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THE NATIONAL UNIVERSITY OF IRELAND
UNIVERSITY COLLEGE CORK

DEPARTMENT OF FOOD TECHNOLOGY

THE FORMULATION, TESTING AND STABILITY OF 16% FAT CREAM LIQUEURS

THESIS

presented by

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for the DEGREE of DOCTOR OF PHILOSOPHY

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ABSTRACT

Cream liqueurs manufactured by a one-step process, where alcohol was added before homogenisation, were more stable than those processed by a two -step process which involved addition of alcohol after homogenisation. Using the one-step process, it was possible to produce creaming-stable liqueurs by using one pass through a homogeniser (27.6 MPa) equipped with "liquid whirl" valves.

Test procedures to characterise cream liqueurs and to predict shelf life were studied in detail. A turbidity test proved simple, rapid and sensitive for characterising particle size and homogenisation efficiency. Prediction of age thickening/gelation in cream liqueurs during incubation at 45 °C depended on the age of the sample when incubated. Samples that gelled at 45 °C may not do so at ambient temperature. Commercial cream liqueurs were similar in gross chemical composition, and unlike experimentally produced liqueurs, these did not exhibit either age-gelation at ambient or elevated temperatures.

Solutions of commercial sodium caseinates from different sources varied in their calcium sensitivity. When incorporated into cream liqueurs, caseinates influenced the rate of viscosity increase, coalescence and, possibly, gelation during incubated storage. Mild heat and alcohol treatment modified the properties of caseinate used to stabilise non-alcoholic emulsions, while the presence of alcohol in emulsions was important in preventing clustering of globules.

The response to added trisodium citrate varied. In many cases, addition of the recommended level (0.18%) did not prevent gelation. Addition of small amounts of NaOH with 0.18 % trisodium citrate before homogenisation was beneficial. The stage at which citrate was added during processing was critical to the degree of viscosity increase (as opposed to gelation) in the product during 45 °C incubation.

The component responsible for age-gelation was present in the milk-solids non fat portion of the cream and variations in the creams used were important in the age-gelation phenomenon Results indicated that , in addition to possibly Ca^{++}, the micellar casein portion of serum may play a role in gelation.

The role of the low molecular weight surfactants, sodium stearoyl lactylate and monodiglycerides in preventing gelation, was influenced by the presence of trisodium citrate. Clustering of fat globules and age-gelation were inhibited when 0.18 % citrate was included. Inclusion of sodium stearoyl lactylate, but not monodiglycerides, reduced the extent of viscosity increase at 45 °C in citrate containing liqueurs.

Dedicated to

My wife

Catherine

and family

Maura

&

David

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Introduction

Cream liqueurs can be defined as products that contain cream, sugar, alcohol, milk proteins as well as permitted emulsifiers, salts, colours and flavours. The ingredients are combined to produce a unique long life dairy-based liqueur.

While cream liqueurs are manufactured throughout the world, the main international brands are Irish manufactured. A guideline (Confederation of Irish Industry, 1991) , defines the term "Irish Cream Liqueur" as an Irish manufactured product that contains > 12 % fat and 15 % v/v alcohol. If the claim "contains whiskey" is made, at least 10 % of the total alcohol present in the product must be obtained from a whiskey source. However these suggestions have no legal standing.

The original commercial product, "Baileys", was developed jointly by British and Irish scientists and was first marketed in 1975. Since that time, cream liqueurs have experienced phenomenal growth in sales. Current annual production of the top three Irish cream liqueurs are approximately ; ca 38, 000 tonnes of Baileys and 5, 5 00m tonnes each of Carolans and Emmetts cream liqueurs. Emmetts and Baileys are owned by Gilbeys of Ireland which is part of the Grand Metropolitan Group. Carolans is owned by C&C Ireland Ltd., who in turn are controlled by Guinness and Allied Lyons. These brands are at the luxury end of market since they contain a relatively high content of butterfat i.e. 15-16 %. They are normally flavoured with chocolate/coffee/cream type additives and are coloured with caramel and/or annatto.

Research publications on the above type products, which first appeared in 1981, have been largely the result of the efforts of two groups of researchers, Banks and co-workers in the Hannah Institute in Scotland and Dickinson and colleagues in Leeds University. However, relatively little original research has been reported since 1989.

Note: there are many other categories of "cream" liqueur on the market e.g. low fat (4-8 %), medium fat (ca 12 %) , "yoghurt liqueurs" etc. A liqueur based on whole milk powder, O'Darbys, was manufactured until recently in this country. These products are not considered in this project.

General objectives of the study

While there are many reports on cream liqueur systems in the literature, many of these are vague on such potentially important aspects as : manufacturing and testing procedures, ingredient variation and description of defects. In addition, there are no reports on cream liqueur manufacture emanating from Ireland.

It was decided to undertake a study to generate more detailed practical information in this economically important area. The general objectives were to

- produce information on formulation techniques using pilot-plant equipment
- develop simple and inexpensive tests to characterise -average particle size, chemical composition and shelf-life stability
- study the characteristics of commercially available cream liqueurs
- to explore the effects of variations and/or interactions in the raw ingredients, in particular- sodium caseinates, surfactants, trisodium citrate, cream and alcohol.

Chapter 1

LITERATURE REVIEW

1.1 Food emulsions

In this section some basic concepts and nomenclature in the area of food emulsion technology are summarised. We will concentrate on properties of the fluid oil-in-water (o/w) systems, since these are of most relevance to the study of cream liqueur emulsions.

Note: Food emulsion science, including developments in the area, has been extensively reviewed in articles by Dickinson & Stainsby (1987), Parker (1987), Darling & Birkett (1987), Kamel (1991) and Narsimhan (1992). A large number of books have also been devoted to the subject of food colloids/emulsions, including recent texts edited by Larsson & Friberg (1990), Dickinson (1991), El-Nokaly and Cornell (1991) and Dickinson & Walstra (1993). In addition, both specialist journals (e.g. *Journal of Colloid Interface Science, Journal of Dispersion Science and Technology*) and food related publications (e.g. *Journal of Food Science, Journal of Dairy Science*) carry articles of interest to those working in the area.

COLLOIDS AND EMULSIONS

The term *colloid* refers to matter where one of the dimensions is between 1nm and 1 mm in diameter. The colloidal matter or internal phase, which has a large specific surface area, is dispersed in a given medium called the external phase. While many various types of basic colloids systems exist, for example foams, aerosols, suspensions and emulsions (see below) , many real foods are complex colloidal (multiphase) systems; e.g. ice-cream is a foam, emulsion and suspension.

An *emulsion* is a colloidal dispersion of one immiscible liquid in another. In food emulsions, the droplet size may vary widely i.e. from 0.1-10 μm. The two phases in emulsions are termed the oil (o) and water (w) phases. The basic character of the emulsion is set by the continuous phase. In oil-in-water (o/w) emulsions, the oil (discontinuous or internal phase) is dispersed in the water (continuous or external phase). Examples of such systems are milk, salad dressing, mayonnaise, cake batter etc. In w/o emulsions, the water (discontinuous phase) is dispersed in the oil (continuous phase; butter and spreads are examples of such systems. The oil phase in

most dairy emulsions is either partially solid (e.g. milk, butter) or solid (ice cream). However, the important detail is that it was liquid during formation. The partial sol-like nature of droplets after formation can have an important influence on such factors as texture and stability.

The fraction of the emulsion, on a volume basis, that is occupied by the dispersed phase is termed the phase volume (ø). The *effective phase volume* may be larger, since this term takes into account the additional volume taken up by emulsifiers attached to the dispersed phase and/or immobilised continuous phase trapped between particles.

The area of contact between the two phases is termed the interfacial area, and the two phases are separated by a third component termed the emulsifier or surface active agent (surfactant).

EMULSIFIERS AND STABILISERS

There is often confusion in the application of the terms emulsifier and stabiliser (Dickinson & Stainsby, 1988; Dickinson, 1989*a*; Das & Kinsella, 1990*b*) In general, *emulsifiers* are substances that enable the production of new interface by the lowering of the interfacial free energy. They are amphiphatic i.e. they contain both "water loving" (hydrophilic) and "water hating" (hydrophobic) areas on the same molecule. *Stabilisers* , on the other hand, are needed when long term stability of an o/w emulsion is required.

Note: The term surfactant, an abbreviation of the term surface active agent, is often used instead of emulsifier, although many researchers restrict this term for meaning synthetic low molecular emulsifiers (see later). In this review, the terms emulsifier and surfactant are used interchangeably.

Emulsifiers in the food industry fall into two main classes; macromolecules or polymeric surfactants (e.g. proteins, gums) and low molecular weight surfactants (usually lipid in nature). The latter group may also be divided into natural (e.g. phospholipids) and synthetic emulsifiers (e.g. mono-diglycerides). The physico-chemical properties of these substances dictates to a large extent the nature of o/w emulsions. In contrast, the properties of w/o emulsions e.g. crystallisation behaviour

in margarine, spreads etc. are dependent more on fat functionality (Dickinson & Stainsby, 1987).

The following section details the general characteristics of the different classes of emulsifiers. Useful reviews in the area of food emulsifiers are those of Krog (1990), Pomeranz (1991), Kamel (1991) and Das & Kinsella (1990*b*).

Proteins

Proteins are the principal stabilisers in many food products, especially o/w fluid emulsions. They function both as emulsifiers and stabilisers by virtue of their amphitatic and macromolecular nature, respectively. Examples include: milk (caseinates, whey protein, whole milk powder), vegetable (soya isolates, pea protein etc), egg (egg yolk, egg albumen) and animal (e.g. plasma, sarcoplasmic and myofibrillar proteins) products. The conformation and hydrophobicity of the protein molecule are two important characteristics in explaining the role of proteins as emulsifiers:

The conformation of the protein (e.g. primary, secondary, tertiary and quaternary structure), which is the result of various covalent and non-co valent forces, influences the shape and hence film-forming behaviour of the molecule. An example of covalent forces are the disulphide bonds in β-lactoglobulin and soy proteins. Most forces are non-covalent (e.g electrostatic, van der Waals and hydrogen bonding) in nature and both pH and ionic strength have a large effect on these. For example, the nearer the pH is to the isoelectric point, the higher the probability of greater attraction between opposite charges (electrostatic effect) and this leads to a more compact structure. The van der Waals forces are non specific, short range forces which are operative between closely opposed groups in adjacent polypeptides.

The hydrophobicity ("water hating effect") of a protein is an important property since the interaction of a protein molecule with the oil surface is essentially hydrophobic. A total or average hydrophobicity value can be obtained by summing up or taking the average of the hydrophobicity values of the constituent amino acid residues of the protein. However, it has been shown that "surface or effective hydrophobicity", calculated as the increase in fluorescence intensity upon binding to hydrophobic probes in protein solutions, correlates better with emulsifying activity. This area has recently been considered by Li-Chan & Nakai (1991).

For a general review on the functionality of food proteins (including milk proteins) in food emulsions, the reader is referred to a review by Tornberg *et al.* (1990). Because of their relevance to the present study, further characteristics of the milk proteins are mentioned in another section.

Other macromolecular species

Gum arabic, a natural protein-polysaccharide, is obtained from the stems and branches of the *Acacia* family of trees. There is strong evidence to suggest that the excellent emulsifying properties of gum arabic are related to both the content (0.1-5.4 %) of bound protein/polypeptide (Dickinson & Euston, 1991; Dickinson *et al.* 1988, 1991) and it's remarkable water solubility (50-55%). Its main use in the food industry is as an emulsifier in liquid and spray dried (encapsulated) flavours/colours and as an ingredient in confectionery.

Due to their predominantly hydrophilic character, other native polysaccharides have low interfacial surface activities and are therefore not expected to form adsorbed layers in the presence of proteins or other surfactants. However, Reichman & Garti (1991) manufactured coarse but relatively stable emulsions with guar and locust bean gum and Bergenstahl (1988) claimed that various hydrocolloids exhibited surface activity when added to diluted preformed emulsions. Modified gums, such as methyl and hydroxypropyl celluloses, possess surface activity as does propylene glycol alginate and modified starches (Tan, 1990).

Synthetic emulsifiers

The industrial processing of modern food products often requires the use of synthetic surfactants in order to produce foods with the desired texture, appearance and shelf life (Krog, 1990). These additives have many advantages over natural emulsifiers (cost, superior functionality) and consequently they are frequently used in foods despite their perceived "unnaturalness". A wide variety of emulsifiers are manufactured by either chemical synthesis or chemical modification of natural materials. Most food grade emulsifiers are either esters of edible fatty acids (originating from animal or vegetable sources) or of polyvalent alcohols like glycerol, propylene glycol sorbitol and sucrose. These products may be further modified by making derivatives with ethylene oxide or by esterification with organic acids like acetic acid, diacetyl tartaric acid, succinic acid, citric acid or lactic acid (Krog, 1977). These synthetic surfactants can be classified according to their - functional groups

(e.g. citric, lactic esters etc) , "dissolving" properties in water (e.g. insoluble, milky dispersion, soluble), charge and/or HLB values (Pomeranz, 1991):

In terms of charge, one can classify emulsifiers into four groups: anionic, cationic, amphoteric and non ionic; the term relating to the ion that is primarily responsible for their surface activity upon dissociation of a salt. On the other hand, HLB theory classifies synthetic emulsifiers according to their relative hydrophil icity/hydrophobicity. The idea was developed by Griffin in 1949 (see Griffin, 1979) to compare the efficiency of blends of hydrophilic and hydrophobic non-ionic emulsifiers. A rough approximation of the HLB number may be obtained by the apparent water solubility of the surfactant. A disadvantage of the concept is that the emulsifiers are examined in isolation from the emulsion system (Boyd *et al.* , 1972). Due to the complexity of food systems, it is better to optimise the composition of an emulsifier blend by experiment rather by calculation.

See Appendix 1 for a list and some data on synthetic surfactants. Some details of the two important types used in the cream liqueur industry are given below:

Mono-diglycerides (MDG)

Monodiglycerides are made from edible fats and oils of animal or vegetable origin and the resulting product is a mixture of mono, di and triglycerides. The monoglyceride portion can be concentrated to about 95 % by distillation and high diglyceride containing products can be obtained by glycerolysis. Commercial blends of MDG contain 35-60 % monoglycerides with the balance comprising of di-and triglycerides. The free hydroxyl groups in MDG can be esterified with organic acids such as acetic, lactic, diacetyl tartaric, citric and succinic acids to form more lipophilic or hydrophilic derivatives. For example, the citric acid ester (CMDG), a very hydrophilic anionic emulsifier, is manufactured by esterification of monoglycerides with citric acid (Krog, 1990).

Stearoyl lactylates (SSL, CSL)

These anionic surfactants are produced by esterification of stearic acid with lactic acid in the presence of sodium or calcium hydroxides to yield sodium or calcium stearoyl lactylates (SSL or CSL), respectively. The sodium content of SSL can be approximately 5 %. SSL is water dispersible at neutral pH, but at pHs below 4-5 it's solubility is limited due to the content of 15-20 % free fatty acids. The reported HLB values of SSL vary widely in the literature ; some authors quote a value of ca. 10 (Kamel, 1991) while others quote values as high as 21 (Das & Kinsella, 1990*b*). CSL is

significantly less hydrophilic. Both compounds are used in the baking industry as dough strengtheners and SSL is used as an emulsifier in many food emulsions e.g. coffee whiteners.

Phospholipid type emulsifiers

Lecithin is the best known product in this class, and the term usually denotes the commercial product rather than the phosphatide *phosphatidylcholine.* Most lecithins are produced are solvent-extraction of crude soyabean oil. The resultant product is a complex mixture of phosphatides, triglycerides and minor components (e.g. phytoglycolipids, phytosterols, tocopherols and fatty acids). Modification of the crude lecithin, by either chemical , physical or enzymatic means, can dictate whether the compound will act as an o/w or an w/o emulsifier. Enzymatic hydrolysis of lecithin, by the enzyme phospholipase A, will produce lysolecithins. These products are excellent o/w surfactants and show less calcium sensitivity than the native lecithin. Several types of soya lecithins and modified lecithin are available in either liquid, plastic or free flowing solid form; see Pomeranz (1991) for details.

Other important natural sources of phospholipids are egg yolk (30 % of yolk solids) and the milk fat globular membrane (MFGM) of milk, which contains a high proportion, ca. 33 %, of phospholipid-type material.

EMULSION FORMATION

The formation of emulsions is an energy demanding process which involves the disruption of the dispersed phase into smaller droplets. This results in the creation of a greatly increased interfacial surface area which is then stabilised by the absorption (and often spreading) of surfactants. Emulsification is facilitated by reduction of interfacial tension between water and oil; this allows for the formation of a greatly enlarged interfacial area using a reduced energy input. However, by far the most important factor in determining initial droplet size is the amount and intensity of the energy input (Dickinson & Stainsby, 1987, 1988, Darling & Birkett, 1987).

Equipment used

A wide variety of equipment and mechanical processes is used in the formation of commercial o/w emulsions. All result in the reduction of the particle size, although the degree of reduction and particle size distribution may vary. The main difference in the devices is the energy densities generated; e.g. 10^4 Wm^{-3} (paddle stirrer), 6×10^9 Wm^{-3} (laboratory valve homogeniser) and $10^{11}/10^{12}$ Wm^{-3} (high pressure homogeniser); (Walstra and Oortwijn, 1982 ; Haque and Kinsella, 1989*a,b*).

High speed mixers / turbomixers

These machines operate at very high speeds e.g. 10,000-20,000 r.p.m. Devices such as the "Ultra-Turrax" consist of a stator and rotor which are separated by a small clearance. Shearing is the main mechanism of homogenisation, with some contribution from cavitation and turbulence phenomena (Tornberg & Lundh, 1978; Tornberg *et al.* , 1990). The resultant particle size distribution curves are broad (see later), with an average diameter usually around 5 μm. The incorporation of air into the mixture is a problem and in severe cases this may cause denaturation and loss of solubility of protein. High viscosity fluids can be handled, although extremely viscous samples receive insufficient turbulence. High-speed mixers are widely used in food production and in basic research studies due to their low cost.

Colloid mills

The colloid mill can be considered a modification of the turbomixer except that the gaps (0.2-0.5 μm) are narrower. The emulsification of the liquid is carried out under strong shearing flow in a narrow gap between a high speed rotor and a non-slotted stator. Uniform particle distributions are obtained and particle diameters in the order of 2 μm can result. A significant temperature rise occurs during processing and cooling is often necessary.

High pressure valve homogeniser (HPVH)

The high pressure valve homogeniser (HPVH) is basically a pump that forces liquids through a very narrow gap at high pressures; normally 3- 30 MPa. This process results in the reduction in size of the liquid oil globules. Only a small fraction (< 0.1%) of the energy input by the homogeniser is stored in the emulsion as free energy; the rest is dissipated as heat.

Droplets are disrupted by a combination of intense laminar and turbulent shear flow, turbulence shattering and cavitation (White, 1981; Mulder & Walstra, 1974; Rees,

1968). However, there are conflicting theories as to which process is the most important in the breakdown of large globules into very fine ones. According to the turbulent theory, the droplets are exposed to turbulent flow field, characterised by velocity fluctuations in the HPVH. The turbulent energy can be pictured as being dissipated by minute eddies of different scales and sizes. (e.g. 0.3 μm) and the size of the droplet will be decided by the size of the eddies (Mulder & Walstra, 1974). In accordance with this theory, droplet size decreases with decreasing viscosity of the continuous phase. In addition, pressure drops leads to cavitation resulting in the formation of small vapour bubbles. These bubbles collapse rapidly producing heavy shock waves in the continuous phase. Shear forces created due to the rapid motion (50-200 m/s) through the orifice, could also play a dominant role in particle breakdown i.e. under the influence of shear, the drops in a coarse emulsion spread out in threads. Small droplets may break away from the pointed ends of these threads.

The mean fat globule diameter for homogenised milk can be described by the empirical equation (Goulden & Phipps, 1964; Walstra, 1975)

$$d = (P_O/P)^q$$

where "d " is the average fat globule diameter (see later) ; P is pressure; "q" is the slope of the graph of log (d) versus log (P), while P_O is the pressure corresponding to unit globule diameter .

Smaller droplets were obtained when the same total energy was supplied at high power as compared to multiple passes at lower power, supporting the role for energy density (Walstra, 1975).

Both Walstra (1975) and Stistrup & Andreasen (1966) compared the efficiency of homogenisers with different type valves. The efficiency, as measured by turbidimetric readings (see later), was liquid whirl > plain > conic designs. The care and proper functioning of pressure gauges , ammeters valves , seats, joints and piston packings of the homogeniser is essential for effective operation (White, 1981).

Laboratory valve homogenisers

In many areas of experimental research it is necessary to use small scale homogenisation equipment to produce emulsions containing small quantities of expensive and scarce ingredients. The construction, operation and theory of a laboratory valve homogeniser has been described in detail by Tornberg & Lundh (1978) and subsequent results obtained by Tornberg and co-workers had been

summarised by Tornberg *et al.* (1990). A different type of mini-homogeniser was constructed and described by Dickinson *et al.* (1987*b*). The premix is forced through a spring loaded ball valve at a known pressure differential (usually 300 bar) by a piston that moves at a constant speed. It is reported that 10 ml of emulsion can be recovered from 15 ml of premix and that the particle size distributions are similar to that produced in a APV GAULIN pilot high pressure valve homogeniser. Kieseker & Aiken (1988) used the homogeniser of a Foss Electric Milkotester to produce samples of recombined evaporated milk for heat stability testing. They estimated that the four homogenisation stages were equivalent to a treatment of 14 MPa. The capacity was 0.5 l/hr. Remillard *et al.* (1993) homogenised milk with the in-built ball homogeniser of a Milkoscan 133B. The resulting homogenisation pressure was 5 MPa. The average particle size decreased from 3.04 to 1.2 μm. In this laboratory, Keohane (1994) compared the homogenisation efficiency of the homogeniser in a Foss Milkotester with that of a Rannie LAB pilot plant homogeniser (liquid whirl valves). Using homogenised milk, two passes of the Milkotester homogeniser corresponded to 13.8 MPa on the Rannie. Using a model emulsion (40% m/m fat), the Milkotester's "cream programme" cycle homogenised the sample more efficiently than the "milk programme".

ultrasonics

Smith & Dairiki (1975*a*) used of a "Branson Sonifier Cell Disrupter" for producing emulsions. This instrument processed 50 ml samples in a batch-type operation. Size distribution data indicated that most globules were less than 1 μm after 9-42 sec sonication. This device was later used by Tornberg & Hermansson (1977) where it was compared with the laboratory valve homogeniser and a high speed mixer. Commercial flow-through ultrasonic homogenisers are available, although they do not appear to be widely used in the food industry.

microfluidisation

The application of this relatively new technology to dairy emulsions, including cream liqueurs and milk, has been described by Paquin & Glasson (1989) and Pouliot *et al.* (1991). The homogenisation device is based on a chamber made up of a system of channels; the milk being pumped by a high pressure pump and split into two streams, which are projected against each other at a high speed at an angle of 180 degrees. During this operation, shear forces, turbulence and cavitation combine to reduce fat globule size (Remillard *et al.*, 1993). The pressures involved during processing are very high e.g. 69 MPa. There are no moving parts in the actual emulsification unit. A small scale microfluidisation type unit has been described by Baurgaud *et al.* (1990).

Formation / adsorption at interface

Milk proteins, especially the caseins have a strong tendency to adsorb at the interface. Following adsorption they stabilise the resulting emulsion by forming a mechanical "steric" barrier which protects the droplets from flocculation or coalescence (see later). As mentioned in an earlier section, it is important to distinguish between the role of the protein at the moment of emulsification (emulsifying effect) and the long term stability of the emulsion (stabilising effect). The first role is favoured by a highly disordered, flexible and hydrophobic protein which is able to rapidly reduce the tension at the o/w interface. The second role is favoured by a more ordered globular structure that is able to form a tightly packed viscoelastic structure at the interface (Dickinson, 1989*a*).

In the manufacture of dairy emulsions by HPVH, mass transport of proteins to the newly formed interface takes place mainly by convection and not by diffusion, as is the case in studies at a planar interface. According to Walstra & Oortwijn (1982) this is the reason why large particles (casein micelles) absorb in preference to smaller particles (submicelles, serum proteins) when milk or cream is homogenised. The theory also predicts that smaller droplets tend to preferentially adsorb the larger protein particles.

The physico-chemical mechanisms and theoretical aspects of the absorption of proteins at the air/water or oil/water interface has been reviewed by Dickinson *et al.* (1988*b*), Leman & Kinsella (1989) and Damodaran (1990).

Competitive absorption

When emulsions containing mixtures of surfactants are homogenised, the most surface active component will tend to predominant at the interface. This phenomena is termed "competitive absorption". Even after emulsion formation, milk proteins can be displaced from the emulsion interface by addition of other surfactants, either other proteins, phospholipids or LMWS. In recent years, this area has been extensively studied by Dickinson and coworkers (e.g see reviews by Dickinson *et al.*, 1989*b*, Dickinson *et al.*, 1993 & Dickinson, 1991*a*). The ease and extent of displacement is dependent on such factors as; relative ratios of the competing species, order of exposure to the interface, the age of the existing layer and molecular nature of surfactant

EMULSION CHARACTERISATION

Freshly formed emulsions should be characterised to establish a base-line against which to measure subsequent long term stability (see later). For example, chemical tests e.g. pH, fat, total solids, density etc, confirm proper formulation and enable calculation of phase volume. Rheological characterisation can also be important and some aspects of this area are discussed in a later section. Other parameters which are often measured include; the amount, nature, thickness, charge and appearance of the protein / emulsifier at the interface (e.g. Walstra, 1988; Britten & Giroux, 1991*b*; Robson & Dalgleish,1987*a*; Fang & Dalgleish,1993; Sharma & Dalgleish, 1993, 1994).

