of skim milk

A: Overall view of protein distribution
BAR = 1.3μm



B: An increased casein micelle cluster is pointed by the arrow " C "
BAR = 0.5 μm



C: Arrow " C " is pointing to an increased casein micelle cluster
BAR = 0.2 μm

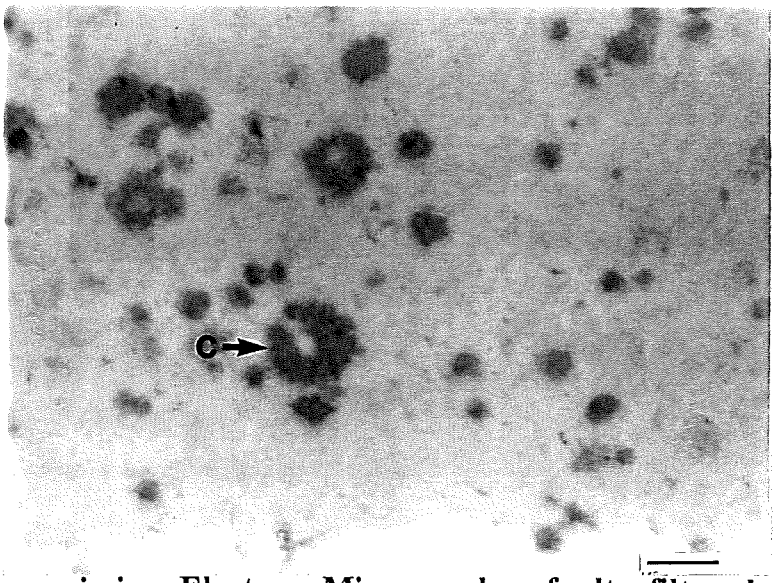


Figure No. 3.18 Transmission Electron Micrographs of ultrafiltered retentate

β- and κ-caseins, which occur in the proportions of approximately 3:1:3:1. The rest is inorganic matter (6%) known as colloidal calcium phosphate, which is composed mainly of calcium, phosphate, magnesium and citrate (Singh, 1988).

3.6.2 Microscopy of ice creams

3.6.2.1 Control ice cream

The control ice cream was made using skim milk powder (10.4% MSNF). It was softer and had an overrun of 67 %.

3.6.2.1.1 Light Microscopy (LM)

Sections of ice cream samples were taken from blocks prepared as described in section 2.4.3.12 of Materials and Methods after they were placed on a slide and stained with eosin for five minutes and then washed out with distilled water.

Control ice cream (See Figure No. 3.19), presented randomly distributed air cells (A) and ice crystal (I) structures. This overall structure is better appreciated with this technique rather than TEM, since the forms and shapes can be appreciated in overview.

Air cells appeared to be approximately circular in cross-section (See arrow in Figure No. 3.19-A and B) and presented sizes from approximately 20 μm to 200 μm. Ice crystals were present normally as angular shapes (See arrow in Figure No. 3.19-C). The ice crystal size ranged approximately from 30 μm to 200μm. The control presented an increased proportion of smaller ice crystals and air cells than the ultrafiltered ice cream. Overall the ice crystals and air cells appeared to occupy a smaller proportion of the structure in the UF-ice cream than in the ice cream control.

3.6.2.1.2 Scanning Electron Microscopy (SEM)

The control microstructure at x 85 and x 210 magnification (See Figure No. 3.20-B and C) presents a normal ice crystal (I), and air cells (A) distribution. It presents air cells ranging approximately from 20 to 150 μm. Ice crystal sizes ranged from 20 to 150 μm. In general ice crystals (angular) and air cells (spherical) presented normal shapes (See arrow ' I ' for ice crystal and small arrow for air cell in Figure No. 3.20-B).

During the sample preparation, the ice cream fracture surface was exposed to sublimation at - 80° C, to enhance the definition of the ice crystals (See section No. 2.4.3.12.1 of Materials and Methods chapter). Etching aids in differentiation of ice crystals in a matrix by revealing crystals boundaries produced as a result of eutectic crystallisation (Caldwell *et al.* 1992). The process removes the ice, which is filling the sockets, making them sharper showing a clearer shape of ice crystal (See arrow 'I' in Figure No. 3.20-B). Ice crystals can then be differentiated from air cells by the flat base of the ice crystals after the ice has been partially etched away (See Figure No. 3.21).

At x 1.150K magnification fat droplets are present between the ice crystals and air cell (See arrows ' f ' in Figure No. 3.20-A.) There is less area of the matrix as compared with the ultrafiltered ice cream. This may be due to the higher proportion of air cells and ice crystals in the microstructure. This figure shows some fat droplets on the surface of the cell, in accord with the observations of Brooker (1993).

This figure shows the presence of different crystal forms inside the air cells as well as small particles from the fracture of the sample. Those crystals

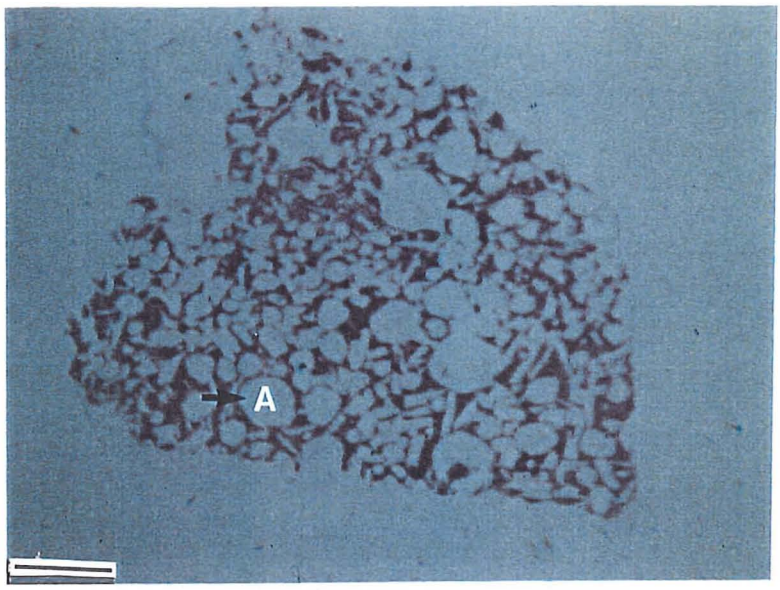
present inside the air cells may be ice, lactose or sucrose crystals, due to its geometrical shape (See arrow ' U ' in Figure No. 3.20-A). The crystals are essentially, hexagonal prisms. Most likely they are ice crystals, although they did not etch readily in the SEM. Whilst there is a possibility that the crystals are sugar, it would seem difficult to suggest a mechanism for their formation within the air spaces; ice crystals could grow in the air spaces by sublimation and condensation. (See 'U' Figure No. 3.22). Crystallisation of water vapour may come from sublimation throughout the matrix, following the increase in temperature. On re cooling water vapour and air could have migrated to the interstices between larger crystals; the water vapour recondensed as the small crystals and the air reformed with its fat membrane around them.

3.6.2.1.3 Transmission Electron Microscopy (TEM)

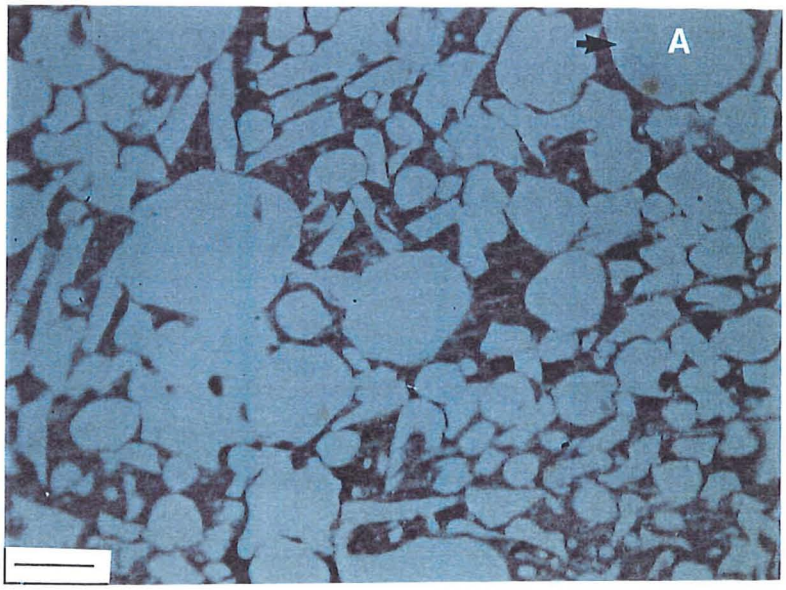
The control ice cream microstructure is presented in Figures No. 3.23 at different magnifications. At x 7500 the general microstructure is presented and comprises mainly air cells (A), ice crystals (I), fat (F), proteins (P) and matrix. Differentiation of the larger air cells and ice crystals on the one hand and smaller air cells and larger fat droplets on the other was not easy, although when viewed in context in the microscope, it was often possible to trace boundaries to sharp angles to identify ice crystals or deduce air cells from the nature of the interface.

The magnifications at x 20,000 and x 50,000 show mainly the casein micelle clusters surrounded by fat "ghosts" and clear matrix. The ' C ' arrows show the casein micelle clusters in Figure No. 3.23-B and C.

A: Arrow is pointing
to an air cell
BAR = 300 μm



B: Arrow is pointing
to an air cell
BAR = 25 μm



C: Arrow is indicating
an ice crystal
BAR = 25 μm

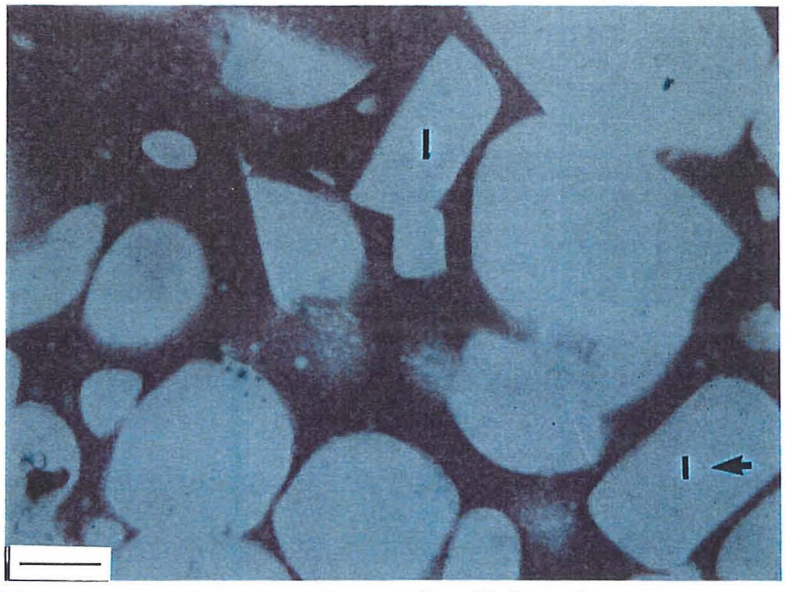
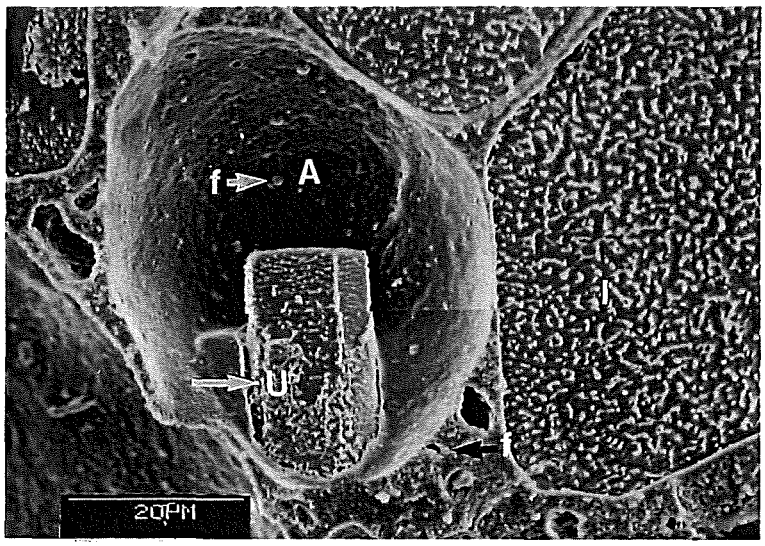
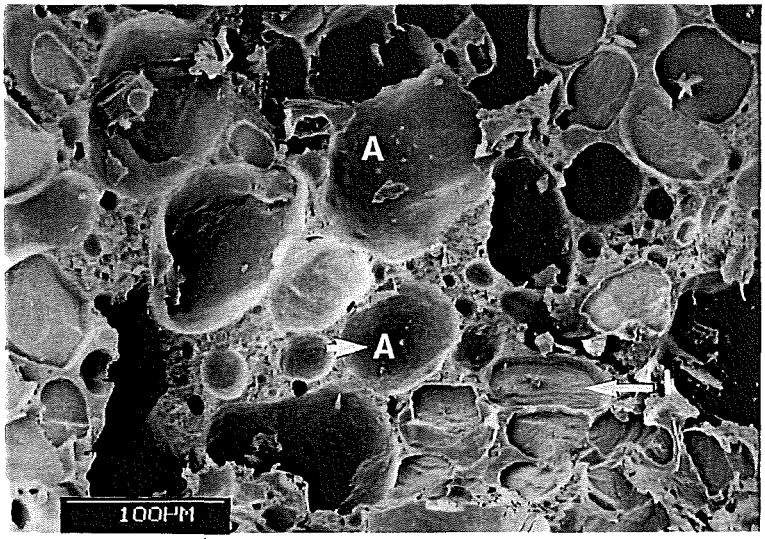


Figure No. 3.19 Control ice cream microstructure using light microscope

A: Fat droplets are indicated by arrow " f " Arrow " U " is pointing an unidentified crystal inside an air cell (A)



B: Arrows " A " indicate air cells
Arrows " I " indicates ice crystals



C: Overall view of the ice cream microstructure

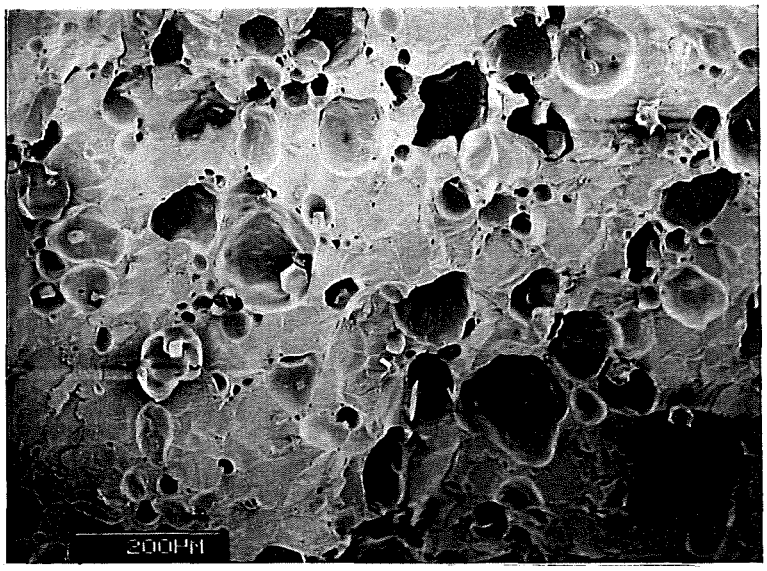
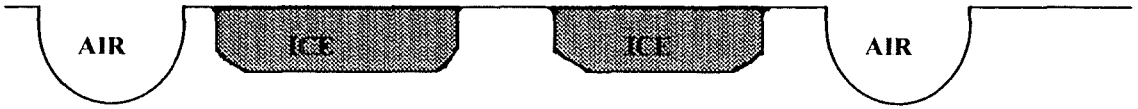


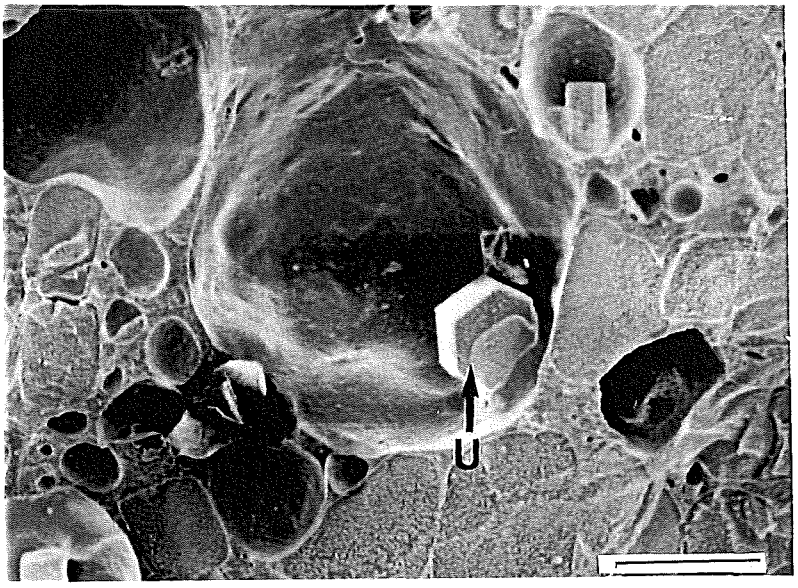
Figure No. 3.20 Scanning Electron micrographs of the control ice cream

Figure No. 3.21 Effect of ice sublimation on the structure of ice crystal in ice cream

a) Frozen fracture



b) After etching (i.e. sublimation of ice)

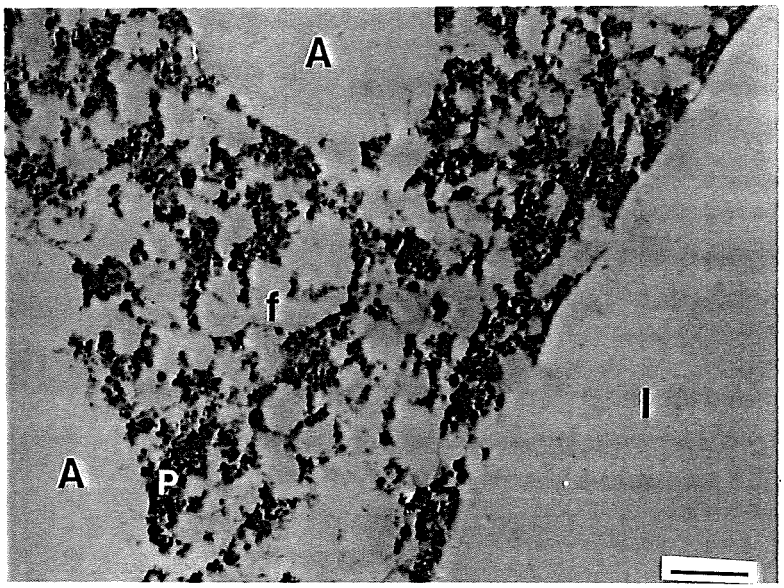


The arrow " U " is pointing one of those crystals formed inside the air cells. NOTE other crystals inside the air cells.

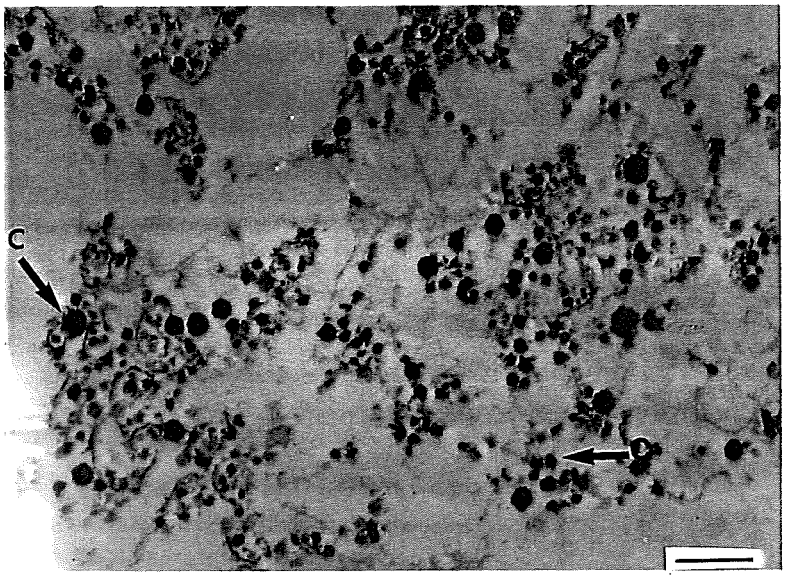
BAR = 50 μm

Figure No. 3.22 Scanning Electron micrograph of an unidentified crystal in control ice cream

A: Ice crystal is represented by the " I ", proteins are indicated by " P ", fat droplets by " f " and air cells by " A "
BAR = 1 μm



B: The arrows " C " are pointing to a small cluster of casein micelles
BAR = 0.5 μm



C: The " C " arrow is pointingto a casein micelle cluster.The " S " arrow is indicating a faint strand, which is most likely to be whey proteins
BAR = 0.2 μm

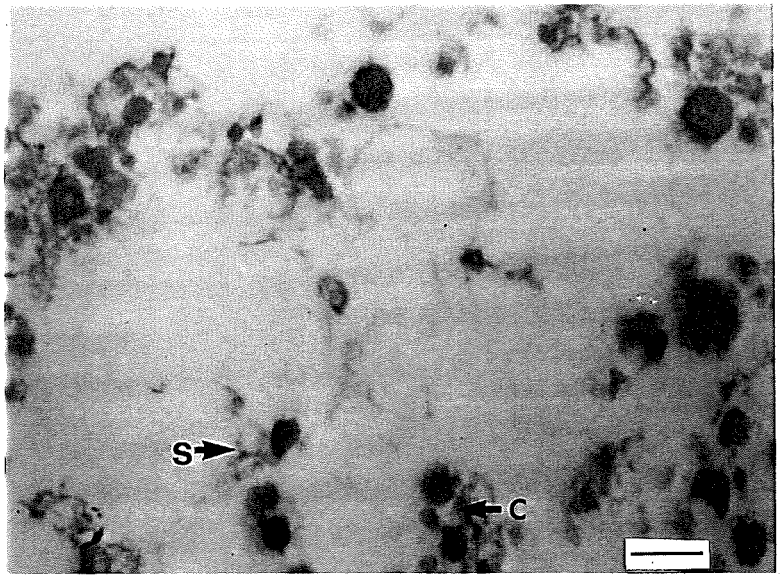


Figure No. 3.23 Transmission Electron micrographs of the control ice cream

Berger and White (1971) mention that the fat in the ice cream mix may be present as small homogenised globules (< 2 μm), improperly homogenised globules (6 - 8 μm), small clumps (about 20μm), agglomerated fat (up to 25 μm), and coalesced fat (> 25μm). In this study an approximate assessment of the fat globule size was carried out. (See Table No. 3.21), indicating that in all cases the fat globule size < 2 μm accounted for about 90% of the droplets. However, some larger sizes of droplets are present in the control. The areas interpreted as fat in Figure No. 3.23, confirm the view that most droplets are less than 2.0μm in diameter.

Proteins are adsorbed at air-water and oil-water interfaces because they contain both polar and non-polar groups (Mitchell, 1986). Proteins in the ice cream microstructure are present in the form of aggregates of different sizes formed by subunits. However some casein micelles are attached to the fat globules as a result of the connection of polar groups on the surface of fat globule and ionised groups of proteins during homogenisation process. Faint strands are seen (See arrow ' S ' in Figure No. 3.23-C). These are most likely whey proteins and emulsifier at the fat/matrix interface and there is a small amount of casein micelle structure associated with these strands. The interface between the matrix and air cells (See Figure No. 3.23-A), showed indents representing fat droplets at the boundary layer. Similar structures were present at ice/matrix interfaces, but were less frequent. A concentration of casein micelles towards the ice/matrix interface produced a compact layer.

3.6.2.2 Ice cream based on skim milk ultrafiltrate

3.6.2.2.1 Light microscopy (LM)

In general, in ice cream from skim milk ultrafiltrate, the air cells were normally spherical (See arrow ' A ' in Figure No. 3.24-A) and presented

different sizes approximately from 20 μm to 100 μm. Ice crystals were normally present in an angular shape (See arrow ' I ' in Figure No. 3.24-B). The ice crystal size ranged approximately from 20 μm to 150μm.

These illustrations confirm from the microscopy point of view, that the ultrafiltered ice cream microstructure is affected by the chemical composition. In this study the ultrafiltered ice cream had 8.83% protein, 13.16 % MSNF and 54.6% overrun. Thus, a closer structure is expected, with a higher ratio of matrix to ice and air than the control.

3.6.2.2.2 Scanning Electron Microscopy (SEM)

Figure No. 3.25, presents x 85 and medium x 210 magnification of ice crystals (I) and air cells (A) distribution. Ice crystal sizes were in the range approximately of 30 μm to 100 μm. Air cell sizes ranged approximately from 20 to 100 μm. Air cells presented a spherical shape (See arrow A in Figure No. 3.25-A and B), meanwhile ice crystals showed in most of the cases an angular shape (See arrows I in Figure No. 3.25-A and B).

At x1.150 K magnification an ice crystal (I) shape is well defined with a size of approximately 40 μm on one axis and 30 μm on the other. The air cell (A) presented a rounded shape with some fat droplets on the surface (See arrow ' F ' in Figure No. 3.25-A). This figure shows a structured matrix (M) having fat droplets (See arrows ' d ' in Figure No. 24-A), with an average size of 1 μm. However, the large arrow (d) in the same figure, shows a possible agglomerate of fat droplets or coalesced fat.

3.6.2.2.3 Transmission Electron Microscopy (TEM)

Figure No. 3.26, presents UF-ice cream microstructure at x 7,500, x 20,000 and x 50,000 magnification. It illustrates a normal structure of ice cream. Ice crystals (I) and air cells (A) are well defined, surrounded by the matrix (M) formed by proteins (See arrow ' p ' in Figure No. 3.26-A, B and C), fat droplets (See arrow ' f ' in A and B), and other components such as sucrose. Air cells are present in a rounded shape, normally with fat globules present in the interface. Proteins are present in a very large amount associated with fat droplets and coalesced fat clusters within the matrix, and normally at the edges of the ice crystals. Fat droplet sizes ranged from 0.5 to 1.5 μm.

The arrows ' f 'in Figure No. 3.26-B and C, are pointing at a component which is most likely coalesced small fat globules. Its size is approximately 2μm.

In summary, ice cream based on milk ultrafiltrate presented an increased proportion of casein micelles within the matrix than the control.