In this study, we are most interested in methods for characterising initial particle size, either by actual measurement of particle diameter or by using some empirical index.

Different particle diameters

One of the most important characteristics of an emulsion is it's particle size distribution, since subsequent stability (next section) and other properties, such as homogenisation efficiency and emulsifying characteristics of ingredients, are dependent on this variable. There are many methods that measure particle size and the area has been reviewed by Walstra *et al.* (1969), Dickinson & Stainsby (1988) and Das & Kinsella (1990*b*).

Various average particle diameters are usually calculated (Rawle, 1993; Walstra, 1965, 1968 & Walstra *et al.*, 1969). The most commonly used average diameters are mentioned below.

The *number average droplet diameter (d)* can be defined as;

$$d = \Sigma d_i N_i / \Sigma N_i$$

where d_i is the average diameter of the i class interval and N_i is the number of globules in that class.

The volume mean diameter or number weight mean (d_v) can be defined as

$$d_v = (\Sigma d_i^3 N_i / \Sigma N_i)^{1/3}$$

The volume surface mean diameter (d_{vs} or d_{32}) is considered the arithmetic mean of the surface weighted distribution.

$$d_{32} = \Sigma d_i^3 N_i / \Sigma d_i^2 N_i$$

This average is useful since it can be used in calculations relating to interfacial area (A) of an emulsion;

$$A\ (m^2/ml) = 6\varnothing / d_{32}$$

where $\varnothing$ is the volume fraction of the fat phase.

The d_{43} is the mean of the volumetric distribution or equivalent volume mean.

$$d_{43} = \Sigma d_i^4 N_i / \Sigma d_i^3 N_i$$

This is also useful to emulsion technologists since it gives an indication where the mass of the system lies. This is important in relation to creaming etc

Other diameters can be described from an understanding of volume frequency size distribution graphs e.g. $d_{v,0.5}$ is the volume median diameter i.e the value which divides a distribution in two equal halves and d_{mod} is the modal average diameter i.e the most common value of the frequency distribution.

Methods for the characterisation of initial particle size

Before characterising the size and distribution of droplets it is important to account for the presence of flocculated particles. Flocculation is usually reversed by using detergent solutions e.g. 0.1 % SDS (Pierce & Kinsella, 1978) or by dissociating agents e.g. urea / mercaptoethanol / EDTA (Robin & Paquin, 1991) or 30 mM EDTA/0.5 % Tween 20 (McCrea, 1994). The light microscope is often used to confirm the presence of flocs in samples, although this can be difficult if the particles are very small. However care should be taken since these agents, being surface active, can cause de-emulsification. Many of the methods described below are unable to detect very small globules (less than 0.2 μm). This is not always a disadvantage, since these particles rarely contribute to creaming.

Microscopy

Traditionally, light microscopy was widely used in emulsion studies to directly assess the droplet size distribution. The method is highly empirical although a trained operator can make a good judgement of the size and shape of the dispersion. The limit of resolution is about 0.2 μm, and accurate sizing ,at a magnification of x1000, can only be carried out above ca. > 0.5 μm (Das & Kinsella, 1990*b*). The use of phase contrast optics is preferable, since this accentuates the visualisation of fat globules due to the difference in refractive index between oil and water phase. A minimum count of ca 300 particles is needed to allow statistically significant results (Becher, 1965; Tan, 1990). The microscope can be interfaced with a computer or screen to aid in the counting and sizing process. Using this set-up, Klemaszewski *et al.* (1989) obtained images with a total magnification of up to 5500 fold and an approximate resolution of 0.18 μm.

The droplet sizes of fine emulsions can be studied with the electron microscope using many different preparation techniques (eg. Liboff *et al.* , 1988; Sargent, 1988; Lee & Morr, 1992). With biological samples, the disadvantages include; the difficulty in sample preparation, reproducibility and artefacts. However, the method is used to standardize latex particles, which in turn are used to validate other instruments.

Spectroturbidimetry

Early research soon established that the measurement of the amount light transmitted/ absorbed through a diluted emulsion was a useful method of determining the homogenisation efficiency of dairy emulsions manufactured by high pressure homogenisation. Spectrophotometers, having both a light source and a detector, are

usually used for measurement purposes; hence the term "spectroturbidimetry". Note; the term "turbidity" is often qualitative indicating the "whiteness" of a solution or it may be a quantitative, correlating directly with absorbance.

Ashworth (1951) found that the transmission (as opposed to absorbance, as commonly used by later researchers), of diluted milk or reconstituted milk samples, measured at 515 μm, increased with increasing homogenisation pressure. These results correlated well with a standard gravitational method of measuring creaming. The light scattering of casein micelles was eliminated by addition of 0.01 M ammonium hydroxide. Deackoff & Rees (1957) also measured transmission of homogenised milks, and found that use of longer wavelengths e.g. 1020 μm, gave better reproducibility and greater sensitivity. The method was very sensitive to increased homogenisation pressure, number of passes through the homogeniser and the type and condition of homogenising valve. Many early researchers (Keeney & Josephson, 1958; Keeney, 1962; Govin & Leeder, 1971) used turbidity both as a measure of the "initial stability" of ice-cream mixes (correlating with emulsion fineness) and of fat agglomeration during the freezing process.

Goulden (1958, 1960) and Goulden & Phipps (1960) elaborated on the light transmittance of diluted homogenised milk emulsions. Goulden (1958) established the following equation for monodisperse emulsion of milk globules measured in a 1 cm light path.

$$OD = 0.143\,(x)\,K/d$$

where OD is optical density (absorbance), x is a constant, d is globule diameter, K is the scattering coefficient.

For non absorbing globules (i.e. the actual fat does not absorb visible light), K is dependant on (1) the ratio of globule size to wavelength (2) the refractive index of the suspended globules with respect to that of the suspending medium. For visible light the maximum specific turbidity occurs with droplets of diameter 3-4 μm ; an equal weight of smaller or larger droplets scatters less light (Dickinson & Stainsby, 1988). In summary, provided there is a suitable optical set-up on the measuring

spectrophotometer (Goulden, 1960), turbidity is solely a function of the fat globule size distribution and hence the degree of homogenisation. Goulden & Phipps (1960) showed that results calculated from turbidity for homogenised milks agreed with results (d_{32}) obtained from sizing by the microscope. Also there was a linear correlation if the relationship between log P (pressure) and log absorbance was plotted. Similar results were obtained by Stistrup & Andreasen (1966) with unfrozen ice-cream emulsions. Thus, methods employing a single wavelength provide an exact globule diameter only when applied to an emulsion in which all of the globules are the same size. However, the effects due to size distribution are often small enough to be neglected e.g. homogenised milk and cream (Goulden & Phillips, 1964).

Since it is possible for a single wavelength reading to correspond to different average particle sizes, many researchers have used the ratio of two readings at different wavelengths as an index of particle size. Kaufman & Garti (1981), Frenkel *et al.* (1982) and Arkad *et al.* (1986) used the ratio of the absorbancies at 800 and 400 nm (A800/A400), which was termed the R droplet size index, for evaluation of emulsions. The lower this ratio, the smaller is the particle size. This method can be expanded i.e. by reading turbidity at many wavelengths it is possible to calculate the slope of the log wavelength / log turbidity plot. Again the stronger the wavelength dependence, the smaller the average droplet size (Das & Kinsella, 1989). Holt *et al.* (1975) had previously used the wavelength dependence of turbidity for estimating the average diameter of casein micelles.

Mulder & Walstra (1974) claim that while the optical density at long wavelengths is proportional to the d_{43} for small globules (not d_{32} as claimed by Goulden & Phipps, 1960), it gives no indication of the distribution width/distribution. A more involved spectroturbidimetric method, that estimates particle size distribution in the range 0.2 - 8 μm, as well as the average particle data, has been described by Walstra (1968). The method involves the comparison of experimental turbidity spectra with theoretical spectra constructed with assumed droplet size distribution functions. It is simple to perform, although the theory behind the method is complicated and the calculations involved can be tedious. The method requires the knowledge of the refractive index of oil and water as function of wavelength (300-1200 μm). A modified spectrophotometer must be used i.e. one with a small angle of acceptance of transmitted light. This method was used to calibrate a simple spectrophotometric instrument (the "Emulsion Quality Analyser") which can then be used to calculate the average particle size (d_{43}) of homogenised milk from a single absorbance reading at 900 nm (see Pandolfe and Masucci, 1984).

Coulter counter

The principle of the Coulter Counter is based on the fact that the volume of a (non-conducting) suspended particle can be determined by variations in electrical impedance as it passes through an aperture between two electrodes. For this to take place, an emulsion is diluted in an electrolyte solution, e.g. 0.9% NaCl. A large number of the suspended particles (e.g. 100,000) are counted in 30-90 seconds, giving a high statistical confidence. The analysis can be performed on particles in the size range 0.4 μm - 1200 μm by varying the size of the aperture from 15 - 2000 μm. Readings are independent of material composition, surface texture, refractive index or light interaction effects but the presence of electrolyte may be a problem with some emulsions since flocculation of particles may occur. Walstra & Oortwijn (1969) examined the suitability of this instrument for estimating particle sizes in o/w emulsions , including milks.

Laser diffraction instruments.

Using laser diffraction analysers, (e.g. Coulter LS, Malvern Mastersizer), the shape of the particle size distribution is determined after measurement of laser light scattered by a diluted emulsion at diffraction angles . Up to 100 detectors capture raw data from various angles and these are processed using a suitable calculation model such as the Mie theory of interaction of light with matter (Rawle, 1993.) The instruments are simple to operate (Muir *et al.* , 1991) and the useful range is normally 0.1-900 μm. However, the refractive indices of the suspending medium and particles, together with a factor to account for any absorption of light by the particles, must be known accurately.

Photon correlation spectroscopy

The principle of operation is based on the sensing of the Brownian motion of suspended particles by photon correlation spectroscopy. For particles in liquid suspension this is a random motion caused by their collision with diluent molecules. The rate at which the particle diffuse is inversely proportional to particle size and the fluctuation in the intensity of the "light" scattered by these particles is measured, usually at an angle of 90 degrees. The time scale of these intensity fluctuations is measured and converted to size data. A knowledge of the viscosity and the refractive index of the suspending medium is required. According to Dickinson & Stainsby (1988) the average particle sizes obtained in polydisperse systems is biased toward the small end of the distribution. Commercial laser light scattering instruments have very

high resolutions and ranges e.g. 4-4000 nm. As with the diffraction technique above, a laser beam is used as the source of electromagnetic radiation.

Emulsion rheology

The viscosity of a fluid emulsion is an important characteristic since it influences such aspects as the rate of creaming (next section), the physical shelf-life of the product (see note on thickening/gelation in next section) and the organoleptic properties of the product. According to Griffin (1979), the viscosity of an emulsion is essentially the viscosity of the continuous phase as long as it presents > 50 % of the volume, and this explains the fact that w/o emulsions are considered thicker than o/w emulsions of similar phase volume. In general, emulsions with small particles are more viscous than emulsions that contain large particles and polydisperse emulsions are considered less viscous than monodisperse emulsions, although there is little hard evidence to back up such statements (Pomeranz, 1991).

Viscosity is defined, in physical terms, as the ratio of the shearing stress to the shear rate in a liquid undergoing laminar flow. In simpler terms it is considered the resistance to flow. Two types of behaviour are important i.e. Newtonian flow where the (true) viscosity does not depend on the shear rate and non-Newtonian flow where the opposite is the case. Most emulsions or dispersions that exhibit non-Newtonian behaviour are so-called "shear thinning", since the (apparent) viscosity decreases with increasing shear rate. The presence of a so called "yield stress" in very concentrated emulsions or dispersions can be understood in terms of a three dimensional structure which has sufficient strength to prevent flow when the applied stress is less than certain values.

Provided there are only hydrodynamic interactions between the droplets (no attraction or repulsion forces), the viscosity of an emulsion can be described by semi empirical relationships such as that of Eilier's (Halling, 1981; Walstra & Jenness, 1984; Dickinson & Stainsby, 1982);

$$\eta = \eta_0 \, (1 + (1.25\varnothing / 1-\varnothing/\varnothing_{max}))^2.$$

where η is the viscosity of the emulsions; η_0 is the viscosity of the liquid in which the particles are suspended e.g. water ; $\varnothing$ is the total volume fraction of the dispersed particles, which according to Walstra & Jenness (1984) should contain values not only for the fat but also protein particles (e.g. casein micelles) and sugars, and $\varnothing_{max}$ is the

hypothetical maximum phase volume for a population of fat globules if packed together whilst keeping the same size distribution. For most dairy emulsions this is normally ca. 0.9.

The above equation predicts the viscosity of skim milk, cream, concentrated milks and milk ultrafiltrate for total ∅ up to 0.6 (Walstra & Jenness, 1984). However, beyond this point emulsions often show viscosities higher than that predicted by the Eilier or other semi-empirical equations. This non-Newtonian behaviour is primarily caused by particle interactions which can lead to aggregation. Other descriptions are needed to distinguish between the contribution of volume fraction and particle interaction to viscosity or changes in viscosity (Jeurnink & De Kruif, 1993). Aggregates behave as if they have a volume greater than the sum of the volumes of the individual globules from which they are constituted, since they contain trapped liquid and contain irregular shapes. Increasing the rate of shear breaks up the aggregates which results in a lower apparent viscosity.

EMULSION STABILITY; DEFECTS AND TESTING.

The primary mechanisms involved in emulsion stability are creaming, flocculation and coalescence. Other defects can be described from these phenomena e.g. Darling (1982) describes aggregation as a collective term for coalescence and flocculation and gelation of commercial fluid dairy emulsions can be a consequence of massive aggregation of protein-covered fat globules. All of the processes described below may occur singly or in combination, as described by Mulder & Walstra (1974).

A wide variety of methods are documented in the literature for the assessment of stability in fluid systems and these have been reviewed by Dickinson & Stainsby (1982), Darling (1982), Leman & Kinsella (1989). The reports mainly concern academic work and while the definitions of stability can vary, most measure the amount of change occurring in a system. In the case of commercial stability, the primary consideration is that these changes should be reversible and not interfere with consumer perception of the product.

Before discussing the principal types of instabilities and their measurement, one should mention theoretical considerations of the forces that operate between droplets. The nature of the interparticle forces between droplets affects the stability; in particular this stability depends on the balance between the attractive intramolecular forces between molecules in the film and the repulsive forces on the outer film surface (Leman and Kinsella, 1989 ; Parker, 1987). These forces may be electrostatic, steric and hydrative in nature. All the interaction forces mentioned above are influenced by the extent of adsorption of proteins and emulsifier, pH, ionic strength etc. (Narsimhan, 1992). Although helpful in the understanding of instability, they cannot predict how long an emulsion will remain stable. Walstra (1993) warns on the dangers of applying classical colloid theory to food systems ; most food particles are not identical, homogeneous hard spheres. Practical assessment of emulsion stability, through the use of empirical type tests, is essential in the development of any individual product.

Creaming

Creaming refers to the gravitational separation of emulsified droplets. This results in the formation of layers with oil volume fractions (Ø) values higher than that of the original emulsion. In practice, this creamed layer is usually visible to the eye e.g. creaming in unhomogenised milk. If creaming occurs in an emulsion, the bottom portions of the sample are lower in dispersed phase and consequently a watery serum

(serum separation) may be visible. Serum separation / creaming is a potential problem in aqueous systems containing droplets of diameter > 0.5 μm (Dickinson & Stainsby, 1988).

In theory, the settling speed (in ms^{-1}) of an isolated sphere of diameter d, undergoing creaming, is given by Stokes equation :

$$v = 2 g \Delta d r^2 / 9\eta$$

where v is the linear velocity, g is the acceleration due to gravity, Δd is the density difference between the particle and the continuous phase, r is the particle radius and η is the viscosity of the continuous phase.

The equation is mostly applicable for fairly dilute emulsions/suspensions. In concentrated systems, the settling rate is slower than that calculated from Stokes Law (Bergenstahl & Claesson, 1990). This is because the particles get in the way of each other, a process called "hindered settling". One of the important considerations not taken into account above is the rheological nature of the continuous phase; it is often highly non-Newtonian and multi phasic (Walstra, 1987). Creaming can be stopped completely if the viscoelastic dispersion medium has a sufficiently high yield stress. Measurement of the apparent viscosity at low shear rates, e.g. 10^{-4} - 10^{-1} s^{-1}, is desirable.

Besides making the droplets smaller, the easiest way to delay creaming is to increase the viscosity of the continuous phase. Hydrocolloids such as gums and starches are commonly used as thickening agents. The use of these compounds rests on their influence on the "microviscosity" (Friberg *et al.* , 1990). An ideal thickener gives a continuous phase which is experienced as a "solid" by the droplets (a high yield value) but which behaves as a liquid when the emulsion is poured. Another way to inhibit creaming is to counteract the density differences between the disperse and continuous phases. The addition of weighting agents e.g. ester gums, to oils of low density (e.g. citrus oils) was commonly used in the beverage industry (Tan, 1990).

Creaming is usually reversible since gentle shaking will usually effect a return to homogeneity. However, if the sample is allowed to stand for a long time or is

excessively centrifuged, extensive aggregation and interdroplet bridging may occur leading to phase inversion or oiling off.

Measurement of creaming .

The amount of visible creaming is usually measured after a given time period and this is expressed as a percentage of the original volume (height) of the emulsion. An example of this is the cream rising index in milk. This visualisation of creaming can be assisted by the presence of dyes in the continuous / discontinuous phase. For very stable emulsions, visible gravity creaming occurs very slowly and it is better to estimate the difference in some constituents (e.g. total solids, fat etc.) between a portion of the bottom of the sample and the original emulsion. Centrifugal methods may be applied to speed up creaming. In theory, applying centrifugal forces of 2,000, 4000 and 12,000 *g* for 60 min would be equivalent to ambient storage for 3, 6, and 18 months (Be cher, 1965) However, acceleration of creaming may result in incorrect assumptions, as this approach may not reflect the complexity of changes occurring during the storage period. Tornberg & Hermansson (1977) and Leman *et al.* (1988) used gentle centrifugal conditions; e.g. 180 -940 g for 15- 30 min.

Recently other methods have also been developed to measure creaming. In an non-invasive ultrasonic method, the velocity of ultrasound is directly related to the relative concentrations of the disperse and continuous phases, provided that the dispersed particles are smaller than the wavelength of the ultrasound (Howe *et al.*, 1986). An instrument can be constructed to measure the volume fraction on an emulsion along the length of a sample tube. A similar type densitometric method measures the opacity profile along a 10 x 0.6 cm tube using ordinary light (Britten *et al.* , 1991). The method could also differentiate between sedimentation, syneresis and coalescence.

Flocculation/aggregation

Flocculation and aggregation both refer to the same phenomenon i.e. that of droplets touching one another without losing individual identity (Das & Kinsella, 1990*b*). If the resultant cluster is only a few droplets, flocculation is the preferred term and this event may be reversible; however when a large number of particles are involved it is often regarded as (irreversible) aggregation/coagulation. Flocculation may be induced in several ways:

1. by adjusting the pH towards the isoelectric point. This reduces the interdroplet electrostatic repulsion

2. by adding excess electrolyte; this screens out the electrostatic repulsion

3. by reducing the solvent quality of the continuous phase for the adsorbed protein

4. by adding adsorbing polymer molecules; this causes bridging flocculation

5. by adding non adsorbing polymer (e.g. gums) to cause depletion flocculation This process can be regarded as a phase separation process for systems containing dispersed particles. The reason for the phase separation is that polymer molecules prefer to be surrounded by like molecules rather than by molecules that are chemically different. In this process, the incompatible biopolymer is "squeezed out" or depleted from space between two droplets (Gunning *et al.*, 1988; Dickinson, 1988; Tolstoguzov, 1990).

The droplets in a stable emulsion are prevented from flocculating either by electrostatic stabilisation or by steric stabilisation or a combination of the two. In the former case, the droplets are kept apart by repulsion between their charged surfaces and in the later by osmotic repulsion between their macromolecular adsorbed layers (Dickinson & Stainsby, 1982). Surface active polyelectrolytes, such as proteins, can provide both of these types of stabilisation and lowering the dielectric constant aids by increasing the electrostatic repulsion (Dickinson & Stainsby, 1988). In colloids of high volume fraction, flocculation may be a positive factor, since creaming will be severely restricted if particles are flocculated into an open but highly connected semi-continuous gel-like structure. These systems may be prone to syneresis i.e the expulsion of water from the gel network (Dickinson, 1988).

Measurement of flocculation.

Darling (1982) observed that there were few if any quantitative measurements on the flocculated state of emulsions. The author went on to describe a semi-quantitative method to measure the homogenisation-induced clustering of cream. The method is based on the time dependent decrease of viscosity that occurs during at a constant shear rate measurement run. The viscosity curves were analysed and described mathematically. Further studies, on the application of a similar type technique, were outlined by Darling & Birkett (1987) ; here the addition of a second surfactant to the emulsion, after homogenisation, reduces the flocculation by displacing protein from bridged droplets, thus decreasing the viscosity. Similarly, Tornberg & Ediriweera (1988) measured of the extent of flocculation by estimating the amount of ionic detergents needed to disperse flocs. These authors also scored the microscopic appearance of the flocs in the undiluted emulsions, as did Dickinson *et al.* (1989*a*) with various protein stabilised emulsions and White (1981) with ice-cream mixes.

Gelation

Gelation is not normally considered a classical defect of emulsions, however in the case of cream liqueurs (Muir & Banks, 1987; Muir *et al.*, 1991) and other long-life emulsions (Harwalkar, 1992) flocculation/aggregation in these systems can lead to full gelation, often followed by syneresis. Factors influencing gelation and methods to prevent it are discussed in a later section; here some rheological aspects will be mentioned.

In simple terms, a gel can be considered to be a continuous three dimensional network of macroscopic dimensions, immersed in a continuous liquid phase (Ziegler & Foegeding, 1990). Although gels are usually considered as showing solid character, some important features of the liquids state may also be present e.g. part of a deformation may not be recovered on removal of a stress (Walstra & Jenness, 1984). The preponderance of elastic over viscous properties varies widely (Walstra *et al.*, 1991).

Important factors in gelation are the polymer-solvent and polymer-polymer interactions, since these influence the waterholding capacity of the resultant gels. For example, the more favourable the protein-solvent interaction the greater the degree of hydration (Ziegler & Foegeding, 1990). Less favourable interactions between the

network and the surrounding fluid may cause excessive particle aggregation and hence syneresis.

Gels can be classified in microstructural terms under four headings (Clark, 1992), with most foods gels falling into the latter two categories:

1. *Lamellar liquid crystalline mesophases e.g. soap gels and phospholipids*
2. *Disordered covalently cross-linked polymeric networks e.g. rubber*
3. *Polymers networks formed through physical aggregation of macromolecules e.g. gelatin and polysaccharide gels*
4. *Particulate networks gels based on colloidal aggregates e.g. milk gels, some globular protein gels*

Polysaccharide and gelatin gels consist of long flexible macromolecules that are cross-linked at some places by either covalent bonds, micro-crystalline domains, entanglements or other linkages. The molecular structure, gelation mechanisms and rheology of such gels are discussed in detail in texts edited by Harris (1990), Dickinson (1991*b*), Pomeranz (1991), Mitchell & Ledward (1986) and articles by Ziegler & Foegeding (1990) and Clark (1992).

Particle gels, which are the type of interest in this study, form by the aggregation of the suspended particles into an irregular continuous network. They differ in respect to classical macromolecular gels in many ways e.g, they are coarser; they have strands which are much shorter relative to their thickness; they fracture at low deformations and the three -dimensional arrangement of individual particles into flocs is important (Walstra *et al.* , 1991). See also review articles and texts mentioned above.

Measurement

Since the process leading to gelation can be a slow and gradual thickening, the actual gelation time may be difficult to quantify. Usually, gelation is judged by the visual examination of containers of stored products, at set time intervals. Alternatively, one

could define a "gelation" time as the number of days for a sample to achieve certain viscosity or some other rheological parameter (Leviton *et al.*, 1963).

Coalescence

Coalescence, an irreversible process, is the increase in particle size due to fusing of individual droplets. It is apparent that flocculation can be seen as the precursor of coalescence. The process can occur at the time of formation of the emulsion (recoalescence) or when the emulsion is subjected to stresses e.g. freeze-thaw cycles, high temperatures or drying (Tornberg and Ediriweera, 1988). Phillips (1981) concluded that the thickness of the layers is more important in the resistance to coalescence than the rheological properties in coarse emulsions. However, many researchers attribute coalescence stability to more spread out and attached films (Das & Kinsella, 1990*b*). Small droplets are more stable than large ones, and the production of same is the best way to prevent coalescence e.g. decreasing the average droplet size by a factor of 2, can decrease the coalescence rate by a factor by 10-100 (Bergenstahl & Claesson, 1990). Coalescence may be encouraged by inclusion of low-molecular weight surfactants; these displace proteins from the interface and this phenomenon is important in the manufacture of ice-cream where controlled deemulsification / agglomeration of the fat particles is desirable during freezing.

An event known as phase inversion can occur if an o/w system turns to an w/o system (or vice versa) and this is the result of massive coalescence. This can occur if the phase volume of the oil is high and is most likely to happen during the emulsification process. If the droplets are partly solid (contain some fat crystals) true coalescence does not result. Instead, a granule or clump of droplets may be formed and this process is called partial coalescence (Darling, 1982; Melsen & Walstra, 1989). Partial coalescence is found to be quickest at 20 °C.

Measurement of coalescence

Many authors measure the extent of coalescence by follow turbidity or changes in turbidity with time. For example, Keeney & Josephson (1958) and Govin & Leeder (1971) followed the deemulsification (coalescence, agglomeration) of ice-cream mixes on freezing by following the changes in specific turbidity with time. However, the situation can be more complex in very fine polydisperse emulsions ; Reddy & Fogler (1981) derived an equation that accurately predicted the increase in particle

concentration of a polydisperse emulsion with time. An initial particle size value is needed but after that the method is relatively simple. The same authors used the above theories to differentiate between different mechanisms of emulsion breakdown (Reddy & Fogler, 1981). Das & Kinsella (1989*a*, 1990*a*) used this method in a study on the coalescence rates in whey protein stabilised emulsions.

Britten & Giroux (1991*a*) measured the susceptibility of various emulsions to coalescence after continuous stirring. The rate of coalescence in this accelerated test was measured by turbidimetric means.

The measurement of free fat or solvent extractable fat has been used to study coalescence stability and it would appear that solvent extraction methods give a good measure of coalescence stability with relatively unstable or damaged emulsions. Stristrup & Andreasen (1962), used chloroform-extracted fat as a measure of the degree of deemulsification in ice cream. Tornberg and Ediriweera (1988) used a 3 hour Soxhlet type extraction with hexane as the solvent. The results of that study demonstrated that the hexane extraction method is capable of measuring coalescence stability over a wide range of instabilities as compared with measuring the increase in particle size. Other researchers used a modified Rose-Gottlieb type extraction (petroleum spirit, diethyl ether) e.g. Castle *et al.* (1988) measured free fat in their study on the stability of concentrated emulsions and Foley & O'Connell (1990) used this method to study caseinate and whey protein stabilised 18 % fat o/w emulsions. Fink & Kessler (1983) used a modified solvent extraction method and a heating/centrifugal method for determination of the free fat in 30 % cream samples; extraction methods give a good indication of the stability of newly formed membranes, while the centrifugal method gives a measure of the membrane-less fat.

1.2 Components of cream liqueurs;

A typical cream liqueur contains ca.: 33-40 % cream (usually of 40-50 % fat), 18-19 % added sucrose, 3-3.5 % sodium caseinate, 12.7 % ethanol, added water and ca 1 % (total) of synthetic emulsifiers /colour/flavours ; see section 1.3 for more details.

In this section, some important aspects of the individual constituents of liqueurs are reviewed, with special emphasis on (1) the main functional component the protein (especially casein) and (2) the most characteristic component, ethanol. Synthetic emulsifiers have been previously mentioned (section 1.1) and the main added stabilising salt, citrate, is also found in milk (see later).

Although important constituents in cream liqueurs, the properties of the added sucrose ("sugar") and the main colour, caramel, are not reviewed. Sucrose, which is a disaccharide of fructose and glucose, functions primarily as a sweetener, bacteriostatic agent and, by virtue of its high concentration, it contributes to the viscosity of the product. Recent research by Cornell *et al.* (1994) indicated that there is no evidence of any chemical interactions between sugar and milk proteins. Caramel is produced from the reaction of sugars (glucose, sucrose) under various conditions to produce a complex macromolecular substance which may be either negatively or positively charged. No literature was found on the interaction of caramel and protein.

CREAM

Cream, which is produced from unhomogenised milk by mechanical separation of the fat globules, is an o/w emulsion where the globules are dispersed in an aqueous (or serum) phase consisting of protein, lactose and salts. In cow's milk these globules range in size from 0.2-20 μm with a d_{32} of 2.5-4.5 μm (Buchheim & Dejmek, 1990). Globules between 1 and 8 μm account for 90 % or more of the total milk fat. The milk fat emulsion has been extensively reviewed by Mulder & Walstra (1974), Buchheim & Dejmek (1990) and the general composition of cows milk has been reviewed in textbooks e.g. Walstra & Jenness (1984), Webb *et al.* (1974) and Wong *et al.* (1988). The composition of bovine milk varies throughout the season (mainly due to changes in diet and stage of lactation) and a comprehensive study of the composition of Irish milk has been published (Phelan *et al.* , 1982; Keogh *et al.* , 1982).

The globules of raw milk are coated by a membrane termed the milk fat globular membrane (MFGM) which is formed when the fat is excreted in the form of small droplets in the mammary gland; this area has been reviewed by Mulder & Walstra (1974), McPherson & Kitchen (1983) and Walstra & Jenness (1984).

Proteins

The proteins (ca 34 g/l) in milk, fall into two categories ; the caseins, which are insoluble at pH 4.6 (isoelectric point) at 20 °C and the whey proteins which are soluble under these conditions. In a spring calving herd, the casein can vary throughout the season from ca 70-80 % of the total protein and the differences are mainly due to the stage of lactation; the contribution decreases in late lactation. Liquid milk herds have an average value of 76 % and this varies little (74.5-76.1) throughout the year (Phelan *et al.* , 1982).

Whey Proteins

The whey proteins comprise 20 % of the milk proteins. Of this figure 50-55% is β-lactoglobulin, 20-25 % is α-lactalbumin, 6% is bovine serum albumin and the immunoglobulins count for 10 % (Doxastakis, 1989; Lee *et al.* , 1992; Muir, 1992). Whey proteins exist in their native state as compact, globular proteins (ca 4 nm in size) which are highly susceptible to heat-induced denaturation and irreversible polymerisation via intermolecular disulfide bond formation.

β- Lactoglobulin (18, 300 daltons) exists as a dimer in solution. It possess two disulphide (S-S) bonds and a thiol (R-SH) group. The thiol group is important since it appears to facilitate R-SH/S-S interactions on heating. The configuration of the β-Lactoglobulin is very pH and temperature sensitive. α- Lactalbumin (14, 000 daltons) is a compact globular protein with four S-S bonds, which makes it more heat stable than β–lactoglobulin. BSA (63, 000 daltons) is a very well characterised molecule because of its presence in blood.

The whey proteins are characterised by their susceptibility to heat. On heating milk above 60 °C, these proteins become associated with the casein micelles through disulphide or hydrophobic interactions and precipitate together with the caseins upon acidification at pH 4.6. Law *et al.* (1994) quantified the extent of denaturation of the individual and total whey proteins in milk using a variety of techniques and found that the extent of denaturation of total protein on heating for 5 mins at 70, 80, 90 °C was

20, 40 and 65 % respectively. Proteose peptones, amounting to ca 25 % of the total whey proteins, were the most heat-stable and did not precipitate with the caseins at pH 4.6. The most heat stable of the true whey proteins was α-lactalbumin; 40 % was still undenatured after 5 mins at 100 °C. The most heat labile proteins were the immunoglobulins; 60 % of which were denatured after only 5 mins at 70 °C.

Casein

Milk contains caseins (24-28 g/l) which largely exist with salts in a unique complex known as the micelle (see later). As mentioned previously, whole caseins can be isolated either by isoelectric precipitation at pH 4.6 at 20 °C with HCl but other methods can also be used e.g. centrifugation at 45000 *g* x 90 min at 37 °C following adjustment by adding 0.07mM $CaCl_2$. However, it should be noted that ca 10% of the total casein is non-precipitatable under the centrifugation conditions i.e. it is present as so called "soluble casein". The physico-chemistry of caseins has been extensively reviewed; see Dalgleish (1989), Lee *et al.*, 1992; Muir, 1992; Doxastakis (1989) and Swaisgood (1993).

In freshly drawn milk, the 24-28 g/l total caseins can be separated into four primary products, α_{s1}, α_{s2}, β and κ, in the approximate ratio 40:10:35:12. In addition, various polypeptides, including γ -casein and proteose peptones, are produced by the action of indigenous alkaline milk proteinase (or plasmin) on caseins. These may account for 3 % of the caseins but can be as high as 10 % in late lactation milks (Fox & Mulvihill, 1990). All the caseins, which can be considered phosphoproteins, are relatively small molecules i.e. 20 000- 24 000 daltons. The α_{s1}, α_{s2}, β and κ, caseins contain 8-9, 10-13, 4-5 and 1-2 mole phosphorus / mole protein, respectively. The phosphate residues, which exist as monoesters of serine, occur mainly in clusters and are capable of binding metal ions, especially calcium, strongly. This can result in charge neutralisation and aggregation.

Micelles The individual caseins mentioned above associate with each other and with calcium phosphate to form colloidal particles called micelles . On a dry weight basis, the micelles consist of ca 93% of protein and 7 % small ions (principally calcium, phosphate, magnesium and citrate) in a complex referred to as colloidal calcium phosphate or CCP (Rollema, 1992). Micelles contain about 25,000 monomers per micelle and have a molecular mass of 10^7-10^9 daltons. They range in diameter from 15-600 nm with an average d_{32} of 100-140 nm (Buchheim & Dejmek, 1990,; Griffin & Anderson, 1983; Donnelly *et al.* , 1984). High values for voluminosity and

hydration e.g. 3-4 g water/g protein (Buchheim & Dejmek, 1990) are reported, which indicates a spongy type particle. The relative contribution of the major caseins to the composition of the micelle depends on their size class; e.g. the smaller the micelle the greater the % contribution from κ -casein (McGann *et al.*, 1980). The monomers (individual caseins) are held together by hydrophobic interactions, electrostatic interactions (mostly as calcium and/or calcium phosphate bridges between phosphoserine and glutamic acid residues) and hydrogen bonds. The aggregation can be influenced by a variety of parameters.

Different models have been have been proposed for micelle structure (Rollema, 1992) although none of these have received universal acceptance. Electronmicroscopy indicates the micelle is composed of submicelles in a "raspberry " type structure and this is favoured by many current researchers (e.g. Walstra, 1990) as best fitting all the available data. The submicelles are stabilised by CCP and hydrophobic protein interactions. It appears that κ–casein plays an important role on the surface of the micellar, with κ-casein deficient submicelles located in within the micelle and κ-casein rich micelles concentrated at the surface. The presence of a "hairy layer" of κ–casein of thickness 10-15 nm is supported by the fact that removal of the hairs by proteolytic action decreases the diameter and voluminosity of the micelle (Walstra, 1990; Walstra & Jenness, 1984). Only 10-15% of the κ–casein is involved in the hairy layer.

The negative charge on casein micelles, at the normal pH of milk, is of primary importance to their stability and this charge is to a large extend controlled by the amount of Ca^{++} bound. Increasing the Ca^{++} concentration in milk increases the amount of bound calcium and reduces the net negative charge of the micelles and thus decreases the energy barrier to coagulation; decreasing Ca^{++} has the opposite effect. At low pH, electrostatic bonds between the positive and negative groups on the caseins keep the micelles together and at the isoelectric point these bonds are quite strong (Walstra, 1993*b*); casein particles at this point are very different from micelles although their size distribution has not changed greatly. Low pH also leads to higher Ca^{++} activity which lowers the negative charge on the micelles. Steric repulsions are also responsible for the stability of the micellar dispersion (Walstra, 1990). The stability or instability of the casein micelles, and factors affecting same, are of importance in commercial processing of milk. For example, the high heat stability of milk at pH values > 7.0 is believed to be due to the high net negative charge; see extensive review heat stability by Singh & Creamer (1992) and recent publication by Law *et al.* (1994). In cheese making, micelles are destabilised when rennin attacks κ-

casein to form a soluble macropeptide and para-κ-casein. Some of the surface charge is lost and these altered and denuded micelles will aggregate in the presence of sufficient Ca^{++} at temperatures > 15 °C. This area has being reviewed comprehensively in a text edited by Fox (1993).

Milk lipids

The lipid content of milk can be arbitrarily defined as the amount of (non-polar) material extracted by the Rose-Gottlieb procedure (e.g. IDF Standard 1 C, 1987). Triacylglycerols (triglycerides) are the predominant lipid class in milk accounting for 97-98 % of the mass and these consist of glycerol molecules each esterified with three fatty acid molecules. The fatty acid composition and their relative positions on the glycerol backbone is complex. The predominant fatty acid is palmitic (C16) at ca. 24 % followed by stearic (C18) at ca. 13 %. Milk fat is characterised by its relatively high content of short chain fatty acids e.g. ca 3.5 % butyric acid. In general, the characteristics of milk fat are highly dependent on the nature of the feedstuff ingested by the cow.

The remaining lipid classes in milk are; phospholipids (ca 1 % of total milk fat), mono and diacylglycerols, free fatty acids (0.35%) and cholesterol and its esters (0.3%); (Jensen & Clark, 1988 ; Walstra & Jenness, 1984). There are relatively few studies on the diglyceride/monoglyceride content of fresh or stored milk and the amounts reported vary widely ; for example Mulder & Walstra (1974) report a diglyceride content of only 0.3 % of milk fat in contrast to the figure of 1.5 % quoted by Berlitz (1987). The phospholipid portion consists mainly of ca phosphatidylcholine (35%), phosphatidylethanolamine (32%) and sphingomyelin (25%) (Jensen & Clark, 1988). Most of the phospholipid is associated with the fat globule but the serum portion can contain 30-50 % of the total. The percentage of phospholipid varies with product type ; for example in 40 % fat cream, 30 % cream and in milk it is 0.5, 0.6 and 0.8 % in the total fat, respectively.

The density of milkfat at 20 °C is about 918 kg/m^3 and the refractive index (sodium D line) at that temperature is ca 1.458. Another important physical aspect of butteroil is its melting characteristic. Milkfat is considered fully liquid at 40 °C and at lower temperatures the solid fat content of milkfat can be calculated to be ca. 45% and 20 % at 5 and 20 °C, respectively. This physical state of the fat is considered to be an

important aspect in the coalescence and partial coalescence of milk emulsions by Walstra (1987).

Other milk constituents

Salts

The mineral system in raw milk comprises of a mixture of calcium (1,200 mg/l), magnesium(120 mg/l), phosphate (3000 mg/l) and citrate (1,800 mg/l) together with the monovalent species sodium (500 mg/l), potassium (1,500 mg/l) and chloride (1000 mg/l). The minerals are partitioned between a colloidal and a diffusible (soluble) phase. The monovalent anions and cations are found almost exclusively in the soluble phase, as is the citrate. Colloidal calcium is either associated with inorganic phosphate or with casein but these cannot be easily differentiated (Walstra & Jenness, 1984). The structure of the colloidal calcium phosphate has been the subject of much research. Overall, the levels of calcium, especially ionic calcium (Ca^{++}) is considered of particular importance to the stability of many dairy products (Muir 1985). Of the 33% of calcium that is soluble about 33 % of this is considered ionic. Holt & Muir (1979) found a high correlation between the soluble calcium and citrate concentrations from creamery milk in Scotland.

Lactose

Lactose is a disaccharide consisting of one residue each of d-glucose and d-galactose and its presence in milk is virtually unique (Walstra & Jenness, 1984). Most cows' milk contains more lactose than any of the other solids (4.5-5.0 %) and it occurs in the final cream liqueur at a level of ca. 1%. Because of the presence of a free aldehyde group, heating lactose in the presence of amino groups results in the Maillard reaction which involves the reaction of the lactose with the amino group of proteins. The results are a brown colour and off-flavours, which are of importance in many dairy products.

Enzymes

Milk contains numerous indigenous enzymes but potentially the most important is the milk proteinase , plasmin. Raw milk contains about 0.3 mg/l plasmin and about nine times more of its precursor, plasminogen. Both of these forms are associated with the casein micelles. Other components of the system are important i.e. plasminogen activators, plasmin inhibitors and inhibitors of plasminogen activators. The plasmin activity of milk is increased upon pasteurisation probably by inactivation of an

inhibitor of plasminogen. β–Casein is hydrolysed by the proteinases more quickly than α_{s1} casein and most authors have shown that κ-casein is relatively resistant to hydrolysis (e.g. Driessen *et al.*, 1981; Kohlmann *et al.* , 1988, 1991 & Manji & Kakuda, 1988).

Heat resistant enzymes can be excreted by various psychrotrophic bacteria especially those of the *Pseudomonas* class. The area has been reviewed by Fox *et al.* (1989), Driessen (1989) and Fairbarn & Law (1986). The presence of these enzymes has been associated with product defects and production losses (Mottar, 1989).

SODIUM CASEINATE

A wide range of casein-rich ingredients can be produced by processing raw milk. These products include milk powders, coprecipitates, caseins and caseinates; see Pomeranz (1991) and Mulvihill (1992). However, the most relevant product to standard cream liqueur manufacture is sodium caseinate, since this is the principle emulsifying protein in that system. In this section, we will consider some characteristics of caseinate and caseinate solutions.

Manufacture and composition

The first step in the manufacture of sodium caseinate is the production of acid casein from skim milk. This is achieved by isoelectric precipitation, usually with hydrochloric acid. The acid casein curd is then washed with water and neutralised with the relevant base or basic salt solution, to a pH of about 6.7. A hot (90-95 °C) solution of ca 15 % total solids is then spray dried. Important steps in process are the dewatering and washing of the casein curd (to remove contaminating milk solids), the amount of time at high temperature and pH and the conditions of drying (Mulvihill, 1992). Typically, sodium caseinate contains ca; 90 % protein (of which 93-95 % is from casein), 4 -6% moisture, 1 % lipids, 3-5 % ash (including 1.1 - 2.0% sodium from sodium hydroxide), some unwashed lactose (0.2-2 %) and milk salts (including 0.01-0.10 % calcium).

Muir & Dalgleish (1987) found that commercial roller dried samples contained on average ca 0.35 % calcium, while Danish and Scottish spray dried products contained ca. 0.07% and 0.02%, respectively. Total and ester phosphate contents showed less variation. Dalgleish & Law (1988), who quantified differences in protein composition

in the above commercial caseinates, reported important differences in fast-protein liquid- chromatographs e.g. broadening of peaks especially in the α_{s2} and κ– casein areas. The differences between the roller and spray dried samples were small but reproducible. They also noted (no data given) differences between batches from the same manufacturer and plant.

Colloidal / aggregation state

The combined acid and alkali treatments received during casein manufacture disrupts the native structure of the casein micelles by solubilizing the colloidal calcium phosphate structure. This results in a protein of much smaller aggregates. and very low calcium levels. Thus, sodium caseinate is widely regarded as a partially aggregated mixture of the four individual caseins (α_{s1}, α_{s2}, β and κ) in the approximate proportions 4:1:4:1. The nature of the aggregation has been investigated by a few authors using a variety of techniques e.g. gel filtration, electron microscopy and particle sizing. However there are few studies on commercial sodium caseinates (Famelart & Surel, 1994) ; most studies utilising laboratory produced material (e.g. Pepper, 1972; Creamer & Berry, 1975; Haque *et al.* , 1993) Results from electron microscopy of non emulsified and emulsified samples containing sodium casein (Bucheim & Dejmek, 1990; Foley & O' Connell, 1990), from solubility data obtained from ultracentrifugtion of solutions of caseinate (Mulvihill & Murphy, 1991; Famelart & Surel, 1994) and protein interfacial load data (e.g. Fang & Dalgleish, 1993) indicate that sodium caseinate is in a "soluble" state with small particle sizes.

Guo *et al.* (1989) demonstrated that the most significant changes brought about by severe heating of caseinate solutions were alterations in the charges of the proteins rather than changes in the colloidal state. Lieske & Konrad (1994) showed that pre-heating caseinate solutions for 90 °C for 5 mins at pH 10 significantly improved the solubility index of these solutions if measured at lower pHs e.g. pH 5. No size data were reported.

The addition of calcium to sodium caseinate solutions has been studied by, amongst others, Zittle and co-workers (Zittle *et al.* , 1956,1957; Zittle, 1961) and more recently by Dalgleish & Law (1988). The increase in turbidity on addtion of calcium to caseinate solutions would indicate the formation of larger insoluble colloidal particles. In studies on the effect of Ca^{++} addition on the aggregation of the individual caseins, Horne (1982) has studied the calcium induced precipitation of α_{s1} casein and while both Dickson & Perkins (1971) and Pappas & Rothwell (1991) have established that the order of binding capacities is α_{s1} > β > κ–caseins. The possible binding sites are

monoester phosphate groups, contributed by serine and threonine residues and carboxyl groups of aspartic acid and glutamic acid (Dickson & Perkins, 1971).

Use of caseinate as emulsifier

As mentioned in a previous section, proteins make excellent emulsifiers by virtue of their amphiphatic nature and ability to spread and remain at the interface. In this respect, sodium caseinate is one of the most efficient and well studied of proteins. It is not possible in this review to explore the large body of work that has been published on non-alcoholic caseinate containing emulsions. However, important work includes that of:

Tornberg and coworkers who studied a wide variety of proteins under controlled conditions (see Tornberg et al., 1990 for review)

Dalgleish and coworkers (e.g. Fang & Dalgleish, 1993) who studied the interfacial composition of sodium caseinate emulsions

Sabharwal & Vakaleris (1972), Vakaleris & Sabharwal (1972), Smith & Dairki (1975b) and Robin et al. (1992) who studied the interaction between LMWS etc and sodium caseinate emulsions

Dickinson and coworkers on the competitive adsorption of mixtures of surfactants; see reviews by Dickinson et al. (1987a; 1989a; 1993) and books edited *by the Dickinson (1991) and Dickinson & Walstra (1993).*

The work of Chesworth et al.(1984), Dickinson et al. (1987b) and Agboola & Dalgleish (1995) who studied the effects of calcium addition *to various caseinate stabilised emulsions*

Britten and co-workers in Agriculture Canada (e.g. Britten et al., 1994) who have studied the emulsifying properties of blends of whey proteins, denatured whey proteins or caseinate etc

The results of these and other relevant research will be discussed in the experimental section. The use of caseinate as an emulsifier in cream liqueur systems is reviewed in a later section.

ETHANOL

Ethanol (or "alcohol") is a unique food ingredient, since its popularity and usage in beverages such as cream liqueurs is due to its pharmacological action on the central nervous system, where it acts as a depressant / relaxant. As a chemical, it is widely used as an industrial solvent where its water solubility and partial solubility with more non-polar solvents is useful. It is especially valuable as a dehydrating agent in the isolation of organic compounds, including proteins. Some important physical properties of alcohol are included in Appendix 2.

Historically, the stability of milk to alcohol was used as an indicator of the milks suitability to severe heat processing; however, in reality it was a fairly accurate indicator of the developed lactic acid rather than the subsequent heat-stability of fresh milk. More recently, elucidation of the mechanism of alcohol coagulation of casein micelles has proved of interest to dairy chemists in researching the physico-chemistry of the casein micelle (Horne, 1992 ; Zadow, 1993*b*). Horne and coworkers have extensively researched the stability of native milk to denaturation by ethanol and this work is reviewed by Horne (1992) and Horne & Muir (1990). Earlier research in the area includes that of Davies & White (1958). Some important points are outlined below.

Note: Most of the work in relation to ethanol and dairy systems focuses on alcohol addition to milk of normal pH to form final solutions of ca 30-55 % v/v. Cream liqueurs contain about 21 % v/v alcohol in a ca 26 % w/v sugar fat-free phase. Thus, despite the important qualitative and quantitative analytical differences between milk and cream liqueurs, it is relevant to consider the considerable literature on the effects of alcohol on milk and other non-cream liqueur systems.

alcohol test

In the original alcohol test, the alcohol stability was defined as the highest strength of ethanol, by volume, needed to cause immediate visible precipitation after mixing equal quantities of milk and alcohol solutions. Horne & Parker (1980) modified the original 1:1 ratio of alcohol solutions : milk to a 2:1 ratio; this allowed for the increased stability of certain samples. The test is highly empirical and recently Zadow (1993*a*) showed that at or near the natural pH of milk, the rate of alcohol addition has a marked effect on results. Donnelly (1987), who measured ethanol stability as a function of calcium addition in a caseinate/sucrose solutions, also found that if stabilities were expressed as

final alcohol content of the mixture at visible coagulation, the results were dependent on the concentrations of alcohol-containing solutions.

Factors affecting alcohol stability of milk

Effect of adjusted pH The alcohol stability of a milk sample varies greatly with pH in the range of 6.0-7.3 (Horne & Parker, 1980). The resultant plot of ethanol vs pH is characteristically sigmoidal with the parameter pK being the most useful; a shift towards higher values indicates decreasing stability and vice-versa; see Fig.1.2.1

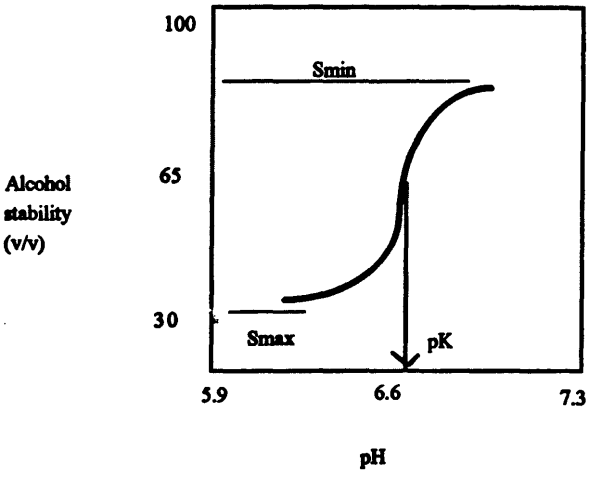


Fig 1.2.1 Typical alcohol stability / pH profile of milk.; the pK value corresponds to the inflexion point of the curve, S_{min} and S_{max} correspond to alcohol values at low and high pH respectively (Horne & Parker, 1980).

Effect of non colloidal phase and added salts. Cross dialysis experiments with milks showing different alcohol/pH stability curves, showed that the position of the curve on the axis is governed by the diffusible components in the serum phase (Horne & Parker, 1981*a*). Thus, the whey proteins had no effect on the ethanol stability, while the removal of the colloidal calcium phosphate (CCP) had only a slight effect.