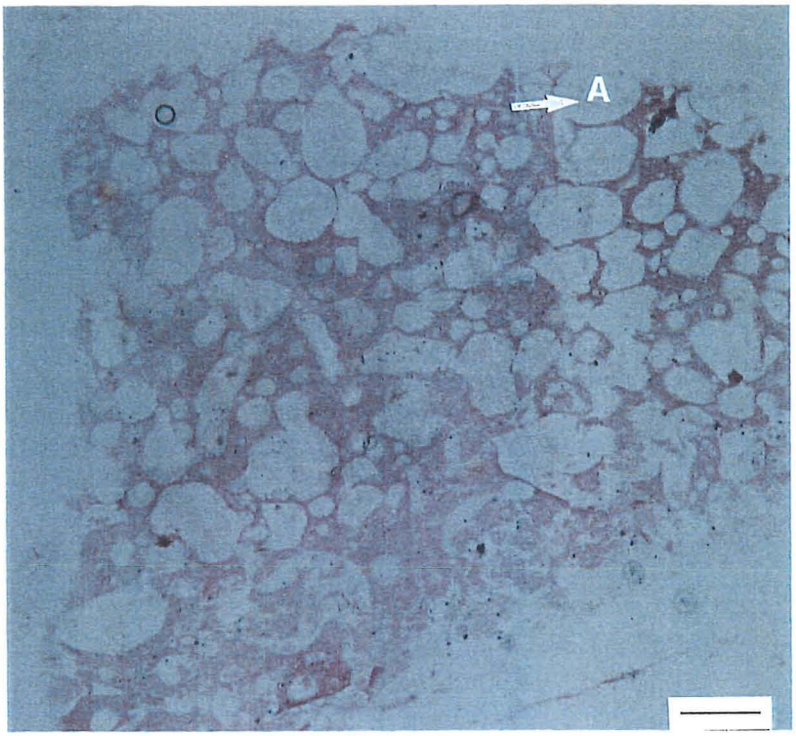
3.6.2.3 Heat shocked control ice cream

3.6.2.3.1 Light microscopy (LM)

After the heat shock treatment, the samples presented a completely different structure. Ice crystals were affected by the temperature cycling, forming larger blocks of ice crystals as a consequence of the combination of small crystals (See arrows in Figure No. 3.27-A and B). These ice crystal blocks are distributed randomly without any specific shape. Air cells in general tended to disappear or to form elongated shapes as a consequence of the squeezing pressure by other components such as ice crystals and matrix. The size of some aggregated crystals are in the order of 500 μm. as shown in Figure No. 3.27-A. The aggregation of ice crystals produced structures where the matrix between ice

A: Arrow " A " is indicating an air cell

BAR = 100 μm



B: The ice crystal block in the micrograph is represented by " I "

BAR = 25 μm

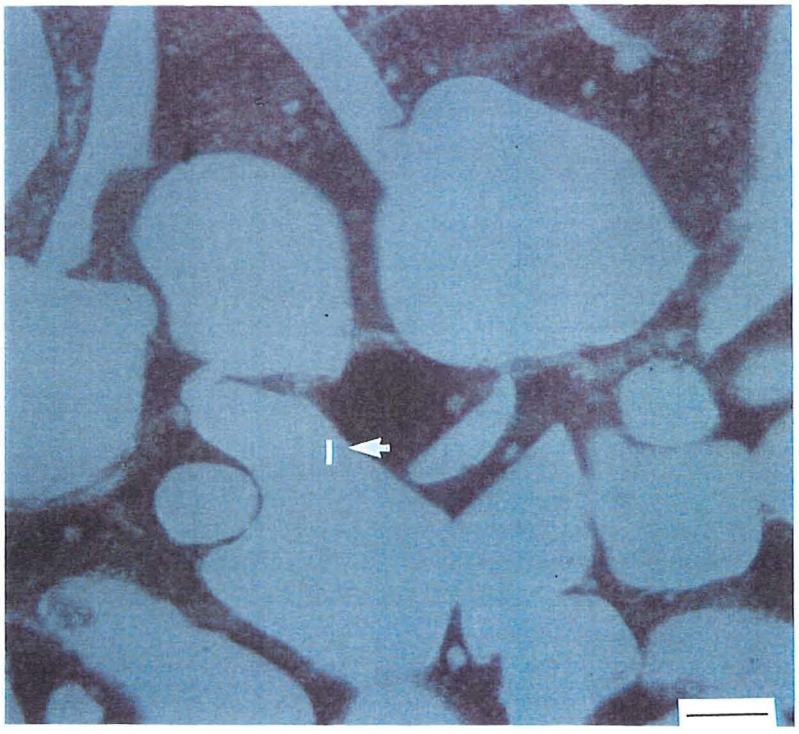
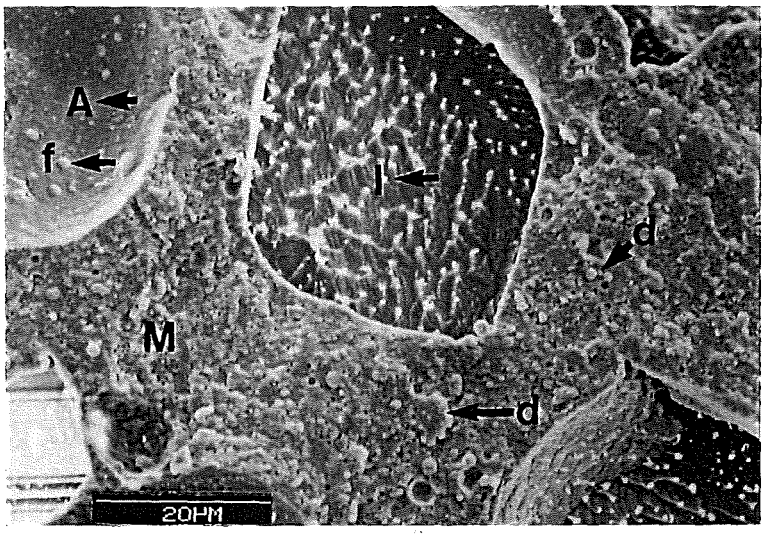
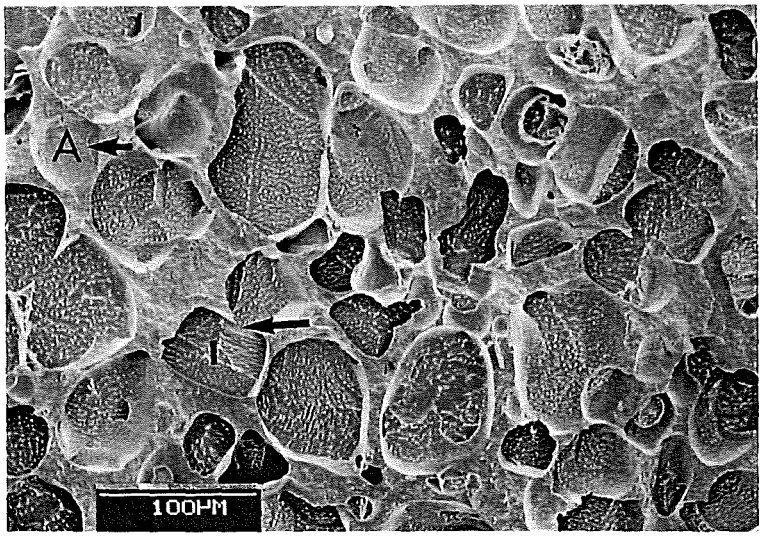


Figure No. 3.24 Light micrographs of ultrafiltered ice cream

A: The arrow " I " is indicating an ice crystal, the arrow " A " an air cell and the " M " the matrix. The arrow " f" a fat droplets and the" d " indicates a possible agglomerate of fat droplets or coalesced fat



B: Arrow " A " is pointing an air cell, and " I " to an ice crystal



C: The arrow is pointing to an ice crystal

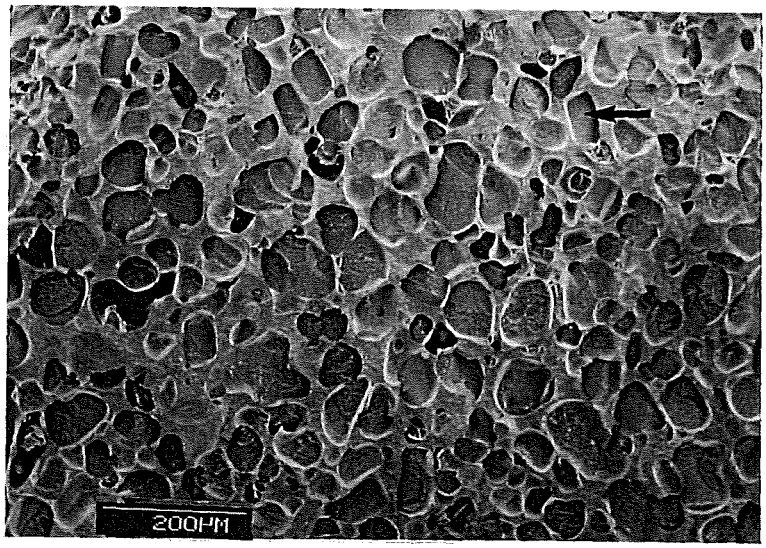
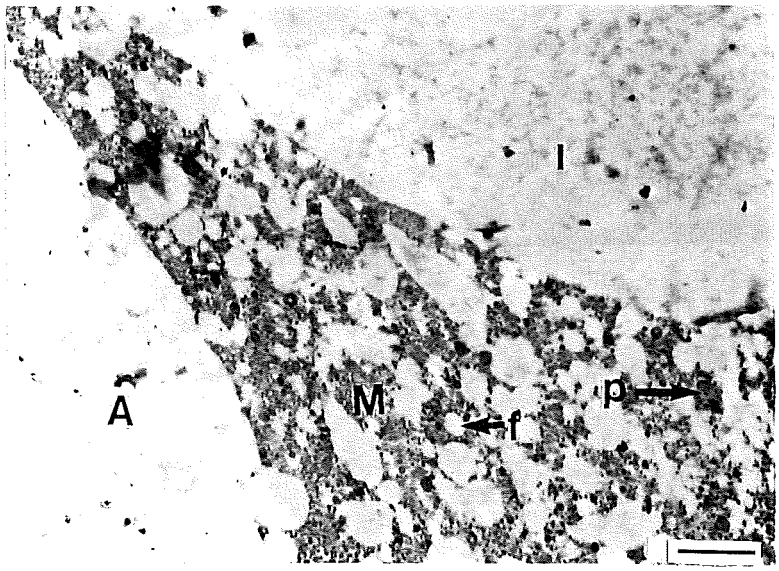
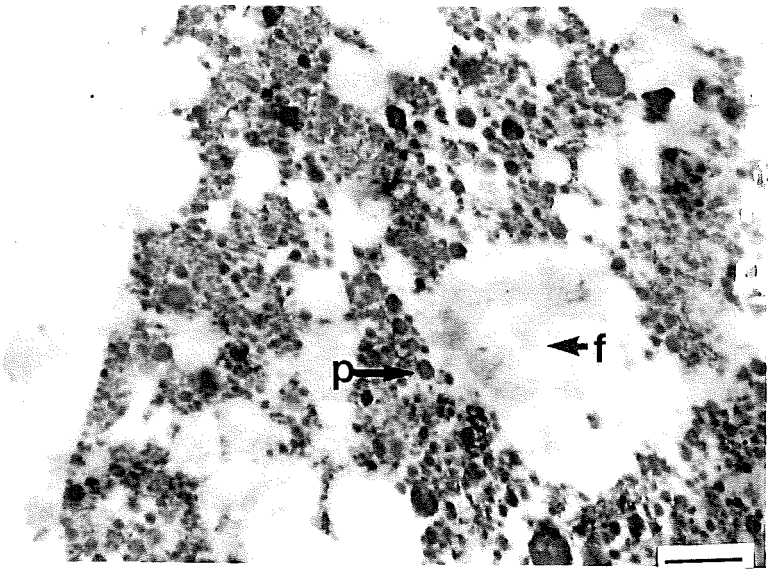


Figure No. 3.25 Scanning Electron micrographs of ultrafiltered ice cream

A: This micrograph is representedby the matrix (M) ice crystals (I), air cells (A), fat droplets (f) and proteins (p)
BAR = 1.3 μm



B: Arrow " f " is pointing to coalesced fat, and to proteins "p"
BAR = 0.5 μm



C: High magnification of coalesced fat "f" and proteins "p"
BAR = 0.2 μm

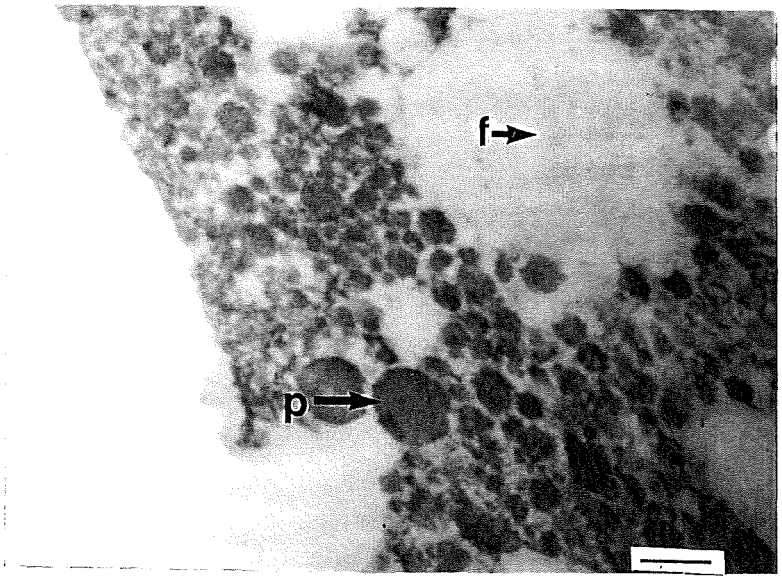


Figure No. 3.26 Transmission Electron micrographs of ultrafiltered ice cream

cells had partly broken to create structures like strings of beads or clover leaves. The presence of large ice crystals in ice cream tends to promote iciness problems in the texture of the products.

3.6.2.3.2 Scanning Electron Microscopy (SEM)

At x 85 magnification the distribution of air cells and ice crystals in the microstructure of ice cream shows air cells ranging from approximately 20 to 200 μm. In some cases air appeared as deformed cells (See arrows ' a ' in Figure No. 3.28-B and C. Ice crystals (See arrows ' i ' in Figure No. 3.28-B and C) have irregular shapes due to the union of individual ice crystals as a result of the heat shock treatment. Ice crystal size ranged from approximately 40 to 150 μm.

At x 210 magnification the elongated shapes of the ice crystals are better observed. Most of them increased in size as a result of the ice crystal union. The average ice crystal size in this figure is 75 μm. Air cells appeared as channels, as a result of the displacement of the cells. In this figure some other unidentified crystals were present inside the air cells as mentioned before.

At a magnification of x 1.150K (Figure No. 3.28-A) Ice crystals (I) are larger than in the control ice cream. Air cells (A) are divided by a very thin matrix layer. Fat droplets of 1 μm are on the surface of the air cell and matrix (See arrow ' f ' in Figure No. 3.28). The large arrow in this illustration is pointing to a probable agglomerated droplets.

3.6.2.3.3 Transmission Electron Microscopy (TEM)

Control ice cream after heat shock treatment is as shown in LM and SEM with large blocks of ice crystal. In Figure No. 3.29-A, B and C, apparently only fat "ghosts", protein and the matrix are showed. The fat appeared to have been

present as an agglomerated droplets (See arrows ' f ' in all illustrations in this figure).

The casein micelles were mostly loosely associated in the matrix with faint fibrous links visible at higher magnification. Some larger micelles had 'fuzzy' edges (See arrow ' c ' in Figure No. 3.29-C) implying a whey protein association at the outer surface of the micelle.

3.6.2.4 Heat shocked milk ultrafiltrate ice cream

3.6.2.4.1 Light microscopy (LM)

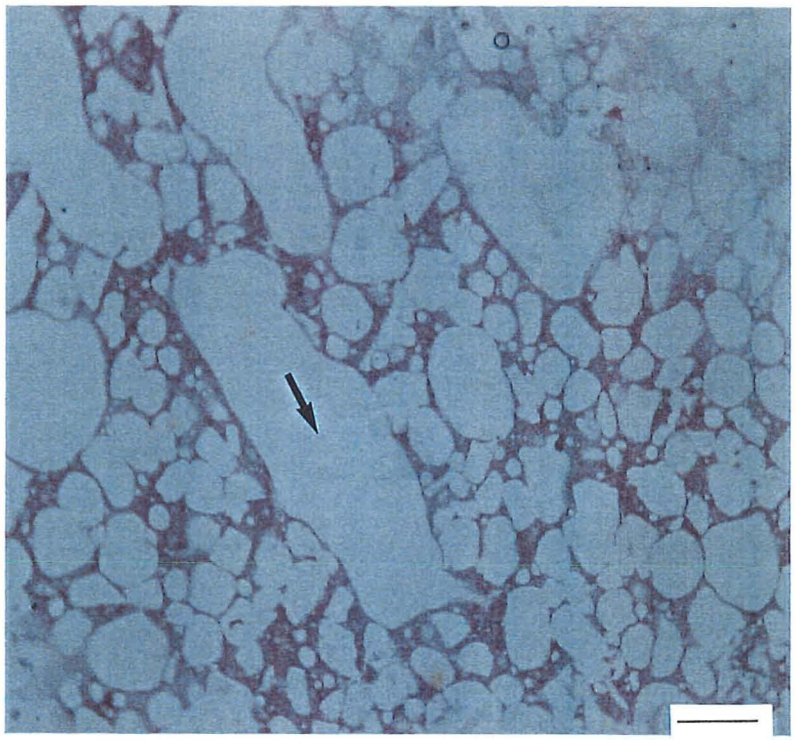
Ultrafiltrate based ice cream after heat shock treatment presented some changes in its microstructure. Ice crystals were enlarged after heat shock treatment, as shown in Figure No. 3.30-A with the arrow, however the size of the crystals was generally smaller compared with the control heat shocked ice cream. In illustration 'B' the arrow is pointing to the union of ice crystals. Ultrafiltered ice cream presented less air cells as a consequence of the lower overrun obtained during its manufacture.

3.6.2.4.2 Scanning Electron Microscopy (SEM)

Figure 3.31-C presents a low magnification x 85 and x 210 view of the microstructure, showing mainly ice crystals and air cells (See arrows ' I ' for ice crystals in illustrations 'B' and 'C'). Ice crystal size ranged from 40 to 130μm and air cells ranged from 30 to 110 μm. This figure shows a modified structure compared with the original sample. The matrix for instance seems to be reduced in area as a result of the expansion of ice crystals and possibly the disappearance of air cells. In some cases ice crystals appear as if two crystals were forming one crystal together.

A: Arrow is indicating a larger block of ice crystal

BAR - 100 μm



B: Arrow is pointing the union of two ice crystals

BAR = 25 μm

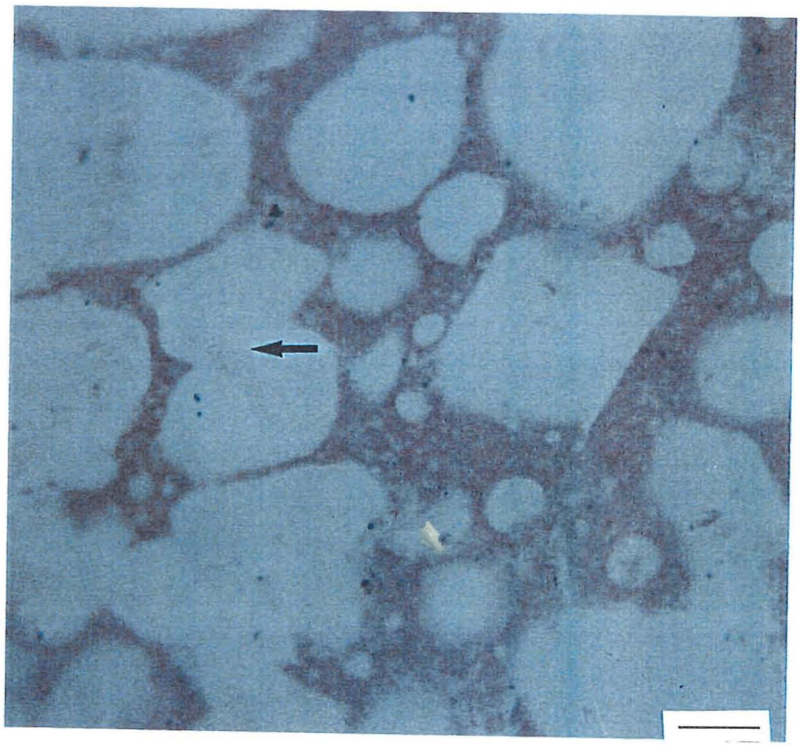
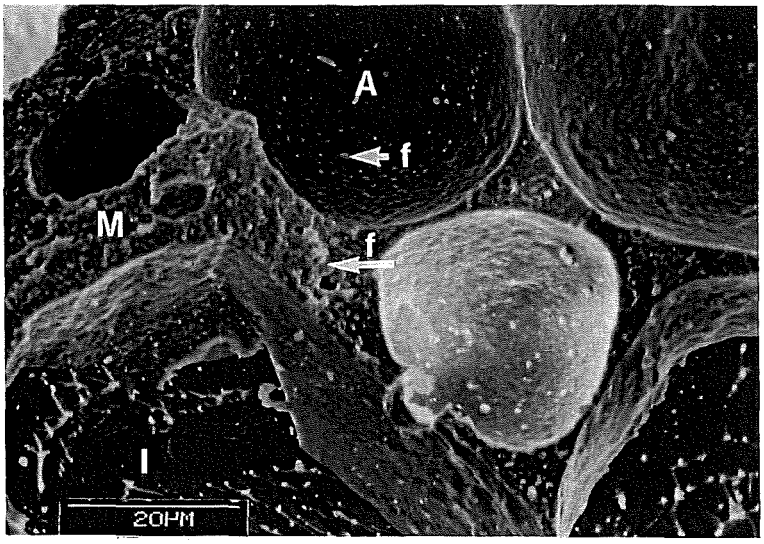
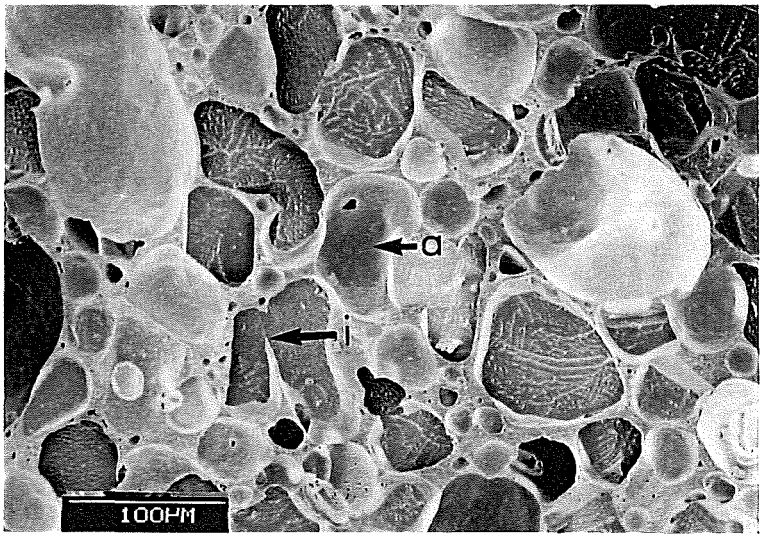


Figure No. 3.27 Light micrograph of heat shocked control ice cream

A: This micrograph is represented by air cells (A), ice crystals (I), fat droplets (f) and the matrix (M)



B: Arrow "a" is pointing an air cell, and "I" to the union of two ice crystals



C: Arrows are pointing to an ice crystal (i) and air cell (a)

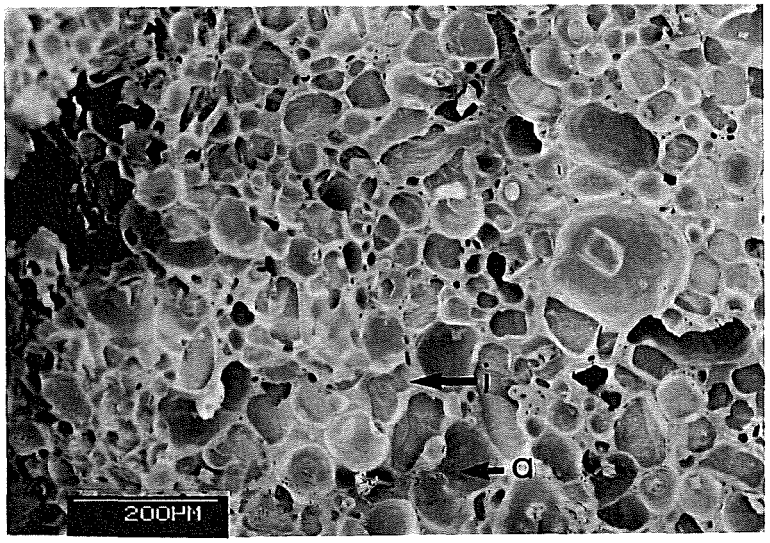
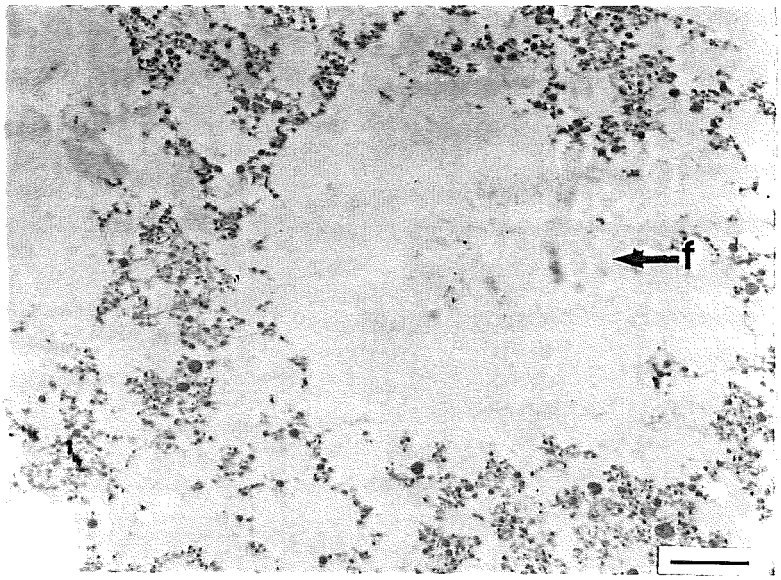
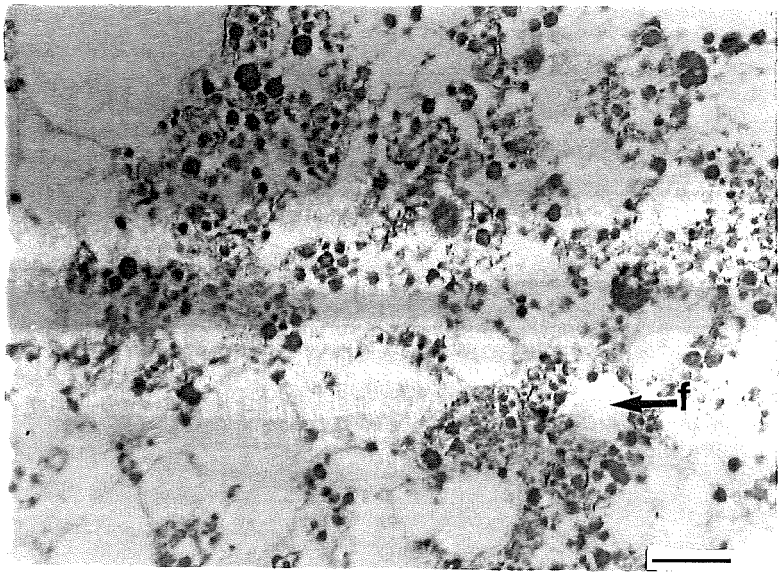


Figure No. 3.28 Scanning Electron micrograph of heat shocked control ice cream

A: The arrow "f" is pointingat coalesced fat
BAR = 1.3 μm



B: The arrow "f" is indicating the presence of coalesced fat
BAR = 0.5 μm



C: The fat is indicated by the arrow "f", and the casein micelle by "C"
BAR = 0.2 μm

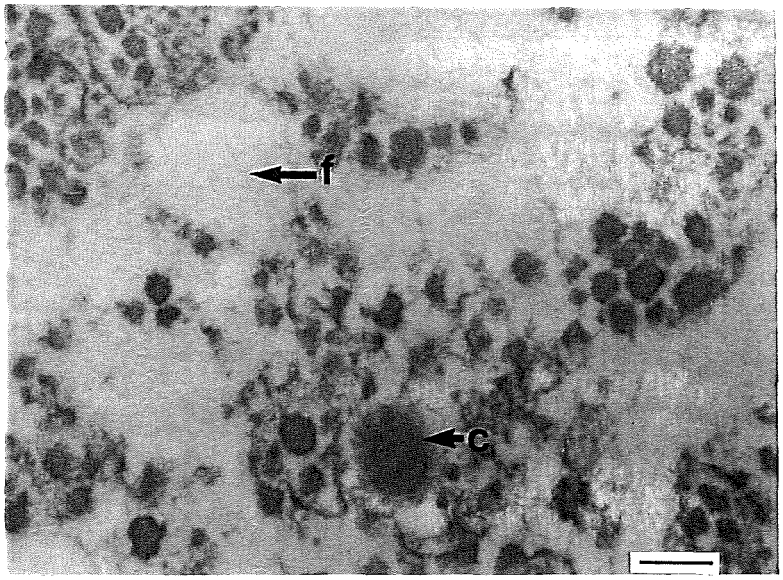
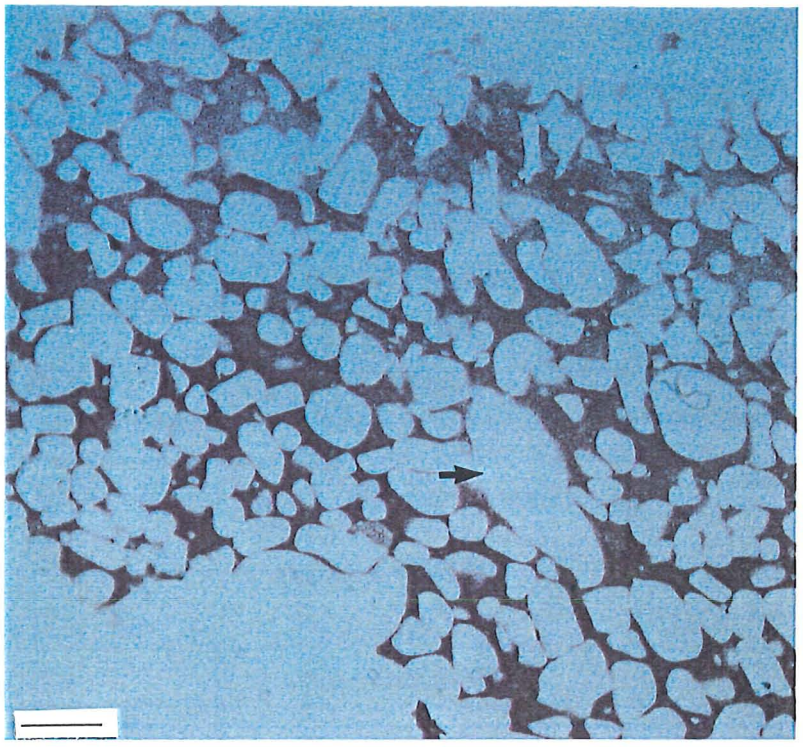


Figure No. 3.29 Transmission Electron micrograph of heat shocked control ice cream

A: Arrow is indicating a larger block of ice crystal

BAR = 100 μm



B: Arrow is pointing at the union of some ice crystals

BAR = 25 μm

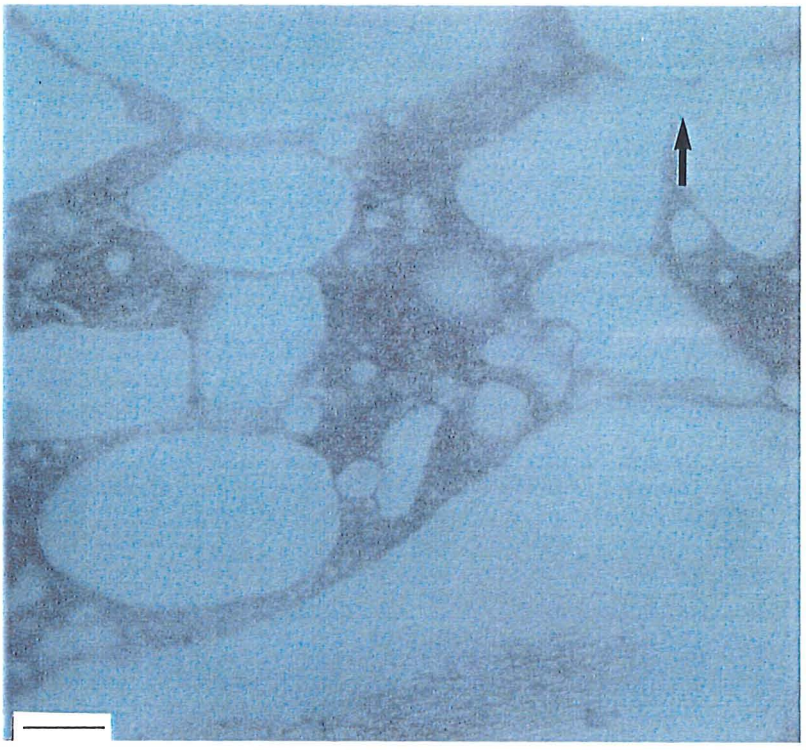
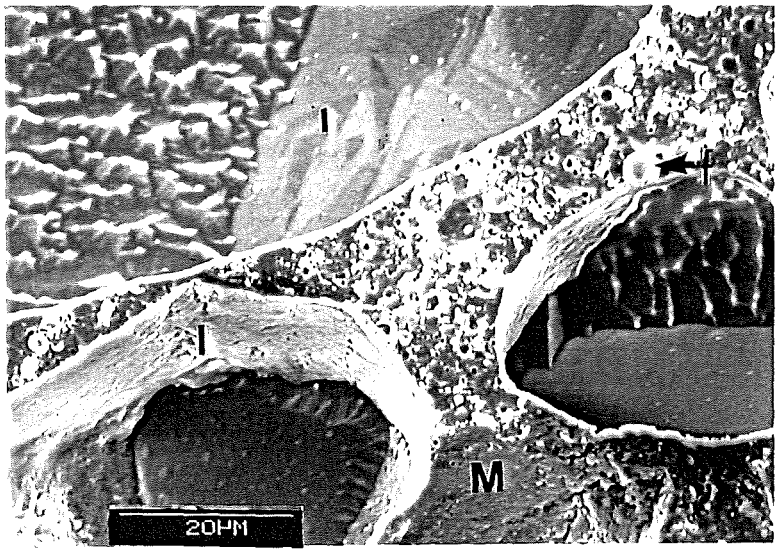
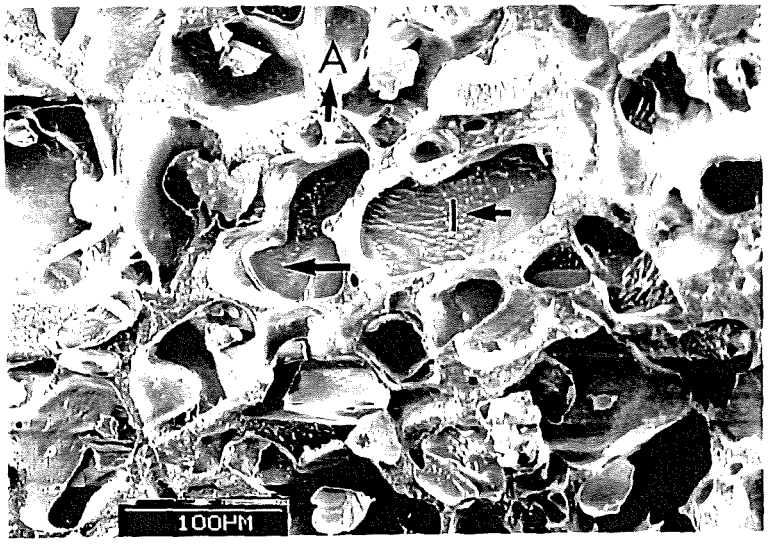