The addition of Ca^{++} had a dramatic effect on the stability of system. For example, the stabilities of a control milk and that of samples with 2 or 4 mM added Ca^{++}, at pH 6.75 , were ca. 90, 75 and 55 % , respectively (Horne & Parker, 1981*a*). Addition of phosphate, EDTA or citrate to these samples improved stabilites (shifted the

alcohol/pH profile) in response to their calcium chelating properties. Removal of soluble phosphate, by dialysis against a phosphate-free synthetic milk serum, resulted in a reduction in stability. Addition of NaCl (20-100mM) also had a destabilising effect, especially at high pHs..

Lactational and compositional Donnelly & Horne (1986) reported data on the ethanol stabilities of bulk and individual milks and sought to correlate alcohol stability with variations in salt composition of milk. The authors also investigated seasonal and lactational variations in different herds. Linear regression analysis showed that the strongest correlation with alcohol stability was with the soluble salt balance i.e. (Ca^{++} + Mg^{++}) - (P_I + Citrate) or the ratio of these two terms. The main component responsible for variable salt balance ratio was usually soluble P_I. Previously, Davies & White (1958) had shown that approximately 60 % of the variation in stability was accounted for by variation in the concentration of ionised calcium. In contrast, the data of Batra & DeMan (1964) did not show any correlations between stability, ions or salt balances.

Davies & White (1958) showed that very early lactation milks were most unstable to ethanol and Horne *et al.* (1986) considered this to be due largely to the natural low pH of milk at this time. Stability increased during lactation although late lactation milks showed a wide variety of stabilities. Donnelly & Horne (1986) reported changes in alcohol stability with lactation in winter/spring, creamery and autumn calving herds at the natural pH of Irish milks. In general, the stabilities of bulk milks from winter/spring and autumn calving herds were lowest in early and late lactation. Horne *et al.* (1986) also reported on lactation and composition effects of Scottish milks. Late lactation milk often contains high levels of plasmin which may cause proteolysis of caseins especially β-casein. However, extensive proteolysis of a micellar casein dispersion, by indigenous plasmin, did not affect its ethanol stability (Grufferty & Fox, 1988). The low ethanol stability of some late lactation milk was probably due to a high concentration of calcium (Donnelly & Horne,1986).

Processing Banks & Muir (1988) reported briefly on the effect of 5 mM added Ca^{++} on the ethanol stability of homogenised milk. Homogenisation at 10.3 MPa, especially at mid/high pH values, had a slight destabilising effect on alcohol stability, as compared to unhomogenised milk.

Forewarming increased the ethanol stability, presumably this is due to deposition of soluble calcium as calcium phosphate on the micelles (Horne & Parker (1981*d*). Mohammed & Fox (1986) studied the effect of forewarming on the ethanol stabilities

of milk and also serum protein-free casein micelles (SPFCM). Preheating skim milk or SPFCM at 120 °C for 6 or 20 min had similar effects on increasing the ethanol stability; hence it appeared that the β-lactoglobulin/κ -casein complex formed during heating had no influence on changes in the stability. Preheating at 140 °C for 13 min significantly increased the ethanol stability of the SPFCM dispersion and also had a dramatic effect on the calcium sensitivity ; for example about 90 % of the control protein was sedimented in a final concentration of 15 mM Ca^{++}, compared to only 20 % for the preheated sample. Hence, the stabilising influence of preheating against ethanol-induced coagulation of milk, appears to be due to improved stability of casein against precipitation by Ca^{++}.

When milk was concentrated to various degrees by Horne & Parker (1983), they found that the stability decreased at high pHs e.g. pH 6.9. When the ethanol stability at fixed pHs were plotted against total chloride (a measure of the degree of concentration), a linear relationship was observed above pH 6.8. This suggested that, above the natural pH of milk, the ionic strength controls the ethanol stability of milk-concentrates. The reduction of ions, by dialysis of the starting milk, resulted in concentrates of improved stability.

Zadow (1993*b*) noted that mixtures of alcohol (> 60 % v/v) and milk became clear if heated to temperatures > 60 °C. These changes were reversible and suggested that the micelle underwent dissociation under such conditions. It was speculated that both hydrogen and hydrophobic bonds were weakened, causing disintegration of the micelle. This effect had also being observed by O'Kennedy (1991).

Effect of species Horne & Parker (1982*f*) found that goats milk is much less stable to alcohol than bovine milk. Cross dialysis experiments showed that the differences were due mainly to the colloidal phase. The authors noted that goats milk contains higher levels of β-casein and lower levels of α_{s1} and concluded that this was the probable cause of the poorer stability of goats milk. However, Schmidt & Koops (1977), using artificial casein micelles of various casein compositions, had found that high β–casein ratios destabilised the system to alcohol precipitation while high κ-casein ratios protected same. The stability of these model micelle systems was further decreased by dephosphorylation (Schmidt & Poll, 1989).

Effect of protein modification Neutralisation of the positive lysine residues of casein by reaction with acetic anhydride increased the ethanol stability of milk (Horne & Parker, 1982*e*). Conversely, reduction of the negative charge by amidation of the

carboxyl groups, destabilised the ethanol stability of milk. Reactions with different aldehydes had variable results.

Physico-chemical effects

Addition of ethanol or other solvents e.g. methanol, acetone, leads to a decrease in the dielectric constant of the medium (Walstra, 1990). This change may result in changes in the physico-chemical equilibrium of the system, such as changes in the ionisation constants for amino acids on the casein molecules. e.g. serine phosphate and glutamic acid. This in turn can influence ion binding effects (Pierre, 1989) and influence the repulsive electrostatic charge on the micelles.

Another consequence of ethanol addition is the decrease in the size of the micelles. According to the most popular model for casein micelle structure, the micelle is stabilised by a protruding hairs of the C- terminal of κ-casein monomer (steric stabilisation). This layer, by nature of its negative charge, also provides electrostatic stabilisation. Horne (1986) and Horne & Davidson (1986) showed, using measurements with photon correlation spectroscopy, that this protective barrier collapsed on exposure to alcohol. This event was reversible and was considered necessary before aggregation could occur. Although the mechanism by which barrier collapse occurs is not known, it could be due to decreased electrostatic repulsion, resulting from the decreased dielectric constant of the medium (Walstra, 1990). All of these studies took place in highly diluted systems i.e. skim milk suspended in buffers of various composition (alcohol, Ca^{++}, etc). Griffin *et al.* (1989) diluted a concentrated skim milk sample to various levels of protein, in the presence and absence of 9.8 % v/v ethanol. The viscosities of the alcohol containing solutions were slightly less than those of the controls, and this was in line with the expected reduction of casein micelle voluminosity which occurs as a result of the of collapse of the outer layer. This reduction in volume fraction was calculated to be ca. 8 %.

Mechanism of alcohol destabilisation

Horne (1986, 1992) proposed a mechanism(s) whereby the precipitation of calcium phosphate, soluble calcium as well as net micellar charge play major roles in controlling ethanol stability.

The deleterious effect of calcium ions can be explained by their reduction of the net negative charge on the micelles, thus lowering the energy barrier to coagulation. The

addition of alcohol enhances the binding of Ca^{++} as a consequence of the change in pK of the ionizable groups on the protein on lowering of the dielectric constant. Chelation of Ca^{++} by EDTA or citrate will obviously reduce Ca^{++} activity. The net micellar charge was also reduced by chemical modification and this explains the reduced stability in this system (Horne & Parker, 1981*e*)

The unique shape of the pH /alcohol stability profile can be explained by the effect of pH on the calcium phosphate solubility. As the pH of milk is increased, the concentration of Ca^{++} decreases due to sequestering by phosphate ions. Also when milk is forewarmed, the resulting precipitation of calcium phosphate would enhance alcohol stability through the decreased availability of calcium. Horne & Muir (1990) suggested that a repulsive electrostatic component must be overcome before aggregation can proceed. This may involve electrostatic effects on the hairy layer which is reduced / flattened on exposure to alcohol.

Effect of ethanol on protein solutions

Alcohol stability of caseinate

Arima *et al.* (1964) examined the alcohol stability of both caseinate and fractionated caseinate solutions produced from normal and low alcohol-stability milks. The sensitivity test involved mixing of alcoholic and protein solutions and centrifugation of the precipitate.

The whole-casein solutions produced from the alcohol-sensitive milks were more prone to precipitation than the normal milks, on addition of alcohol solutions (final concentration 8-28 % v/v) and 5 mM Ca^{++} . Fractionation of caseinates on DEAE cellulose resulted in two groups of differing stability; the κ-Casein-rich fractions were the most stable and α-s and β-rich fractions were more unstable to added alcohol. These results, together with stability data obtained using a purified κ–casein, indicated that κ-casein (the most stable of the caseins to alcohol), stabilised whole casein in solutions containing ethanol and calcium ion.

As part of their studies on the alcohol stability of milk, Horne & Parker (1981*b*) measured the alcohol stabilities of 2.5 % solutions of sodium caseinate (in 50 mM NaCl) in the presence of added Ca^{++} or inorganic phosphate (P_i). In the absence of Ca^{++}, sodium caseinate shows the linear increase in stability with increasing pH. This is

consistent with the increasing net negative charge on the casein as the serine phosphates are ionised. On addition of Ca^{++} (0 -7.5mM), the alcohol stability is decreased significantly but the linear behaviour with increasing pH is maintained. Adding 15 mM P_i to the calcium containing systems increased their stabilites only at high pHs i.e. > 6.75. The shape of the alcohol /pH curves became more like that of milk. Increasing the phosphate concentration at a constant (7.5mM) Ca^{++} addition, significantly increased alcohol stability e.g. the stability of the system at 0, 10 and 20†mM phosphate addition was 40, 60 and 95 % at pH 7.0. No results for the effect of citrate addition to this system were reported.

Alcohol stabilities of caseinate / sugar solutions.

Donnelly (1987) studied the alcohol stability of a skim milk, a caseinate/skim/sucrose solution (31 % sucrose, 36% skim milk ;4.5 % caseinate) and a homogenised sample of the latter mixed with cream (an alcohol -free base). The caseinate containing sample was more stable than the original skim milk, but homogenisation of the skim/sugar/ caseinate sample with cream, destabilised it towards alcohol, especially at pHs > 6.2. The author also examined the effect of added KCl (0-50 mM) on the ethanol stability of a simple system of water/sucrose/sodium caseinate- (50/23/4.15), at various levels of added calcium (7-15mM); the pH was maintained at 6.85. In general, stability decreased with increasing calcium and further decreased with KCl concentrations (0-50mM). A significant increase in stability was noticed, at high Ca levels (no added KCl), if the caseinate concentration was increased. This was due to the calcium chelating properties of the caseinate.

Donnelly (1987) then studied the effect of added inorganic phosphate (0-10mM) on the ethanol stability of a fat-free bases of various calcium (Ca) concentrations. Levels of up to 8 mM inorganic phosphate increased alcohol stability especially at high Ca levels. The effect of added citrate (0-4 mM) on the ethanol/ Ca curves was also measured (a constant level of 8mM inorganic phosphate, 78 mM KCl at pH of 6.85 was maintained). Even at 1 mM addition of citrate, good ethanol stability was achieved up to 16 mM Ca. It should be noted that, although all the added calcium was added as $CaCl_2$, the Ca level quoted in these curves is not free Ca^{++}.

Precipitation of a casein rich protein with ethanol

Hewedi *et al.* (1985) precipitated the proteins of previously pasteurised or heated skim milks, by adding ethanol to a final concentration of 30 v/v at pH 6.3 at room temperature. About 12-13 % of the recovered protein was whey protein and the dry

sample contained 1.6% calcium and 0.95 % phosphorous. The properties of these ethanol precipitated proteins (EPP) were compared to those of sodium caseinate:

The size distribution of the dispersions, measured by differential centrifugation, showed that the EPP were similar to micellar casein. Tests on the calcium sensitivity of 3 % w/v protein solutions showed that sodium caseinate was more unstable to added Ca^{++} than EPP up to 6 mM. However, while there was no further loss in stability for the sodium caseinate at Ca^{++} ion levels greater the 6 mM , the stability of the EPP continued to decrease with the increasing Ca^{++}. The protein recovered from the severely heated milk was more sensitive to increasing concentrations of Ca^{++} than that recovered from pasteurised milk. The sensitivity of the ethanol precipitated protein to calcium indicates that the ability of κ–casein to stabilise the other caseins is lost on precipitation of milk protein by ethanol.

Effect of alcohol on caseinate/sugar systems.

The measurement of the ethanol stability of milk by methods outlined previously can be regarded as the result of short term equilibrium conditions. However, in the shelf-life of a product e.g. a cream liqueur, long term kinetic factors are important. With this in mind, Muir (1987) suggested that the measurement of the rate of increase in viscosity of a caseinate containing solution , at 45 °C and in the presence of alcohol, for up to 28 days, would be a better indicator of stability than the traditional test. An index of stability, the daily viscosity increment (% DVI)., was calculated as ;

$$\%\ DVI_{(\text{after y days})} = (V_y - V_0) / V_0 * (100/y.)$$

where, V_y ; is the viscosity after 'y' days of incubation, V_0 ; initial viscosity, y ; number of days of incubation. This is a measure of the rate of increase in viscosity, normalised to take into account different starting viscosities.

The author formulated a model cream liqueur analogue system to study the reaction of sodium caseinate to ethanol for 28 days incubation at 45 °C. This system contained the sugar, caseinate, alcohol and water components of a cream liqueur (see later).

Effect of calcium , citrate and salt addition

The liqueur analogue system was used to investigate the effects of variables found to influence the ethanol test in milk (Banks & Muir, 1988). Addition of as little as 1 mM Ca^{++} increased the % DVI about 4 fold, but this was largely reversed by addition of 10 mM trisodium citrate. The addition of citrate alone had no effect on %DVI. The results from this system mirror those obtained in the alcohol stability test i.e. the use of a calcium sequestrant, citrate, increased the ethanol stability.

Banks & Muir (1988) reported that the instability of high alcohol (15.7 - 21.4-m/m) analogue systems was markedly influenced by the presence of 25 mM sodium chloride. For example, the %DVI ranged from 1.7 - 2.6 for the control samples but was 5.5-19.8 for the salt containing samples; ionic strength had been shown to be an important factor in the ethanol stability of concentrated milk systems (Horne & Parker, 1983).

Different caseinates in liqueur analogue

Muir & Dalgleish (1987) and Banks & Muir (1988) reported on the viscosity increases in analogue systems made with various commercial sodium caseinates. There were marked differences in the behaviour of these model liqueur analogues using the standard viscometric test described above.

Roller dried samples, which contained calcium levels of ca 3500 mg/kg, showed very little increases in viscosity (% DVI 0.2-3.5), while spray dried samples (122-800 mg Ca/kg) showed larger but more variable responses (% DVI ; 0.93-3.5) Among the spray dried caseinates, there were no correlations between total calcium and degree of viscosity increase. The best correlation to viscosity increase was with the β– casein content of the powders as measured by fast protein liquid chromatography.

Model emulsions systems containing ethanol

Dickinson *et al.* (1989*b*) stated that while the presence of alcohol (10 wt%) has a small influence on the steady state tension of a casein film adsorbed at o/w interface, its effect on the short time dynamic surface tension, such as during homogenisation, leads to emulsions with finer droplets and much better stability towards creaming. Bullin *et al.* (1988) manufactured 15 % wt hexadecane- in - water emulsions using sodium caseinate (protein/fat ratio = 0.067 and 0.200, pH 7.0 = 0.005M;phosphate buffer, pressure 30 MPa by high pressure homogenisation). Ethanol, 0-38 % wt, was incorporated before homogenisation. Moderate levels of alcohol, 10 -20 wt%, improved the stability to creaming and this was considered to be due to a reduction in droplet

size. Concentrations of > 30 wt % led to instability due to aggregation. The authors also mentioned that if alcohol (up to 20 %wt) was added to emulsions after homogenisation, there was no change in stability. They also studied the effects of electrolyte addition. Emulsions, stabilised by the 1 % level of sodium caseinate (f/p = 0.067), and with > 5 wt % ethanol, were flocculated by the addition, at a 1:1 ratio, of 2 M NaCl.

Burgaud & Dickinson (1990) confirmed that the decrease in droplet size in emulsions was due to added ethanol (0-30 wt %). They choose processing conditions to produce droplet sizes within the range of the Coulter Counter (> 0.6 μm). The oil phase was 10% volume tetradecane and the emulsifier level was 0.25 % i.e. a protein/fat ratio of 0.025. In addition to caseinate, whey protein isolate, a gelatin and gum arabic were evaluated. When gum arabic was used as emulsifier, the average droplet size increased with increasing ethanol concentration, whereas when the proteins were used, the average droplet sizes decreased at first and then increased at higher concentrations of ethanol. Results obtained with sodium caseinate and whey proteins were similar. The more hydrophilic gelatin always produced coarser emulsions and showed more susceptibility to higher ethanol levels.

1.3 Cream liqueurs

Cream liqueurs can be defined as products that typically contain; cream, sodium caseinate, sugars, potable alcohol, flavours, colours and, often, low molecular weight surfactants. While commercial products were initially marketed in 1974/75, the precursors of cream liqueurs were home-made cocktail-type drinks incorporating fresh cream or milk and alcohol. For example, Banks & Muir (1988) mention that a home made drink, based on sweetened condensed milk, alcohol and a flavouring agent (e.g. instant coffee) was popular in Australia. Irish coffee is another example of such a home-made cream alcoholic concoction. There are also similarities between cream liqueurs and another protein/fat containing liqueur, advocaat, where instead of cream, egg- yolks are used. Unfortunately, there would appear to be no research reports in the literature on this product.

The leading commercial brands are reported to contain around 39-40 % solids including ca: 15-16 % butterfat, 3 % added sodium caseinate, 19% added sugar and 1.5 % milk solids-non fat (Muir, 1988*b*). The alcohol content is usually 17 % v/v, corresponding to 12.7 % m/m (a value of 14% m/m is widely quoted in the literature but this is incorrect). The apparent viscosity of these cream liqueurs is about 20-30 mPa.s (e.g. Muir, 1987,1988*b*; Dickinson *et al.* , 1989*c*) and the average particle diameter, d_{32}, is considered to be about 0.2 μm (Nahran, 1987; Bucheim & Dejmek, 1990; Paquin & Glasson, 1989).

The first research publication was that of Banks *et al.* (1981*b*). Subsequent major studies have largely been the results of the work of Banks and co-workers in the Hannah Institute in Scotland (their work has been reviewed by Banks *et al.*, 1983; Muir & Banks 1985, 1987 and Banks & Muir (1988), and that of Dickinson and colleagues in Leeds University e.g. Dickinson et al. (1989*c,d,e*).

GENERAL TECHNOLOGY

Process schemes

The technology of cream liqueur manufacture is similar to that of other dairy emulsions e.g. ice-cream, recombined milks etc. Important items of equipment are a high shear mixer for incorporation of powders / solids, a high pressure valve homogeniser (HPVH), holding tanks and a mixer (batch or in-line type) for incorporation

of the alcohol. Widmar *et al.* (1985), in a patent application, outlined the design of a plant for cream liqueurs manufacture. The method used was basically that of Banks *et al.* (1982).

Typical schemes for the production of 15-16 % fat cream liqueurs are shown in Fig 1.3.1 In both procedures, the caseinate is added as a solution (with or without sugar) to the cream before homogenisation. Other authors have dissolved the caseinate directly into the cream (Abbott & Savage, 1985; Paquin & Giasson, 1989; Dickinson *et al.*, 1989c; Given *et al.* , 1993). Either way, the protein must be adequately dissolved and mixed before any product is homogenised. This is usually accomplished by a high shear mixer, e.g. of the Silverson type, and such a mixture is often termed the premix or, inaccurately, a "pre-emulsion". Emulsifiers, where used, can be incorpor ated in molten form to the premix.

The major variation in the processing of cream liqueurs is the stage of incorporation of the alcohol (Fig. 1.3.1). Basically, the alcohol may be added before or after homogenisation (Banks *et al.* , 1982).

In a *one-step or 1-S process*, alcohol was added to the premix to a final level of ca 12.7 m/m before the product is homogenised at ca 25-30 MPa. The alcohol can also be added as a solution with the sugar (Dickinson *et al.*, 1989c; Given *et al.* , 1993).

In the *two-step or 2-S process* , a concentrated alcohol-free base (AFB) was first homogenised twice at ca 27.5 MPa. This AFB was then cooled to 10-15 °C and an alcohol solution (75-85 g alcohol per 100 g solution) was added to bring the product to specification. Products often receive multiple homogenisation i.e. more than one passage through the homogeniser. This is achieved by collecting all of the first pass product and re homogenising it.

Assuming the product is homogenised more than once, a third process variation is possible. This is termed an intermediate process (INT), and it is a combination of the 1-S and 2-S procedures; here a non- alcoholic base is first homogenised, after which the alcohol is added and the product re-homogenised (Abbott & Savage, 1985; Muir, 1987).

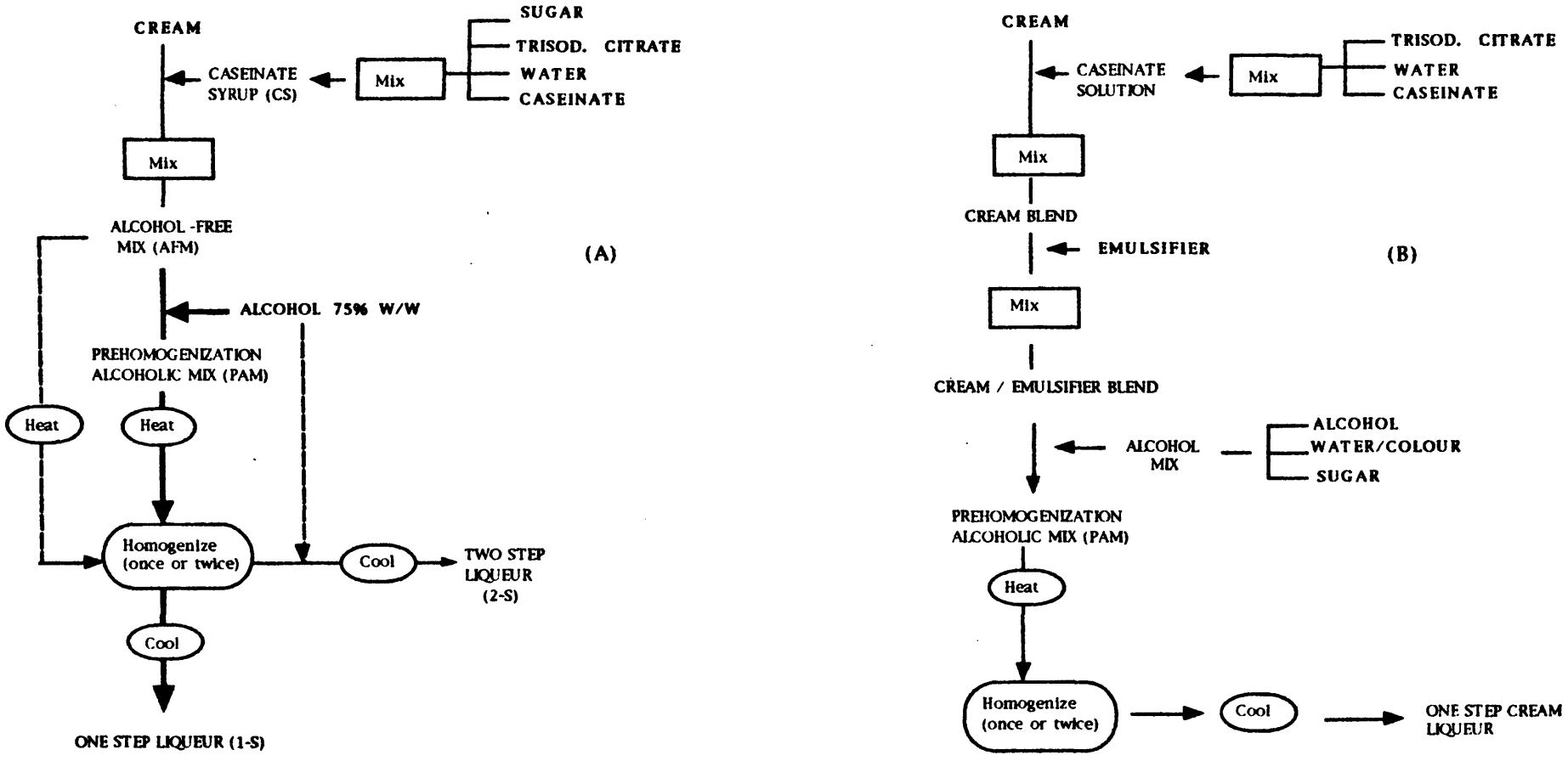


Fig. 1.3.1 Typical process schemes for the manufacture of cream liqueurs. (A) Method described by Banks *et al*. (1982) to produce one step (1-S) or two step (2-S) products. (B) Method described by Dickinson *et al*. (1989c,d) for the production of a one -step product.

Initial characterisation of liqueurs

Compositional analysis

Banks *et al.* (1981*b*) and Dickinson *et al.*. (1989*e*) measured total extractable fat by the Rose-Gottlieb method. Later, Muir (1988*a*) used the Gerber method (homogenised milk procedure) for total fat in high fat liqueurs. Protein, total solids and pH have been measured by these researchers using conventional methods. There are no reports in the literature on the ash or total mineral contents of liqueurs, however, Dickinson *et al.* (1989*e*) measured free calcium ion with an ion selective electrode.

Physical analysis

Banks *et al.* (1982) measured the viscosity of emulsions with a Brookfield viscometer at a shear rate of 79 s^{-1} at 30 °C. Banks *et al.* (1981*b*) had previously demonstrated the slight thixotropic nature of cream liqueurs, especially at shear rates of < 50 s^{-1}. Typical viscosities of the standard liqueur are ca. 20 mPa.s at 30°C (Banks *et al.*, 1981*b*).

The Coulter Counter has been widely used to estimate the amount of fat present in the larger particles. Banks *et al.* (1982) reported the proportion of fat (wt %) contained in globules less than 0.84 or 1.5 μm, while later Banks & Muir (1985), reported the wt % fat below 0.5 or 1.0 μm. Banks & Muir (1988) considered that not more than 3 % wt of the fat should be present in particles with diameters greater than 0.8 μm. Dickinson *et al.* (1989*c,d,e*) using similar techniques, showed that the presence or absence of 17 vol % alcohol in the suspending electrolyte made no difference to the results.

Muir *et al.* (1991) used a laser diffraction instrument to measure particle size distribution in liqueurs, after the sample have been diluted with electrolyte. The average particle size was described as volume surface average diameter (d_{32}), but the authors also calculated the diameters at the upper and lower deciles i.e. $d_{v,0.1}$ and $d_{v,0.9}$ (these corresponds to the diameters below which 10 % and 90 % of the fat lies, respectively). Narhan (1987) reported average particle sizes for cream liqueurs using dynamic light scattering as did Paquin & Giasson (1989). The dilution media were not mentioned.

Bucheim & Dejmek (1990), in a review, showed an electronmicrograph of a cream liqueur (freeze-fracture type preparation) and reported an average particle size (d_{32}) of 0.15 μm, but it is not clear whether these authors calculated this from their own

studies. Dickinson *et al.* (1989*d,e*) examined samples by light microscopy in order detect any large droplets or particles.

Measurement of instability

Four main types of defect have been found in cream liqueurs; creaming, neckplugs, granular precipitation and age thickening/gelation. General aspects of creaming and gelation have been discussed previously (section 1.1).

Age thickening/gelation.

The most serious shelf life problem effecting cream liqueurs and other long life dairy emulsions, such as ultra-high temperature (UHT) treated , sterilised and sweetened condensed milks, is age thickening and gelation. This general area has been extensively reviewed in recent times by Harwalkar (1992) for non-cream liqueur products. The gelation and thickening behaviour of cream liqueurs is described below along with factors effecting this phenomenon:

In the early stages of gelation, the liqueur appears thick and aggregated. This progresses rapidly to gelation of the whole product, with subsequent syneresis to produce a clear liquid at the bottom. Banks *et al.* (1981*b*) monitored these changes in product stability by incubation of freshly produced samples at 45 °C. The shelf life was taken as the time elapsed before visible separation of clear serum was observed at the bottom of the sample container. Hence this test monitors the tendency of the product to show syneresis and age thickening. A commercially stable product should have a minimum shelf life of at least 30 days using this test (Banks *et al.*, 1983) ; this could equate to ca 1 year at ambient temperature (Banks & Muir, 1988). This test was also used by Dickinson *et al.* (1989*a,b*).

The above test can be subjective and is insensitive to small changes in thickening/aggregation. Hence Muir (1987) suggested that measurement of the increase in viscosity (measured at 20.0 °C) of the incubated liqueurs would be a more objective and quantitative test. Muir (1988*a*) defined an index, the Daily Viscosity Increment (DVI), which quantified the rate of viscosity increase during incubation; this test has been previously defined for non-emulsions systems ; see section 1.2 (ethanol).

The increase in the number of the aggregated particles in the thickening liqueurs can also be measured by Coulter Counter (Banks & Muir, 1985) or laser light diffraction methods (Muir *et al.* , 1991). Using the latter method, there was a very good correlation between the average particle size of the uppermost decile ($d_{v,0.9}$) and viscosity of the

thickened samples. The authors suggested that particle size estimation can be substituted for viscometric measurements as a means of estimating the extent of age gelation during accelerated shelf-life tests of cream liqueurs.

Creaming

Muir & Banks (1985, 1987) noted that the most commonly occurring defect in early samples of cream liqueurs was the development of a fat layer in the neck of bottles during storage at room temperature. This defect was probably a consequence of poor homogenisation conditions and/or the use of skim milk powder as the main emulsifier.

Banks *et al.* (1982) measured the type and amount of creaming after placing 50 mls of liqueur in a stoppered tube (25 x 170 mm) for 150 days at 30 °C. They used terms such as "firm, soft or very soft fat plugs and slight cream ring" to quantify the extent creaming. Dickinson *et al.* (1989c) recorded the degree of creaming of liqueurs by storing 25 ml samples in tightly sealed graduated measuring cylinders at ambient temperatures. The visible cream layer was expressed as a % volume of the sample. Most of the creaming occurred within 2 weeks (Narhan, 1987). Paquin & Giasson (1989) measured gravity creaming after storing samples at 38°C for 6 months. They expressed the % fat (a "Milko-Scan" was used) in the bottom one-third of the storage container as a percentage of the original fat of the liqueur. The same authors used a similar approach to calculate creaming due to accelerated centrifugation (16,300 ***g*** x 30 min).

Neckplugs

Neckplugs, as a defect, have been described as semi-solid lumps of fat, 2-3 cm in size, that occur in the tops of bottles (Dickinson *et al.* , 1989*e*). They resemble unsalted butter in composition and structure Their fat is mostly extractable (85-99.5 %) and hence these structures are considered inverted o/w emulsions. While creaming is obviously a precursor to the defect, the phenomenon has been found in samples that have been apparently well homogenised (Muir & Banks, 1986*b*; Dickinson *et al.* 1989*e*). The presence of neckplugs was noted by visible inspection of the bottle.

Granular precipitation

A crystalline precipitate often forms on prolonged storage of cream liqueurs. Chemical analysis showed it to be composed of calcium and citrate (Muir & Banks, 1987). Storage at high temperature was found to accelerate formation of the deposit, corresponding to the decreased solubility of calcium citrate at high temperatures.

Lowering the level of trisodium citrate and adding it as late as possible in the manufacturing sequence, were reported to reduce the problem (Bank & Muir, 1988) although the former remedy may reduce shelf-life somewhat. Methods for measurement of deposits, other than visual observation, are not mentioned.

PHYSICO-CHEMICAL ASPECTS OF 15-16 % FAT CREAM LIQUEURS AS INFLUENCED BY VARIOUS PARAMETERS

Effects of processing methods

Banks *et al.* (1982) investigated the effect of different homogenisation conditions using the 1-S and 2-S methods of manufacture. The 1-S process gave greater homogenisation efficiency than the 2-S method as judged by Coulter Counter e.g. two homogenisation passes at 27.6 MPa (1-S) gave a finer emulsion than two passes at 31.0 MPa (2-S). However, the shelf lives of either sample at 45 °C were similar. Except in the case of coarse dispersions, the shelf life at 45 °C, in contrast to % creaming, was not effected by homogenisation pressure.

Muir (1987) investigated the differences in storage stability between the two processes using the viscometric technique. The results showed that the finer 1-S produced-emulsions were less prone to thickening / aggregation than the 2-S produced ones. An intermediate product, i.e. a product homogenised once without the alcohol and for the second time with the alcohol added, showed thickening behaviour between the other two samples. The use of the 2-S or the intermediate method necessitates the transport of an alcohol-free base (AFB) from a dairy to a bottling plant. These bases can be kept at least 4 days at 2 °C before alcohol addition (Muir, 1987).

Widmar *et al.* (1985) described a product and process essentially similar to the 1-S procedure of Banks *et al.* (1982). The higher homogenisation pressure (2 x 31.0/3.5 MPa) and temperature (70 °C) used in processing were claimed to result in a product more stable to viscosity increases, although no data was reported.

There was no real benefit to product stability or decreases in particle fineness in homogenising a 1-S product more than twice (Muir & Banks, 1986). Similar results were found by Dickinson *et al.* (1989*e*), in their attempts to reproduce, in the laboratory, the neckpluging defect which occurred commercially. The latter authors

processed monodiglyceride containing samples with double stage homogenisation (17.2 / 3.5 MPa) and found that, besides producing slightly smaller droplets following the second pass, repeated homogenisation (10 times) of a liqueur did not have any effect on the sample. Even very poorly homogenised samples (double stage: 7.0/3.5 MPa), which exhibited very large creaming values (50 %) , did not show neckpluging.

Effect of pH

In their preliminary work, Banks *et al.* (1981*a*) reported that non-citrate liqueurs produced by the 2-S method, were very unstable at 45 °C. Most samples showed serum separation after 4-10 days. Banks *et al.* (1981*b*) adjusted the pH of these samples, after homogenisation, with various sodium dihydrogen phosphate/trisodium phosphate combinations (100 mM total inclusion). Adjusting the pH from 6.5 to 7.8 led to a marked improvement in the stability i.e. from 4 to 28 days at 45 °C. However, the stability attained at pH 7.8 by this method was still unsatisfactory; see next heading for details.

Samples of MDG-containing cream liqueur mixtures (average pH 6.84) were adjusted to pH's 5.4 -10.1 prior to homogenisation by the 1-S method (Dickinson *et al.*, 1987*e*). After homogenisation, there was little change in the initial viscosity (ca. 27 mPa.s.), creaming (ca. 2 %) or ambient shelf-life in samples between pH 6.4 - 7.5. Samples below pH 6.0 thickened due to flocculation and samples above 7.5 showed increased creaming at ambient temperatures. The stability of the emulsions with respect to serum separation at 45 °C was very pH dependent, showing optimum stability (ca 125 days) at the natural pH of the liqueur ca 6.9. Stability was only ca. 60 days at pHs < 6.2 and > 7.3.

Given *et al.* (1993), in a patent application, stated that pH control during the manufacture of a commercial product (Baileys) was the most important parameter with regard to subsequent liqueur and emulsion stability. The optimum pH of the finished product was 6.7-6.9 with values around 7.0 leading to creaming problems. To overcome variations, the authors controlled the pH of the cream/caseinate mix between 6.5-6.8 using a sodium caseinate of the appropriate "pH" . The pH of the caseinate can be controlled at production by adjusting the amount of alkali added for solubilisation of the casein curd.

Serum phase and added calcium

Initial research by Banks *et al.* (1981*b*) studied the role of the serum phase in an effort to improve the stability of liqueurs manufactured by the 2-S process. They removed this component either by washing the cream thoroughly with water or by using anhydrous butterfat. The results indicated that serum component(s), and not the pH value *per se*, were the major cause of instability in the system. The anhydrous milk fat sample had an 8 fold longer incubated shelf-life than the non-citrate cream sample (control), despite the lower pH. Adding citrate, (10mM i.e. 0.25 m/m trisodium citrate) to the control samples, after homogenisation, improved the shelf -life dramatically. These results were confirmed by Banks *et al.* (1982). This evidence suggested that ionised calcium (which could be chelated by citrate) was the main cause of gelation. (Note; we will assume that literature references to trisodium citrate mean the amount of the dihydrate salt, although this is not clear; the amount used by Muir and co-workers ranged from 0.19 to 0.25 % i.e. ca 8-10 mM).

Dickinson *et al.* (1989*b*) added Ca^{++} (0-10,000 mg/kg of final liqueur) before homogenisation to 1-S produced liqueurs containing 0.16% trisodium citrate and 0.34 % mono-diglyceride (MDG). Samples with added Ca^{++} in the range 0-40 mg/kg showed identical Coulter Counter droplet size distributions; a shift towards larger particles occurred at concentrations greater than 40 mg/kg. Addition of < 10 mg Ca^{++}/ kg had no effect on shelf life at 45°C but adding 100 mg/kg reduced the shelf life , as compared to the controls, from 120 to 60 days . The calcium ion concentration of samples containing no added calcium, as measured by a calcium ion electrode, was typically 11 mg/kg Ca^{++}.

Use of different caseinate levels and different proteins

The protein:fat ratio in a 16 % fat liqueur, without any *added* protein, is 0.038 as compared to a ratio of 0.166 for an unhomogenised 16 % fat cream. Hence, it is necessary to add extra protein during homogenisation to achieve adequate stabilisation. According to Muir & Banks (1987), it is probable that non-fat milk powder was used as a source of emulsifier in the early days of cream liqueur manufacture. This may account for the poor stability of such products. However, there are no reports in the literature on the use of this ingredient. The initial work of Banks *et al.* (1981b,1982) established sodium caseinate as an excellent functional

protein for the system i.e. it exhibited good emulsifying/stabilising properties, a bland taste and desirable viscosity.

Banks *et al.* (1983) published some of their earlier work on optimising the protein:fat ratio in liqueurs manufactured by the 2-S procedure. The use of 3.0% as opposed to 2.5 % caseinate, increased the relative shelf life of the products from 47 to 76 days. Later, Muir & Banks (1986) homogenised samples at normal, intermediate and low protein:fat ratios, under standard and extreme homogenisation conditions. The results indicated that it would be extremely difficult to over homogenise a liqueur of normal protein:fat ratio (0.19-0.22).

The effect of increasing the protein: fat ratio from 0.169-0.592 in a calcium and citrate free "butteroil liqueur" (1-S process) and its fat-free analogue was studied by Muir (1988*a*). The fat-free analogue was prepared with all the ingredients except the fat and was mixed rather than homogenised. A comparison of the responses of the butteroil liqueur and its fat-free analoque indicated that, as previously suggested by Donnelly (1987), homogenisation had a significant destabilisation effect of these systems.

The stability of butter liqueurs products, measured as % DVI over a 28 day period at 45 °C storage, was governed by a property of the protein itself. A minimum amount (low protein:fat ratio) should be used in the butteroil liqueurs, commensurate with the prevention of creaming and the attainment of desirable viscosity. In contrast, Muir (1988*a*) stated that with normal cream liqueurs , increasing caseinate above that required for emulsification/viscosity increased stability by counteracting the calcium induced aggregation via calcium complexation.

While caseinate is suitable as an emulsifier at neutral pH values, it is susceptible to precipitation by calcium and acids (e.g. fruit juices, carbonated drinks). With this "problem" in mind, Banks *et al.* (1983) reported on the use of egg albumin and an animal bone protein to improve the acid stability of cream liqueurs. Little details are given but a 2-S process at 55 °C was used. The alcohol-free egg albumen based emulsion appeared very heterogeneous after the first homogenisation but was normal appearing after the second pass. However, the mixture coagulated immediately on addition of alcohol. On the basis of this (albeit limited) report, it would seem that egg albumen is not a suitable emulsifier for use in cream liqueurs. The acid-stable protein, extracted from various animal bones, produced coarse emulsions that creamed rapidly. This was the case even when the protein:fat ratio was increased. It can be concluded

that this protein was too hydrophilic to be a good surfactant and/or is an extremely poor stabiliser in the prevention of coalescence.

Kaustinen & Bradley (1987) investigated the use of a demineralised whey protein concentrate in various formulations and processes. The basic processing method of Banks *et al.* (1982) was used, although the mixes were first pasteurised at 68 °C for 30 mins before homogenisation at 27.6 MPa (twice). Stable and satisfactory cream liqueurs where produced using 6 % added WPC when the fat originated from 42 % cream or 62% washed cream and that the alcohol was added before homogenisation. However, increasing the level of milk-solid non-fat, on top of the WPC level, decreased the shelf life at 40 °C. Unfortunately, the usefulness of this report is limited since neither the protein content / demineralisation levels of the WPC nor the exact fat level of the final liqueurs are clear.

Effect of low molecular weight surfactants (LMWS)

Banks *et al.* (1983) investigated the used of monoglyceride type emulsifiers in liqueurs manufactured by the 2-S process. Preliminary results, using a normal 2-S process, showed that 0.2% glycerol monostearate (GMS) could induce creaming and, if citrate was added afterwards, oiling-off , in incubated samples. Changes in the sequence of processing and processing temperature lead to a remarkable reversal of the results i.e. there was a synergistic stabilising effect to due to the addition GMS or glycerol monooleate (GMO) in the presence of citrate.

Banks *et al.* (1983) also replaced about two thirds of the sodium caseinate with various levels of GMS and / or the citric acid ester of GMS (GMS-cit). The exact 2-S process scheme used is not clear and we assume that no citrate was added to the samples. Only samples containing GMS-citrate alone showed medium to long term stability. The viscosities at the 0.73 % GMS-cit or GMS containing samples were 14 vs 53 mPa.s, respectively, and the higher values indicated extensive clustering with the GMS containing samples. The complete replacement of all the protein in a liqueur could be accomplished by using a GMS-cit to butteroil ratio of ca 0.072-0.140. The products had a reasonable viscosity and a very good storage stability. However, despite containing no protein, the product formed a fine precipitate when mixed with acidic drinks.

Effect of variations in emulsifier concentration.

Dickinson *et al.*(1989*c,d,e*) published a series of papers based on the thesis by Narhan (1987), on the use of LMWS in the manufacture of cream liqueurs using the

1-S process. Samples were homogenised once with double stage homogenisation at 55 °C and 0.16% trisodium citrate was added before homogenisation.

The influence of increasing the total added emulsifiers (caseinate + MDG in a 9/1 ratio) in the range 0 - 3.42 m/m was reported by Narhan (1987) and Dickinson *et al.* (1989e). Cream liqueurs with no added emulsifiers had a higher viscosity than those with the total added emulsifier levels greater than 1.7% e.g. 32 versus 27 mPa.s, respectively. The zero and low level emulsifier samples also had significantly increased ambient temperature creaming values i.e. 50 % and 15 % creaming for 0 and 1.7 % added emulsifiers respectively, compared with only 2 % creaming for the 3.42 % wt control (Narhan, 1987). Reducing the total added emulsifier content from 3.42 to 2.57 % was found to significantly increase the proportion of large droplets in the cream liqueur, as measured by the Coulter counter (Dickinson *et al.* , 1989c).

The effect of added MDG on rheological and gravity creaming properties was also reported. Creaming decreased at the higher inclusion levels, due mainly to development of a weak network which was evidenced by the yield stress data. For example, increasing the added MDG level from 0.34 to 0.5 % had a marked effect on increasing yield stress and decreasing creaming, while having relatively little effect on the apparent viscosity. Viscosity did increase dramatically when concentrations of surfactant were > 1%. This was considered to be due to protein/surfactant interaction at the interfaces and in the aqueous phase.

The effect of varying the MDG and the SSL concentrations in the range 0-2%, whilst keeping the caseinate concentration fixed at 3.1 %, was reported by Dickinson *et al.* (1989c). There was no apparent change in the distribution of particles above 0.6 μm at low concentrations of the LMWS (< 0.5%) as compared to the control. There was an increase in larger particles at high levels of surfactant (2%), especially the with the MDG containing liqueurs. The authors also measured the displacement of caseinate from the fat droplet interface by the surfactants. With no added LMWS, it was shown that most (ca.75 %) of the total protein in the system is attached to the fat. The addition of 0.34 % emulsifiers (shown to the optimal level for 45 °C stability) increased in the aqueous phase protein content from 1.0 to 1.5 % protein. However, even at 2 % added LMWS, complete displacement of protein was never achieved; in this extreme case Dickinson *et al.* (1989c) speculated that the primary interfacial layer contained only ethanol and LMWS, with the protein adsorbed in a secondary sterically-stabilising layer.

Addition of an optimum amount of LMWS (ca 0.34 %) effectively doubled the shelf life at 45 °C to ca 110 days as compared to non-LWMS controls samples. Further addition of LMWS (e.g. 1-1.5 % total level) decreased stability, which in this experiment was measured by the first appearance of serum (syneresis). The authors observed that one should not expect a direct correlation between shelf-life at 45 °C and shelf life at room temperature; at the higher temperatures- (i) the milk fat is completely liquefied (ii) many chemical reaction occur more rapidly and (iii) some calcium salts, especially calcium citrate, may be less soluble.

PHYSICO-CHEMICAL ASPECTS OF NON STANDARD CREAM LIQUEURS

High alcohol levels

Banks & Muir (1985) manufactured 16 % fat cream liqueurs with high alcohol contents (< 25.9 % v/v) using the normal 1-S process. Liqueurs containing 22.4 or 24.2 % v/v ethanol were highly unstable; both gelation and creaming were observed within 1-3 days of storage at 45 °C. The creaming phenomenon had not been seen previously and it appeared to be an aggregation process. The authors established that adding either citrate or another calcium sequesterant, sodium hexametaphosphate, to 25.9 % v/v samples, after homogenisation, actually further decreased the stability of the system (Banks & Muir, 1985). The results indicated that it is the presence of ions, rather than the presence of ionic calcium per se, that is the cause of the decreased stability of high alcohol liqueurs made by the one-step procedure. This was emphasised by Horne & Muir (1990) who measured the viscosity in 19.2 %wt alcohol liqueurs with added NaCl (0-100mM).

However, Banks & Muir (1985) reported that it is possible to produce high alcohol samples, using the normal 1-S process, if washed cream or butteroil was used instead of ordinary cream as the source of fat. All the samples were stable up to 22.4 % v/v but after that the order of stability was washed cream > butteroil > washed cream + 10 mM citrate. The washed cream based liqueurs consistently gave the greater stability over the butteroil samples, indicating that the milk-fat globular membrane contributed somewhat to stability. The addition of citrate to washed cream resulted in

a decrease in stability, in accordance with the contention that ionic material leads to emulsion instability at high alcohol contents (see above).

It was possible to make stable liqueurs up to 24.2 % v/v, alcohol using ordinary cream, if the 1-S procedure was modified slightly. This involved manufacturing a 17 % product of slightly higher total solids, in the normal 1-S fashion, and then adding the extra amount of high strength alcohol, to achieve the final desired alcohol and total solids concentration, after homogenisation. This had dramatic results on the stability as compared to the normal 1-S process. However, initial viscosity and particle size results suggest that aggregation is taking place as the alcohol content is increased. The initial viscosity of such a 24.4 % v/v alcohol product is much greater than the corresponding product made with washed cream + citrate. However, the product manufactured by the modified method was more stable to 45 °C than the washed cream + citrate sample i.e. 61 versus 15 days, respectively. Banks & Muir (1985) concluded that it would appear that a serum component(s), not necessary ionic calcium, caused a deleterious reaction during the emulsification process in the presence of high alcohol concentrations.

It is possible to manufacture a very stable high alcohol (19.2 % wt) butteroil liqueur using the 1-S process if the fat level is < 10 % and the total solids is kept at 30.0 % (Muir, 1988*a*). In such a "calcium free"/low ionic strength system, it is the concentration of sodium caseinate that determines the rate of thickening. For example, increasing the amount of caseinate from 20 to 40g/kg increased the %DVI five-fold.

Cream liqueur containing neutralised wine.

Muir & Banks (1986*a*) formulated 15-15.5 % fat cream liqueurs where up to 11 % of the product alcohol was from white wine. The wine was first neutralised to pH 7.0-7.5 with solid sodium bicarbonate before blending with the desired amount of neutral spirit. The mix, which also contained about 0.25 % trisodium citrate, was then processed with the 1-S procedure. The products had an initial viscosity of about 20 mPa.s. and were extremely stable to incubation e.g. a shelf life of 240 days at 45 °C was attained. This was probably due to their high pH and high citrate level.

Different fat levels.

The effect of different fat content on the properties of liqueurs with a constant protein:fat ratio and total solids value, was investigated by Banks *et al.* (1981b).

Samples were processed by the 2-S procedure and 10 mM citrate was added to half of the samples after homogenisation.

Decreasing the fat content of non-citrate liqueurs from 16 % to 8.2 % increased the stability at 45 °C from 2 to 34 days ; the corresponding increase in stability for citrate containing samples was from 101 to 134 days . It was also found that there was no difficulties in manufacturing a citrate containing cream liqueur with a fat level of 20 % wt. However, it was not possible to manufacture a cream containing product containing a level of 25 % fat, using the standard 1 or 2-S processes.

Muir (1988*a*) sought to produce a high-fat alcoholic emulsion suitable for pouring as a top layer on hot coffee. This would simulate the effect of an Irish Coffee type beverage. Preliminary experiments showed that liqueurs containing 40 % fat (from anhydrous milkfat), ca 10-12 % wt alcohol, 3.5 % sodium caseinate and a small amount of sugar, were stable during storage tests. The total solids of the product was 46 %. The density of the product was low enough to float on hot, preferably sugared, coffee. The manufacturing process used was an intermediate type scheme i.e. the butterfat was first emulsified with the caseinate at 65 °C but without alcohol present. Alcohol was then added to this base at 60 °C and the mix rehomogenised.

The effect of increasing the level of caseinate from 25 to 50 g/kg a 40 % butterfat and 12 % alcohol formulation was then investigated. The initial viscosities of the products increased in a linear fashion from 120-320 mPa.s. Despite the initial high viscosities, the samples did not thicken as much as normal cream liqueurs at 45°C; some even decreased. The extent of thickening at 45 °C was proportional to increases in the protein content. Storage at 30 °C and 20 °C did not lead to viscosity decrease, as happened at 45°C. No particle size data was reported in this study.

Abbott & Savage (1985), in a brief report, described a method for the formulation of a 12.5 % fat product containing 0.31 % MDG ("Cremodan Super"). No citrate was mentioned in the recipe and the process, an intermediate one, involved 3 homogenisations*ie*. the alcohol-free base was homogenised twice at 80 °C and, after alcohol addition, the liqueur was rehomogenised at 30 °C. The authors mentioned that, as well as enhancing viscosity, mouthfeel and uniformity of taste, the presence of MDG considerably improved the shelf-life. The levels of ingredients were said to be important, but, other than the optimum recipe, no other data was reported.