Figure No. 3.30 Light micrograph of heat shocked ultrafiltered ice cream

A: This micrograph is represented by ice crystals (I),matrix(M), and fat droplets (f)



B: The arrow "A" is indicating an air cell, and "I" an ice crystal. The sole arrow is pointing the union of two ice crystals



C: The arrow "I" is pointing an ice crystal, and the "A" to an air cell

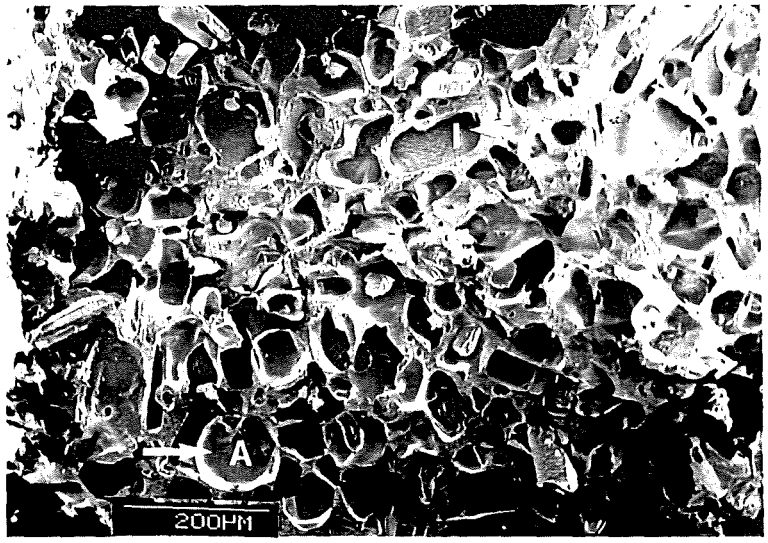
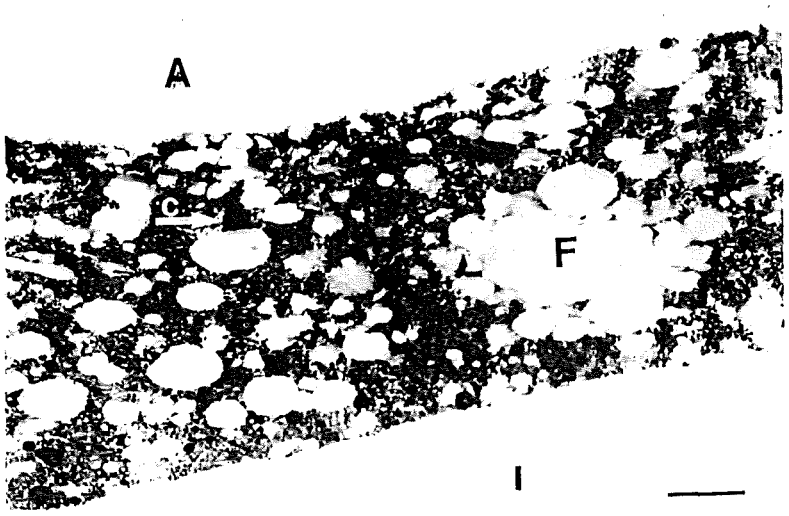
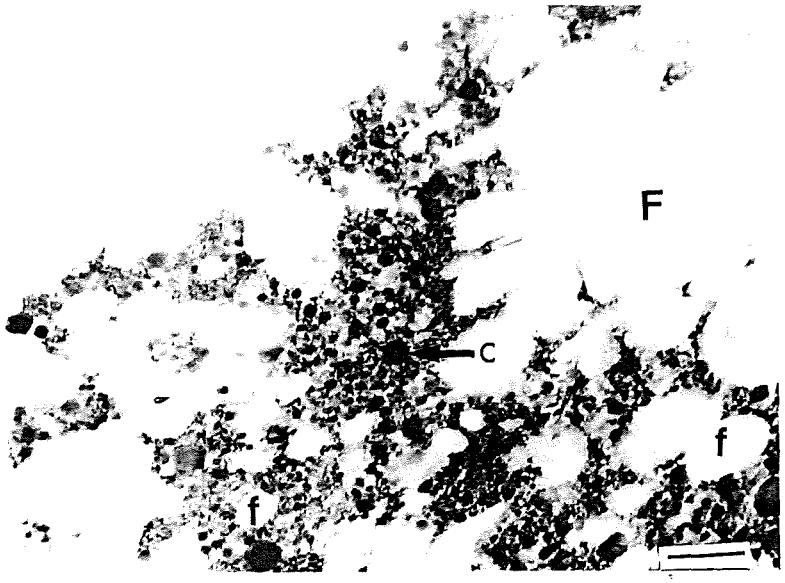


Figure No. 3.31 Scanning Electron micrograph of heat shocked ultrafiltered ice cream

A: Ice crystal is represented by "I", air cell by "A" and the coalesced fat group by "F". The casein micelle is pointed by the arrow "c" BAR = 1.3 μm



B: The ice cream components are represented as described above BAR = 0.5 μm



C: The micrograph, basically shows fat droplets "f" and casein micelles "c" BAR = 0.2 μm

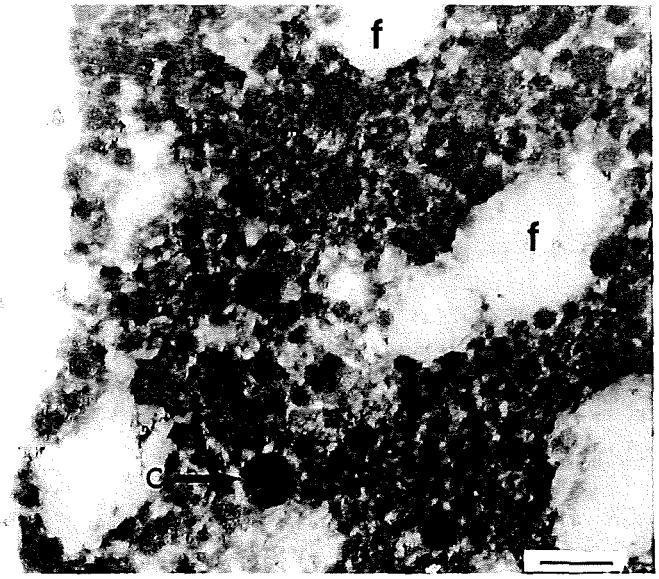


Figure No. 3.32 Transmission Electron micrographs of heat shocked ultrafiltered ice cream

Figure 3.31, shows a higher magnification x 1.15 K of the microstructure. It presents fat droplets (See arrow ' f ' in Figure 3.31-A) between the ice crystals (I) and air cell (A). The matrix (M) area is more disrupted than the unheated sample due to the merging of air cells and refrozen ice crystals.

3.6.2.4.3 Transmission Electron Microscopy (TEM)

Ultrafiltrate based ice cream after heat shock treatment presents an increase of the ice crystal sizes (See Figure No. 3.32-A), due to the ice crystals refreezing. Air cells in this illustration shows similar size to the ice crystals. The fat (F) tended to form coalesced groups and droplets are present within the matrix. Casein micelles seem undisturbed, but show a higher density of packing to either the control or the normally stored ultrafiltrate based ice cream (See arrow ' c ' pointing at a casein micelle). Micelle sizes, ranged approximately from 0.005 μm to 0.2 μm.

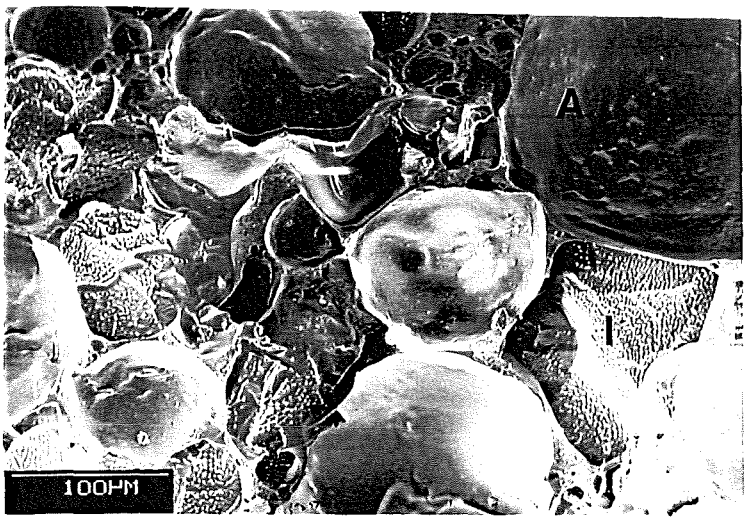
At magnifications (x 20,000 and x 50,000 respectively), the illustrations 'B' and 'C' present mainly fat droplets (f), coalesced fat (F) and casein micelles (See arrows ' c ' pointing to the casein micelles). The fat droplets present a size ranging approximately from 0.1 μmm to 1 μm. The coalesced fat size is approximately 4 μm. The normal range size in an properly homogenised mix is from 0.1 μm to 1 μm. Again fat droplets were more prominent at those interfaces judged to be air/matrix compared with those judged to be ice/matrix.

3.6.2.5 Commercial ice cream

3.6.2.5.1 Scanning Electron Microscopy (SEM)

An ice cream purchased locally was included in this analysis in order to make a comparison with a commercial brand.

A: This illustration shows basically, air cells "A" and ice crystals "I"



B: The air cell is represented by "A" and ice crystals by "I"

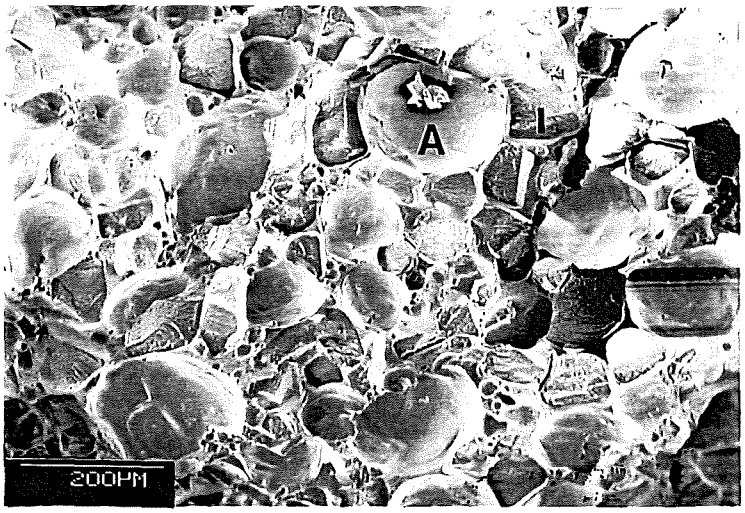


Figure No. 3.33 Scanning Electron micrographs of a commercial ice cream (high overrun)

Figure No. 3.33, at x 85 and x 210 magnification presents the ice crystal (I) and air cell (A) distribution of local purchase ice cream. Ice crystal size are in the range of 25 to 75 μm. Air cells are more prominent than the in the ultrafiltrate based ice cream and the control, due to the fact that commercial ice cream normally has higher overrun. The maximum air cell size in this figure corresponds to approximately 200 μm.

3.6.3 Summary/Discussion

Examination of the figures from light microscopy, scanning electron microscopy and transmission electron microscopy techniques reveal that ultrafiltered-ice cream and control ice cream have an air cell and ice crystal structure within a matrix formed mainly by an aqueous solution entraining proteins and sugar. The matrix in the ultrafiltered ice cream was more densely packed than the control. This is due to the presence of more protein from ultrafiltered retentate.

The microstructures of heat shocked ice creams were changed. In general ice crystals increased in size showing in some cases an elongated shapes as a result of the union of ice crystals. Air cells presented in some cases a modified channel shape. The heat shocked control showed the presence of small crystals inside the air cells, due maybe to the recrystallisation of ice crystals from water vapour within the air cells surrounded by the re-diffused air. Apparently the proteins are not affected in their structure.

The different microscopical approaches have different benefits and drawbacks.

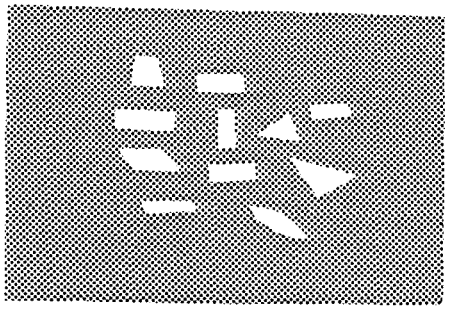
Light Microscopy (LM).- Limited resolution but shows overall pattern of ice and air surrounded by matrix.

Cryo-Scanning Electron Microscopy (SEM).- Allows better differentiation of air and ice than LM and shows a bit more detail of matrix. It only involves rapid freezing and no chemical dehydration.

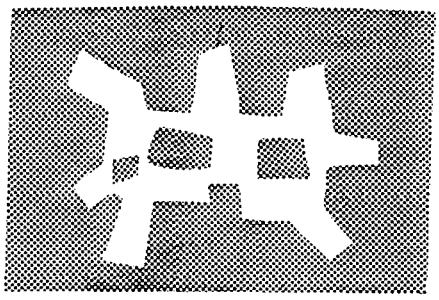
Transmission Electron Microscopy (TEM).- It gives best detail of matrix but it is difficult to see overall patterns in structure.

Freeze-substitution is not previously reported for TEM of ice cream. In TEM more association of casein in matrix is revealed, and may be due to the higher concentrations of Ca^{++} bridging the casein micelles. Hence, they tend to be a densely packed layer at interfaces, more so in UF-Retentate and ice cream.

The microscopy approaches give essentially 2D information, but in combination it is possible to imagine a 3D structure, particularly in relation to the ice crystals.



Initial separate ice crystals



During storage

This is as seen in 2D but, will inevitably occur above and below the plane of view and hence the most likely consequence is that the ice crystals will develop into a 3D network through the block. Poor storage conditions will lead to a stronger network with larger crystal blocks. This implies that a more profund change can occur in ice cream during storage, and especially during poor storage. The structure changes from one of dispersed ice and air embedded in a eutectic matrix to one where the ice and possibly even the air may form parallel matrices to the eutectic.

3.6.4 Fat droplet analysis

Fat droplets in ice cream mixes, according to Berger and White (1971), would exist in different forms such as small homogenised globules, improperly homogenised globules, small clumps, agglomerated fat and coalesced fat.

An approximate assessment of the fat globule size distribution in melted ice creams was carried out, using a differential interface contrast (DIC) technique in a light microscope. A computerised software programme (Optimas ®) was used to count and size the fat droplets in each ice cream sample.

According to Berger *et al.* (1971) the fat globule size in ice cream extends below 0.1 μm. In this situation the results from a light microscopy analysis of fat are only from 0.2μm upwards. Berger *et al.* (1971) deduced from combined light and TEM studies that the ' tail ' of the distribution was predictable from light microscopy and for routine purposes light microscopy sizing was sufficient.

Table No. 3.21, and Appendix A.5, show the dimensional characteristics of fat droplets in the ice creams for this study. Arrows in Figure No. 3.34-A and B, present a representation of some fat droplets in the ice creams.

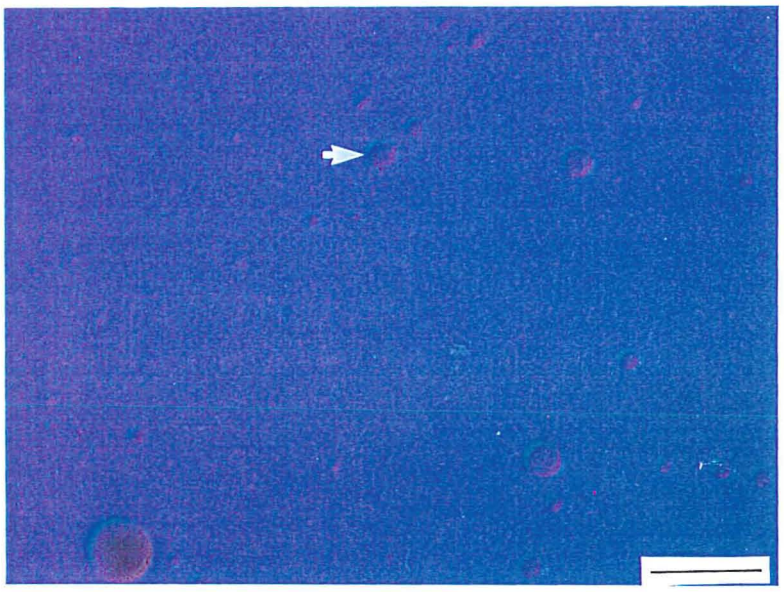
TABLE No. 3.21 DIMENSIONAL CHARACTERISTICS OF FAT DROPLETS IN ICE CREAMS

DIAMETER (μm) Less Than	UF-ICE CREAM* (%)	HS-UF-ICREAM* (%)	CONTROL (%)	HS-CONTROL* (%)
0.8	33.0	36.5	46.5	41.0
1.1	27.8	26.6	23.2	28.3
1.4	15.4	11.7	10.6	12.3
1.6	9.9	8.5	5.4	7.8
1.8	5.7	4.3	5.0	4.6
2.0	2.8	3.6	1.7	2.6
2.1	2.2	2.4	1.4	1.3
2.2	1.3	1.9	0.6	0.4
2.4	0.3	1.4	1.6	0.4
2.5	0.5	0.5	1.1	0.4
2.6	0.2	0.3	0.7	0.3
2.8	0.3	0.4	0.1	0.3
2.8	0.2	0.4	0.3	0.0
3.0	0.1	0.6	0.1	0.0
3.1	0.1	0.6	0.2	0.1
3.7	0.1	0.3	0.6	0.1
4.5	0.1	0.1	0.7	0.1
4.8	0.0	0.0	0.1	0.0
7.0	0.0	0.0	0.1	0.0

* Heat shocked ice creams

** Ultrafiltered ice cream

A: The arrow is pointing at small fat droplet of approximately 3 μm
BAR = 10 μm



B: The arrow is indicating at a big fat droplet of approximately 8 μm
BAR = 10 μm

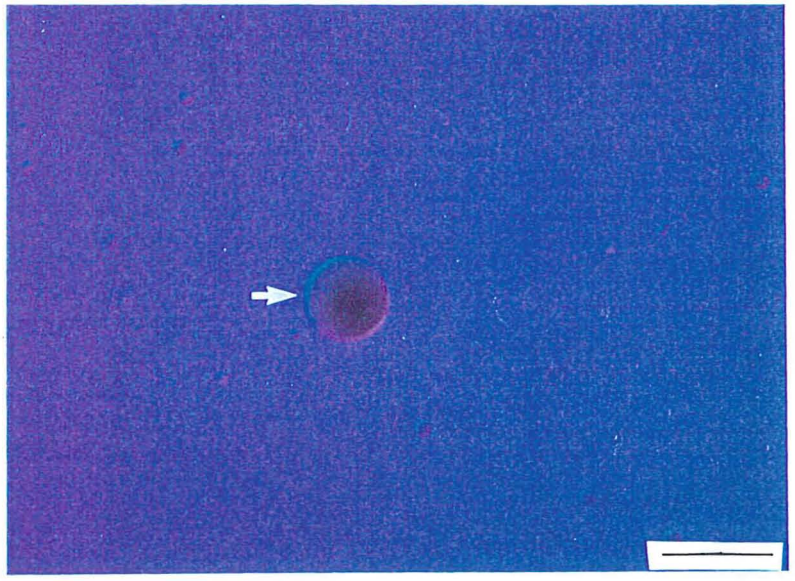


Figure No. 3.34 Light micrograph of fat droplets in ice cream mix

Ultrafiltrate based ice cream presented a higher proportion of smaller fat globules in the range up to 1.4μm, but in all samples sizes below 2μm are around 90%. However, there are a few fat droplets with size extended to 4.5 μ m. On the other hand, the control had the same tendency, but with 80.3% of fat droplets up to 1.4 μm, it showed an increase in the number of fat droplets extending to 4.5 μm.

After the heat shock treatment, the fat droplets in the ice creams apparently tended to keep the same characteristics.

The normal droplet size in all samples were mostly around 0.5 μm. However, some droplet sizes extended in all cases above 2μm giving an indication that the fat in these ice creams may be present as improperly homogenised globules, agglomerate fat or coalesced fat.

3.7 Conclusions

The source and the amount of MSNF used in the formulations, affected the chemical, physical, sensorial and microstructure characteristics of the final products. Ash, Protein, Calcium, Phosphorus and Magnesium were increased and Lactose, Potassium and Sodium were decreased by using ultrafiltered retentate as a replacement of skim milk powder in ice cream formulations.

Products made using ultrafiltered retentate were harder and needed more time for melting. However, they showed low overrun and higher extrusion temperature, than the control. UF-mixes before freezing were more viscous than the control due to higher protein content.

Ultrafiltered products showed better consistency in body and texture, when exposed to warmer temperatures and refrozen again.

Ultrafiltered products showed smaller ice crystals sizes, more protein presence and tended to more stable after heat shock treatment.

Heat shocked ice creams presented a network of ice crystals as a result of the ice crystals refreezing during heat shock treatment.

Transmission Electron Microscopy technique can be used for studying the microstructure of ice cream.

CHAPTER FOUR

ULTRAFILTRATION IN CAJETA MANUFACTURE

CHAPTER FOUR: CAJETA MANUFACTURE

4.1 ULTRAFILTRATION FOR CAJETA MANUFACTURE

4.1.1 Introduction

As explained in section 1.3.2, manufacture of cajeta often leads to sandiness because of the high concentration of lactose leading to crystallisation. Lactose crystals which can be up to 1500μm (Hough *et al.* 1990). Efforts have been made to resolve this problem by breaking down lactose by using bacteria or enzymes, and by seeding with lactose microcrystals. Reliable results were obtained, but all of them are highly costly (Sabioni *et al.* 1984a; Sabioni *et al.* 1984b, and Martinez *et al.* 1990).

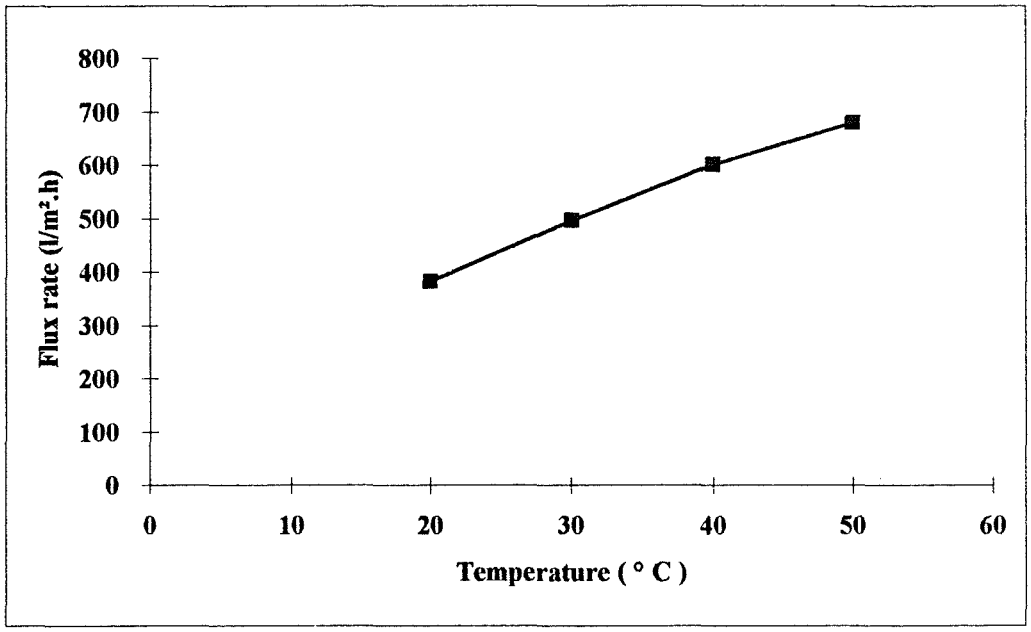
Using ultrafiltration can lead to a reduced concentration of lactose. Thus, the ultrafiltration process may be adapted to produce a low lactose cajeta product, and by reducing the heat processing time, the production costs may be lowered (Caric', 1994).

4.1.2 Ultrafiltration process

A 25.23 kg (3.19 kg dry matter) batch of whole milk from SAC-Auchincruive farm was ultrafiltered with a target of 55 % volume reduction. To obtain the ultrafiltered retentate, a pilot-scale ultrafiltration unit was used (See Section 2.3.2 of Materials and Methods Chapter). The permeability of the membrane was checked before the process by comparing its flux rate, at different temperatures with water at 50°C. (See Figure No. 4.1). Appendix A.6, shows the means for the flux rate of water at different temperatures.

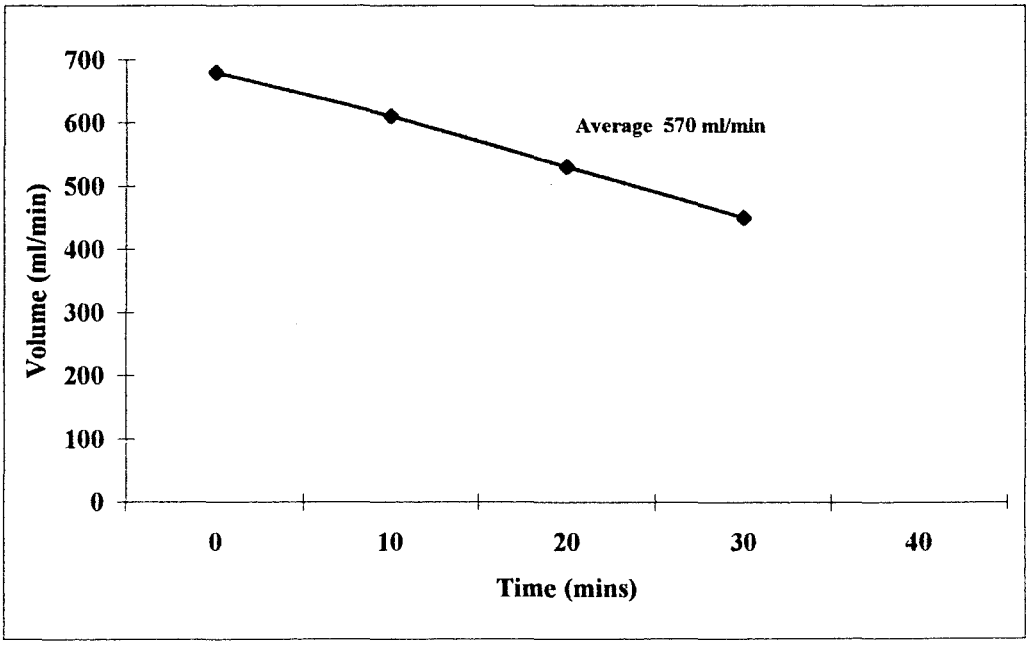
During the UF-process of whole milk the flux rate of the ultrafiltered

FIGURE No. 4.1 FLUX RATE OF UF-ROMICON MEMBRANE USING WATER AT DIFFERENT TEMPERATURES *



* Membrane of 50,000 Daltons of Nominal Molecular Weight Cutoff with Inlet and Outlet pressures of 0.15 and 0.12 MPa (gauge) respectively

FIGURE No. 4.2 FLUX RATES OF WHOLE MILK IN TWO ULTRAFILTRATION PROCESSES FOR CAJETA MANUFACTURE AT 50° C *



* Membrane of 50,000 Daltons of Nominal Molecular Weight Cutoff with Inlet and Outlet pressures of 0.15 and 0.12 MPa (gauge) respectively

permeate was checked at 10 minutes intervals giving a total average of 570 ml/min (See Figure No. 4.2). Appendix A.7, shows the flux rate values for the process. The flux is lower than the ice cream ultrafiltration process, because whole milk was used instead of skim milk, and fat probably obstructed the permeation rate in the ultrafiltration process.

4.1.3 Chemical Composition of UF-Ingredients for Cajeta Manufacture

Whole milk, ultrafiltered retentate and ultrafiltered permeate were chemically analysed in duplicate. Table No. 4.1, shows the means of the chemical composition.

The mass balance for every component (See Table No. 4.2), gave recoveries which ranged from 84.4% to 99.5% , due mainly to the layer formed on the ultrafiltration membrane during concentration, and the loss of milk constituents inside the ultrafiltration plant.

The fat level was 3.77% in whole milk and 9.30 % in the ultrafiltered retentate, giving a 99.5 % recovery. However, fat in the cajeta formulation can be manipulated, since it can be added from another source after the ultrafiltration process. Consequently the alternative approach of ultrafiltration using skim milk would increase the flux rate. The fat can be added after the ultrafiltration stage and provides an alternative processing option.