Paquin & Giasson (1989) also used an intermediate process to manufacture a lower fat (13.0 %) liqueur, which also contained 0.25 % glycerol monostearate and 0.38 % trisodium citrate. The authors compared the efficiency of a microfluidizer and a

conventional homogeniser to produce the liqueurs. Particle size and accelerated and gravity creaming parameters were measured: Microfluidization was superior to conventional homogenisation in reducing particle size in this trial (no details of the high pressure homogenisation, other than pressure, were given). For example, the average particle size (d_{43}) of product processed at 30 MPa, measured with dynamic light scattering, was 0.21 μm for high pressure homogenisation versus 0.13 μm for the microfluidized product. However, despite this, the conventionally homogenised product was slightly more homogenous after incubation at 38 °C for 6 months. Initial creaming patterns were similar after centrifugation of product at 16 300 ***g*** x 15 min.

Whole milk powder based liqueurs

Roche (1993) manufactured whole milk powder based liqueurs. The basic product contained: 14% whole milk powder (giving a final product of ca 4 % fat), 24 % sucrose and emulsifiers. The alcohol level was 17 % v/v and there was no added sodium caseinate. Industrial experience with this type system had shown that only powder manufactured from September milk (bulk creamery source), was suitable for incorporation into liqueurs. The stability of the products were assessed by a commercial manufacturer (the project sponsor) using a microscopic grading technique, of which no details were given. It would seem that the main problem related to lactose/salt crystallisation (D. Crowley ;personal communication).

Roche (1993) attempted various procedures in order to produce acceptable liqueurs using unsuitable milk powders. The author tested stability by measuring the viscosity increase of the samples at 37 °C, although there was not information as to whether this test measured the same phenomenon as the microscopic test. The main results were

- microscopic examination of freshly produced samples was the best indicator of liqueur quality and subsequent shelf-life.
- the following procedures increased the stability of 2-S manufactured product; pH adjustment to 7.0, addition of 10 mM trisodium citrate, increased pressure and temperature of homogenisation and utilisation of LMWS i.e. monodiglycerides and especially fatty acid esters of lactic and citric acids.
- incorporation of previously rejected powders into acceptable cream liqueurs was possible only by using an intermediate rather than a 2-S process. (a 1-S procedure was not attempted in this study). The

conditions used for processing the alcohol free base were; homogenisation at 24.3 MPa at 80 °C, cooling to 30 °C and rehomogenisation at 24.3 MPa.

- the shelf life of lecithinated milk powders was very short i.e. emulsion breakdown was obvious on incubation at 37 °C.

The author also measured the composition and ethanol stabilities of milks and milk powders throughout the season. However the factor(s) or balance of factors, determining stability was not clear. The ethanol stability of milk, which was maximal in June, was not a good indicator of suitability for use in liqueurs. The ethanol stability of reconstituted whole milk powder was higher than the corresponding milk, probably as a result of β-lactoglobulin/κ- casein interactions during preheating of the milk before drying.

Other powder based cream liqueurs

Burgess & Early (1986) described, in a patent application, a powder that could be used as the basis for the production of cream liqueur beverages. This approach could have two advantages ; the consumer can concoct his own product using sugar and spirit, or a manufacturer, without a supply of cream, could use the mix as the non-alcoholic component of a product. The ingredients consisted of cream, caseinate, hydrolysed starch, emulsifier/stabiliser and non-fat milk solids. These were mixed, pasteurised, homogenised twice using double stages (17.2/3.5 MPa) and spray dried. The particle sizes should be between 0.8-2.0 μm. The above cream liqueur base could also be reconstituted at home with a liquidiser by adding sugar, water and alcohol to yield a final fat of ca 12 %. It is claimed that the above procedure yields a product that is stable (no fat separation) for up to 2 weeks at 45 °C. A homogenisation step could be substituted for the mixing step above, if a commercial liqueur were to be the end product.

Low pH cream liqueur

van de Hoven *et al.* (1986), in a patent application, outlined procedures for the manufacture of stable alcoholic beverages based on soured milk. The low pH was achieved by either addition of acid or fermentation by bacteria. Pectin was added during the process, presumably to aid stability. An advantage of these type products is that they are a suitable base for addition of acidic type flavours and fruit juice. Products that contained low levels of alcohol were sterilized.

Low pH liqueurs are more prone to oxidation than normal ones (van den Hoven & Ermens, 1991). Replacing the butterfat with hardened vegetable fat reduces oxidation but leads to a product prone to fat clustering at refrigeration temperatures. In this patent application, the authors found that the use of a special oil high in medium chain triglycerides (MCT oil) gave a storage stable liqueur without any physical defects at low temperatures. These liqueurs were also claimed to be more resistant to hydrolytic attack by lipases. This resulted in less soapiness, which was the main off-flavour associated with this defect. The MCT oil had a typical saponification number of 325 and a maximum iodine number of 5. Fatty acids with more than 12 carbons as well as unsaturated fatty acids were nearly completely absent. Butyric and lauric acids were present at very low levels.

CHAPTER 2
General Materials and Methods

Note: In this chapter, details of routine materials and methods used in the study are outlined. More particular details of non-routine materials and methods are described in the relevant experimental sections (Chapters 3 & 4). Where significant work was done in developing and evaluating methodology, this is reported as experimental work in the preliminary experimental section (Chapter 3).

2.1 Materials and testing thereof.

CREAM

Cream was produced using the University milk supply or purchased (normally at ca 48 % fat) from a local dairy (Dawn Dairies, Ballinahina). In one case a batch of frozen cream was used (Imokilly, Co-Op).

The following procedure was typical of that used for the production of cream from raw "University " milks. (These milks were from single herds, either from creamery suppliers numbers 6 , 17 or 72 or from liquid-milk suppliers , numbers 304 and 350:

Bulk milk was received on a Monday night. It was held overnight at 5-6 °C and separated at 35 °C to a fat content of 47-55 % fat the following morning. Approximately 50 litres of the cream were flash pasteurised (cream brought to 90-95 °C) to produce 35 litres of cream and this was immediately cooled to 8-10 °C. The fat content of the cream was checked using the Gerber methods for cream (Irish Standard IS: 68:1955) and it was standardised to ~ 48 % fat with ca. 20 % fat cream. The standardised cream was stored at 4 °C and normally used within 36 hours.

Each batch of cream in the study was given a number, starting with the first batch used. This number constituted the first number of the codes of the cream liqueur produced from that batch. The source of these creams was noted as was the pHs of the creams (Radiometer PHM 82); see Appendix 3. Occasionally, the creams were tested for pasteurisation efficiency by the peroxidase test as outlined by Foley *et al.* (1974).

WATER

Water used in the formulation of the emulsions was normally distilled or demineralised. Demineralisation was performed on a Milli-R06 reverse osmosis unit (Millipore Ltd., Watford, U.K). On a few occasions town tap-water was used. The degree of mineralisation/demineralisation was assessed by conductivity measurements (model CDMZF , Radiometer, Copenhagen)

SUCROSE (SUGAR)

Granulated white sucrose (Irish Sugar PLC) was obtained in 25 kg bags from a local wholesaler. More than one batch was used during the 3 year study.

ETHANOL (ALCOHOL)

High grade neutral spirit or HGNS was purchased from B.P. Chemicals (Ireland). This is a rectified alcohol derived from a fermentation process and is ca 96 % v/v or 94 m/m (g of alcohol per 100 g solution). The relationship between density, m/m and v/v can be found in Appendix 2.

SODIUM CASEINATES

The majority of the cream liqueurs samples manufactured in the study were produced using a single batch of sodium caseinate, obtained at the beginning of the project. This caseinate ("Kerrynor", 4 x 20 kg bags, production code R101 HN6 KH18) was obtained from Kerry Ingredients, Tralee, and was referred to as **K1**. In addition, other "Kerrynor" batches were used as well as caseinates from other commercial manufacturers. Samples of laboratory produced caseinates were also used. More details on these materials are given in the relevant experimental sections.

EMULSIFIERS, STABILIZING SALTS AND COLOUR

Samples of commercial sodium stearoyl lactylate (SSL), Admul 2003, and a mono-diglyceride (MDG), Admul 4223 , manufactured by Quest Ltd, U.K. were supplied by Chemcon Ltd., Dublin. A single sample of each was used throughout the study and these were kept in a freezer (-15 °C).

A 25 kg bag of trisodium citrate 2 H_2O ("trisodium citrate") was received from ADM Ltd., Ringaskiddy, Cork. This stabilizing salt was used throughout the study and it contained ca 88% trisodium citrate/65 % citrate on an as-is basis.

A 25 kg sample of caramel colour, code SCS LV, manufactured by Universal Flavours Ltd., U.K., was supplied by P.K. Chemicals Ltd., Dublin.

2.2 Formulation and processing of cream liqueurs

The method used for the formulation and processing of the cream liqueurs was based on that of Banks & Muir (1982) and Banks & Muir (1985). The basic procedures used in this project are summarised in Fig. 2.2.1. Two main methods of manufacture were used; a one step process (1-S) and a two step process (2-S).

In both the 1-S and 2-S procedures , a syrup containing caseinate/sugar/citrate was made by dissolving the ingredients in very hot water (95 °C). When this had cooled and deaerated, it was added to the cream (~ 48 % fat) and the mixture stirred. This mixture was homogenised either with the alcohol added before (1-S) or after (2-S) the homogenisation step. A modified two-step process (2-Sb) was also used. In this case the caseinate /citrate were mixed directly into a hot cream/water mix, which was subsequently homogenised. The sugar/alcohol/caramel, in the form of a syrup, was added to this emulsion after it had cooled.

The processes are outlined in more detail in the following sections. Slight modifications to the processes used during the course of the investigation (e.g. omission of citrate), are detailed in the relevant experimental sections.

FORMULATION

In this study, the term "cream liqueur" means a 16 % fat product unless otherwise stated. A typical specification (Table 2.2.1) was used for most of the experimental samples. The exact amount of ingredients in a formulation depends on the raw material analysis, the product specification and the final weight of products desired. An example of a typical spreadsheet, used to calculate formulations is shown in Table. 2.2.2.

Ingredients were prepared and carefully weighed out. The minimum amount of product that could be homogenised depended on the number of passes through the homogeniser; 2.5 kg for one pass and 4.8 kg for two pass products. For example, Table 2.2.2, column D, shows a typical recipe for a single step (1-S) product containing caseinate syrup, cream and alcohol solution.

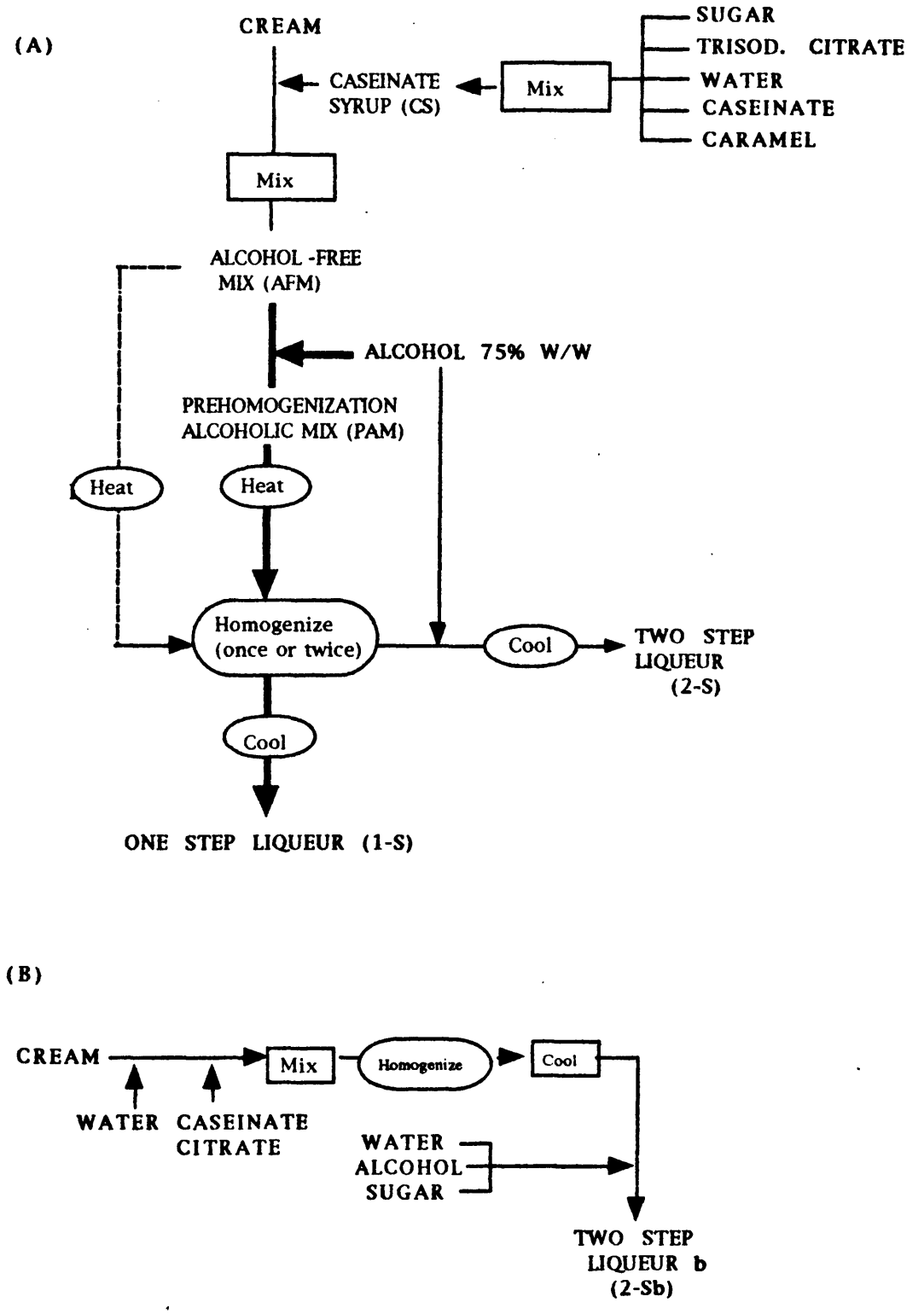


Fig 2.2.1 General process schemes for the production of cream liqueur samples..A) Dry ingredients are dissolved in hot water (ca 95 C) with high-shear mixing to produce a caseinate syrup. This is added to double cream , the mixture heated and high shear mixed. This mixture may be homogenised and the alcohol added after (2-S process) or the alcohol added before homogenisation (1-S process). (B) In this two step process (2-Sb) dry caseinate / citrate is dissoved in the cream . An alcohol / sugar mixture is added after homogenisation. See text for further details of the procedures

Table 2.2.1 Specification for a typical cream liqueur, including sources of the components..

Components	Analysis (% m/m)	Analysis (% v/v)	Ingredient source
Fat	16.0	18.2	Obtained from cream of average fat 48 %. The emulsifiers also contribute to this value.
Ethanol	12.7	17.0	From food grade alcohol (96% v/v)
Sugar	20.0	-	Mostly from added sucrose . Lactose from milk serum, contributes ca. 0.8% in the 20 %
Protein	3.0	-	Mostly from added caseinate . Serum proteins contribute ca. 0.6%, in the 3.0 g/100g
Ash	0.5	-	Contributions from the serum, caseinate, stabilising salts and emulsifiers.
Total solids	40.0	-	

Table 2.2.2. Example of a spreadsheet used for the formulation of cream liqueur recipes by 1 or 2-S processes . *Column A;* input raw material analysis and cream liqueur batch size required. *Column B;* enter the desired specifications of the final product. *Columns C &D* calculate the amounts of ingredients to add for 100 g or the batch size choosen. *Column E* gives extra analytical data on the recipe.. Details of abbreviations used in colums are given below..

A		B		C		D			E	
RAW MATERIAL ANALYSIS (m/m)		PRODUCT SPECIFICATION (m/m)		INGREDIENTS		BATCH WEIGHTS (g)			PRODUCT ANALYSIS	
cream	48.0	fat	*16.0*	cream	*33.3*	cream	33.3	833	water	47.3
sucrose	100.0	sugar	19.2	sucrose	*19.2*	cas.syrup	49.2	1230	t.s.	40.00
alcohol	75.0	protein	*2.8*	sod.cas.	*3.1*	sucrose	0.0	0	t.s.-sug.	20.83
TSC	30.0	TSC	*0.2*	TSC soln.	*0.5*	TSC soln.	0.5	13	serum	17.33
sod.cas.	90.0	caramel	*0.5*	caramel	*0.5*	caramel	0.0	0	msnf	1.47
		emuls.	*0.0*	emulsif.	*0.0*	x water	-0.0	-0	whey prot.	0.11
LIQUEUR		alcohol	*12.7*	alcohol sl.	*16.9*	emul.	0.0	0	micellar ca	0.45
BATCH SIZE (g)		other	*?*	water	*26.5*	alcohol	16.9	423	tot. prot	3.31
	2500	total sc	*40.0*						p/f (wt.)	0.207
				Total	*100.0*	total	100.0	2500	vf fat	0.18

A; analyses are; cream- fat. sucrose- sucrose, alcohol.; ethanol content of alcohol solution TSC; trisodium citrate content of solution sod.cas. (sodium caseinate); protein content.

B; emuls.- emulsifier content. total sol.- total solids. ;

D. cas. syrup.- caseinate syrup. x.wate.- extra make-up water.

E; t.s.- total solids content; t.s.-sug- total solids - sugar; serum- serum content of cream liqueur. msnf- milk solids not fat content. whey prot. -whey protein.; micellar ca.- ; micellar casein content. tot. prot.- total protein. p/f;- protein fat ratio. vf fat- volume fraction fat.

PROCESSING; EQUIPMENT AND CONDITIONS

The sections below provides more detail on the general process schemes outlined in Fig 2.2.1. All the operations were controlled and standardised by using a digital thermometer (Digitron 3228K, supplied by Manotherm, Dublin) and a stop watch.

Liqueur manufacture by single step (1-S) and two-step (2-S) procedure.

Caseinate syrup production

A typical caseinate syrup formulation is shown in Table 2.2.3. Typical batch sizes were 14-25 kg.

- The dry ingredients (sugar, sodium caseinate, trisodium citrate) were dry blended. (Note, when referring to the concentration of trisodium citrate , we mean the anhydrous salt ; the salt is supplied as the dihydrate. Citrate may be omitted and/or added at another stage).

- Caramel was weighed into a stainless steel or plastic bucket.

- The required amount of water (~ 95 °C), previously boiled in a 25 litre steam kettle was added to the bucket containing the caramel. The liquid was agitated with a Silverson AXR mixer with a general purpose emulsification head and the dry ingredients were added rapidly. Mixing , at full speed, was continued for about ten minutes. The bucket was covered with aluminium foil during and after the operation.

- The mixture was allowed to cool, deaerate and hydrate for about 2-4 hours at room temperature, after which it was stored in a cold-room (4-7 °C). The syrup was usually used during the following two days.

Table 2.2.3 A typical recipe for a caseinate syrup. The inclusion level in the 1-S or 2-S products was typically 40 % m/m of the final product.

Ingredient	% w/w
Sucrose	39.0
Sodium caseinate	6.2
30 % w/w trisodium citrate..$2H_2O$ [1]	1.2
Caramel colour	1.0
Water	52.6

1. The citrate solution can be added after processing if desired; 30 % w/w sucrose solution is added if citrate is not used

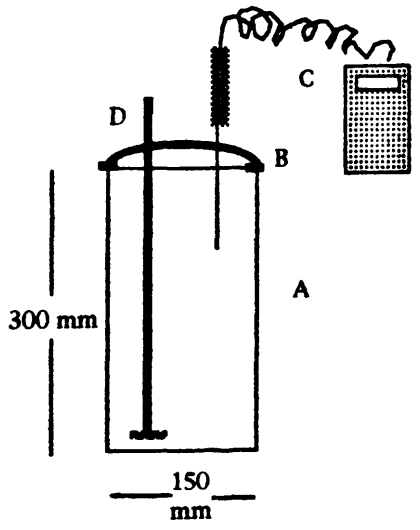


Fig. 2.2.2 Diagram of the container used for weigh ing and processing the cream liqueur samples. A ; Stainless steel can. B ; Removable lid, C Temperature probe entering through a hole in lid D; Stainless steel hand /agitator entering through lid.

Heating and mixing of cream , caseinate syrup

- The cold cream (~ 48 % fat) and cooled caseinate syrup were weighed into a suitable 5 litre stainless steel container (Fig.2.2.2) . Citrate or any other water soluble additive was added at this stage if desired. This mix was termed an"alcohol-free mix" (AFM).

- The can was covered and the temperature probe and hand-stirrer inserted. It was then placed in a steam-kettle , which was half-filled with very hot water (90-100 °C) or in a water-bath (60-100 °C). The temperature of the AFM was brought to ~ 45 °C , with constant stirring / agitation.

- The can was removed and the mix was given a high-shear mix with a Silverson AXR mixer with a square hole emulsor head. Mixing time was normally 30 sec / 2 kg of the AFM. This was to simulate high shear mixing that may occur in industrial practice. This inevitably resulted in some aeration of the mix. The AFM was usually allowed to deaerate on standing for a short period of time (2-10 min).

- *Emulsifier addition (where used)* ; AFM was brought to the approximate melting point of the emulsifier (55-65 °C) . The required amount of emulsifier (plus some extra to account for losses), previously melted inside a glass bottle by boiling water, was added into the mix, with high-shear mixing.

1- S process only; Addition of alcohol solution before homogenisation .

- A 75 % m/m (~ 81.3 % v/v) solution of alcohol was prepared using 94 % m/m alcohol and the relevant process water. An exact amount of this solution, necessary for a cream liqueur sample, was weighed into a labelled Universal glass bottle (500 or 1000 ml)

- The alcohol solution (at ambient temperature) was added quickly and with very efficient and vigorous stirring, to the AFM. A plastic spatula was used as the stirrer. The AFM was normally at ca 45 °C , but sometimes higher temperatures were used for samples containing emulsifiers. At this stage the product can be referred to as the "prehomogenised alcoholic mix" (PHM)

Heating and holding of AFM or PHM before homogenisation..

- The can of AFM or PHM was replaced into the boiling steam kettle/water bath and the liquid brought to the relevant temperature. The can was placed in a water-bath at the processing temperature, if holding of the PHM / AFM before homogenisation was desired

Homogenisation / homogenisers

- Two high-pressure valve homogenisers were used during the study. Most of work was performed on a three piston single-stage Rannie LAB homogeniser (ca 150 litre/ hour). This machine was equipped with a liquid whirling (corrugated) homogenising valve and was capable of a maximum pressure of ca 31 MPa (4500 psi). The other homogeniser was a Manton-Gaulin double-stage machine, equipped with flat (poppet) valves (maximum pressure was 20.7 MPa) and a throughput also of ca 150 l/hr.

- The homogeniser was brought to the processing temperature by flushing hot water, at the appropriate temperature, through it. The PHM/ AFM was poured into the bowl of the homogeniser as the last of the water was visible. The homogenisation pressures were applied as the effluent from the homogeniser started to turn milky. At this point about 1.5 -2 litres of milky liquid product were allowed to go to waste before a sample was collected (see below).

- Cleaning of homogenisers; the homogenisers were cleaned at the start of every production run by recirculation with, in turn, 0.5 % NaOH, hot water, 0.5 % nitric acid and hot water. All solutions were at ca 60-70 °C. Hot water was used to clean the homogeniser between samples. Hot detergent solution was used occasionally to clean the machine between a series of samples.

Collection and cooling of samples

- *Sampling*: One pass products , about 0.5 l of sample was collected from the original 2.5-2.7 l . The sample was usually collected in the now empty glass bottle used for the alcohol addition. For two pass products, ~ 2.5-2.7 l were collected from the original 4.5-4.7 l sample after the first pass. A small sample (~0.1- 0.5 l) of this first pass product was then taken, before the remaining product was homogenised again. A sample of the second pass product was then taken as before.

- Cooling: The glass bottles, containing the samples, were immersed in water (8-11 °C) and left, with frequent inversions, for about 40-60 min.

2- S process only; Addition of alcohol solutions to homogenised AFB ;

When the homogenised alcohol free mixture had cooled to ambient temperature, the required amounts of AFB and 75 % m/m alcohol were weighed out in separate beakers. The alcohol solution was stirred into the AFB with a wide-bladed spatula or a laboratory mixer, until a completely homogenous mixture was obtained. Typically 500 -1000 g of final product was produced.

Liqueur manufactured by the 2-Sb process

Production of a cream emulsion.

A mixture of cream and water was heated in the stainless steel cans as before. Sodium caseinate and citrate (if required) were dissolved with high shear mixing; This was accomplished at ~ 45 °C or, if emulsifiers were used, at 55-65 °C. This mixture (termed the cream emulsion; see Table 2.2.4) was homogenised and cooled as before.

Production of alcohol syrup for 2-Sb products.

All of the sugar and alcohol and some of the water were added after homogenisation. A mixture (alcohol syrup) of alcohol, sugar, caramel and the remaining water is made up. A recipe for the alcohol syrup is also shown in Table 2.2.4. The sugar and caramel are first dissolved in the heated water and after cooling, the alcohol is added to

this mixture. This solution was added to the aforementioned cooled cream emulsion to produce the final liqueur. Other ingredients e.g. citrate could also be incorporated at this stage, if desired.

Table 2.2.4 Typical recipe for cream emulsion and alcohol syrup used in the production of cream liqueur by two step process 2-Sb.

Ingredient	% w/w
Cream emulsion (e.g. 50% w/w inclusion)	
Cream (48 % fat) [1]	67.0
Sodium caseinate	5.5
30 % w/w trisodium citrate.. $2H_2O$ [2]	1.2
Water	26.3
Alcohol syrup (e.g. 50 w/w addition)	
Sugar	38.0
94 % w/w alcohol	27.0
Caramel	1.0
Water	34.0

1 Emulsifier can be added to this portion if desired
2 The citrate solution can be added at another stage if desired. A 30 % w/w sucrose solution is added if citrate is not added.