Lactose was determined by difference. The results shown that it was slightly increased in ultrafiltered retentate and ultrafiltered permeate with 5.01% and 5.10% respectively from 4.9% in milk. However, the mass balance shows that the partition of 1.236 kg of lactose in the milk was divided into 0.511 kg in

TABLE No. 4.1 CHEMICAL COMPOSITION OF RAW MATERIAL IN CAJETA MANUFACTURE

SAMPLE DESCRIPTION	ASH (%)	PROTEIN (%)	LACTOSE * (%)	FAT (%)	TOTAL SOLIDS (%)	M.S.N.F (%)
WHOLE MILK	0.76 a	3.22 a	4.90 a	3.77 a	12.65 a	8.88 a
UF-RETENTATE	1.20 b	7.30 b	5.01 b	9.30 b	22.81 b	13.51 b
UF-PERMEATE	0.46 c	0.37 c	5.10 c	0.00 c	5.93 c	5.93 c
SEDifference	0.015	0.037	0.0	0.017	0.082	0.086
RECOVERY (%)[1]	97.3	97.8	99.0	99.5	98.7	

a,b,c Means within the same column followed by the same letter are not significantly ($P< 0.05$)

* Determined by difference

[1] Recovery on dry matter basis

TABLE No. 4.2 MASS BALANCE FOR UF-CAJETA INGREDIENTS

	WM*	UF-R[1]	UF-P[2]	RECOVERY (%)[3]
VOLUME (kg)	25.23	10.19	14.08	
MASS (kg)	3.2	2.3	0.8	98.7
Ash %	0.8	1.2	0.5	
Mass (kg)	0.19	0.12	0.06	97.3
Protein%	3.2	7.3	0.4	
Mass (kg)	0.81	0.74	0.05	97.8
Fat%	3.8	9.3	0.0	
Mass (kg)	0.95	0.95	0.00	99.5
Lactose%	4.9	5.0	5.1	
Mass (kg)	1.24	0.51	0.71	99.0
T.Solids%	12.7	22.8	5.9	
Mass (kg)	3.19	2.32	0.83	98.7
Ca (mg/100g)	901.2	967.0	221.0	
Mass (g)	28.75	22.43	1.83	84.4
P (mg/100g)	675.9	699.2	686.6	
Mass (g)	0.04	0.01	0.02	94.0
Mg (mg/100g)	83.0	65.8	118.1	
Mass (g)	0.30	0.15	0.12	90.2
K (mg/100g)	1209.5	681.7	2678.0	
Mass (g)	0.02	0.01	0.01	96.9
Na (mg/100g)	347.8	199.1	691.5	
Mass (g)	0.07	0.05	0.02	98.6

* Whole milk

[1] Ultrafiltered retentate

[2] Ultrafiltered permeate

[3] Recovery on dry matter basis

ultrafiltered retentate and 0.714 kg in ultrafiltered permeate, giving a recovery of 99.0%.

The percentage of total solids, on the other hand, is increased by the level of concentration process, so as concentration increases, percentage of total solids are increased. The results show the total solids were increased from 12.65% in milk to 22.81% in ultrafiltered retentate and 5.93% in ultrafiltered permeate with a percent of recovery of 98.7 % from the mass balance, hence the relative concentration of lactose in the retentate was considered reduced.

The mineral content (dry basis), in the raw material are shown in Table 4.3. The rate of mass recovery for most of the minerals ranged from 90.2% to 98.6%, the exception was calcium, where the recovery was 84.4%. This difference is due mainly to the decrease in calcium phosphate solubility and the concentration at the ultrafiltration membrane as discussed previously in section 3.3.2 of the ice cream chapter.

The calcium content originally in the milk was 901 mg/100g. It was increased to 967 mg/100g in ultrafiltered retentate compared with 221 mg/100g in the ultrafiltered permeate. Potassium and sodium levels increased from the original milk to the ultrafiltered permeate. Magnesium and Phosphorus levels decreased in ultrafiltered retentate. The reasons underlying the partitioning of minerals during the ultrafiltration of milk have already been discussed in section 3.3.2.

TABLE No. 4.3 MINERAL CONTENT (Dry Basis) IN RAW MATERIAL FOR CAJETA MANUFACTURE

SAMPLE	CALCIUM (mg/100 g)	PHOSPHORUS (mg/100 g)	MAGNESIUM (mg/100 g)	POTASSIUM (mg/100 g)	SODIUM (mg/100 g)
WHOLE MILK	901 a	676 a	83 a	1209 a	348 a
UF-RETENTATE	967 b	699 b	66 b	682 b	199 b
UF-PERMEATE	221 c	686 c	118 c	2678 c	691 c
SEDifference	1.44	1.09	3.11	5.31	0.79
RECOVERY (%)[1]	84.4	94.0	90.2	96.9	98.6

a,b,c Means within the same column followed by the same letter are not significantly different ($P<0.05$) significantly different ($P < 0.05$)

[1] Recovery on dry matter basis

4.2 Cajeta Manufacture

4.2.1 Methodology

Cajeta was made following a normal formulation (See Table No. 4.4) for the control using whole milk. To produce the ultrafiltered cajeta a modified recipe was used, and it was brought about from some preliminary trials that were carried out varying the level of ingredients and finally by replacing some of the sucrose by glucose syrup. In both cases sodium bicarbonate was added to neutralize the warm (e.g. 30° C) milk or ultrafiltered retentate to pH 7.0 in order to avoid protein precipitation during processing. The quantity of milk used for the control and ultrafiltered cajeta was initially the same, but the milk for ultrafiltered cajeta was subjected to a 55% volumetric reduction by ultrafiltration process before use. A higher volume reduction can be achieved, but changes in formulation may be expected since some chemical components are lost during the UF-process due to the loss of lactose, minerals and some non-protein nitrogen.

TABLE No. 4.4 FORMULATIONS FOR CAJETA MANUFACTURE

INGREDIENT	MILK CAJETA		UF-CAJETA	
	(kg)	(%)	(kg)	(%)
WHOLE MILK	3.5	83.3		
UF-RETENTATE			2.1	69
SUCROSE	0.5	12.5	0.7	23.2
GLUCOSE SYRUP	0.2	4.2	0.2	7.7
VANILLA	0.004	0.01	0.004	0.1
SODIUM BICARBONATE*	0.003		0.004	

* For adjustment to pH 7

The processing procedure of cajeta manufacture (See Table No. 4.5) was carried out, placing the milk or ultrafiltered retentate in a steam-heated boiling pan. The temperature was controlled throughout the process using a thermometer (previously described in section 2.3.6.1 of Materials and Methods). Glucose syrup was added in both cases at 48° C, and sucrose at 60° C, thus, avoiding possible problems with lactose crystallization at the beginning of the process, as well as achieving adequate solubility of both the glucose syrup and sucrose. The processing temperature ranged from 96 to 98° C with constant stirring.

To determine the final concentration of the product a hand sugar refractometer was used, and then the batches were checked to a final reading of approximately 70 % concentration, as recommended by Hough *et al.* (1990). Moro and Hough (1985) mention that there is not any other practicable technique to check the final concentration of the product, other than by using a hand refractometer. However, the refractometer although easy to use only has an accuracy of about ±2 % for measuring the final concentration of the total solids in the cajeta product when taking the clarity of the scale interface into account. Once the product was ready, and before cooling, 4 ml of vanilla was added for flavouring and the cooling process continued with constant stirring to 50° C for packing in 100g food-grade plastic, screw-top containers.

4.2.2 Processing time

The time taken for each process is given in Table No. 4.5. The results are an average of duplicated batches using the same formula in each case. In the UF-cajeta process the ultrafiltered retentate ingredient contained 22.8 % of total solids. at 30° C. Total solids of the mixture of ultrafiltered retentate, glucose syrup and sucrose were determined from a sample at 60° C (46.70 %). The

TABLE No. 4.5 CAJETA PROCESSING SUMMARY

STAGES	UF-CAJETA			MILK-CAJETA		
	TIME (h:min)	TEMP. (° C)	T.SOLIDS (%)	TIME (h:min)	TEMP. (° C)	T.SOLIDS (%)
STARTING TIME	0:00	30	22.81	0:00	34	12.65
ADDITION OF GLUCOSE SYRUP	0:06	48		0:02	48	
ADDITION OF SUCROSE	0:08	60	46.70	0:04	60	27.21
FINISHING TIME	1:40	94 - 98	70.00 *	2:40	94 - 98	70.00 *
TOTAL PROCESSING TIME	1.40			2.40		

* Determined by sugar refractometer

mixture was taken to temperatures ranging from 94° C to 98° C until the final concentration was reached (approximately 70%). The time taken for this process was 1 hour 40 minutes.

For milk cajeta the process was similar to UF-cajeta. However, the starting temperature of the milk was 34° C with 12.65 % total solids. Glucose syrup and sucrose were added at the same temperature as for UF-cajeta and the total solids at 60° C were 27.21 %. The concentration was carried out at temperature ranging from 94° C to 98° C to approximately 70 % total solids. The time taken for the process was 2 hours 40 minutes.

The processing time for UF-cajeta starting with ultrafiltered retentate was less than milk cajeta. At certain production rates UF-cajeta may be less costly to produce than milk cajeta, when the total processing time and energy requirements are considered.

4.2.3 Chemical Characteristics of Cajeta

The chemical composition results for UF-cajeta and the control are shown in Table No. 4.6. They were statistically different ($P < 0.05$).

In UF-cajeta formulation, the proportion of ultrafiltered retentate used was equivalent to the proportion of milk used for the control batch since both processes started with the same amount of milk.

The total solids of the control (70.49%) is 1.78 % higher than that of the UF-cajeta (69.26%). The ash content of the control (1.76%) is almost 24% higher than that in the UF-cajeta, demonstrating the demineralising effect of the

TABLE No. 4.6 CHEMICAL COMPOSITION OF CAJETAS

SAMPLE DESCRIPTION	ASH (%)	PROTEIN (%)	SUCROSE* (%)	LACTOSE * (%)	OTHER * CARBOH. (%)	TOTAL * CARBOH. (%)	FAT (%)	TOTAL SOLIDS (%)
UF-CAJETA	1.42 a	7.20 a	34.90	5.21	11.63	51.74	8.90 a	69.26 a
CONTROL	1.76 b	6.91 b	32.21	10.52	10.74	53.47	8.35 b	70.49 b
SEDifference	0.032	0.122					0.102	0.800

a,b Means within the same column followed by the same letter are not significantly different ($P<0.05$)

* All carbohydrates were determined by calculation of ingredient added

ultrafiltration treatment of the whole milk. Sucrose levels were similar as required by the product formulation, but the lactose content of the UF-cajeta (5.21%) was 50.5% lower than that of the control (10.52%). The lower lactose level in the UF-cajeta is the main reason for the significantly reduced sandiness in this product (See Figure No. 4.13).

The protein content of the UF-cajeta was marginally higher (4.2%) than the control, and the fat content was 6.6% higher mainly due to variations in the formulations arising from the use of different ingredients.

Using the total amount of carbohydrate in the formulation manufacturers specifications and other chemical analysis, a mass balance of each component was carried out (See table No. 4.7). Lactose in milk cajeta was 10.5%, and in UF-cajeta 5.2% as a result of the ultrafiltration process. Sucrose in milk cajeta was 32.2 % and 34.9 % in UF-cajeta, due to the high level used in formulation. UF-cajeta had 2.1 % of glucose compared with the control with 1.9. Maltose in UF-cajeta was 3.3%, in milk cajeta it was 3.0%. Other sugars (other carbohydrates present in the cajeta syrup, but not mentioned in the product chemical specifications) in UF-cajeta were 6.3% and in milk cajeta were 5.8%.

The mineral content in the cajetas was affected by the chemical partition effect during UF-Process, separating some of them into the ultrafiltered retentate and some of them into ultrafiltered permeate giving to the product less minerals compared with the milk used to make the control with the exception of Calcium and Phosphorus. The results (See Table No. 4.8) show that UF-cajeta and the control were statistically different ($P< 0.05$). For instance Calcium in UF-cajeta was higher than the control with 414 mg/100g and 217 mg/100g respectively.

TABLE No. 4.7 MASS BALANCE FOR CAJETA MANUFACTURE AT 70% TOTAL SOLIDS CONCENTRATION

COMPONENT	MILK (kg)	GLUC-SYRUP (kg)	SUCROSE (kg)	UF-R** (kg)	GLUC-SYRUP* (kg)	SUCROSE (kg)	MILK-CAJETA (kg)	MILK-CAJETA (%)	UF-CAJETA (kg)	UF-CAJETA (%)
MASS (kg)	**3.5**	**0.175**	**0.525**	**2.1**	**0.235**	**0.705**	**1.63**		**2.02**	
ASH (%)	0.76			1.2			0.027	1.6	0.025	1.2
PROTEIN (%)	3.22			7.3			0.113	6.9	0.153	7.6
FAT (%)	3.77			9.3			0.132	8.1	0.195	9.7
LACTOSE (%)	4.9			5.01			0.172	10.5	0.105	5.2
SUCROSE			100			100	0.525	32.2	0.705	34.9
GLUCOSE		18			18		0.032	1.9	0.042	2.1
MALTOSE		28			28		0.049	3.0	0.066	3.3
HIGH SUGARS		54			54		0.095	5.8	0.127	6.3
TOTAL SOLIDS (%)	12.65	100	100	22.81	100	100	1.143	70.1	1.419	70.2

* Glucose syrup
** Ultrafiltered retentate

TABLE No. 4.8 MINERAL CONTENT (Dry basis) IN CAJETA PRODUCTS

SAMPLE	CALCIUM (mg/100 g)	PHOSPHORUS (mg/100 g)	MAGNESIUM (mg/100 g)	POTASSIUM (mg/100 g)	SODIUM (mg/100 g)
UF-CAJETA	414 a	286 a	23 a	189 a	88 a
CONTROL	218 b	174 b	34 b	271 b	108 b
SEDifference	30.22	17.28	0.78	13.43	3.18

a,b Means within the same column followed by the same letter are not significantly different ($P<0.05$)

Phosphorus was higher in the UF-cajeta with 285 mg/100g than the control with 174 mg/100 g of product. By contrast, magnesium was higher in the control than the UF-cajeta with 34 mg/100g and 22 mg/100 g of product respectively. Potassium and sodium were higher in the control with values of 274 mg/100g and 107 mg/100g of product respectively.

As previously mentioned in section 3.3.3, the 19% reduction in sodium levels should have a dietary benefit for consumers of the product, as well the enhanced calcium and phosphorous levels.

4.3 Physical Characteristics of Cajeta

4.3.1 Consistency

The consistency of the cajeta samples was measured in terms of the penetration resistance of a given probe in Newtons. The measurement was carried out in duplicate for every sample at 10° C, 20° C, 30° C and 40° C. Table No. 4.9, gives the average readings.

TABLE No. 4.9 CONSISTENCY (Newtons) OF CAJETAS AT DIFFERENT TEMPERATURES **

SAMPLES	10° C	S.D*	20° C	S.D.*	30° C	S.D*	40° C	S.D.*
UF-CAJETA	0.35 a	0.017	0.30 a	0.017	0.26 a	0.0	0.21 a	0.017
CONTROL	0.26 b	0.017	0.20 b	0.017	0.16 b	0.017	0.12 b	0.0
SEDiff	0.007		0.007		0.005		0.005	

a,b Means within the same column followed by the same letter are not significantly different ($P<0.05$)

* Standard deviation

** Probe type TA-16 at 15 mm penetration distance and speed penetration of 1.0 mm/sec

The UF-cajeta showed more resistance to penetration by the probe. The warmer the temperature the softer the product and vice versa in each case. However, comparing the two samples, UF-cajeta was always harder than the control. This may be, due to the microstructure of the UF-product being built up with slightly more proteins, sucrose and fat, as well as 50% less lactose and about 8% more of other carbohydrates than the control (See Table No. 4.7). This created a plastic compact glassy matrix, which will give more resistance to penetration by a probe. The protein/carbohydrate ratio is about 7.7% higher in the UF-cajeta and this may have enabled the protein to establish a firmer network in the matrix. Overall this affected the organoleptic characteristics of the product, giving a more sticky texture as shown in Figure No. 4.14 later.

4.4 Microbiological Quality of Cajeta

The microbiological quality of the cajetas was examined, even though they are supposed to be low bacterial growth products, due the high sugar concentration.

4.4.1 Yeasts and Moulds, Coliforms and Total Viable Count

Yeasts and moulds are the only microorganisms that may grow in cajeta and, in most cases, due to external contamination. One day after processing the products were analyzed for yeast by checking the presence of gas being produced. The results (See Table No. 4.10) show that all the samples were absent.

After 8 months storage, the products were analyzed for coliforms, total viable count and yeast and moulds using two media. One was prepared with Ringers solution containing 20 % sucrose and the another one was prepared with Ringers solution with no sucrose. The reason for this was to check if

TABLE No. 4.10 YEAST AND MOULDS IN CAJETA AFTER ONE DAY OF PROCESSING

SAMPLE	A (10 g)		B (1 g)	
	INCUBATION TIME AT 25° C			
	5 Days	10 Days	5 Days	10 Days
UF-CAJETA-1	Abs.*	Abs.*	Abs.*	Abs.*
UF-CAJETA-2	Abs.*	Abs.*	Abs.*	Abs.*
MILK-CAJETA-1	Abs.*	Abs.*	Abs.*	Abs.*
MILK-CAJETA-2	Abs.*	Abs.*	Abs.*	Abs.*

A - 5 Tubes with 10g sample in 90ml Malt Extract Broth (Dilution 7% sugar)
B - 5 Tubes with 1g sample in 9ml Malt Extract Broth (Dilution 7% sugar)
* Absent

TABLE No. 4.11 MICROBIOLOGICAL QUALITY (CFU/g) OF CAJETA AFTER EIGHT MONTHS (RINGERS WITH 20% SUCROSE)

SAMPLE	TOTAL BACTERIAL COUNT*	COLIFORM*	YEAST AND MOULDS**
UF-CAJETA-1	< 10 a	< 10 a	Est. 5
UF-CAJETA-2	< 10 a	< 10 a	2000
MILK-CAJETA-1	< 10 a	< 10 a	< 10 a
MILK-CAJETA-2	< 10 a	< 10 a	< 10 a

a No growth at 10^{-1} dilution
* At 30° C for 3 days
** At 25° C fot 5 and 10 days

TABLE No. 4.12 MICROBIOLOGICAL QUALITY (CFU/g) OF CAJETA AFTER EIGHT MONTHS (RINGERS ONLY)

SAMPLE	TOTAL BACTERIAL COUNT*	COLIFORM*	YEAST AND MOULDS**
UF-CAJETA-1	< 10 a	< 10 a	< 10 a
UF-CAJETA-2	< 10 a	< 10 a	Est. 20
MILK-CAJETA-1	< 10 a	< 10 a	Est. 5
MILK-CAJETA-2	<10 a	< 10 a	< 10 a

Results are the average of two determinations performed on the sample
a No growth at 10^{-1} dilution
* At 30° C for 3 days
** At 25° C fot 5 and 10 days

TABLE No. 4.13 THREE MPN* TEST FOR COLIFORM IN CAJETA AFTER EIGHT MONTHS STORAGE

SAMPLES	UF-CAJETA-1	UF-CAJETA-2	MILK-CAJETA-1	MILK-CAJETA-2
	COLIFORM **			
DILUTION	-1 -2 -3	-1 -2 -3	-1 -2 -3	-1 -2 -3
POSITIVE TUBES	0 0 0	0 0 0	0 0 0	0 0 0
MOST PROB. NUMBER*	< 3/g	< 3/g	< 3/g	< 3/g

* Most probable number

** Three tubes MPN at 30° C for 3 days

microorganisms are affected by changes in sugar concentration of their habitat. The total bacterial count and coliform tests using Ringers solution with 20 % of sucrose (See Table No. 4.11) in all the samples was always negative. But the yeast and moulds test in one of the UF-cajeta duplicates gave 2000 CFU/g but, as it was only one of the duplicates the results may be due to external contamination during packing. The results using Ringers solution without sucrose (See Table No. 4.12) showed that coliforms, total bacterial count and yeast and moulds were all absent with, in most cases, results of < 10 CFU/g and some other cases estimations of 20 and 5 CFU/g. According to the results for both media, microorganisms are largely affected by changes in sucrose concentration and false results can be obtained if this is not considered. Thus Ringers with 20% sucrose should be used for routine microbiological analysis of cajeta.

In addition coliforms were checked again using the most probable number (MPN) method (See Table No. 4.13) and in all cases they gave results for MPN of < 3/g .

According to the analysis of results, cajeta is a safe product from the point of view of microbial contamination, but the control of the growth of osmophilic yeast and moulds have to be considered in the manufacture of such product.

4.5 Microscopy Analysis of Cajeta

As it was previously described (Section 3.6 of chapter three), Microscopy plays a very important role in the food industry. In this case cajeta samples were analyzed by using Light Microscopy (LM) and Transmission Electron Microscopy (TEM) in order to characterize the microstructure.

4.5.1 Light Microscopy Analysis (LM)

Lactose can be the cause of sandiness in cajeta. Light microscopic analysis of cajeta was used in this study to analyze the number, rate of growth and structure of the lactose crystals in six fields using a x 100 magnification (As described in section 2.4.4.13).

4.5.1.1 Number of Crystals

The number of crystals present in UF-cajeta and the control cajeta were kept under inspection during 125 days storage at 4° C and 30° C.

Table No. 4.14, shows the average results of six fields taken every fifth day up to 65 days and then monthly. In UF-cajeta stored at 4° C no lactose crystals were found in the fields from the first to the last day. The same product stored at 30° C showed only one crystal in the fields initially, but this did not increase in size during storage and no new crystals were formed. The control samples stored at 4° C did not show crystals initially, but after the tenth day crystals started appearing with 39 visible on the 35th day when the number was constant until the end of the trial. The milk cajeta stored at 30° C showed a similar pattern but to a lesser extent; three crystals appeared on the 10th day, and these increased to 21 crystals by day 50 and then remained constant to the end of the trial.

Figure No. 4.3, shows the representation of UF-cajeta at the 10th day at 4° C after processing, and no lactose crystals were found at any temperature. However in the control cajeta (Figures No. 4.4-A and B), the numbers of lactose crystals were different for the control stored at 30° C and 4° C. Figure A appears to have slightly less lactose crystals compared with illustration B which was stored at 4° C, which agrees with the average values shown in Table No. 4.14.

The storage temperature of 4° C at this stage seems to promote the growth of crystals. This is may be due to the low lactose solubility at this temperature, since at higher temperatures lactose increases its solubility (See Figure No. 1.3 of Literature Review Chapter).

After 65 days storage at the same temperatures new photographs were taken of the ultrafiltered cajeta and the control. In UF-cajeta there were no observable lactose crystals at x100 magnification at either 4° C or 30° C (See Figure No. 4.5). However the milk cajeta images presented an increase on the number of lactose crystals (See Figure No. 4.6). Illustration B shows crystals of similar size formed at 4° C whereas illustration A at 30° C, indicates a wider range of lactose crystal sizes. This may be due to the higher solubility of lactose at 30° C, leading to preferential growth of larger crystals rather than formation of new, smaller crystals. In general conditions favouring slower crystal growth will result in fewer but larger crystals.

A new form of lactose crystal was found after 30 days of storage at 30° C for the cajeta control. This new form has a spherulite shape (See arrows in Figures No. 4.7 and 4.8) which has a central intercept point for all the elongated components (See arrows in Figure No. 4.9). This new form does not follow any specific pattern of the distribution and the size of the elongated components. The only common characteristic is the central intercept point. The new crystal form does not correspond to any other carbohydrates because they do not achieve saturation levels in the cajeta (See Figure No. 1.3). The control cajeta has approximately, 35.6 g of lactose in 100 g of water compared to approximately 19 g in 100 g of water at room temperature.

One possible explanation is that, during the crystallisation of lactose,

α-lactose monohydrate is the usual crystalline form obtained from aqueous lactose solutions. However if crystallization takes place at high concentration of lactose, the solution could be supersaturated with respect to both α and β-lactose. Theoretically, then both forms may crystallize, irrespective of the temperature (Roetman, 1981). When crystallisation takes place, it goes from a saturated solution to a glass state, increasing the viscosity of the solution.

Warburton and Pixton (1978) mention that there are several shapes of lactose α -hydrate crystals and, which one is formed depends on the conditions of growth. They mention that when precipitation pressure is high and crystallization is forced, prism shapes are produced. The form changes with decreasing pressure to diamond, pyramid, tomahawk and 13-sided crystals, but irregular crystals may be found due to the presence of impurities in some dairy products. However, there is no information about β-lactose crystal forms.

4.5.1.2 Size of Crystals

The sizes of the lactose crystals were inspected by detecting randomly the crystals in the microscope slide using a x 100 magnification. The number of useful fields of view on the slide varied when measuring crystals in UF-cajeta, particularly when the incidence of crystals was rare.

UF-cajeta crystal size was more stable than the control (See Table No. 4.15). When the UF-cajeta products were stored at 4° C and 30° C in both cases the average result was one crystal of 10μm size from the first day and keeping the same size constant to the 65th day. The presence of a lone crystal may be due to the presence of a dust or gas bubble nucleus. Brennan *et al.* (1976) mention that alternative crystals of similar structure to the solute crystals,

TABLE No. 4.14 NUMBER OF LACTOSE CRYSTALS IN CAJETAS*

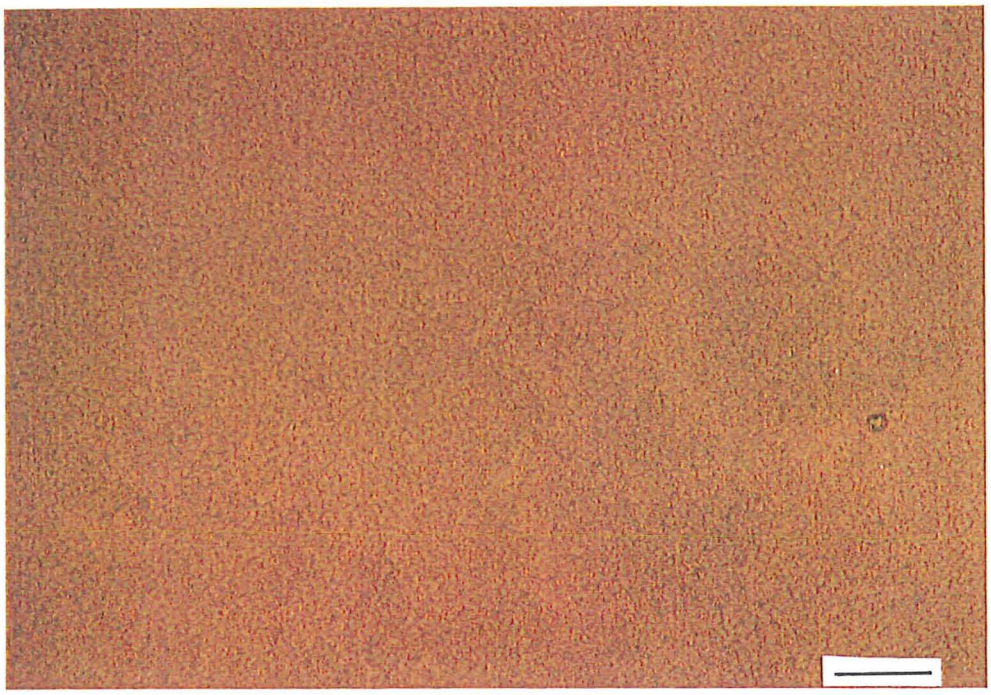
	UF-CAJETA		CONTROL	
DAY	**4 ^{O} C**	**30 ^{O} C**	**4 ^{O} C**	**30 ^{O} C**
1	0	1	0	0
5	0	1	0	0
10	0	1	9	3
15	0	1	21	6
20	0	1	30	10
25	0	1	35	13
30	0	1	38	17
35	0	1	39	19
40	0	1	39	20
45	0	1	39	20
50	0	1	39	21
55	0	1	39	21
60	0	1	39	21
65	0	1	39	21
95	0	1	39	21
125	0	1	39	21

* Results are an average of six fields in every slide and were taken from the average of every fifth day

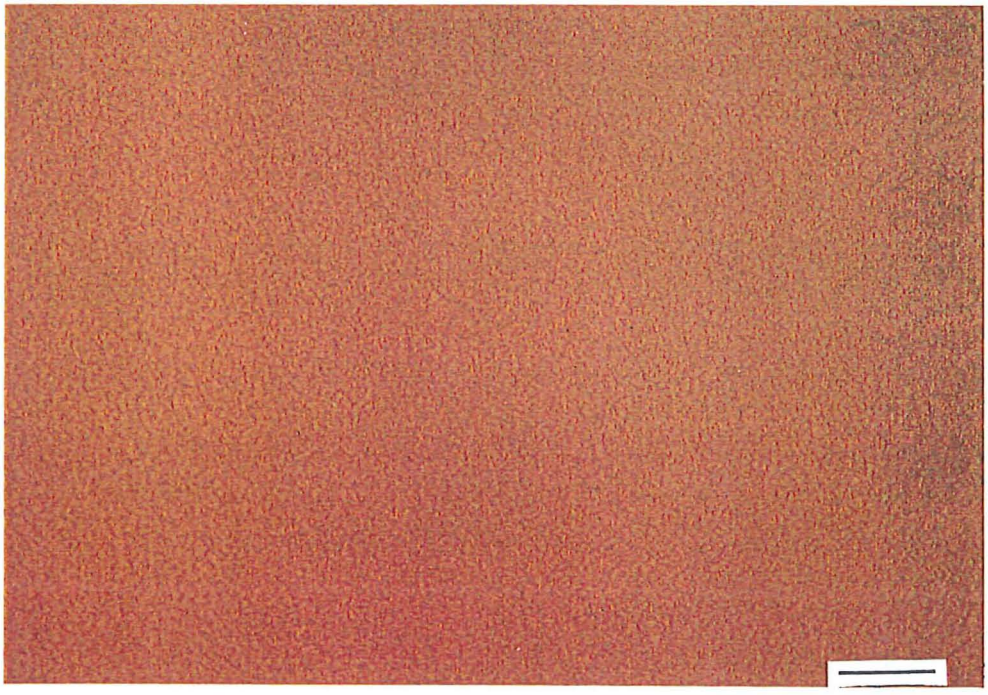
TABLE No. 4.15 SIZE (Microns) OF LACTOSE CRYSTALS IN CAJETAS *

	UF-CAJETA		CONTROL	
DAY	**4 ^{o} C**	**30 ^{o} C**	**4 ^{o} C**	**30 ^{o} C**
1	10	10	0	0.0
5	10	10	0	28
10	10	10	59	69
15	10	10	89	119
20	10	10	129	208
25	10	10	158	247
30	10	10	178	297
35	10	10	198	317
40	10	10	198	327
45	10	10	208	327
50	10	10	228	337
55	10	10	228	337
60	10	10	228	337
65	10	10	228	337
95	10	10	228	337
125	10	10	228	337

* Results are an average of six fields in every slide and were read every fifth day

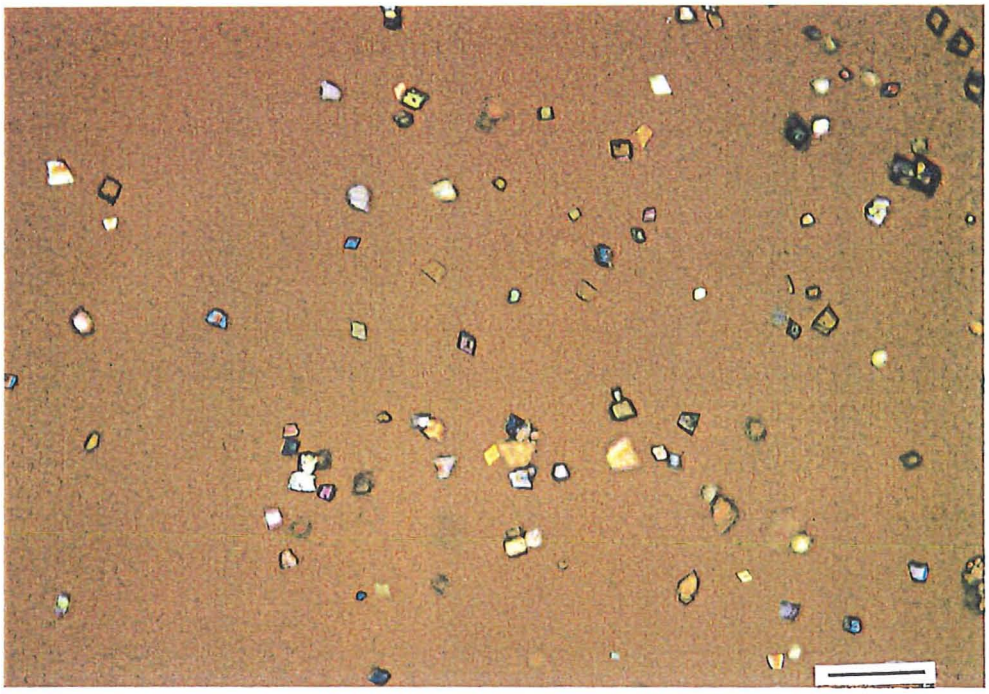


A: Storage temperature 30° C, BAR = 300 μm

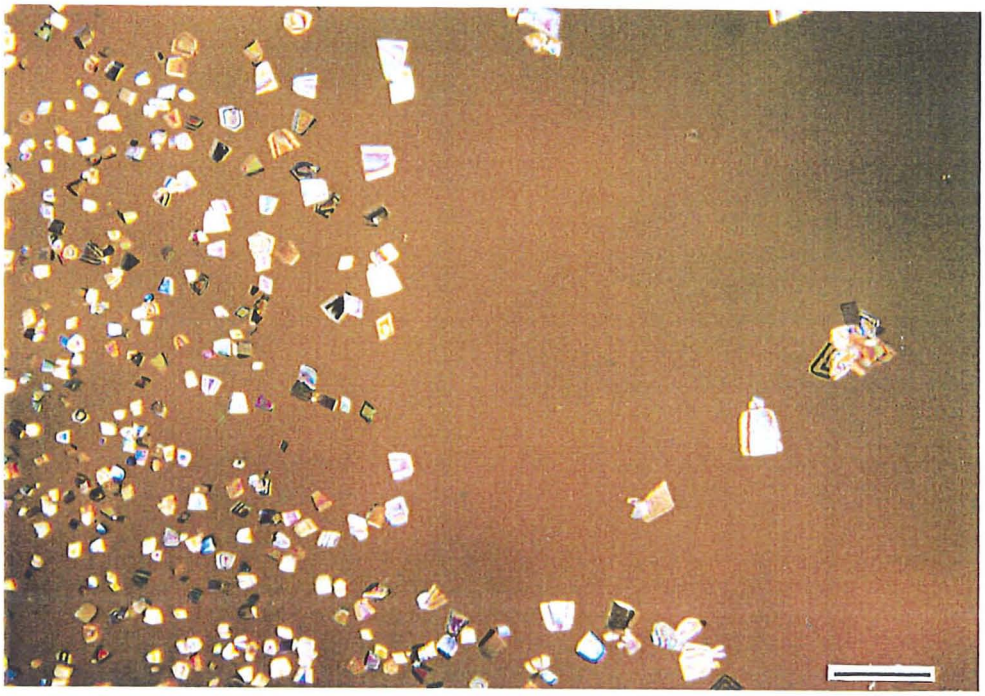


B: Storage temperature 4° C BAR = 300 μm

Figure No. 4.3 Ultrafiltered cajeta after 10 days of storage at different temperatures



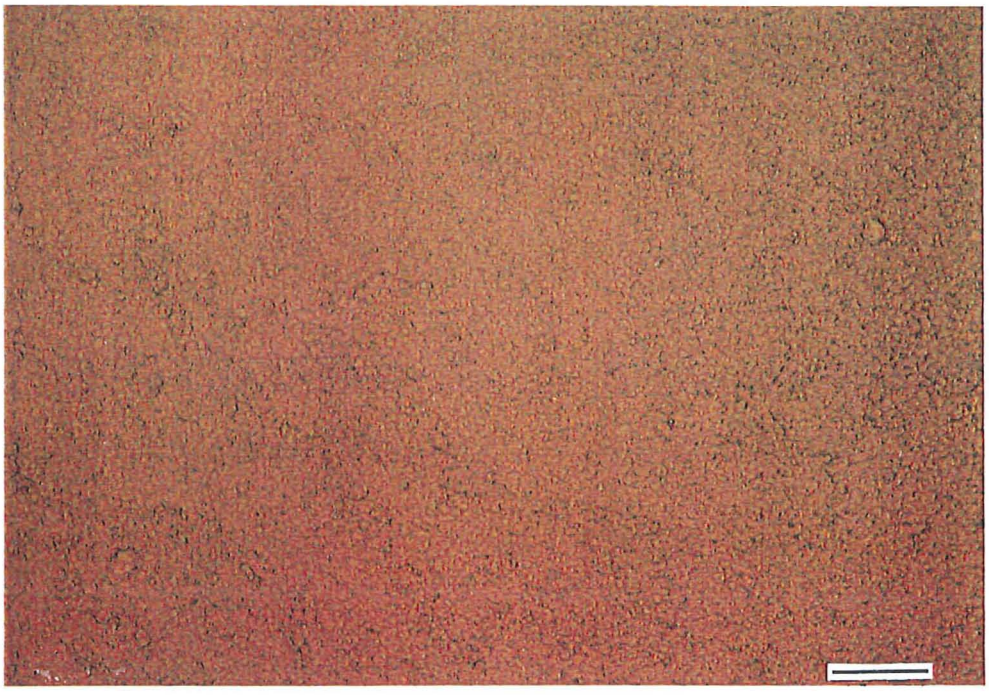
A: Storage temperature at 30° C, BAR = 300 μm



B: Storage temperature at 4° C BAR = 300 μm

Figure No. 4.4 Milk cajeta after 10 days of storage at different temperatures

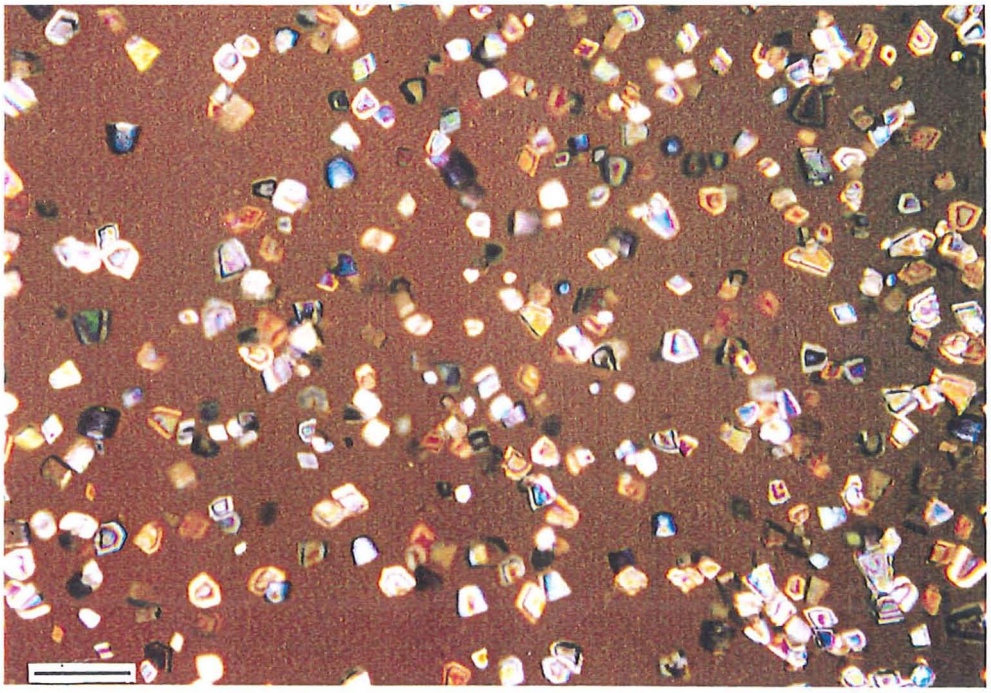
A: Storage temperature at 30° C, BAR = 300 μm



B: Storage temperature at 4° C BAR = 300 μm

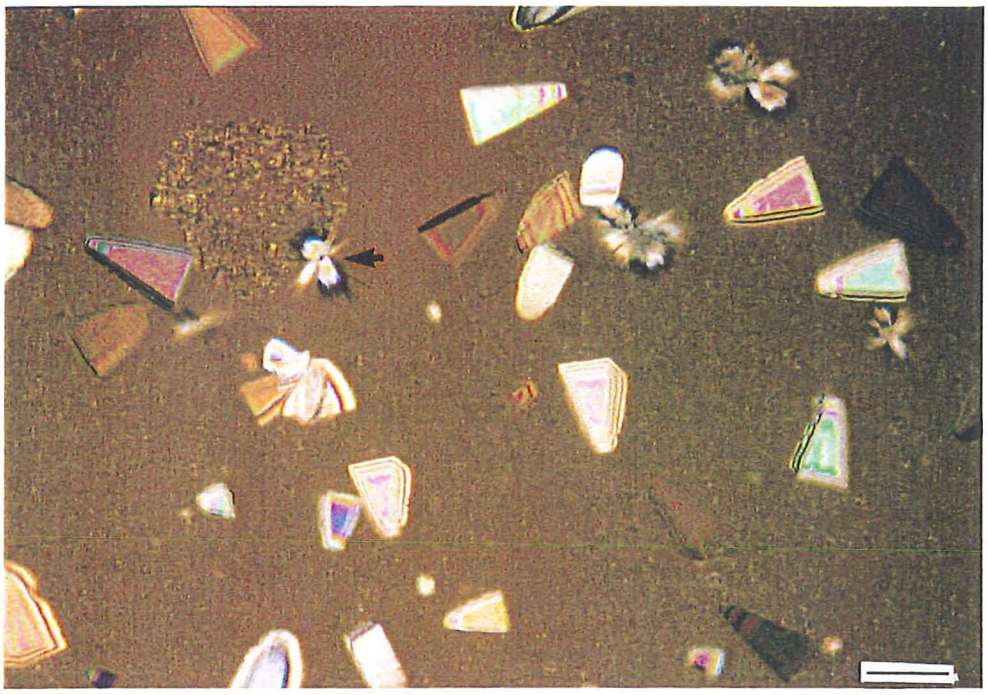
Figure No. 4.5 Ultrafiltered cajeta after 65 days of storage at different temperatures

A: Storage temperature at 30° C, BAR = 300 μm

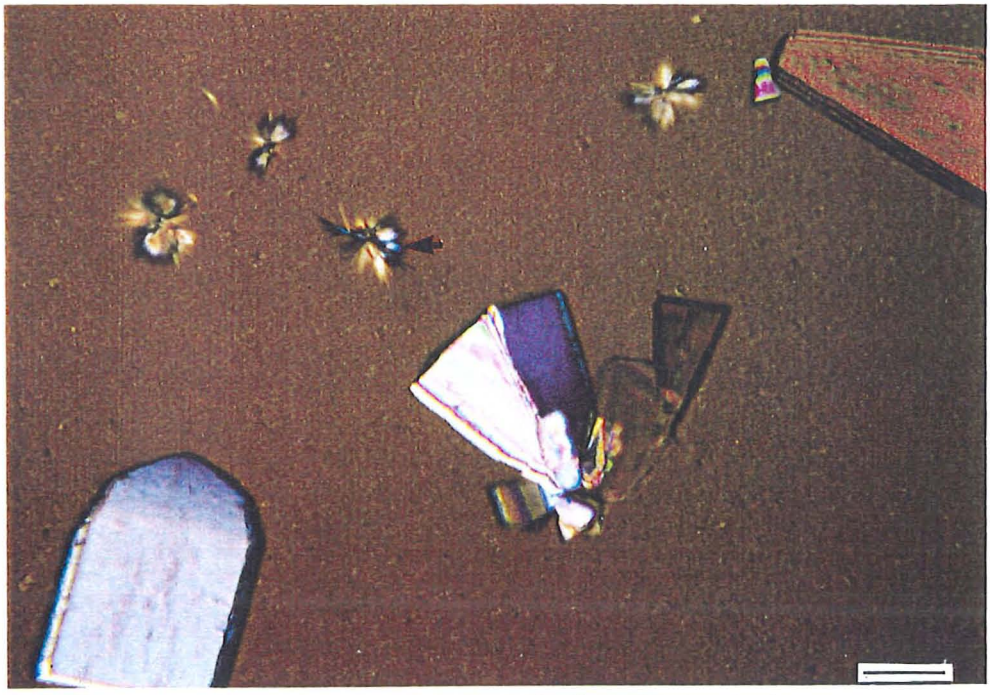


B: Storage temperature at 4° C BAR = 300 μm

Figure No. 4.6 Milk cajeta after 65 days of storage at different temperatures

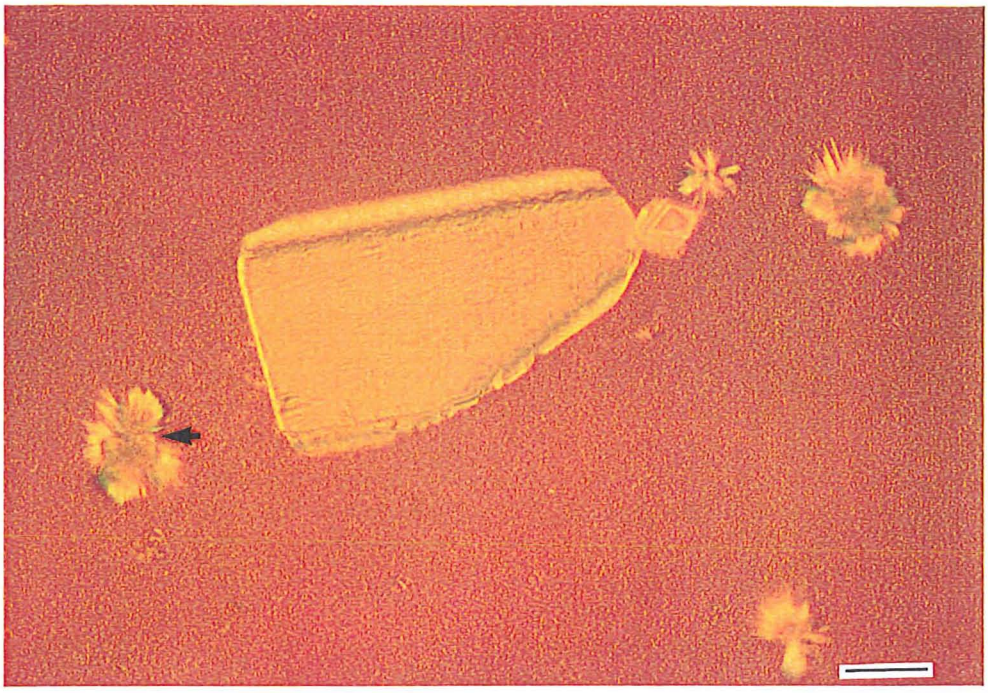


A: Storage temperature at 30° C, arrow is pointing the new form of crystal BAR = 100 μm

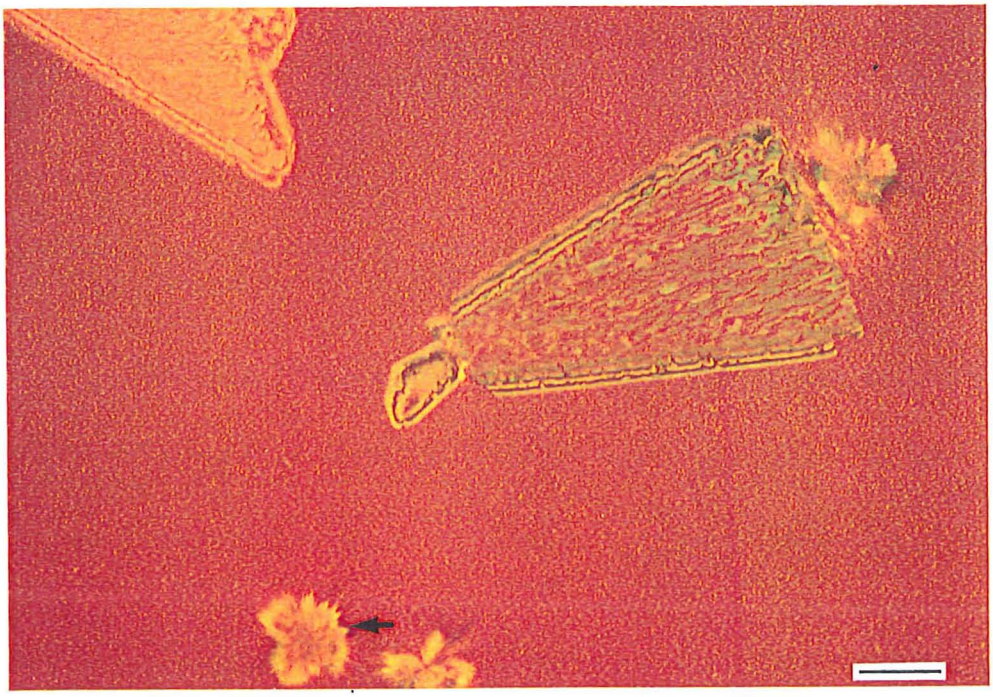


B: Storage temperature at 30° C, arrow is pointing the new form of crystal BAR = 100 μm

Figure No. 4.7 Milk cajeta showing a new form of crystals



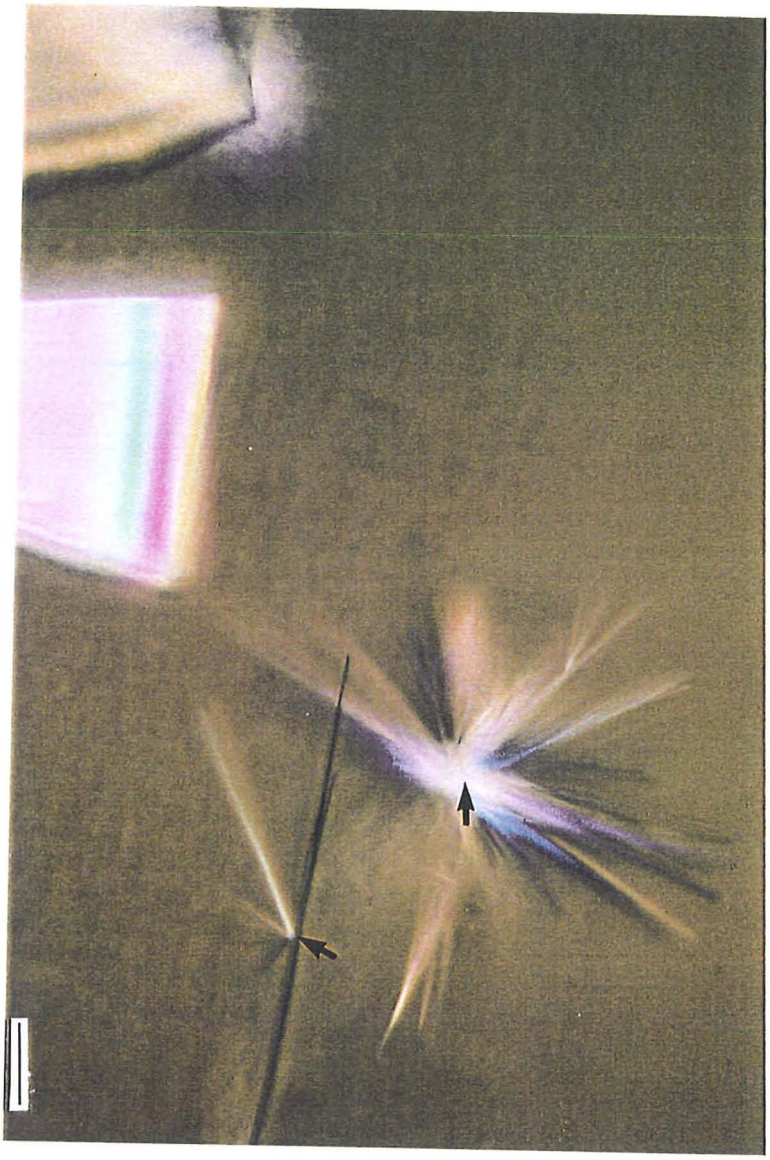
A: Storage temperature at 30° C, arrow is pointing the new form of crystal BAR = 100 μm



B: Storage temperature at 30° C, arrow is pointing the new form of crystal BAR = 100 μm

Figure No. 4.8 The new form of crystals in milk cajeta

Figure No. 4.9 The new form of crystal in milk cajeta stored at 30° C



Arrow is pointing to the central intercept of the elongated components of the new form of crystal
BAR = 100μm

dust or gas bubbles may also initiate crystallization, as may the application of mechanical shock or ultrasonic vibrations. But on the other hand, the milk cajeta at 4° C showed no crystal growth until the 10th day with an average of 59μm. It kept growing until the 50th day with a final size of 228μm. Then the size remained constant until the end of the trial. In the control stored at 30° C the first crystals were detected on the 5th day averaging 28 μm, and they grew until the 50th day to 337μm, thereafter remaining constant to the final day.

According to the results for both the number of crystals and the size of the crystals, UF-retentate as an ingredient in cajeta manufacture helps to prevent the presence of lactose crystals.

4.5.2 Transmission Electron Microscopy (TEM)

Samples of both UF-cajeta (Protein 7.20 %) and milk cajeta (Protein 6.91 %) were prepared as described in section 2.4.3.12.1.

The stability of the casein micelles, is influenced by several treatments such as acidification, heating and addition of Ca^{++}. During heat treatment various physical and chemical changes occur in casein micelle, whey proteins, lactose and salts, affecting their functionalities in milk products.

Creamer and Matheson (1980) mention that when milk is heated in the temperature range of 90° C to 140° C at pH values below 6.7, denatured whey proteins complex on to the micellar surfaces, involving κ-casein, but at higher pH values, denatured whey proteins remain in the intermicellar fluid as fibrous strands. Dalgleish *et al.* (1987) suggest that the increase in the casein micelle diameter on heating milk is thought to be due to deposition of denaturated whey proteins on to the micellar surfaces and precipitation of calcium phosphate.

Carroll *et al.* (1971) have noted a doubling of casein micelle size in sterilised concentrated milk (26% solids) compared with fresh milk, and this implies increased aggregation of casein micelles. They suggested that the increased level of calcium in concentrated milk may lead to calcium bridging between micelles with a subsequent increase in micelle size. In this study the calcium content in UF-cajeta was almost double than in the milk cajeta. (See Table No. 4.8).

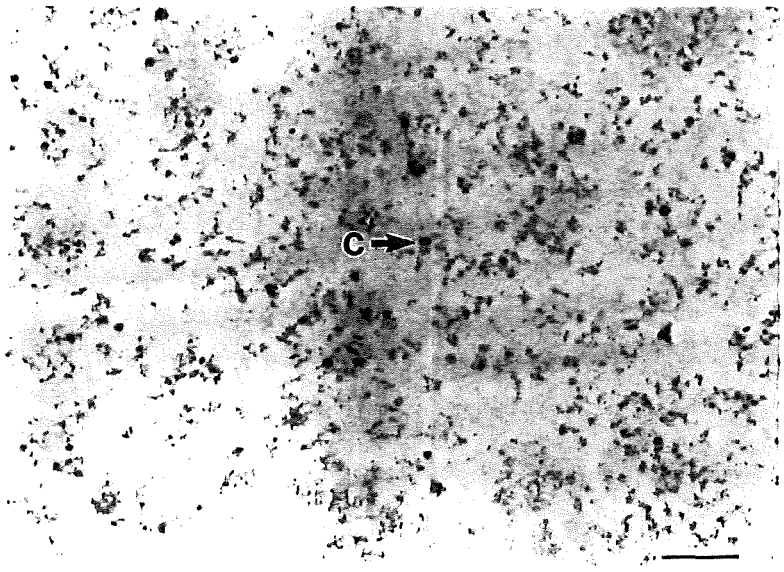
The increase of casein micelle size in the UF-cajeta does not affect the stability of the product, since it is formed mainly by a glassy sugar matrix, where casein micelles take a secondary role in the microstructure of the product.

Figure No. 4.10, shows images of milk-cajeta at x 7,500, x 20,000 and x 50,000 magnification. The three images present a general overview of milk cajeta microstructure formed mainly from proteins. Casein micelles are forming aggregates and are distributed randomly (See arrows ' C ' on illustrations in Figure No. 4.10). However, in Figure C as indicated with arrow ' W ' faint fibrous strands are seen on the surface of the casein micelle. These strands are likely to be mainly denaturated whey proteins.

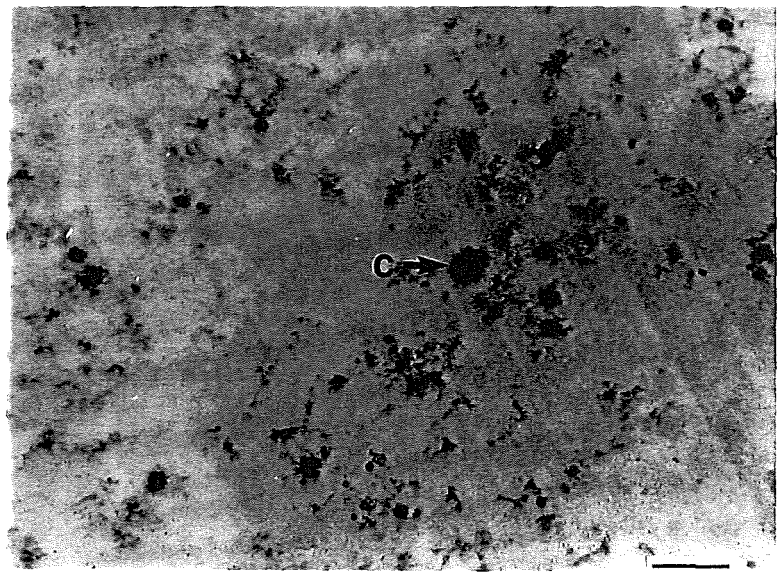
Figure No. 4.11, shows x 7,500, x 20,000 and x 50,000 magnification of UF-cajeta microstructure. In all illustrations proteins are the main components (See arrows ' C ' on all illustrations in Figure No. 4.11), probably consisting of denatured whey proteins on the surface of the casein micelle (See arrow ' W ' on illustration C). In this case proteins are in slightly more prominent, forming more extensive clusters.

In neither UF-cajeta nor milk cajeta were carbohydrates, fat or minerals evident because the samples were prepared for protein fixation.