2.3 Testing of emulsions

Fig 2.2.3 summarises the test procedures carried out on the cream liqueur samples. The most important tests were total solids, pH , viscosity, shelf life tests (outlined below) and spectroturbidimetry (see later).

CHEMICAL TESTING

Protein

% Protein was measured by Macro-Kjeldahl (Kjeltech system).

Fat

Gerber method

A modified Gerber method was used:

1. Three grammes (2.99-3.01g) of cream liqueur was weighed into a cheese butyrometer which already contained 10 ml of sulphuric acid. (The liqueur should be dispensed gently down the side of an angled butyrometer). Amyl alcohol (1ml) and water (8 ml) were added in quick succession. The sample was completely dissolved with the minimum of gentle swirlings and inversions. Shaking should not be used.

2. The butyrometer was completely immersed (stem upwards) in very hot water (80-90 °C) for 10 min. At this stage at least 3 mm of free fat should be visible. The sample was then spun in a heated Gerber centrifuge at ca 200 *g* x 15 min. Occasionally, the immersion and centrifugation procedure was repeated for a second time.

3. The reading of the fat column in the Gerber tube was estimated to the nearest 0.2 % at 65 °C. Duplicate or triplicate samples were performed.

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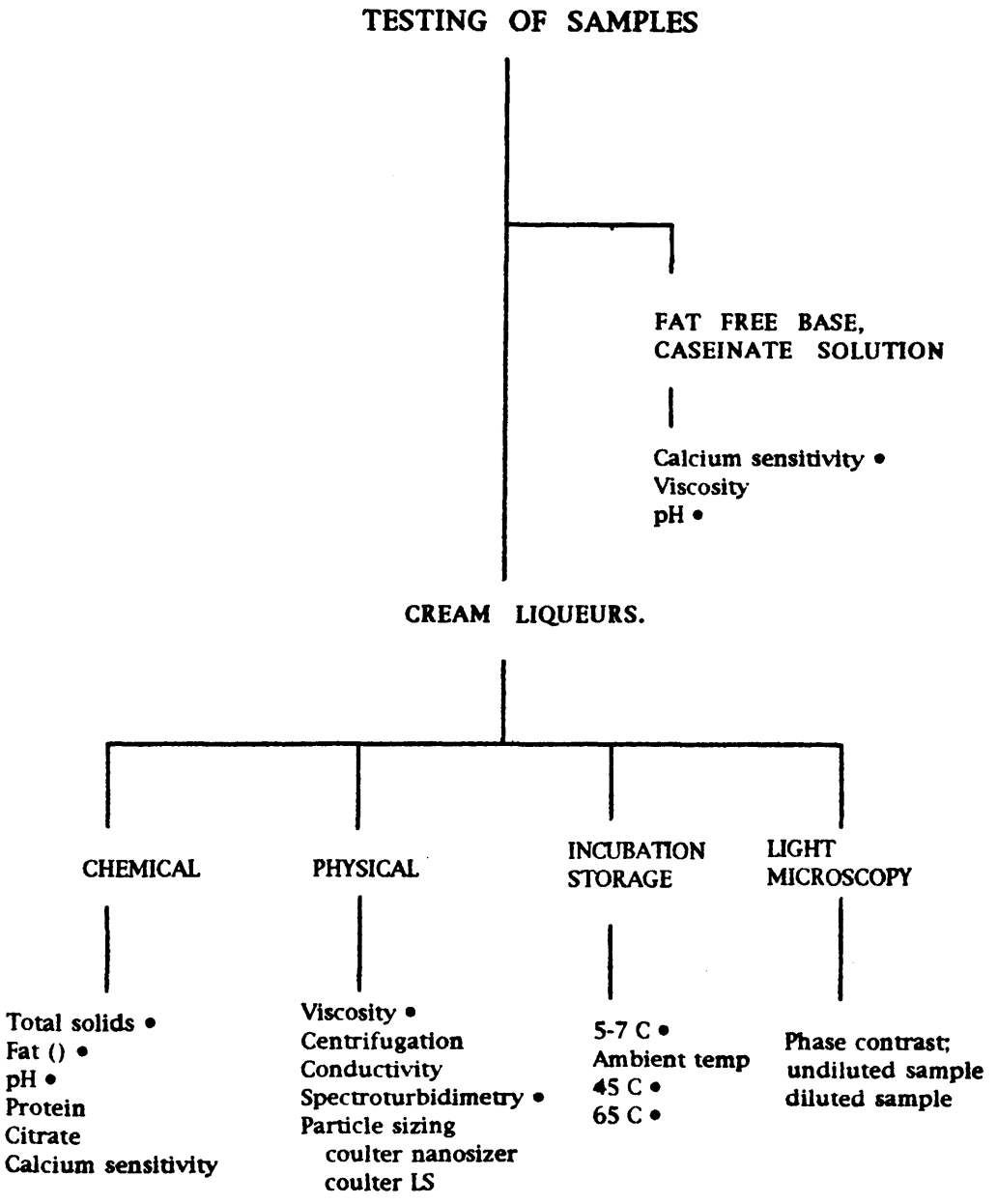


Fig 2.2.3 Scheme summarising the test procedures used in the study. Details of the methods can be found in the General Methods or Experimental Sections.. Only tests marked (•) were used on a routine basis.

Rose-Gottlieb Method.

The standard milk Rose-Gottlieb procedure for milk was used (IDF 1C;1987). To allow for the higher level of fat in cream liqueurs, ~ 2.5 g cream liqueur and 7.5 ml of water were used instead of 10 ml of milk in the procedure. A few drops of red dye aided visualisation of the solvent interface. A 3 decimal balance was used for weighings, hence the results are reported to one decimal place.

Total solids

A hot plate / vacuum oven drying technique was used. Approximately 1.5 g of sample (to 3 decimal places) was added to a metal solids dish and ~ 2 ml of water added. The mixture was swirled before the excess liquid was boiled off on a hot plate (~ 180 °C). The sample was transferred to a vacuum oven at 68 kPa at 100 °C for 1 hour. Samples were cooled in a desiccator for 30 min before weighing. All weighings were to 3 decimal places and results were reported to 1 decimal place.

pH

The pHs of samples were read at 20 °C, with a Radiometer PHM82 meter. The meter was calibrated with pH 4.00 and 7.00 buffers (Reagecon, Limerick) prior to testing and thereafter after 4-5 samples. The samples were allowed to equilibrate for ~ 30-45 s before reading. Final equilibration probably takes 2-3 min but it was not feasible to wait for this period with large numbers of samples. Any errors would have been small and consistent.

Ash

Approximately 1.5- 2.0 g of sample was weighed into a crucible and this was charred carefully over a bunsen. It was removed from the flame when no more smoke was visible. The crucible was placed in a muffle furnace at 550 °C for 4 hours. It was cooled in a dissector and weighed on a 4 place balance.

Citrate

An enzymatic bioassay (Citric Acid UV Test; Boehringer Mannheim) was used to estimate the amount of citrate ion in the cream liqueurs. The principle of the test is as follows: Citrate is broken down to oxalacetate and acetate by citrate lyase. These are reduced with NADH , to malate and lactate, by malate and lactate dehydrogenase

respectively. The disappearance of NADH is monitored by a decrease in UV adsorption, and this is proportional to the amount of citrate originally present. The extraction and testing method used was basically that described for bread, cheese etc in the test method literature (Boehringer Mannheim, 1992). See Appendix 4 for details.

PHYSICAL TESTING

Viscosity

The viscosity of samples was measured with a Brookfield RVT Synchrolectric viscometer equipped with a UL adapter and coaxial cylinder type spindle.

All sample bottles were inverted gently to ensure a homogenous sample for analysis. Samples (16-17 g) were weighed into the special cup which, when fitted to the viscometer, was surrounded by a beaker of water maintained at 20.0 +/- 0.1 °C. All fresh liqueurs were measured at a shearing rate of 122.3 s^{-1}. Maximum viscosity under these conditions was ca 66 mPa s. Thicker samples necessitated measurement at lower shear rates. The accuracy of the instrument was checked against a Brookfield calibration fluid of viscosity 29.9 mPa s (supplied by Lennox, Dublin). All readings were multiplied by a factor (1.03) to account for an error present in the instrument throughout the study.

Calcium sensitivity test

The calcium sensitivity of cream liqueurs was measured by quantifying the turbidity or increase in turbidity, on addition of samples into an alcoholic/sugar solution containing 1mM Ca^{++}. Measurements were made at one or more of the following wavelengths; 400, 600 and 800 nm.

A Ca^{++}-containing solution was made up of ; 1 mM Ca^{++}, 25 % w/v sucrose, 21.7 % v/v alcohol and 20 mM NaCl. An Analar $CaCl_2$ solution (1M) was used as a source of Ca^{++}. A control solution contained no calcium. The solutions were adjusted to pH

6.6 or 7.0 with 0.1 M NaOH. The conductivity of the above solutions was about 500 mMHO. The composition of the above solution simulated the continuous phase of a cream liqueur.

Normally, 0.1ml (or 0.01ml) of cream liqueur was added to 100 ml (or 10 ml) of solution. The samples were mixed by inversion and read after 10 minutes. The Shimazu spectrophotometer mentioned previously was used.

Conductivity

The conductivity (mho) of samples was measured with a Radiometer CDMZF instrument. The probe was rinsed with distilled water before each reading.

Creaming tests

Undiluted emulsions, or emulsions diluted by half with water, were centrifuged at 1000 ***g*** x 30-60 min in graduated tubes. The amount of visible creaming and serum/sediment was observed.

The amount of creaming, after leaving samples undisturbed in glass bottles at ambient temperature, was also noted.

STORAGE / INCUBATION TESTS

The freshly processed samples (0-12 hours old) were transferred to a series of 50 or 150 ml Universal glass bottles. Samples were stored, undisturbed, in the dark at ambient temperature and at 5 °C in a cold room. They were observed for defects at regular intervals. Such defects consisted of rings, lines and collars of creamy material at the top of bottles, watery serum at the bottom, redispersible cream caps/layers and gelation (with or without syneresis).

Samples (20 or 50 ml) were incubated at 45 °C for 28 days and observed for defects and/or measured for viscosity. Initially, the sample was replaced into the incubator after the first measurement for use in further readings. However, most of the samples in the study were read with previously undisturbed samples.

Another test (heat test) involved heating ca. 2 ml samples in a capped test-tubes in a waterbath at 65 °C. After 1 hour the samples were cooled in cold water and examined for degree of thickness

MICROSCOPY

Occasionally, phase-contrast microscopy was used for observation of liqueurs. A Leitz DMR microscope (Leica Microscopes, Wetzlar, Germany) with a Leica Wild MP S48 exposure unit was used.

Either undiluted samples or emulsions that had been diluted to ~ 2 % fat with a 5 % gelatin solution (pH 7.0) were used. In each case, a 5 µl drop of sample was placed on a clean slide. A clean cover slip was placed over the drop and this was pressed gently to spread the drop over the area of the coverslip.

Samples were observed for appearance under various magnifications and these were photographed at short exposure times (~ 0.2-0.8 s) when the Brownian movement had disappeared. This occurred quickly (5 min) for the gelatin -containing samples but took up to 20 min for the undiluted samples. Samples were prevented from drying out by sealing the edges of the coverslip with nail-varnish or "Tipex".

EXPERIMENTAL

CHAPTER 3

Preliminary experiments.

Experiment 3.1 *Simple methods for assessing particle size in cream liqueurs.*

INTRODUCTION AND OBJECTIVE.

Emulsions, once formed need to be characterised. Most of the tests or techniques use established commonly available procedures/instruments and these needed little adaptation or investigation for the purposes of this study. However, for the estimation of particle size, which is one of the most important characteristics of an emulsion, most of the dedicated instrumentation is very expensive and is not found in commercial dairy laboratories.

Since it is one of the objectives of this study to use simple techniques and commonly available instruments to characterise cream liqueurs, it was decided to explore the use of simple spectroturbidimetry in this area. Spectroturbidimetry has been widely used as a means of characterising the properties of many dairy emulsions (see Literature Review), but there is little or no relevant data on its application with very fine food emulsions e.g. cream liqueurs. Monodisperse latex particles and real emulsions (including cream liqueurs) were used to study the accuracy and significance of the data produced by a laboratory spectrophotometer. The results from a Coulter Nanosizer were also evaluated.

MATERIALS AND METHODS

Polystyrene (latex) particles

Polystyrene latex beads (diameters 0.114, 0.303, 0.605, 0.825 and 2.967 μm) were obtained from Sigma (Poole, England). The numbers of the particles per unit weight, or volume, of solid latex relative to the 2.967 μm size (which was arbitrarily assigned a value of unity, i.e. 1) can be calculated to be ; 0.114 μm (17,629), 0.303 μm ; (939), 0.605 μm ; (118) and 0.825 μm ; (46.5). These preparations were obtained as 10 % w/w suspensions and they were diluted to a concentration of 0.016 % w/v e.g. 16 mg solids /100 ml in diluent. The suspensions were ultrasonicated before measurement of absorbance, to ensure complete dispersion.

Artificial polydisperse suspension systems were produced by combining a number (2,4 or 5) of the aforementioned monodisperse latex suspensions. In the first set of samples, three different bimodal suspensions were formulated containing various quantities of two lattices. All these contained the 0.114 μm latex, along with a quantity of either of the 0.605, 0.825 or 2.967 μm ones (see later). In the second set, mixtures of four or five suspensions were combined to produce a more "bell-shaped" unimodal distribution.

Emulsions

Commercial cream liqueur samples (Emmetts and Baileys Irish cream liqueurs) were obtained from the marketplace. Other liqueur samples were manufactured for this study.

Raw milk (University supply) was homogenised at various pressures at 50°C in a Rannie LAB single stage homogeniser (150 l/hour).

A coarse vegetable oil emulsion was produced by mixing 40 % by volume of vegetable oil with 60 % by volume of a 2.5 % w/v sodium caseinate solution (Kerrynor, Kerry Ingredients, Tralee). The mixing was performed with a benchtop Silverson Mixer, type STD-1 (Silverson Machines, Chelsam, England) at full speed for 1 min at 50°C.

Diluents

The following diluents (at 20 +/- 2 °C) were used to disperse emulsions or suspensions.

* *distilled water; this was the most commonly used diluent*

* *a so called "EQA" solution The EQA solution is a very weak sodium hydroxide solution that contains some EDTA and sodium tetraphosphate. It is a proprietary product and is made up from a concentrate according to the manufacturer's instructions (APV, England). It is used to solubilise casein micelles which interfere with the measurement of fat particle size in homogenised milk samples using the 'Emulsion Quality Analyser" instrument (see later).*

* *a detergent containing solution e.g. ; 0.1 % of sodium dodecyl sulphate , SDS, or the non-ionic detergent DP14 (Sigma, Poole, England)*

* *a solution which simulated the continuous phase of a cream liqueur i.e. 22 % v/v ethanol (alcohol), 25 % m/v sucrose*

Fat analysis

Fat was measured in cream liqueurs by Rose-Gottlieb method (see General Methods & Materials section), in milk by the Gerber method (I.S 65;1955) and vegetable oil emulsions by the Gerber method (I.S. 68; 1955). Note: the density difference between vegetable oil and butteroil did not significantly alter the results using this method.

Spectroturbidimetry , turbidity value and ratio index.

The absorbance of latex suspensions or emulsions, at various wavelengths, was measured with a Shimazu UV-120 spectrophotometer. The samples were left for at least 5 mins before reading, and reported values were the average of at least two but usually three dilutions. Matched glass cuvettes were used, with the blank cell containing the diluent.

For the purposes of this study, empirical values or indices were arbitrarily defined from absorbance data. Two such indi ces are outlined here:

"Turbidity value" (TV) can be defined as the absorbance, in 1 cm glass cuvettes and at a given wavelength, of an emulsion/suspension diluted to 0.016 % m/v. (Note: this is approximately the concentration of milkfat recommended for used with the "Emulsion Quality Analyser"; a spectrophotometric based method for determination of average particle size in homogenised milk).

Normally a mass of sample that contained 16 mg fat was quantitatively transferred to a 100 ml volumetric flask with the appropriate diluent. For emulsions, this entailed a knowledge of the fat content of the sample; e.g. 100 mg of 16 % fat liqueur was commonly used to produce dilutions containing 16 mg fat /100 ml at 20 °C.

Note; the term "absorbance value", AV, was not used in order to avoid confusion with the abbreviation for another light-scattering index, AI , which will be defined latter. Turbidity and absorbance are directly proportion, at any given wavelength, in any given optical set-up, provided that none of the scattered light reaches the photodetector and the particles are non-adsorbing (Pearce & Kinsella, 1978).

Ratio value or "R" this parameter can be defined as the TV at 800 nm divided by the reading at 400 nm. It gives an indication of the wavelength dependence of the turbidity value. (Since R is calculated as a ratio, the concentration of the disperse phase is not critical).

Calculation of different particle sizes of polydisperse latex particles mixtures.

As mentioned previously, polydisperse latex particle suspensions were produced by mixing (by weight or volume), different monodisperse suspensions. The weights (or volumes) were chosen arbitrarily.

In this situation, we know the parameters d_i (i.e. diameter of the latex particles) and the relative volume (V_R) contributed by each of different particle sizes. From this data we can calculate the relative numbers (n_i) of each of the particle sizes such that the correct relative volume distribution is obtained.

For example, the relative amounts of 5 monodisperse suspensions are shown in the right hand column below (the target values). Correct values of n_i (a, b,c, d and e) must now be inserted, such that the calculated relative volumes correspond to the target values. This was done by trial and error, using a standard spreadsheet package which contained the equations below .

d_i (um)	n_i	v_i (volume of particles)	Calculated relative volume distribution V_{CR},	Target relative volume distribution V_{TR}. (i.e the relative amounts weighted out)
0.144	a	$= a * 4/3\pi (0.144/2)^3$	$= (v_i / \Sigma v_i) * 100$	5.7
0.303	b	$= b * 4/3\pi (0.303/2)^3$	"	10.7
0.605	c	$= c * 4/3\pi (0.605/2)^3$	"	67.9
0.825	d	$= d * 4/3\pi (0.825/2)^3$	"	10.8
2.967	e	$= e * 4/3\pi (2.967/2)^3$	"	5.0
		Σv_i = sum of above		

In the above case, the relative values of a, b, c, d and e were found to be ; 20,000, 2000, 1600, 100 and 1, respectively. The various particle diameters can now be calculated since d_i and n_i are known for the suspension.

The *volume surface diameter* (d_{vs} or d_{32}) is calculated using the equation;

$$d_{32} = \Sigma d_i^3 N_i / \Sigma d_i^2 N_i =$$

$$\frac{[(0.144)^3 * 20{,}000] + [(0.303)^3 * 2{,}000] + [(0.605)^3 * 1{,}600] \ .....}{[(0.144)^2 * 20{,}000] + [(0.303)^2 * 2{,}000] + [(0.605)^{2*} 1{,}600) \ .....} = 0.472 \ \mu m$$

Similar ly, the *number average droplet diameter* (d) can be defined as;

$$d = \Sigma d_i N_i / \Sigma N_i$$

The *mean volume or weight diameter* (d_v) can be defined as ;

$$d_v = (\Sigma d_i^3 N_i / \Sigma N_i)^{1/3}$$

The *mean diameter of the volume size diameter* or *volume related mean diameter,* is d_{43} ;

$$d_{43} = \Sigma d_i^4 N_i / \Sigma d_i^3 N_i$$

Measurement of particle size with various instruments

The average particle size, d_{43}, of homogenised milk samples was calculated with the Emulsion Quality Analyser (APV, England), according to the manufacturer's instructions. In brief this involved the measurement of the absorbance of a diluted solution of milk (ca 16 mg/100 ml in EQA solution) at 900 nm and the conversion of this figure into the average diameter in microns (μm) using a chart.

The average particle sizes of selected cream liqueur samples and latex particle suspensions was estimated in the laboratory using photon correlation spectroscopy ("Coulter Nanosizer", Coulter Electronics, Luton, England). The instrument gave the following readings (in brackets) for the latex bead standards ; 0.114 (0.115), 0.303(0.307), 0.605(0.609), 0.825(0.850) , 2.967(2.920) μm . Emulsions and suspensions were diluted such that the gain level obtained on the instrument read 25 %.

A few samples of cream liqueurs were analysed by a laser diffraction particle size analyser, i.e. a Coulter LS130, in the manufacturer's laboratory in Luton, England The data generated by this instrument was analysed with either of two treatments; a Fraunhofer model (F) and a specific optical model (OMD). The OMD model was calculated assuming the refractive indices of water (diluent) was 1.333 and milkfat (particle) was 1.462 (Muir et al, 1991). The company's own patented "polarisation intensity differential scatter" (PIDS) operating system was also used.

Photomicrographs were taken (see General Methods and Materials) mostly in order to visualise and compare particle sizes but, in the case of raw milk and the vegetable oil emulsions, the technique was used to give a rough estimate of particle size. This involved counting the number of fat globules in a given size band using the light microscope (mag. x1000). This raw data, i.e. number n_i and diameter d_i, was used to calculate statistical average particle diameters (e.g. d_v, d_{32}, d_{43} etc) according to the formulae outlined above for the latex particles.

RESULTS

Turbidity values of suspensions and emulsions

Latex particles

The turbidity value (TV) of mondisperse latex beads (16 mg of beads /100 ml), depended on the particle size and the wavelength of measurement. This is illustrated on linear (Fig. 3.1.1) and log/log plots (Fig. 3.1.2). The TVs for some of suspensions were very high but multiple light scattering did not seem to be a problem, since the values decreased linear ly when diluted further (data not shown).

For particles < 0.85 μm , the TVs at long wavelengths decreased with decreasing particle size. For example, the A800 readings for 0.825 and 0.114 μm suspensions were 1.256 and 0.045, respectively. Readings also decreased with increasing wavelength. The rate of this decrease is best judged in the log/log plot (Fig. 3.1.2). These slopes (the slope parameter) were calculated and plotted against particle size (Fig. 3.1.3). The slope parameter was inversely proportion to the particle size. Other indices correlating to particle size in these suspensions were also plotted in Fig. 3.1.3. These included the TV at 400 and 800 nm (A400, A800) and the wavelength ratio index, R (i.e. ratio of the absorbencies at 800/400 nms).

Except for the TV (A400), there was a good linear relationship between the indices and average particle size for the smallest four particles. Equations relating particle size in the range 0.114 - 0.825 μm with TV(A800) and R for these monodisperse suspensions are given below;

$$Size\ (\mu m) = 0.089 + 0.548 * TV_{A800} \quad (r^2 = 0.98) \qquad 3.1$$

$$Size\ (\mu m) = 0.047 + 1.217 * R \quad (r^2 = 0.99) \qquad 3.2$$

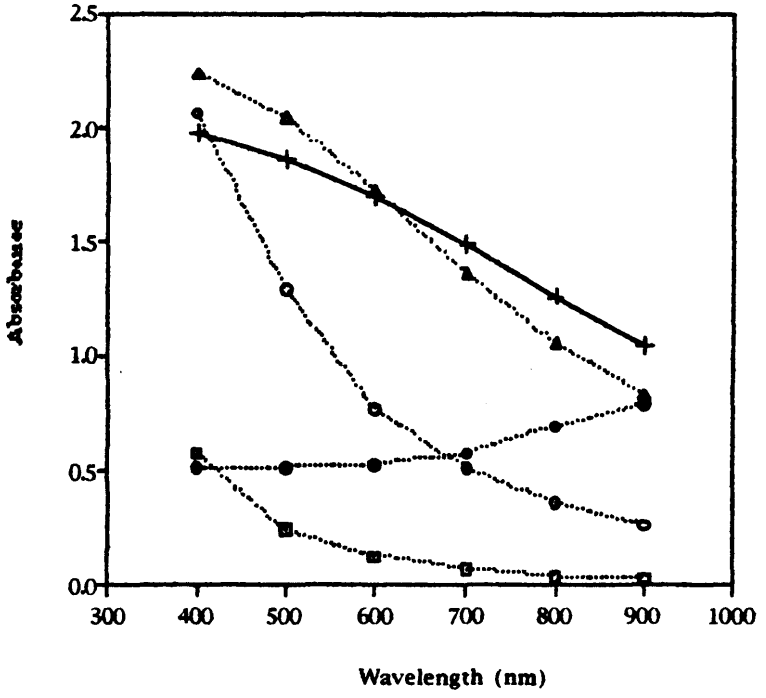


Fig 3.1.1 The effect of increasing wavelength on the turbidity value of latex suspensions. of various sizes. • ; 2.967 , +, 0.825 Δ,: 0.605 o; 0.303 and o ; 0.114 μm.. In all cases ,the concentration of the latex particles was 16 mg/ 100 ml water.

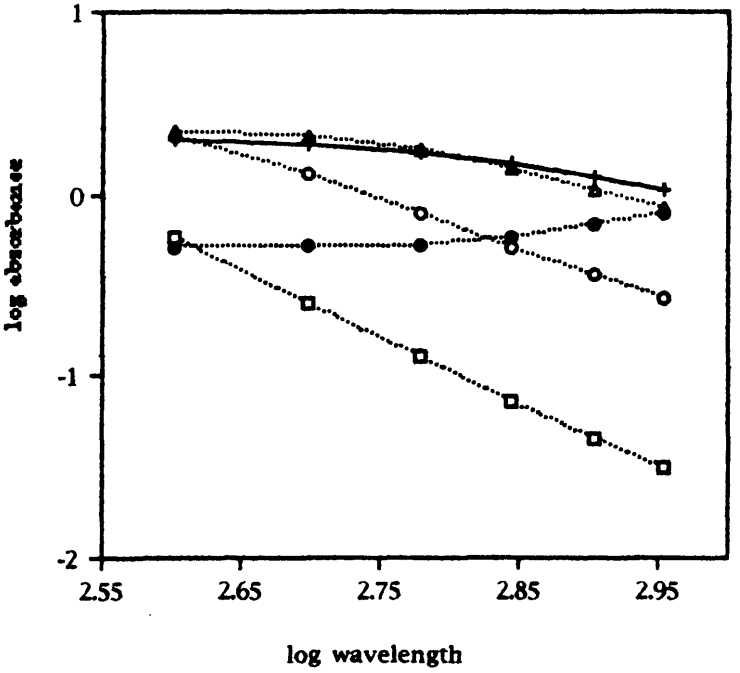


Fig 3.1.2. Log / log plot of turbidity values versus wavelength for the latex suspensions shown in Fig. 3.1.1. Legend is as above..