A: Overall view of protein distribution
BAR = 1.3μm



B: An aggregate of casein micelle
BAR = 0.5μm



C: Arrow "W" is pointing a faint strands, which are likely to be denatured whey proteins. Casein micelle is indicated with "C".
BAR = 0.2μm

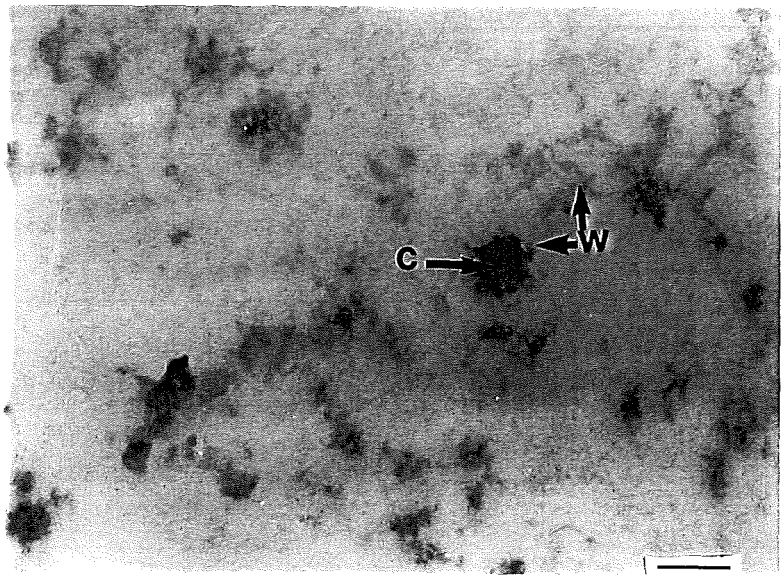
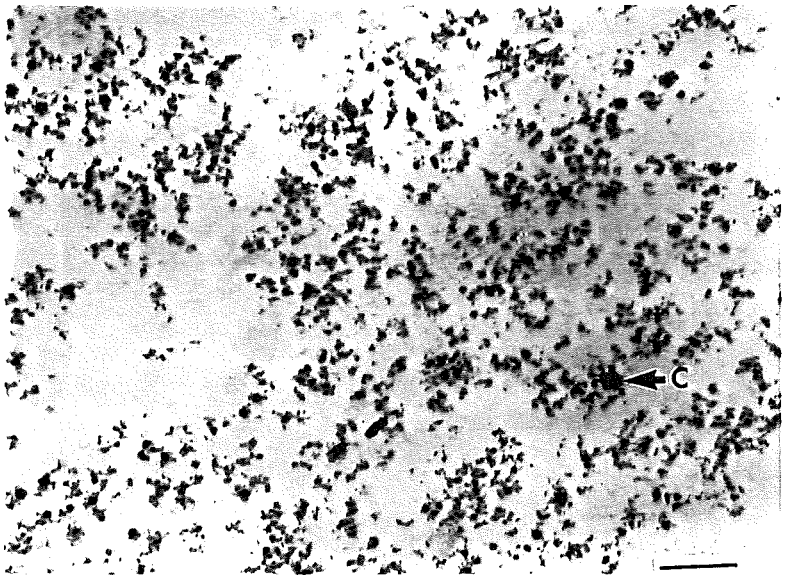
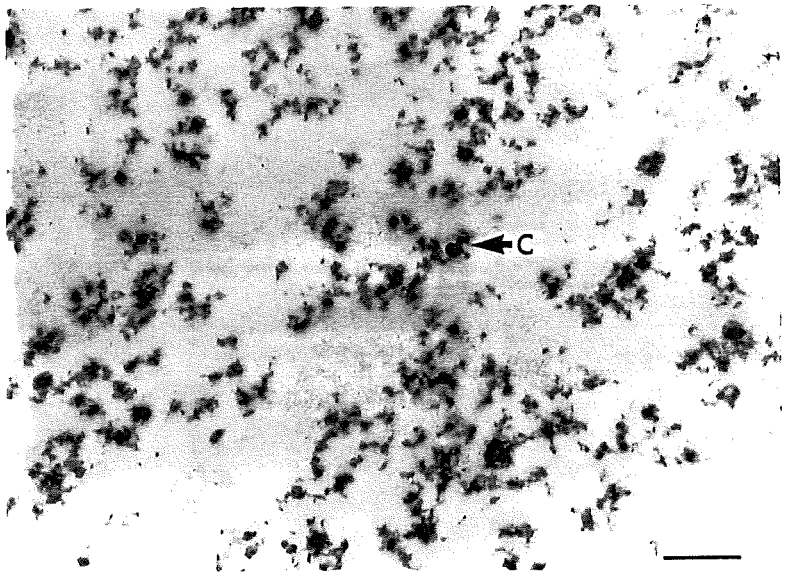


Figure No. 4.10 Transmission Electron micrographs of milk cajeta

A: Overall view of protein distribution
BAR = 1.3μm



B: A casein micelle cluster
BAR = 0.5μm



C: Arrow "W" is pointing a faint strands, which are likely to be denatured whey proteins. Casein micelle is indicated with "C".
BAR = 0.2μm

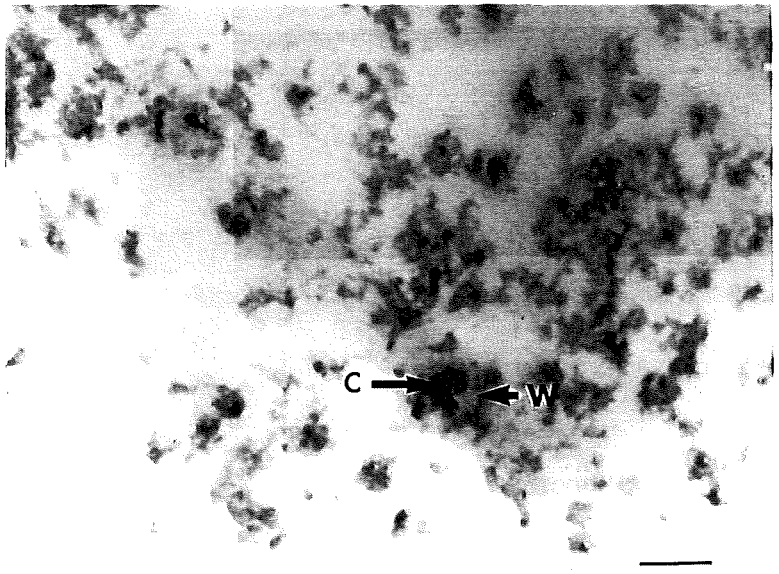


Figure No. 4.11 Transmission Electron micrographs of ultrafiltered cajeta

4.6 Sensory Characteristics of Cajeta

Cajeta has not been organoleptically characterised before. Only a few scientific articles have been published giving a brief description of cajeta mainly concerned with sandiness. In this study five attributes were used to describe the products (See Figure No. 4.12). A REML statistical method was used to fit a mixed model to the data. Random effects of judge and time within judge variations were estimated in the analysis. Effects of presentation order of samples were considered (See Section 2.4.4.15 of Materials and Methods).

4.6.1 Storage Periods

Cajeta samples were organoleptically evaluated after one, five and nine weeks of storage at 4° C. The means scores are shown in Appendix A.8. Statistical differences in this section are all at the level of $P<0.05$.

Lactose crystal sizes are increased by storage when concentrations of lactose are too high. In this study most organoleptic scores were statistically different. UF-cajeta was slightly more sandy after one week with 1.0 and 0.7 compared with the control. But after five weeks of storage UF-cajeta was more stable scoring 1.4 against 5.9 in the control. After nine weeks, the cajeta control scored only 0.6 but UF-cajeta was 9.7 (See Figure No. 4.13). Obviously, the organoleptic results confirm the results of chemical analysis, and the microscopic examination of the cajeta products. The UF-cajeta has half of the lactose content of the control (See Table No. 4.6), and as would be expected, it showed lesser quantity of lactose crystals as illustrated in Figure No. 4.6.

Stickiness was also statistically different in the samples. After one week of storage UF-cajeta was stickier with 6.0 than the control with 3.9. After five weeks storage the UF-cajeta was slightly stickier than the control with 6.0

FIGURE No. 4.12 SCORE CARD FOR CAJETA EVALUATION *

NAME_____

DATE_____

SAMPLE No_____

SANDINESS

None Extremely

STICKINESS

None Extremely

SMOOTHNESS

None Extremely

FLAVOUR

Unacceptable Acceptable

ACCEPTABILITY

Poor Excellent

COMMENTS

_____

_____

* **Lines 150 mm long**

and 5.4 respectively. Finally after nine weeks storage UF-cajeta was less sticky than the control with 5.3 and 6.9 respectively (See Figure No. 4.14). An increase in consistency of the cajeta might have caused some panellists to have scored higher for stickiness.

Smoothness in the products was statistically different only after one and five weeks storage. UF-cajeta was marginally less smooth than the control with 11.9 and 12.2 respectively after one week storage. But after five weeks UF-cajeta becomes smoother than the control with 11.5 and 8.0 respectively. However, after nine weeks the products were not different, scoring roughly the same. UF-cajeta scored 8.1 and the control 8.4 (See Figure No. 4.15).

Smoothness is largely affected by the milk components of the dairy ingredient. The ultrafiltered retentate (i.e. low lactose content) used to substitute whole milk for cajeta manufacture seems to slightly affect the smoothness characteristic of cajeta products.

Flavour was not statistically different in time except after nine weeks. After one and five weeks the control scored 12.2 and 11.1 for flavour and UF-cajeta with 11.8 and 11.1 respectively. After nine weeks storage UF-cajeta had a better flavour score with 8.9 against 8.0 in the control (See Figure No. 4.16). Apparently, the variation in the dairy ingredient used for the manufacture of cajeta did not alter the flavour in the cajeta products significantly, though this would be masked by the added flavour.

The acceptability of the products was not statistically different after one week of storage but was after five and nine weeks. After one week UF-cajeta scored 11.1 against 11.6 for the control. In the second evaluation at five weeks,

UF-cajeta had a better score against the control with 10.8 and 8.4 respectively. However after nine weeks storage the acceptability of UF-cajeta was maintained but the control worsened with scores of 10.7 and 3.2 respectively. According to the results, acceptability of the products is largely affected by the presence of the sandiness problem. This, is because sandiness when detected, has a strong influence on the panellists overall assessment of the product (See Figure No. 4.17).

4.6.3 Principal Component Analysis (PCA) of Cajetas

In order to visualise the relationships between the 4 cajetas and their interrelationships with 5 attributes, a principal components analysis was used and a PCA Biplot was produced. In this analysis (See Figure No. 4.18) the first principal components accounts for 84% of the total variation, and the second accounts for 9% to get an accumulated representation of 93% of the total variation of the data. The vectors describing the products are sandiness, smoothness, flavour and acceptability.

Milk cajetas one and two at 5 and 9 weeks tended to be more sandy and were set apart from the acceptability area. They were separating clearly from UF-cajetas one and two at five and nine weeks. On the other hand, UF-cajetas tended to be less smooth through the evaluations, being allocated after nine weeks at the upper left side of the bi-plot as a result of good acceptability, and a regular degree of stickiness and flavour.

FIGURE No. 4.13 ORGANOLEPTIC RESULTS FOR CAJETA FOR TIME

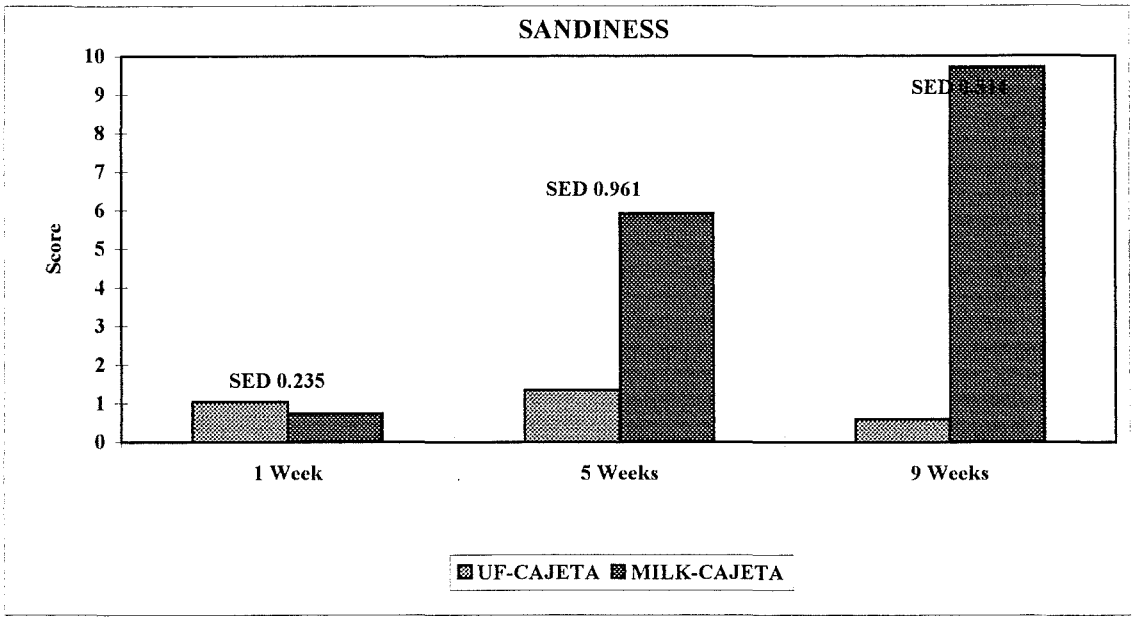


FIGURE No. 4.14 ORGANOLEPTIC RESULTS FOR CAJETA FOR TIME

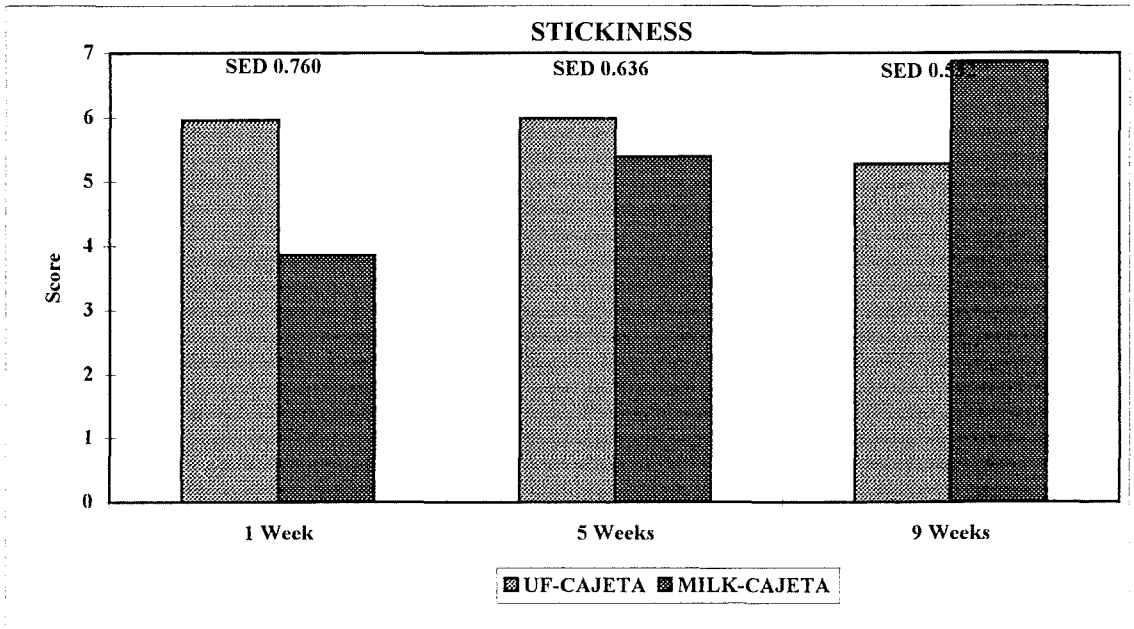


FIGURE No. 4.15 ORGANOLEPTIC RESULTS FOR CAJETA FOR TIME

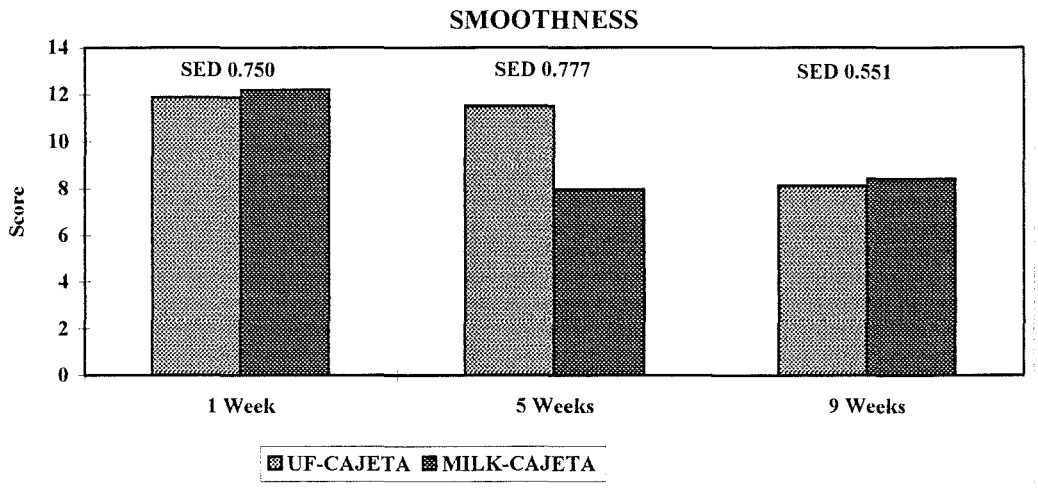


FIGURE No. 4.16 ORGANOLEPTIC RESULTS FOR CAJETA FOR TIME

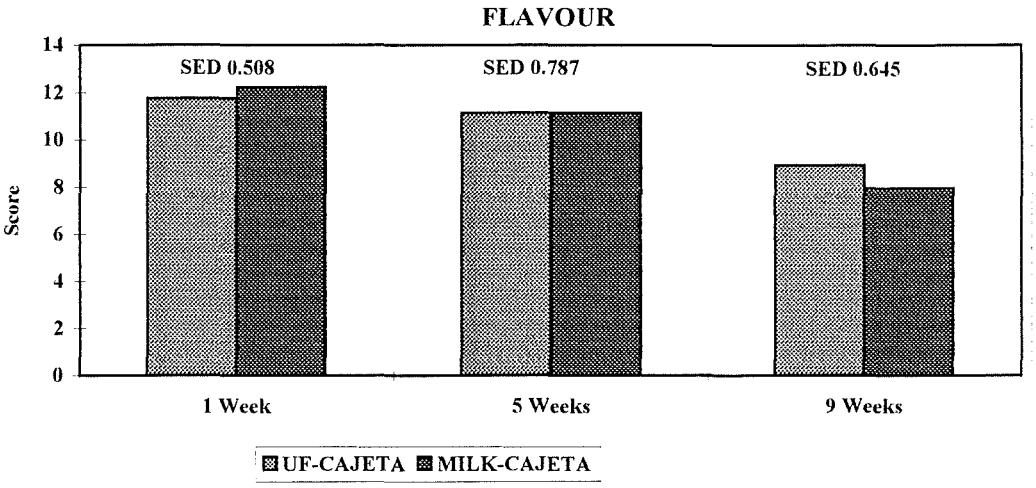


FIGURE No. 4.17 ORGANOLEPTIC RESULTS FOR CAJETA FOR TIME

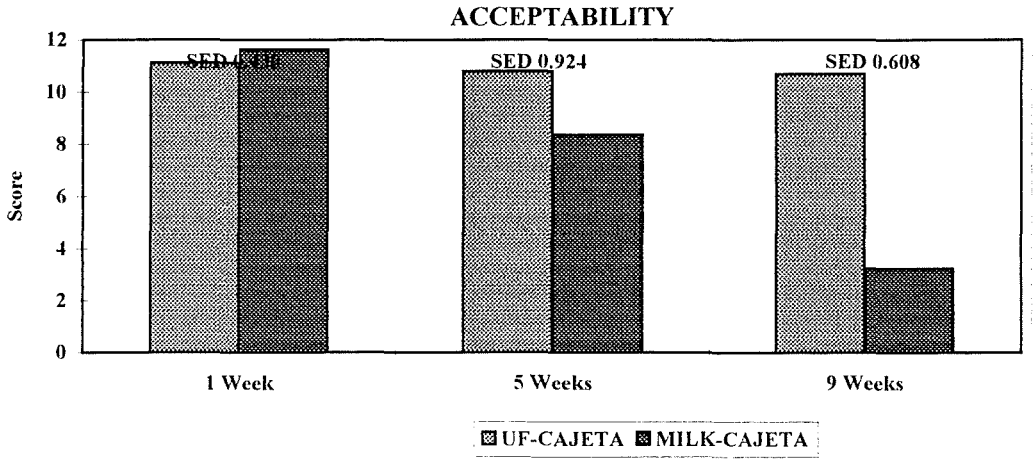
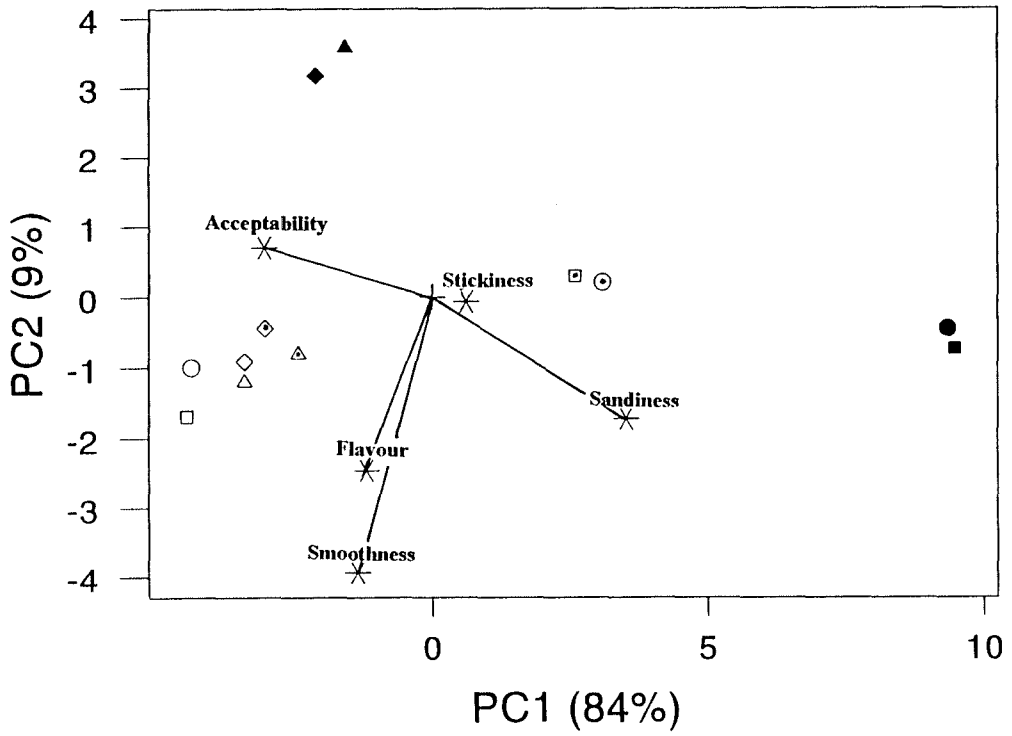


FIGURE No. 4.18 PRINCIPAL COMPONENTS ANALYSIS OF ORGANOLEPTIC EVALUATION OF CAJETA AT 1, 5 AND 9 WEEKS STORAGE AT 4° C



○ Milk Cajeta-1 after 1 week, □ Milk Cajeta-2 after 1 week, ◊ UF-Cajeta-1 after 1 week, Δ UF-Cajeta-2 after 1 week
⊙ Milk Cajeta-1 after 5 weeks, ⊡ Milk Cajeta-2 after 5 weeks, ◈ UF-Cajeta-1 after 5 weeks, ⧋ UF-Cajeta-2 after 5 weeks
● Milk Cajeta-1 after 9 weeks, ■ Milk Cajeta-2 after 9 weeks, ♦ UF-Cajeta-1 after 9 weeks, ▲ UF-Cajeta-2 after 9 weeks

FIGURE No. 4.19 SCORD CARD FOR VISUAL EVALUATION OF SANDINESS IN CAJETA

NAME_____

DATE_____

Please evaluate the cajeta samples, detecting the presence of grains using sight or touching with the fingers. Keep to the order in which they are presented and follow the description below.

1	2	3	4
UNDETECTABLE	SLIGHTLY SANDY	MODERATE	VERY SANDY

SAMPLE _____

SAMPLE _____

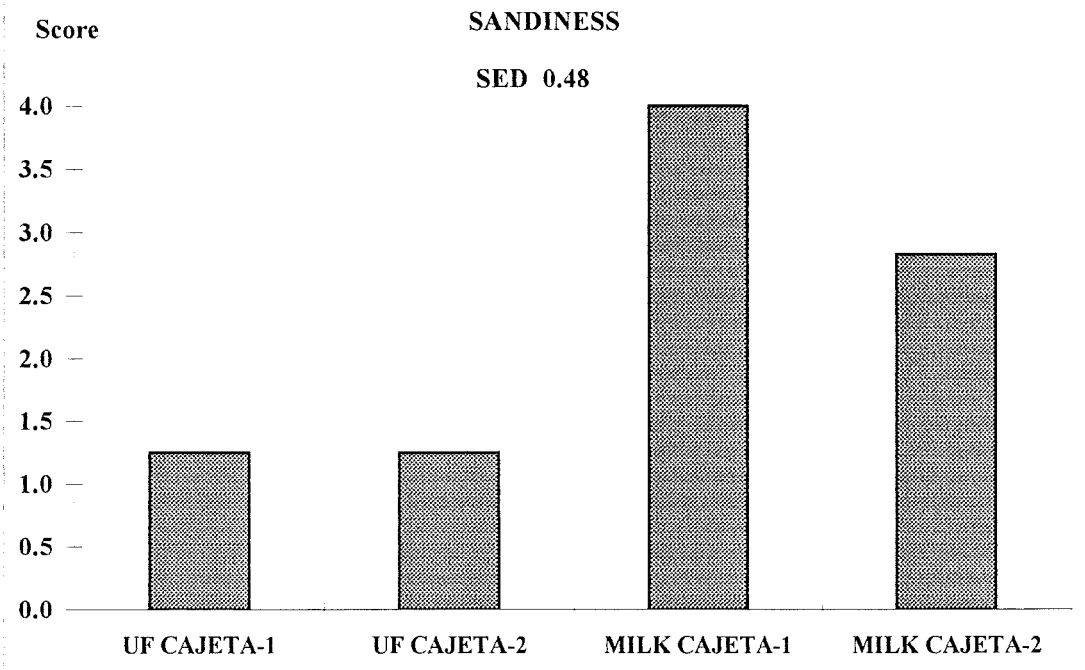
SAMPLE _____

SAMPLE _____

C
COMMENTS

_____
_____
_____
_____

FIGURE No. 4.20 VISUAL SCORES OF SANDINESS IN CAJETA AFTER 8 MONTHS OF STORAGE AT 4° C



4.6.4 Visual Evaluation of Sandiness

This evaluation was carried out after the microbiological analysis of the products after eight months in storage. As previously described in section 4.4.1, one of the UF-cajeta duplicates was found to have yeast and mould due, maybe, to external contamination. So for that reason the products were not used for sensory analysis. However, as sandiness was clearly detected by sight it was inspected in all samples by 12 people who had been used as taste panellists in this study. A score card was used to assess the presence of sandiness (See Figure No. 4.19). The results are shown in Appendix A.9, and Figure No. 4.20 presents a graphic representation of sandiness in the products, showing that sandiness was easy to detect in the control with a score of 3.41 which was statistically different ($P > 0.05$) from UF-cajeta with a score of 1.25.

4.7 Conclusions

Ultrafiltered retentate in cajeta manufacture reduces the processing time (at certain stage of the process), consequently, less energy may be required for the concentration process and there may be less heat damage to the product.

The use of ultrafiltered retentate in cajeta manufacture slightly increases the protein and decreases the lactose content significantly.

Ultrafiltered cajeta has enhanced Calcium and Phosphorous content and the Potassium and Sodium levels are decreased, compared with the traditional product.

UF-Retentate in cajeta manufacture reduces the degree of lactose crystallisation preventing the sandiness problem.

UF-cajeta is more acceptable after several months storage as a result of reducing sandiness.

UF-cajeta is a microbiologically safe product which is stable during storage. Sucrose enriched diluents may be used in evaluating microbiological contamination.

The ultrafiltration process is suitable for the manufacture of good quality cajeta.

CHAPTER FIVE

DISCUSSIONS AND CONCLUSIONS

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

5.1 ULTRAFILTRATION OF MILK

Protein rejection coefficients in whole milk and skim milk are similar. The partition of ash and minerals indicates that the rejection of ionic components is not governed by the rejection characteristics of the membrane alone but will be modified by interaction between the mineral and protein components. The gel layer of protein and fat also acts as a series resistance for the transport of microsolutes.

Fat was expected to be retained. In the Ultrafiltration of whole milk, the milk fat interfered with the separation process reducing the flux rate of UF-Permeation. The lactose content in the retentate was similar for whole milk and skimmed milk filtration.

On the manufacture of some dairy products volume reduction of the permeate phase during the ultrafiltration process can be used as a variable to determine the level of concentration of total solids or protein in the ultrafiltered retentate.

The components of the retentate might be expected to be in a relatively unchanged state. However the change in mineral balance, in particular the concentration, can influence the functionality of protein components. In this case there are some signs of casein aggregation in the ultrafiltered retentate. Other studies have suggested that reducing calcium levels (e.g. by ion exchange) reduces the casein micelle size and that this in turn improves the emulsifying properties of the milk. Hence the effect of ultrafiltration could be to reduce the

emulsifying ability. In neither of the products studied was the emulsifying capacity pushed to its limit and so this change would not be revealed in these studies. If the ultrafiltered retentate were to be used in a rôle where a high emulsifying capability were required (e.g. mayonnaise, pâté) then some attention may need to be given to reducing the effects of calcium (e.g. by ion exchange or sequestration). With UF-Processes, however this seems to be expensive and other inexpensive raw material may give the effects, such as skim milk powder addition.

5.2 ICE CREAM MANUFACTURE

UF-Retentate as a substitute for skim milk improved some ice cream characteristics.

The use of ultrafiltered retentate in ice cream manufacture increased the protein and decreased the lactose content. Thus higher levels of MSNF from ultrafiltered retentate in ice cream formulations can be used without the risk of promoting the sandiness problem caused by the growth of lactose crystals.

UF Ice creams were harder and melted more slowly. They had lower overrun, higher extrusion temperature and were more viscous. High viscosity improved the perceived qualities of the frozen product and reduced ice crystal growth during frozen storage, but excessive mix viscosity can reduce heat transfer rates during pasteurization and freezing. Using ultrafiltered retentate in ice cream formulations could possibly reduce or eliminate the need to utilize other viscosity building agents, as in the case of some manufactures who utilize stabilizer systems to impart pseudoplastic or shear thinning rheological properties to the mix. Further studies evaluating the economic aspects of using

different levels of emulsifiers and stabilisers in ice cream formulations using UF-Retentate are recommended.

As mentioned above UF Ice Creams had lower overrun. The overrun differences between UF Ice Creams are attributed to the mix composition mainly the protein content in MSNF. The reduction in air incorporation may reflect a loss of functionality in the proteins of ultrafiltered retentate. Possibly this relates to changes in the casein micelles such as aggregation and surface properties which may in turn relate to the increased calcium levels. This air incorporation reduction in turn, would have negative effects from the economic point of view, since the ice cream makers require products with a good percent of overrun (e.g. 100 %), which will mean in more economic benefits at the end.

The organoleptic characteristics of UF-Ice creams in some cases were improved compared with the control made using skim milk powder. In a consumer preference study, both products were similar, the control having better preference the first half hour, but after that UF-Ice cream was better than the control. The slow melting characteristic of UF-Ice creams are attributed to the higher protein content in the mixes, which have water binding effects. Supermarkets are a major outlet for ice cream and this means that there may be some time between purchase and home storage in a freezer. In these circumstances the use of ultrafiltered retentate may be advantageous in resisting damage by abuse. However, in other cases the slow melting could be a disadvantage. In ' fast food' restaurants where food is delivered shortly after ordering it would clearly be unhelpful if the ice cream needed to stand for half an hour before coming to eating consistency. These results, however provide an extra method to controlling ice cream properties within a balanced recipe.

Further work needs to be done to investigate the functionalities of other levels of retentate in ice cream formulations with different levels of stabilizers or with blends of skimmed milk powder and ultrafiltered retentate.

The application of heat or other treatments to ultrafiltered retentates may improve product functionalities.

5.3 CAJETA MANUFACTURE

In the manufacture of cajeta a large quantity of water is removed by evaporation during a certain time period. This process is normally costly since evaporation is carried out by applying steam. This increases production costs in cajeta manufacture. Ultrafiltration process as a means to provide a dairy concentrated product (UF-R) can decrease the time taken by evaporation of water, since ultrafiltered retentate can be concentrated to different degrees. If the process is carried out 'on farm' the transport costs can be reduced and the permeate can be used as animal feed.

Ultrafiltered retentate as a dairy ingredient in cajeta manufacture will produce a low lactose product preventing the formation of large lactose crystals which cause sandiness in the product. As cajeta is subjected to a heat concentration process, lactose is also concentrated. Lactose crystals in cajeta start appearing if the concentration of lactose exceeds its solubility in solution. Once sandiness is present in the product it tends to reduce consumer acceptance as a result of the presence of a grainy texture. In this product an important role of the milk is in the development of colour and flavour and in assisting to hold moisture within the sugary matrix. Ultrafiltered retentate appears to perform well in both aspects.

The overall organoleptic attribute of cajeta is strongly influenced by sandiness. Ultrafiltered retentate in cajeta manufacture is an ingredient which supplies a low amount of lactose, which improved the organoleptic characteristics of cajeta after being in storage for some months in this study.

The work presented gives a base for cajeta manufacture although individual manufacturers would need to undertake some product development to achieve their individual product requirements. In particular some attention would need to be given to establishing the levels of volume reduction

5.4 FUNCTIONALITIES OF ULTRAFILTERED RETENTATE

The results of this programme need to be considered against the background of the functionalities of milk constituents. In this way it is possible to assess the observed changes in the products made with ultrafiltered retentate in terms of the predictions that would be deduced from the changes in balance produced by ultrafiltration. The introduction to the thesis considered the various properties of milk and its constituents in the manufacture of dairy products.

In general terms lactose forms part of the matrix or syrup phase in products and as such changes in the sugar balance within these phases can be expected to affect the mechanical properties of the product, its relative sweetness and the stability of the product. These effects were reflected in the results ; the ice cream manufactured with ultrafiltered retentate was harder and this is probably largely related to the change in sugar balance in the matrix. Overall increasing the sugar levels will decrease the hardness as the ratio of eutectic to

ice at a given temperature will increase. In the same way the improved stability of the UF-cajetas is a reflection of the decreased lactose levels.

Proteins have a wide range of functionalities including gel forming, emulsifying, water binding and foaming. In broad terms the concentration of proteins with little heating effects could be expected to improve these properties in the retentate and emulsifying and water binding properties in ice cream. This benefit is reflected in the improved heat shock performance. However the functionality of the proteins is also affected by mineral balance and the change, in particular to calcium concentration, can also affect emulsification and foaming properties in the retentate, as well as increasing stickiness by additional cross linking between casein micelles.

The use of ultrafiltered retentate in this study offered the possibility of adjusting the mass ratios of different milk constituents without adversely affecting their physicochemical characteristics and the functionalities of the processed products. The relative levels of milk proteins, lactose and minerals in the retentate depended on the extent to which milk was processed by ultrafiltration process and the conditions used.

The general effects of ultrafiltered retentate on the functionalities of products in this study (ice cream and cajeta) were mainly related to the concentration of proteins and the reduction of lactose. However, as a result of this chemical fractionation, other characteristics were enhanced such as hardness, melting resistance and the heat shock stability in ice cream. In cajeta the main advantages were the reduction of the processing time and the reduction of sandiness in the product. However, as a result of lactose reduction in both UF-products, lactose crystallisation was prevented. Additionally, the UF-

products were superior on storage. Microscopy analysis corroborated the absence of lactose crystals normally in UF-cajeta. Ultrafiltered retentate will improve the quality and shelf life of cajeta without the necessity of using enzymatic methods to hydrolyse the lactose.

There are indications in this work that foaming and emulsifying properties may be reduced in some circumstances and clearly some attention may need to be given to the use of ultrafiltered retentate where these properties are required. Hence it might be expected that ultrafiltered retentate will not perform well in aerated desserts, instant whipped cream or in dairy based dry mixes for whipped products. The hypothesis that the reduction in functionality is related to calcium induced protein aggregation suggests that a reduction in available calcium may improve performance.

In other circumstances protein aggregation is an integral part of the product. Yogurt and cheese are examples of products where protein aggregation is a key part of the process and it would be expected that ultrafiltered retentate would be useful in these cases. It is perhaps not surprising that these two products have been widely reported as suitable for the use of ultrafiltered retentate.

In conclusion ultrafiltered retentate is most suitable for cajeta manufacture and can be used in ice cream manufacture with some modifications of the ice cream properties. The work has provided pointers to alternate uses of ultrafiltered retentate, although the value of some of these suggestions still need to be demonstrated.

5.5 MICROSCOPY ANALYSIS

5.5.1 Ice Cream

The thesis presents a novel approach through the application of freeze-substitution for light and electron microscopy of ice cream samples. This has complemented and extended the observations made by cold-stage SEM. This part of the work illustrates the benefits of using a range of microscopy techniques where each technique provides part of the overall picture of the product. Thus, cold stage SEM shows ice-crystals and air cells in some detail but does not allow detailed assessment of the protein within the matrix. TEM shows in more detail the role taken by proteins during manufacture and storage of the products and light microscopy gives a wider view of the ice-crystals and matrix structures, in particular revealing the linking of ice crystals during storage.

The general SEM observations confirm previous observations concerning the dimensions of air and ice-crystals in ice-cream and also show an unusual crystal growth within the air spaces of ice-cream on storage. The regular hexagonal nature of these crystals suggests that they may have formed by deposition of water vapour from the air spaces during temperature fluctuations in the storage of ice-cream. The SEM observations also supported the view that the UF-ice cream was more stable during temperature abuse.

Light and TEM studies also revealed improved stability in UF-ice cream, but additionally revealed that the protein in UF-ice cream had a more compacted structure and this was most likely related to the improved stability. The light microscopy in particular suggested that the view of increased ice crystal size being the simple cause of change on storage may be oversimplistic, and there is

evidence to suggest that during storage ice-crystals are fusing to form a network of interconnected crystals through the ice cream mass. This implies that where changes in coarseness have previously been linked to individual ice crystal size a more complete view may be obtained by examining the size of clusters and the extent of cross crystal bonding.

5.5.2 Cajeta

Polarised light microscopy clearly showed lactose crystal growth and this related well to increased granularity on storage. Not unexpectedly lactose crystal growth was effectively prevented by the use of ultrafiltered retentate in cajeta. In the conventional cajeta some interesting observations were made in connection with storage at 4° C and 30° C. The consistency of cajeta is such that crystallisation will not be delayed to any great extent by the viscosity of the matrix (as might be the case in high sugar boilings or in milk powders) and consequently lactose crystallisation is inevitable over a relatively short timescale. That crystallisation occurred more rapidly at 4° C confirms the view that lactose insolubility is the rate determining process, that the 30° C stored sample also produced large crystals is consistent with a lower driving force for crystallisation.

It is curious however that 30° C storage produced a second type of crystal. The majority of crystals at both temperatures of storage were of the characteristic truncated "tomahawk" shape associated with α-lactose monohydrate. At 30° C and 30 days storage a spherulite type crystal form was also seen which may imply that the change in composition of the matrix as the lactose is removed by crystallisation has produced conditions where a higher hydrate of lactose or possibly some β-lactose has crystallised. In technological

terms this is of little immediate significance but it indicates the complexity of crystallisation phenomena in complex mixtures and illustrates how fairly small changes in composition can affect the behaviour of these systems. Lactose removal is a key role for ultrafiltration in terms of controlling the functionality of products this work has shown that in addition to the anticipated changes that the complexity of mixed systems retain some mysteries.

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APPENDICES

Appendix A1 Analysis of carbohydrates by High Performance Liquid Chromatography

Introduction

The analysis and characterisation of food carbohydrates have always been important to food science. Carbohydrates have been analysed by different methods such as enzymatic methods and polarimetric techniques, but they are slow and time consuming. Rapid analysis of carbohydrates can be achieved by HPLC techniques.

In HPLC analysis all the components of a sample mixture will have characteristic retention times within the column of the instrument. Solvent from a external reservoir is pumped at high pressures to an injector, which introduces the sample into the solvent stream. The solvent and the sample enter the column, where separation of the components of the sample takes place. The resolved components are detected by a mass detector ACS 750/14 by gravity, fed to a basic integrator and a computer software windows based (Chrom Perfect, supplied by Justice Innovations, Inc. U.S.A.). A known amount of internal standard (Lindsay, 1992) is used (Xylose) as a reference for the chromatogram response factors for each component of interest.

Skim milk and Ice cream

The skim milk was extracted as described in section 2.4.1.6 of materials and methods chapter using xylose at 1% as an internal standard, and acetonitrile/water (50:50 v/v). A response factor of 0.624 obtained from a calibration standard was used to calculate the concentration of the component in the following formula (Lindsay, 1992).

$$C_u = A_u \times r \times \frac{C_s}{A_s}$$

Where:

C_u = Concentration of the component

A_u = Peak area

C_s = Concentration of internal standard

A_s = Peak area of internal standard

r = Response factor

A value of 4.95% for lactose was obtained (See Figure A1.1). However HPLC analysis of duplicated samples of UF-Retentate and UF-Permeate gave values of 0.65% (± 13%) and 2.07% (±0.34%) respectively which were much lower than expected and insufficiently accurate (See Figure A1.4 and A1.5). Some samples of milk were spiked with lactose at 4.17% and 8.33% (See Figures A1.2 and A1.3) to check the repeatability of the skim milk results. Further HPLC analyses of ice cream still using acetonitrile/water extraction gave lactose levels much lower than expected and of wide variability.

A number of the earlier analyses gave chromatograms with unexpected peaks for unknown components (See Figure A1.6) , despite attempts to clear the extract with a Whatman nitrocellulose membrane filters with a pore size of 0.45 μm and 47 mm of diameter.

It was decided to use trichloroacetic acid solution (20%) to precipitate any interfering substances such as protein. However the HPLC analyses were still unreliable. Polarimetric analysis for lactose gave reasonable results for skim milk (4.72%) and UF permeate (4.82%) but a low figure for lactose in the UF retentate (1.6%). It was decided to use results previously obtained for lactose in skim milk, UF-retentate and UF-permeate and ice cream by an enzymatic method (See section 2.4.2.4 of Materials and Methods). Lactose levels were also calculated by difference from the analysis of ash, protein, fat and total solids in skim milk , UF-retentate, UF-permeate the ice cream mixes. The results for lactose analysis are summarised in Table A1.1.

Cajeta

No reference to the analysis of carbohydrates in cajeta was found in the literature. Preliminary analysis showed that it was difficult to precipitate the proteins in the cajeta, even when the proportion of acetonitrile in water was varied from 0 to 100% of the extracting solution.

No satisfactory analysis of the constituent carbohydrates could be obtained with acetonitrile/water extraction.

Another method for analysis of carbohydrates in cajeta was tried where proteins and fat were removed from the solution by using TCA (20%) for precipitation and NaOH (20%) as a neutralising agent.

The best result for carbohydrate analysis gave a sucrose level of 28.6%

(± 0.45%) against a formula value of 34.9% by mass balance of the ingredients in UF-cajeta (See Figure A1.7). This assumed no chemical change of the sucrose. There was 5.21% lactose by mass balance in the UF-cajeta and the HPLC analysis gave a value of 2.86% (±0.23%) in the final product. Some of the lactose may have changed to glucose and galactose and there also may have been some lactose used in Maillard Browning reactions. Lactulose may have also been formed. Berger and Boekel (1994) found that on heating milk to between 110° C and 150° C for some 20 min, there are two path ways of lactose degradation: the Lobry de Bruyn-Alberda van Ekenstein (LA) transformation and the Maillard reaction. The LA transformation gives lactose isomerisation into lactulose with subsequent degradation into galactose, formic acid and C5/C6 compounds. The Maillard reaction in which lactose interacts with protein bounding lysine residues to form protein-bound lactulosyllysine.

Carbohydrate in cajeta recovery by HPLC analysis ranged from 33% to 82% .

Key aspects requiring further investigation are the removal of interfering substances during the extraction process, the selection of standards, the type of chromatographic column and the operational conditions for the column

TABLE A1.1 LACTOSE VALUES IN ICE CREAM TRIAL USING DIFFERENT TECHNIQUES

SAMPLE	POLARIMETRIC [4] (%)	ENZYMATIC [5] (%)	HPLC (%)	DIFFERENCE * (%)
SKIM MILK	4.72	4.72	4.95	4.77
UF-RETENTATE	1.60	4.82	0.65	4.90
UF-PERMEATE	4.82	4.58	2.07	4.67
UF-MIX1[1]		3.29	0.86	3.94
UF-MIX2[2]		2.91	0.74	3.19
CONTROL[3]		5.06	1.60	5.68

* Values obtained by difference of chemical components

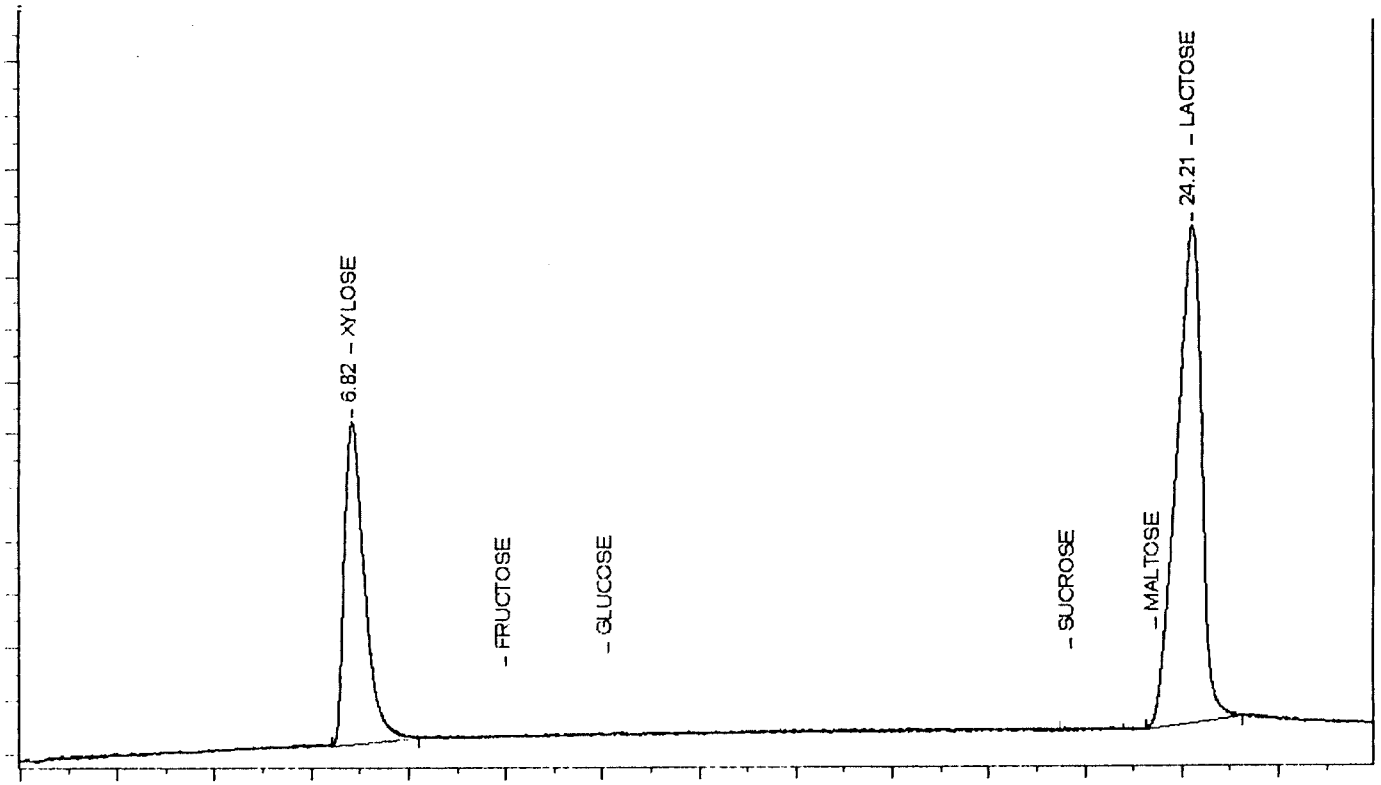
1 Ultrafiltered ice cream mix 1

2 Ultrafiltered ice cream mix 2

3 Control ice cream

4 Biggs and Szaijarto (1963)

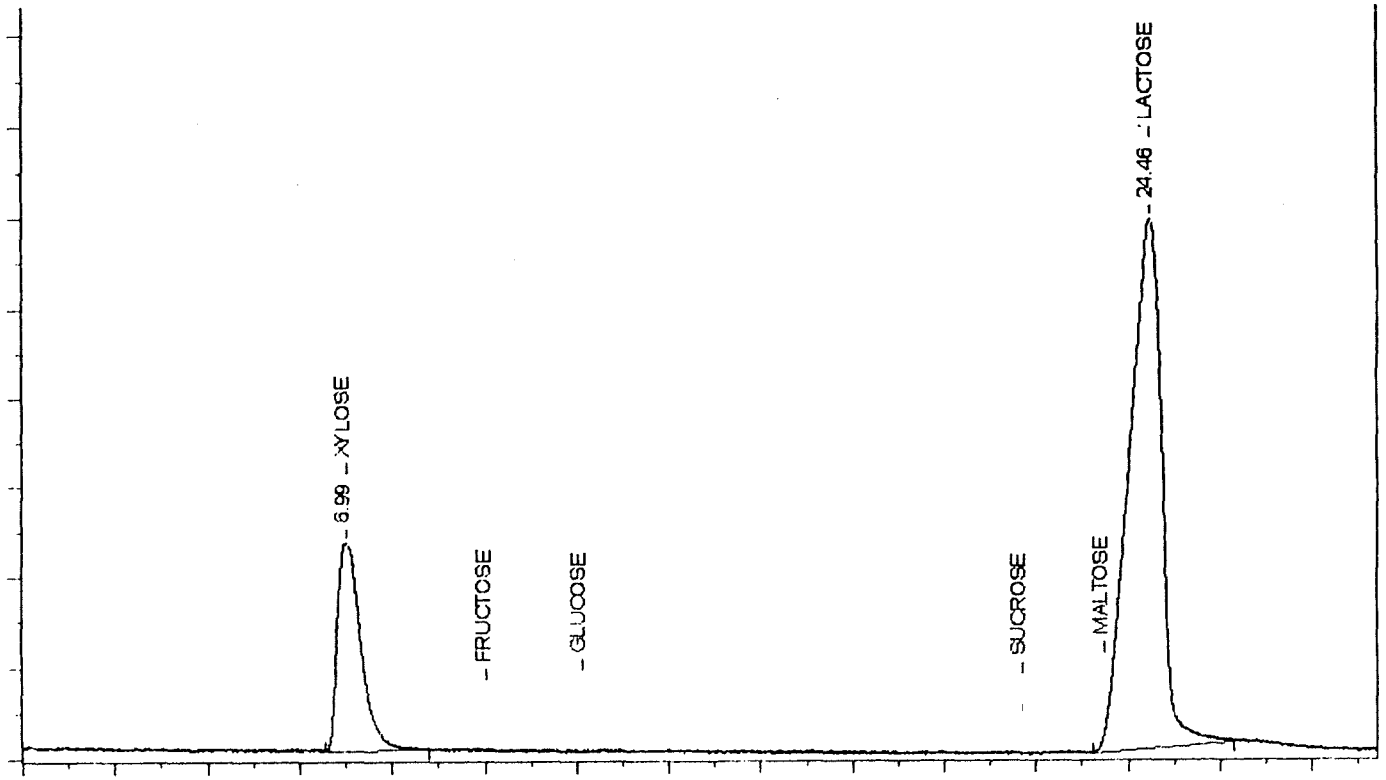
5 IDF 79B:1991



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	6.822	XYLOSE	1.0000	20.201	86768.7	51.223	BB	0.477	3029.70	64.692
2	24.210	LACTOSE	4.9502	100.000	169394.7	100.000	BB	0.603	4683.25	100.000

Total Area = 169394.7, Total Amount = 4.95, Total Height = 4683.25

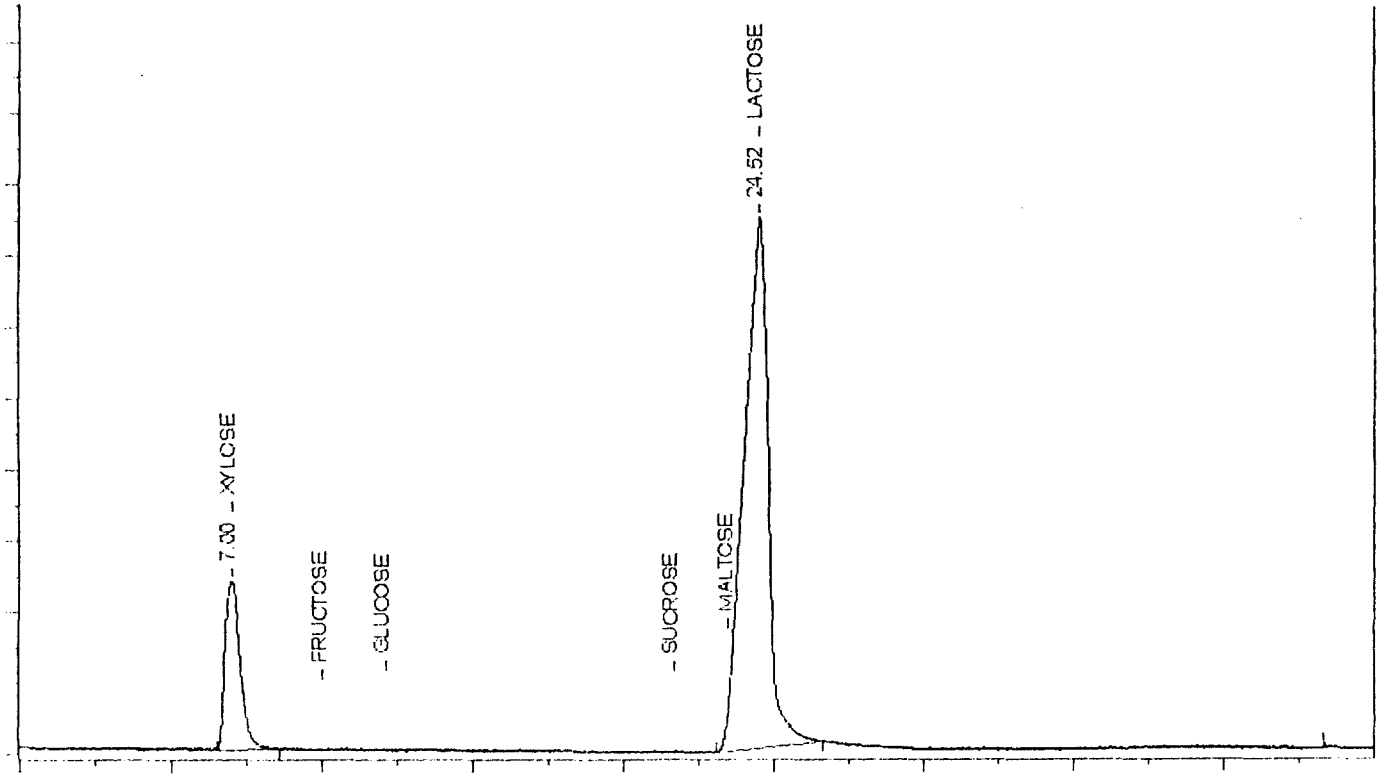
Figure A1.1 Chromatogram of skim milk (Response Factor 0.624)



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	6.987	XYLOSE	1.0000	11.099	81808.4	28.142	BB	0.593	2300.66	38.948
2	24.464	LACTOSE	9.0100	100.000	290695.4	100.000	BB	0.820	5906.96	100.000

Total Area = 290695.4, Total Amount = 9.01, Total Height = 5906.96

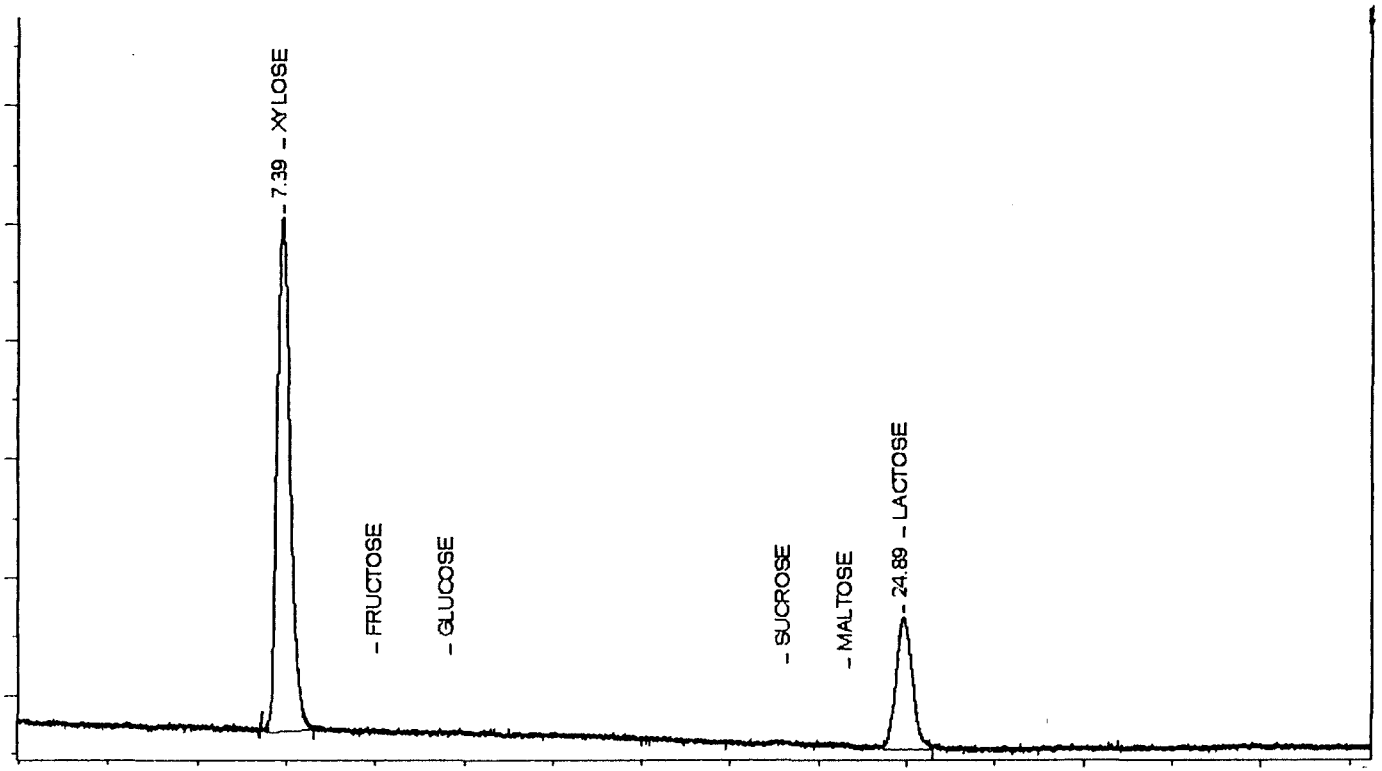
Figure A1.2 Chromatogram of skim milk spiked with 4.17% lactose (Response Factor 0.624)



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	7.000	XYLOSE	1.0000	7.845	82342.4	19.892	BB	0.578	2388.19	31.950
2	24.517	LACTOSE	12.7472	100.000	416469.3	100.000	BB	0.929	7474.67	100.000

Total Area = 416469.3, Total Amount = 12.747, Total Height = 7474.67

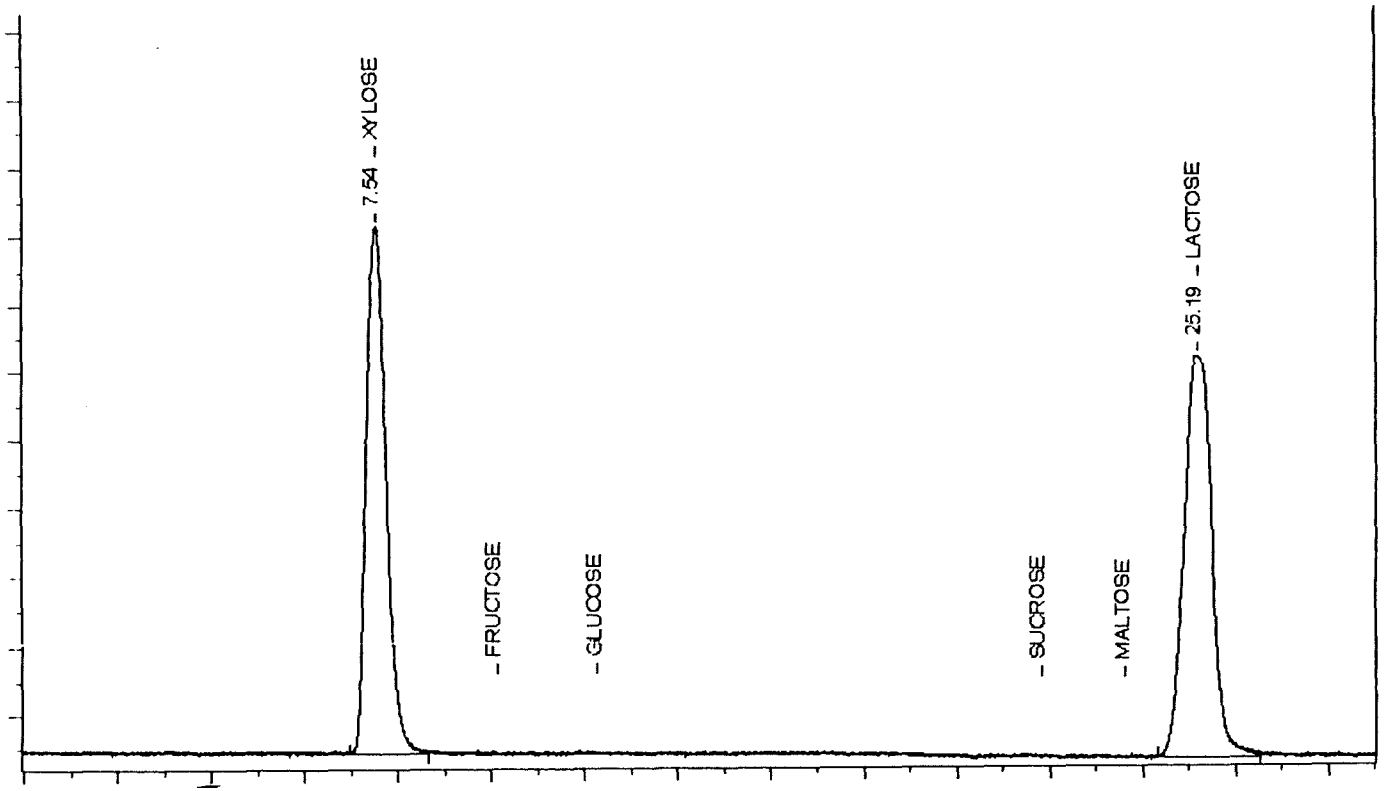
Figure A1.3 Chromatogram of skim milk spiked with 8.33% lactose (Response Factor 0.624)



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	7.389	XYLOSE	2.0000	17.816	56453.6	314.165	BB	0.432	2180.08	388.110
2	24.893	LACTOSE	11.2258	100.000	17969.4	100.000	BB	0.533	561.72	100.000

Total Area = 17969.4, Total Amount = 11.226, Total Height = 561.72

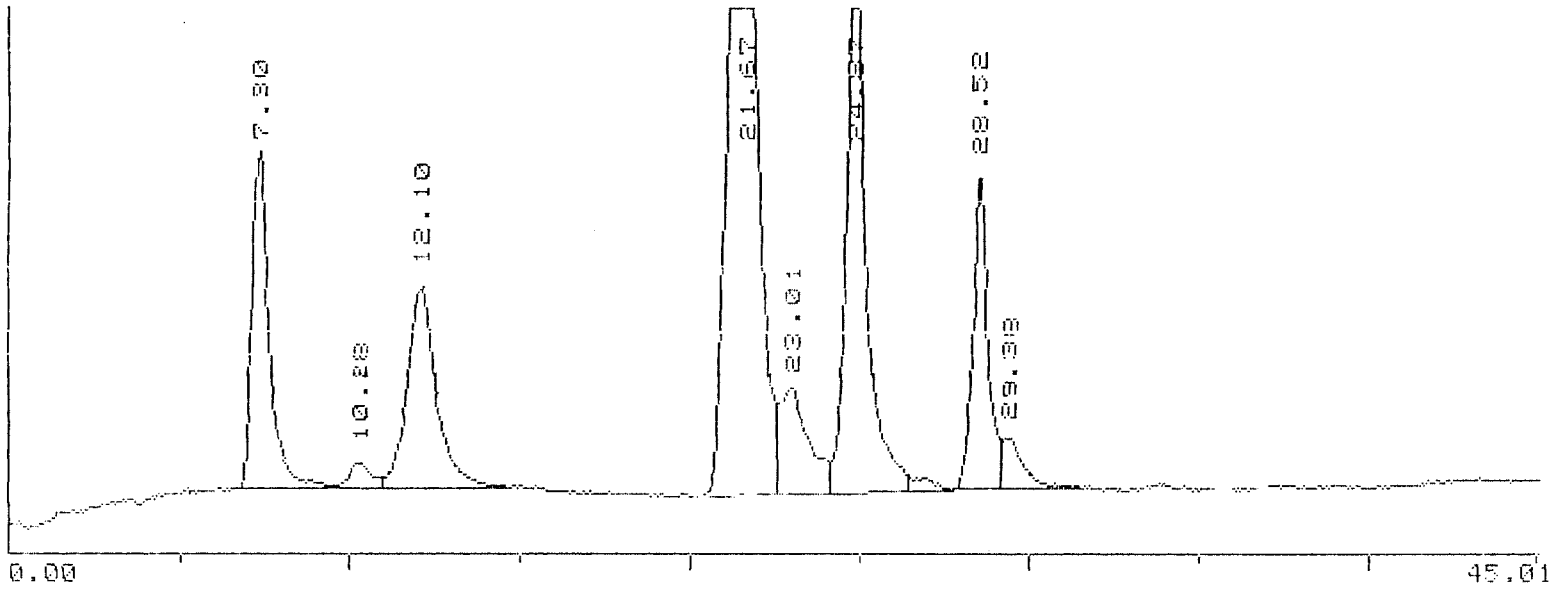
Figure A1.4 Chromatogram of ultrafiltered retentate (Response Factor 0.390)



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	7.543	XYLOSE	2.0000	5.228	112676.3	92.195	BB	0.486	3865.19	130.811
2	25.187	LACTOSE	38.2532	100.000	122214.9	100.000	BB	0.689	2954.80	100.000

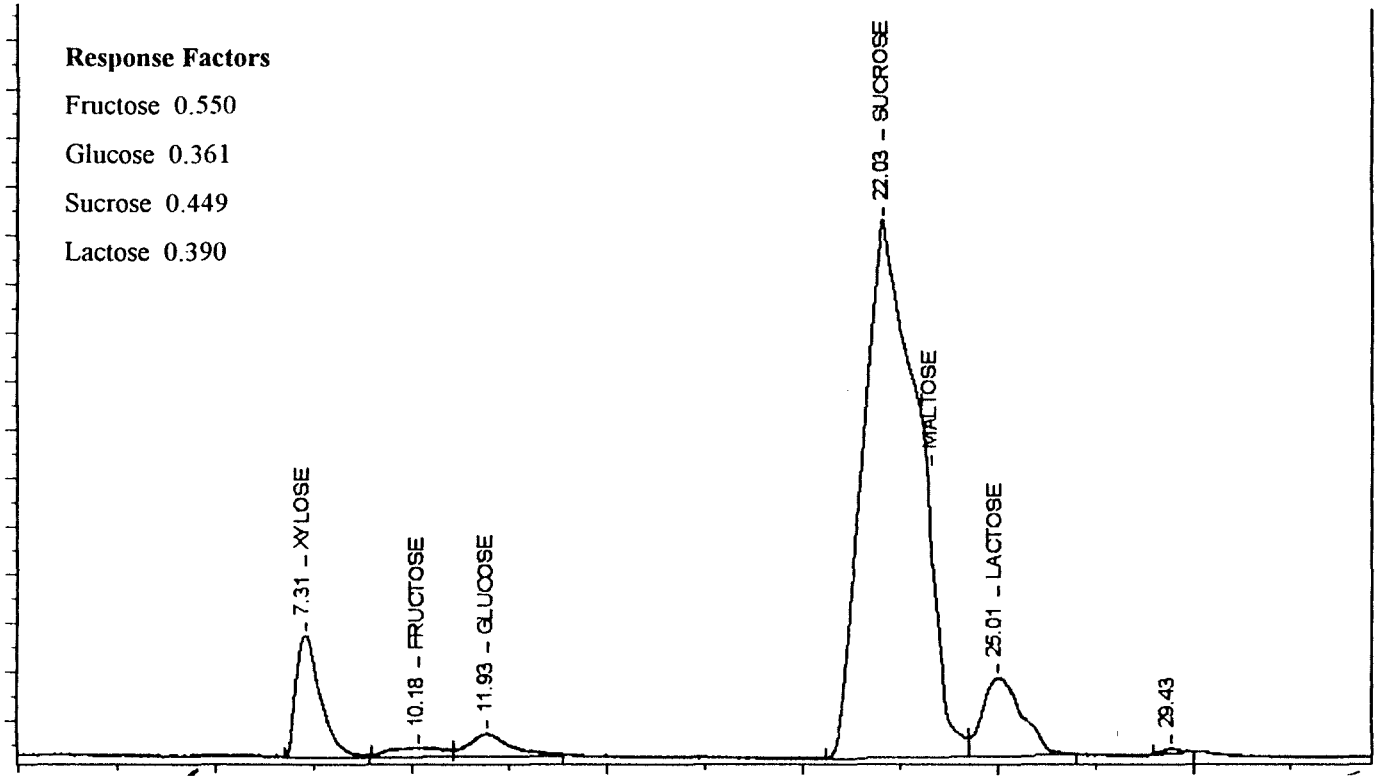
Total Area = 122214.9, Total Amount = 38.253, Total Height = 2954.8

Figure A1.5 Chromatogram of ultrafiltered permeate (Response Factor 0.390)



NAME		RT	AREA	BC	RF
XYLOSE	INTERNAL STD	7.3	88405	01	
2	0.443	10.28	8179	00	0.389
GLUCOSE	4.864	12.1	88892	00	0.393
SUCROSE	21.727	21.67	464616	03	0.336
5	2.496	23.01	46068	01	0.389
LACTOSE	9.353	24.87	172621	00	0.389
7	4.059	28.52	74908	00	0.389
8	0.895	29.38	16515	00	0.389

Figure A1.6 Chromatogram of ice cream mix



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
1	7.312	XYLOSE	2.0000	0.274	106218.6	8.470	BV	0.713	2482.13	18.526
2	10.181	FRUCTOSE	11.4099	1.560	16527.8	1.318	VV	1.522	180.95	1.351
3	11.928	GLUCOSE	14.5894	1.995	32518.8	2.593	VB	1.181	458.83	3.425
4	22.029	SUCROSE	651.5895	89.110	1087987.0	86.758	BV	1.643	11035.08	82.364
5	25.008	LACTOSE	53.6274	7.334	113630.0	9.061	VB	1.177	1608.98	12.009
6	29.429		0.0000	0.000	3379.6	0.269	BB	0.493	114.16	0.852

Figure A1.7 Chromatogram of cajeta

Appendix A 2 FLUX RATE OF UF-ROMICON MEMBRANE USING WATER AT DIFFERENT TEMPERATURES *

TEMPERATURE (° C)	PRESSURE[1] (kPa)	FLOW RATE (l/h)	FLOW RATE (l/ m^2/h)
50	120	900.00	692.30
40	120	800.00	615.38
30	120	654.54	503.49
20	120	514.28	395.60

[1] Gauge

* Membrane of 50 000 Daltons of Nominal Molecular Weight Cutoff.

Appendix A3 FLUX RATES OF SKIM MILK IN THE ULTRAFILTRATION PROCESS AT 50o C *

TIME (mins)	FIRST PROCESS (ml/min)	SECOND PROCESS (ml/min)	AVERAGE (ml/min)
0	960	920	940
10	900	880	890
20	800	720	760
30	600	620	610
40	460	400	430
Average ml/min	744	708	726

* Two processes were carried out to get the final volume required using a membrane of 50 000 Daltons of Nominal molecular weight cutoff and inlet and outlet pressures of 0.15 and 0.12 mPa (gauge) respectively

Appendix A4 MEANS FOR UF-ICE CREAM-1, UF-ICE CREAM2 AND CONTROL AFTER ONE, FOUR AND TWELVE WEEKS OF STORAGE ON SENSORY EVALUATION

ICINESS	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	9.5 a	17.1 b	11.4 a	5.62
	4	14.6 a	16.0 b	20.2 c	1.38
	12	16.9 a	17.5 a	17.5 a	5.93

SANDINESS	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	9.3 a	10.5 a	10.1 a	2.51
	4	9.5 a	11.6 b	17.6 c	0.84
	12	8.8 a	10.7 b	10.4 b	3.33

GUMMINESS	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	22.3 a	25.9 a	17.3 b	1.17
	4	39.1 a	30.1 b	13.5 c	3.78
	12	52.1 a	37.9 b	35.9 b	7.26

WATERY	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	38.1 a	41.8 a	39.5 a	7.32
	4	37.5 a	55.2 b	37.9 a	9.73
	12	34.3 a	49.8 b	40.0 a	7.50

FLUFFINESS	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	21.0 a	18.8 a	29.9 b	5.42
	4	20.5 a	22.5 a	34.4 b	7.58
	12	14.8 a	17.8 a	35.5 b	7.44

FLAVOUR[1]	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	60.7 a	57.8 b	83.9 c	2.89
	4	56.8 a	46.2 b	77.1 c	1.12
	12	48.0 a	44.9 a	61.6 b	3.97

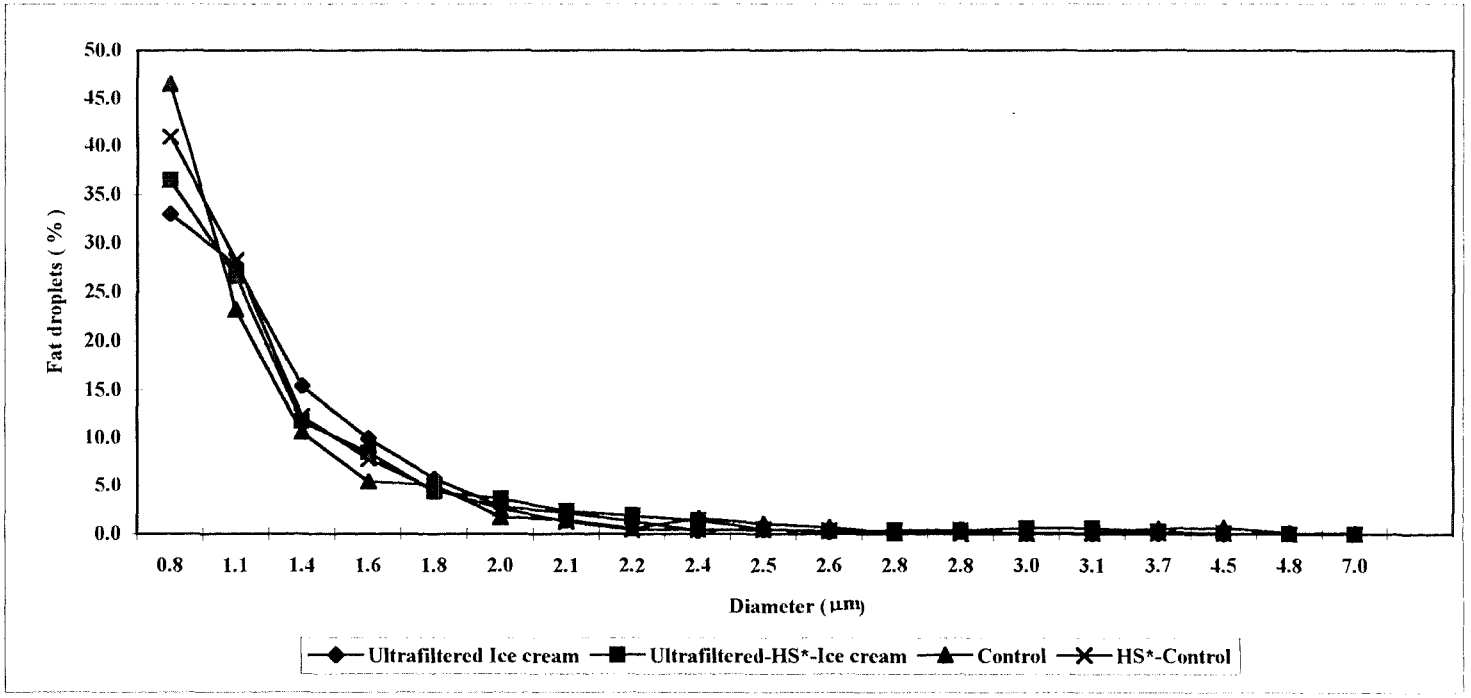
COLOUR	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	64.1 a	61.7 a	59.2 b	3.94
	4	56.7 a	48.4 b	53.2 c	2.48
	12	51.2 a	49.4 a	51.8 a	2.81

ACCEPTABILIT	WEEKS	UF-1	UF-2	CONTROL	SEDiff
	1	44.5 a	54.8 b	47.6 c	1.95
	4	57.7 a	73.6 b	39.3 c	1.52
	12	65.8 a	74.2 a	51.4 b	11.94

a.b.c Means within the same row followed by the same letter are not significantly different ($P<0.05$)

[1] Flavour strength

Appendix A5 DIMENSIONAL CHARACTERISTICS OF FAT DROPLTES IN ICE CREAMS



* Heat shocked ice cream

Appendix A6 FLUX RATE OF UF-ROMICON MEMBRANE USING WATER AT DIFFERENT TEMPERATURES *

TEMPERATURE (° C)	FLUX RATE (l/m².h)
20	381.70
30	495.20
40	600.28
50	678.25

* Membrane of 50,000 Daltons of Nominal Molecular Weight Cutoff with Inlet and outlet of 0.15 and 0.12 mPa (gauge) respectively

Appendix A7 FLUX RATES OF WHOLE MILK IN THE ULTRAFILTRATION PROCESS FOR CAJETA MANUFACTURE AT 50° C *

TIME (mins)	FLUX (ml/min)
0	680
10	610
20	530
30	450
Average/min	567.5

* Membrane of 50,000 Daltons of Nominal Molecular Weight Cutoff with Inlet and Outlet pressures of 0.15 and 0.12 Mpa (gauge) respectively

Appendix A8 ORGANOLEPTIC MEANS SCORES FOR CAJETAS AFTER ONE, THREE AND NINE WEEKS STORAGE AT 4° C

SANDINESS

SESSION	UF-CAJETA	MILK-CAJETA	SEDiff
1 WEEK	1.0 a	0.7 b	0.235
5 WEEKS	1.4 a	5.9 b	0.961
9 WEEKS	0.6 a	9.7 b	0.673

STICKINESS

SESSION	UF-CAJETA	MILK-CAJETA	SEDiff
1 WEEK	6.0 a	3.9 b	0.760
5 WEEKS	6.0 a	5.4 b	0.636
9 WEEKS	5.3 a	6.9 b	0.532

SMOOTHNESS

SESSION	UF-CAJETA	MILK-CAJETA	SEDiff
1 WEEK	11.9 a	12.2 b	0.750
5 WEEKS	11.5 a	8.0 b	0.777
9 WEEKS	8.1 a	8.4 a	0.551

FLAVOUR

SESSION	UF-CAJETA	MILK-CAJETA	SEDiff
1 WEEK	11.8 a	12.2 a	0.508
5 WEEKS	11.1 a	11.1 a	0.787
9 WEEKS	8.9 a	8.0 b	0.645

ACCEPTABILITY

SESSION	UF-CAJETA	MILK-CAJETA	SEDiff
1 WEEK	11.1 a	11.6 a	0.430
5 WEEKS	10.8 a	8.4 b	0.924
9 WEEKS	10.7 a	3.2 b	0.608

a,b Means within the same row followed by the same letter are not significantly different ($P<0.05$)

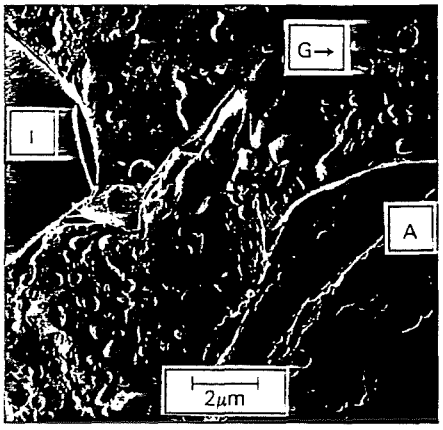
Appendix A9 VISUAL SCORES OF SANDINESS IN CAJETA AFTER 8 MONTHS OF STORAGE AT 4° C

PRODUCT	SANDINESS
UF-CAJETA1	1.25 a
UF-CAJETA2	1.25 a
MILK-CAJETA1	4.00 b
MILK-CAJETA2	2.83 c
SEDiff	0.48

a,b,c Means within the same column followed by the same letter are not significantly different ($P<0.05$)

ARTICLES

Membrane separation processes in ice cream



I = Ice crystal G = Fat Globule A = Air Bubble

Figure 1. Microstructure of ice cream

A study of the advantages of using ultrafiltration in ice cream manufacture, by H Garcia-Nevarez and V N Wade, Scottish Agricultural College

According to Kessler (1), ultrafiltration is used for the separation of large molecules from a liquid using membranes with pore diameters from 2-10nm. In the dairy industry, such molecules are mainly proteins and fats, and ultrafiltration may also be used to concentrate enzymes and micro-organisms.

The concentrated portion, containing mostly protein and fat, is termed the retentate. Other components of smaller molecular size, such as lactose and minerals, pass through the membrane into the permeate. The molecular size of various milk constituents is given by Kessler (Table 1). The process has been used in the dairy industry as a method of milk protein concentration.

Recently, interest has developed in using ultrafiltered retentate (UF-R) as a source of milk solids non fat (MSNF) in ice cream production. This ingredient provides a source of MSNF that has only been subjected to mild heat treatment and also opens the possibility of manipulating the functional properties of ice cream.

Structure of ice cream

Ice cream consists basically of a mixture of milk, fat, sugars, stabilisers, emulsifiers, flavour and colour, see Table 2 (2). It has a complex structure. In essence, air cells, ice crystals, casein micelles and fat droplets are dispersed through a eutectic glass containing sugars and other soluble components. The ice crystals and air bubbles form a coarser dispersion than the fat globules which in turn are coarser than the casein micelles. The serum phase of ice cream surrounds the ice crystals and air bubbles, and results from a freeze-concentration process as water is removed from the solution in the form of ice (Figures 1 and 2) (3,4).

The extent to which casein micelles are dispersed into sub-units greatly influences the emulsion stability; this can be deduced from a consideration of the relative surface areas presented in Table 3 (5). This balance between micelle and sub units will be mainly influenced by the heat treatment of the milk used and the salt balance. The emulsifying agents assist in stabilising the fat/protein interaction at the fat/serum interface and other parts of the ice cream structure.

Table 1. Molecular sizes of milk components (1)

Component	Relative molecular mass (kg/kmol)	Diameter (nm)
Water	18	0.3
Chloride ion	35	0.4
Calcium ion	40	0.4
Lactose	342	0.8
αLactalbumin	14500	3.0
βLactoglobulin	36000	4.0
Blood serum albumin	69000	5.0
Casein micelles	107-109	25-130

Many factors, such as the mix composition, quality of ingredients used in the mix, and processing parameters, are involved in the stabilisation of the ice cream complex.

Physical characteristics have an important effect on the quality of the product. For instance, the ice cream is expected to melt down slowly to give a liquid, which is exactly like the mix from which it was made (6). Flack (7) gave several factors that influence the melting resistance of ice cream such as fat content, MSNF, amount of stabiliser and sugar, as well as processing factors.

For example, the fats used in the ice cream mix are a mixture of trigiycerides, each of which will have a different melting point, and this leads to a melting range rather than a single melting point. Some hard fats may have too high a melting range and impart a waxy and sticky mouthfeel to the ice cream. Alternatively, vegetable oils may cause difficulties in freezing, resulting in an 'oily' taste in the product as well as a weak body to the structure of the ice cream.

The hardness of the product at any given temperature depends on the proportion of water frozen at that temperature. This relates principally to the freezing range of the ice cream mix which is itself governed by the level and nature of soluble solids in the mix. As the freezing range of the mix is reduced, more water will remain unfrozen and the final product will seem softer (8).

By using UF-R in ice cream mixes as a partial or full source of MSNF, it is possible to vary the protein and lactose content in the final product.

It has been reported (9) that the lactose content in ice cream can be reduced by 75% using UF-R. Thus, it is possible that lactose-sensitive people will be able to eat it. In addition, the product will be less likely to exhibit sandiness from lactose crystallisation.

Table 2. Typical ice cream formulation (2)

Component	(%)
Fat	10
MSNF	11
Sugar	13
Stabiliser	0.2
Emulsifier	0.5
Water	65.3

Chemical composition

Ice cream made from skim milk UF-R has a higher protein and lower lactose content than the control using other MSNF sources (10,11,12). These findings suggest that skim milk UF-R can be utilised to make a modified ice cream product.

When whole milk is used, the fat in the ice cream mix may be supplied almost completely by the UF-R, since it can be increased to levels such as

9.5% by the UF process (11).

The high protein content in the UF-R will improve the water binding capacity of the ice cream mix and might reduce the amount of the stabiliser needed (10).

There are a few reports regarding the use of UF-R in ice cream (11,12,13,14). In one case the viscosity of the mix was progressively increased when UF-R was used to replace the MSNF from skim milk powder at levels varying from 25-75% replacement (11). In another case (12) only a very small increase in the viscosity of the ice cream mix was found, even though the protein content was increased from 4.1% in the control to 7.1% in the mix, using ultra-filtered skim milk for MSNF replacement.

Table 3. Calculated dimensions of ice cream components (5)

Component	Average size (µm)	Surface per litre of ice cream (m2) (a)
Fat globules	0.6	560
Air cells	60.0	45
Ice crystals	40.0	20
Casein micelle	0.1	900
Casein sub-units	0.01	9000

(a) Calculated from average size in: 10% fat, 11% MSNF, 15% sugar, 0.6% emulsifier/stabiliser and 100% overrun target

Geilman and Schmidt (10) found that UF-ice cream initially released liquid more slowly on melting than traditional ice cream. On the other hand, Lee and White (11) found that the time to collect the first 10ml of melted liquid decreased as the UF-R replacement level increased from 25-75%. However, they pointed out that the freezing point is a major factor in influencing melting resistance and suggested that the constant sugar level in the mixes was the reason for the low variation between melting characteristics.

Testing hardness

Information about the hardness of ice cream has been reported (10). The control made with SMP was softer than ice cream made from a mix containing ultrafiltered whole milk. Some UF-mixes were also tested where lactose, glucose and fructose were used to replace some of the lactose lost during UF.

This concluded that the type of sugars added to low lactose UF-ice cream affected the hardness of the product, fructose tending to give a harder product. However, the changes in hardness of the UF products could not be explained by the effect of calculated freezing point depression resulting from differences between fructose and glucose. Tong et al (13) also found that ice cream made from UF-R was harder than mixes formulated with condensed milk. It has also been suggested that other interactions, such as those between carbohydrates and protein, influence the hardness of the product (10). The overrun and ice crystal size distribution will also influence the hardness.

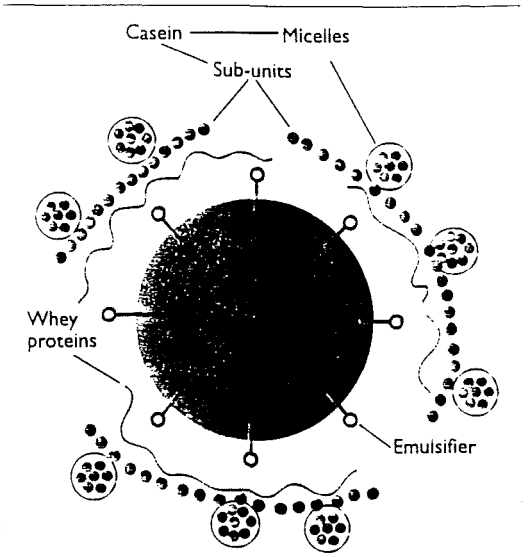
Most investigators seem to conclude that the use of UF-R in ice cream mixes as a source of MSNF results in a product which has enhanced protein content which gives it improved nutritional attributes. The increase in protein content appears to impart improved body and texture to the ice cream.

Some investigators have suggested that the higher protein content improves water binding and that, as a consequence, reduced amounts of stabilisers may be used in the mix. However, this possibility needs to be properly investigated. There also appears to be a lack of investigation into the effect of enhanced protein levels on matters such as flavour releases.

The reduced lactose content could probably assist in the control of sandiness in the final product. There may also be some potential for UF-R ice cream to be marketed in areas where lactose intolerance is a significant problem. However, further investigations would be necessary to judge the acceptability of the product in clinical as well as sensory trials.

Table 4. Functions of some ingredients in ice cream mixes

Component	Function
Protein	Bind water
Fats	Provide smoothness
Carbohydrates	Affect sweetness and freezing temperature
Stabilisers	Bind water
Emulsifiers	Increase fat dispersion



Representation Fat/Protein/Water Interface

Figure 2. Diagram of ice cream structure (7)

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24TH INTERNATIONAL DAIRY CONGRESS

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BRIEF COMMUNICATIONS AND ABSTRACTS OF
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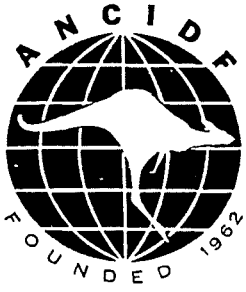
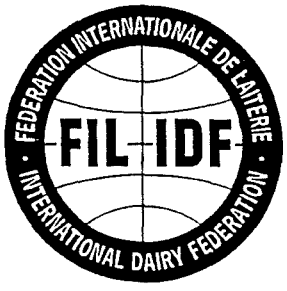
"DAIRYING IN A NEW GLOBAL ENVIRONMENT"





Kb8

Ultrafiltration for Ice Cream Manufacture: Part I (Chemical & Physical Properties)

H. Garcia-Nevarez and V.N. Wade, SAC Auchincruive, Food Science & Technology Dept. Ayr, KA6 5HW, Scotland, U.K.

1. Ultrafiltration (UF) has been used in the Dairy Industry as a means to fractionate and concentrate proteins. However, in the production of ice cream, it can be used to provide UF-Retentate (UF-R) to supply milk solids non fat (MSNF). In this study, the objective was to characterise physically & chemically, products made using UF-R and skim milk powder (SMP).
2. Milk was ultrafiltered to obtain Retentate to be used as a substitute of SMP. Two levels of MSNF were used in two different ice cream formulas and made in duplicate. One similar to the one used for the control (10.92% of MSNF), and another using 13% of MSNF. Samples were analysed in duplicate to determine protein, lactose, ash, total solids, mineral content, and hardness, overrun, extrusion temperature, viscosity and melting properties.
3. Results showed, that products made using UF-R had an increase in protein, ash, calcium, phosphorus and magnesium and a decrease in lactose, potassium and sodium compared against the control using SMP. Likewise, UF-Products were harder, more viscous and took longer to release the first melted drop of ice cream, but had lower overrun and extrusion temperature compared with the control.
4. The use of UF-Retentate as an ingredient to provide MSNF in ice cream manufacture, can lead to the development of products rich in protein and calcium and low in lactose and sodium as well as products with some physical properties enhanced. This type of product may be used for lactose intolerant people, children and elderly people who need protein for growing and calcium to fortify bones, as well as harder products for warmer countries.

Kb9

Ultrafiltration for Ice Cream Manufacture: Part II (Sensory Characteristics)

H. Garcia-Nevarez and V.N. Wade, SAC Auchincruive, Food Science & Technology Dept. Ayr, KA6 5HW, Scotland, U.K.

1. Ultrafiltration process was used to produce ice cream (part I). Products made using Ultrafiltered Retentate had high protein and calcium content and low lactose and sodium content, as well as some enhanced physical properties, such as hardness, viscosity, and melting characteristics. UF-Products may be suitable for lactose intolerant people, children and the elderly.
2. Milk was ultrafiltered to obtain Retentate to be used as a substitute of SMP. Two levels of MSNF were used in two different ice cream formulas and made in duplicate. One similar to the one used for the control (10.92% of MSNF), and another using 13% of MSNF. Sensorial analysis was carried out after one, four and twelve weeks. Presentation order was fixed. A sensory vocabulary, comprising seven attributes, in a 150 mm scale with anchor points. Samples were subjected to a heat shock treatment and the sensory panel evaluated them for the three characteristics: iciness, flavour and acceptability. The scores from both the sensory and the heat shock experiments were modelled by a mixed model using the Residual Maximum Likelihood (REML) technique in Genstat.
3. Results showed that, products were affected in their sensory characteristics by the source of MSNF used in the formulations, UF-Products had better scores in most of the cases, but for some characteristics the control was slightly better, such as gumminess and overall acceptability. Heat shock results, showed that, UF-Products had better resistance to the changes in temperature against the control using SMP.
4. The use of Ultrafiltration process in ice cream manufacture had a favourable effect in the sensorial characteristics of the products, and also, can improve the products resistance to improper handling by the consumers.