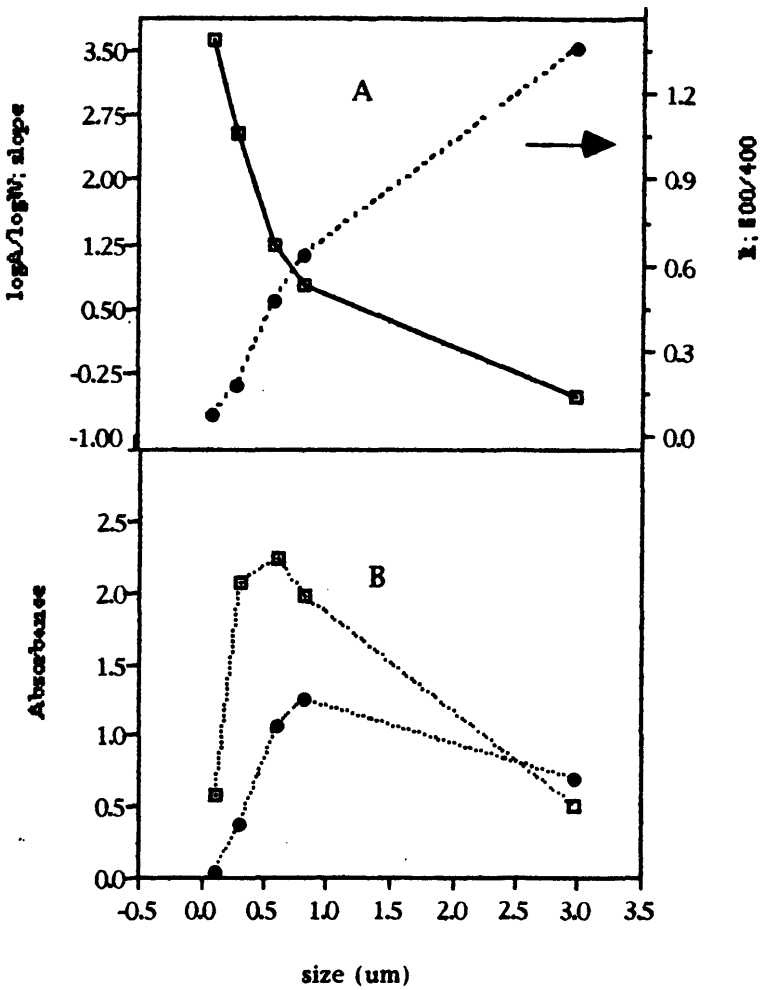


Fig. 3.1.3 The effect of increasing particle size on various indices , calculated from light-scattering data shown in Fig. 3.1.1 A Particle size plotted against the log/log slope parameter (□) or the R value (●). B Particle size plotted against the absorbancies A800 nm (●) or A400 nm (□).

The response of the 2.960 μm suspension was very different from that of the smaller particles (Fig 3.1.1). The TVs were relatively independent of wavelength between 400-700 nm but, in contrast to the trends shown by the particles < 0.825 μm , they increased slightly with increasing wavelength thereafter. The slope and R value of this suspension show better correlation to particle size than do individual readings at A400 or A800 (Fig. 3.1.3).

Emulsions

Absorbance data were obtained using five food emulsions which were considered to be of varying size and size distribution as indicated from photomicrographs (Fig. 3.1.4) and literature data (e.g. see Literature Review and also Keohane, 1994). These emulsions were classified as - very fine (cream liqueurs; d_{43} ca. 0.2-0.4 μm), fine (homogenised milk; d_{43}; 0.63 μm), coarse (unhomogenised milk; d_{43} ca 1.5-2.0 μm) and very coarse (Silverson-mixer produced vegetable emulsion d_{43} 13 μm).

The turbidity values of the five different emulsions, as a function of wavelength, are shown in Fig 3.1.5. The responses fell into the categories noted previously for the latex particles. The finer emulsions (the cream liqueurs and the homogenised milk), gave curvilinear responses to increasing wavelengths while the coarse emulsions (unhomogenised milk, vegetable-oil emulsion) showed little change in absorbance with change of wavelength.

The R and the TV (A800) values of the emulsions are presented in Fig. 3.1.6. As expected, the A800 and the R value decreased with decreasing average particle size for the four emulsions in the range 0.2-2.0 μm. Whilst having a very large R value, the very coarse vegetable oil emulsions had a very low turbidity values.

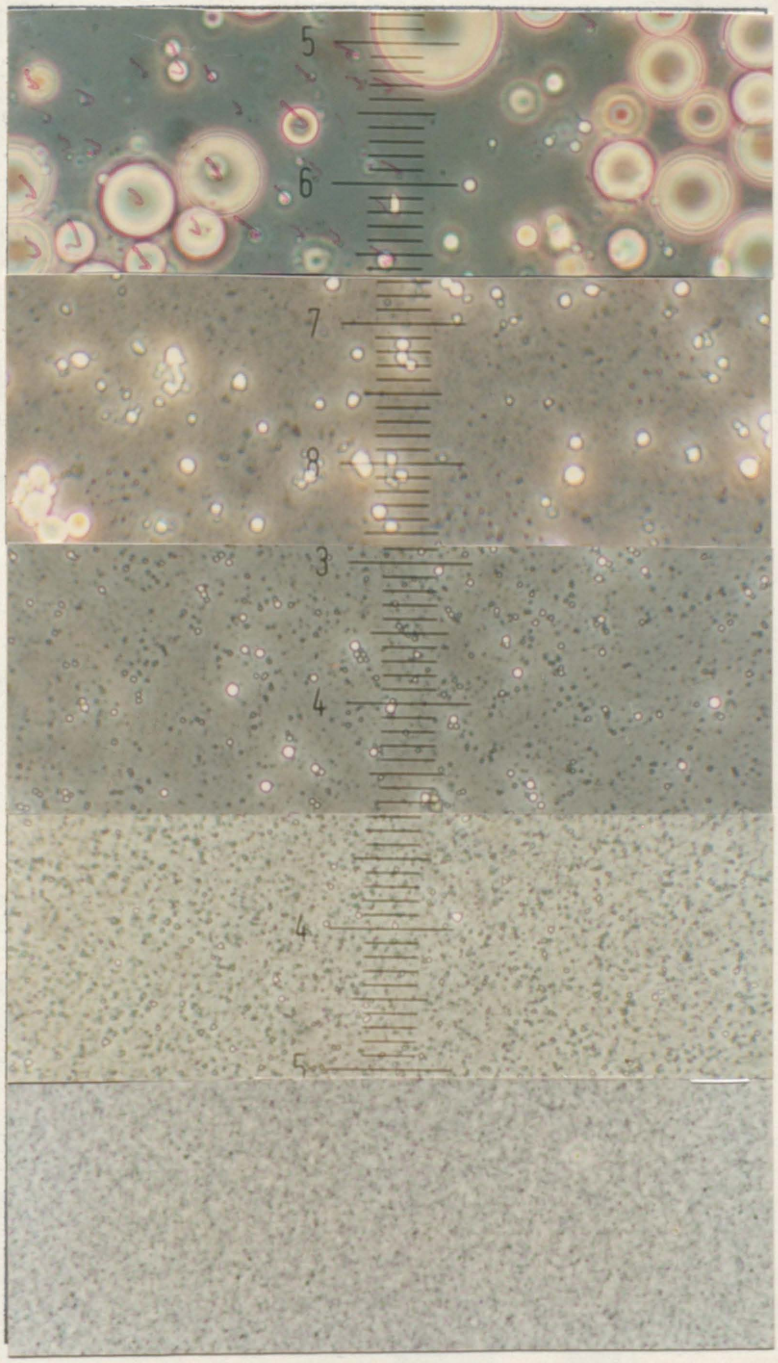


Fig. 3.1.4. Photomicrographs of emulsions with different particle size distributions. a) coarse vegetable oil emulsion b), c), and d) milk homogenised at 0, 6.9 and 24 MPa repectively, with the Rannie homogeniser and e) a cream liqueur. Magnification x 400. Bar indicates scale.

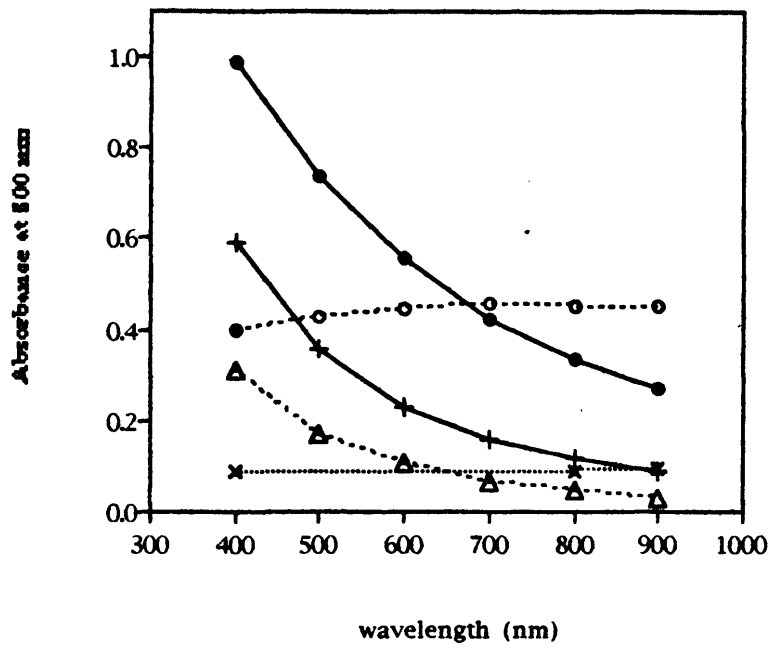


Fig. 3.1.5. The effect of wavelength on the turbidity values (16 mg fat / 100 mls EQA) of various emulsions.. Legend; • homogenised milk (17 MPa) + Emmetts , Δ, Baileys , * coarse emulsion (mixer) o unhomogenised milk.

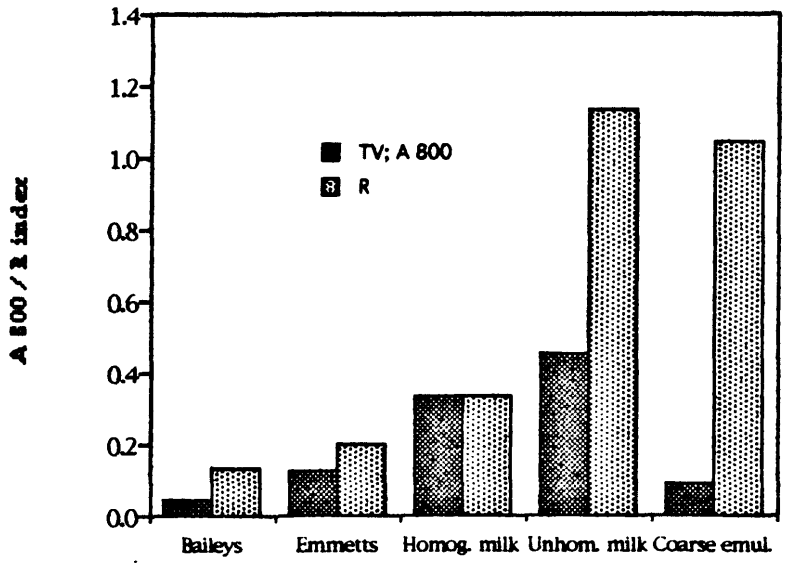


Fig 3.1.6 A comparison of the turbidity (A_{800}) and R values for the 5 emulsions shown in Fig. 3.1.5 .

Comparison of turbidity values and particle diameters obtained by other methods.

mixtures of latex beads

In the first experiment, three different suspensions containing the 0.114 μm latex and one other latex size were formulated (Table 3.1.1.). These bimodal suspensions could be calculated to contain an algebraic average weight diameter of 0.303 μm e.g. for sample 1 (0.114 μm x 0.61) + (0.605 μm x 0.39) = 0.305 μm .

Of the average diameters *calculated* using the d_i and n_i data for the above suspensions, the number diameter (d_n), volume-number diameter (d_v) and volume-surface diameter (d_{32}) were all very low (ca. 0.114- 0.167 μm) compared to the average weight diameter (d_{43}), which corresponded exactly to 0.305 μm .

Experimental average diameters were also derived using TV, R and Nanosizer readings. The diameters obtained from the TV(A800) readings (equation 3.1 , earlier), were in relatively close agreement with the calculated d_{43} for systems 1 and 2 but with d_{32} for system 3. The diameters derived from the R value (equation 3.2, earlier) were greater than the d_{43} for systems 1 and 2 and but were intermediate between d_{32} and d_{43} for system 3. Nanosizer readings correlate well enough with d_{32} for systems 2 and 3 and a value between d_{32} and d_{43} for system 1 (Table 3.1.1).

In the second experiment, different calculated and experimental particle diameters for the more unimodally distributed suspension mixtures , A and B, were obtained Table 3.1.2. The experimentally found Nanosizer diameters were closest ca (10-15 % higher), to the calculated d_{32}. The results calculated from spectrophotometric data were closer to the d_{43}, but by varying degrees. For example, the values calculated from the R value were 6 % higher and 12 % lower than the d_{43} for suspensions A and B respectively while the diameters calculated by TV(A800) were 23% and 8 % higher than the d_{43}.

Table 3.1.1. The different average *calculated* diameters * (dn, dv, d32, d43) and *experimentally* found diameters ** (using Nanosizer, R value and TV values) of three different bimodal latex mixtures . The latex suspensions were formulated by mixing the stated volumes of the individual latex suspensions.. The relative number of particles and the calculated diameters were estimated as outlined in the Materials and Methods section of this experiment.

Sample	Latex (μm)	Relative no. of particles	% Volume (weight)	average diameters (μm)						
				* d_n	* d_v	* d_{32}	* d_{43}	** nanosizer	** R value	** TV A800
1	0.114	234	61.0							
	0.605	1	39.0	0.114	0.134	0.167	0.305	0.233 (6)	0.372	0.335
2	0.114	1031	73.1							
	0.825	1	26.9	0.115	0.126	0.149	0.305	0.179 (5)	0.424	0.286
3	0.114	243655	93.3							
	2.967	1	6.7	0.114	0.117	0.122	0.307	0.115 (2)	0.234	0.137

d n	number average diameter
d v	volume mean diameter
d 32	surface -volume mean diameter
d 43	equivalent volume mean
nanosizer	average particle size found by Coulter Nanosizer ; number in brackets is an index of the polydispersity of the distribution (0-9)
R and TV (A800)	average particle sizes calculated from R values or turbidity values (A800) ; see Equations 3.1 and 3.2.

Table 3.1.2 Two model suspensions were formulated (upper half). The calculated (*) and experimental (**) average diameters are reported (lower half). Calculated diameters were estimated as outlined in the Materials and Methods .

Latex size (μm)	% Volume (weight)	
	Sample A.	Sample B
Formulation		
0.114	18.4	5.7
0.303	30.6	10.7
0.605	32.5	67.9
0.825	18.5	10.8
2.967	0.0	5.0

Results

diameter type	diameter (μm)	
	Sample A.	Sample B
*d n	0.136	0.166
*d v	0.194	0.280
*d 32	0.296	0.472
*d 43	0.463	0.687
** Nanosizer	0.340	0.505
** TV	0.570	0.740
** R	0.490	0.602

d n	number average diameter
d v	volume mean diameter
d 32	surface -volume mean diameter
d 43	equivalent volume mean diameter
Nanosizer	average particle size by Nanosizer ; number in brackers is an index of polydispersity distribution (0-9)
R and TV	average particle sizes calculated from TV A800 or R values ; see Equations 3.1 and 3.2.

Emulsions

Milk was homogenised at different pressures to produce emulsions of varying particle sizes (Fig 3.1.7). Nanosizer diameters were ca 0.51-0.66 % of the d_{43} results, which were calculated with the Emulsion Quality Analyser; the correlation increased with decreasing d_{43}. There was a very good correlation between average particle size (d_{43}) and the turbidity values from the laboratory spectrophotometer; equation 3.3 describes a relationship using TV(A 900).

$$d_{43}\ (\mu m) = 0.0185 + 2.458 * A900 \quad (r^2 = 1.00) \qquad \textbf{3.3}$$

There was a good also relationship between the TV (A800) and Coulter Nanosizer average particle size for a wide variety of cream liqueurs (Fig.3.1.8). Note that for a ca 50 % increase in Coulter particle diameter there is a 3.5 fold increase in TVs. Equations 3.4 and 3.5 describe the correlations for a set of 10 commercial and 13 laboratory- produced liqueurs, respectively. The laboratory samples covered a wider range of values. These readings were taken with water as the diluent.

$$\text{Nanonsizer diameter } (\mu m) = -0.15 + 1.06 * TV_{A800} \quad (r^2 = 0.96) \qquad \textbf{3.4}$$

$$\text{Nanosizer diameter } (\mu m) = -0.341 + 0.0019 * A800 \quad (r^2 = 0.91) \qquad \textbf{3.5}$$

The relationship between the nanosizer average particle diameter and results obtained on the Coulter LS190 was less obvious (Fig 3.1.8). There was a relatively good relationship, especially for commercial products, between the d_{32} (LS190 data) and the Nanosizer diameter (assumed also to be d_{32}). LS 190 readings were lower than Nanosizer readings for particles less than 0.23 μm but significantly higher otherwise. The LS 190 can generate volume distribution curves in the range 0.1-1000 μm (Fig. 3.1.9), along with different average particle data. The amount of fat present in

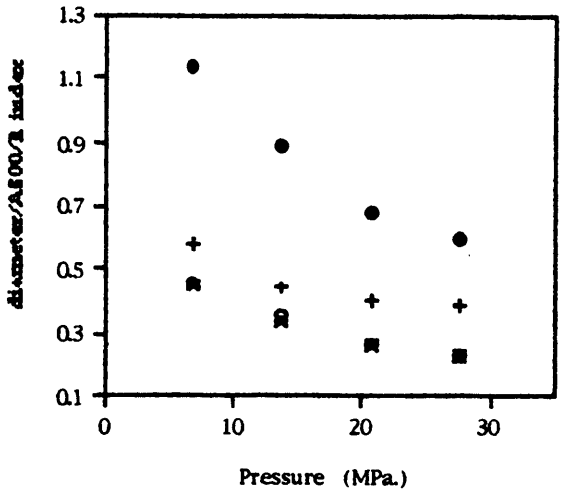


Fig. 3.1.7 The effect of increasing homogenisation pressure on the average particle size of homogenised milk samples. Readings were taken with the Emulsion Quality Analyser (•, d43) or with the Coulter Nanosizer (+). The turbidity values at A900 (*) and the R value (o) from the Spectrophotometer are included for comparison. Samples were homogenised on the Rannie LAB machine.

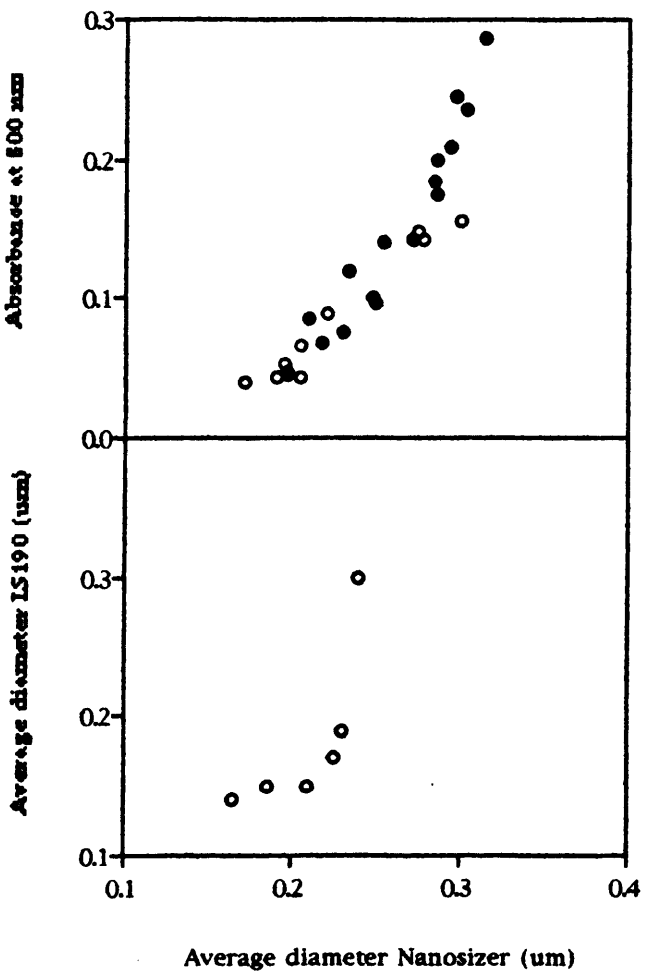


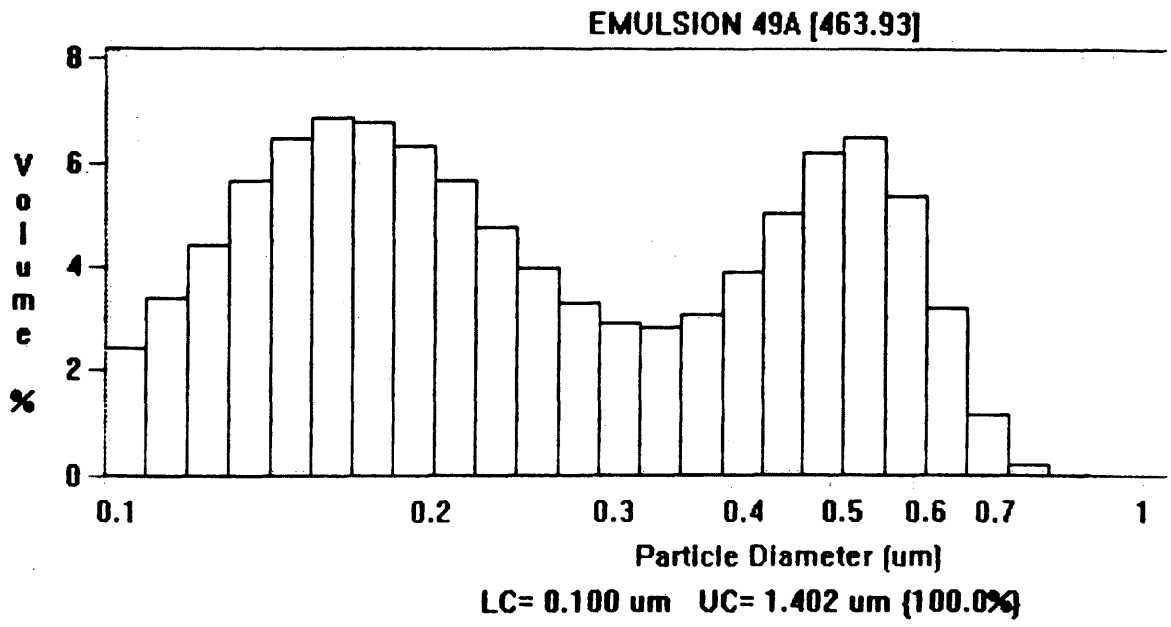
Fig.3.1.8 Upper graph; Relationship between turbidity values and Nanosizer readings for- (o) commercial cream liqueurs and (•) various laboratory liqueurs. Lower graph ; Relationship between Nanonsizer and Coulter LS190 average diameters for laboratory liqueurs..

particles below 0.1 μm , which can be significant in some cases, can only be estimated by extrapolation.

Effect of different diluents on turbidity

The TVof a .605 μm latex suspension and a cream liqueur emulsion depended on the suspending diluent (Fig. 3.1.10). There were significant decreases in the TV when alcohol and especially sugar or sugar/alcohol, were used as diluents. The decreases with cream liqueurs (11-20 %, 40 and 55 %, respectively) were much larger than the corresponding decreases with latex suspensions i.e.10, 25 and 32%. In contrast, the use of these diluents had only a slight (decreasing) effect on the R value.

In a second test, the TVs of two liqueurs , of similar gross composition, were assessed using five diluents of essentially the same refractive indices (Fig.3.1.11). One liqueur was of normal viscosity (B) while a clustered/aggregated sample (A) was about 3 times that thickness. Results were most striking for the thickened sample, A. The use of detergents (0.1% level) or EQA solution in the diluents decreased the TV(800) by about 60 % and the R value by about 100%, as compared to samples diluted with water. Adding detergents and other solutions to the unclustered sample, B , while decreasing the TV by about 10 %, had no effect of the R value. The use of detergents, EQA solution or weak NaOH had no effect on readings with latex beads (data not shown).



Mean volume diameter d43;	0.26 μm
Mean surface volune diameter d32	0.22 μm

Fig. 3.1.9. A particle diameter /volume frequency distribution for a cream liqueur (produced by the two stage process) obtained using the Coulter LS190 laser diffraction particle sizer. See Materials and Methods section for more details . The optical model was Fraunhofer with PIDS. Note that the average particle sizes reported above were reported assuming that there was no fat below 0.1 μm..

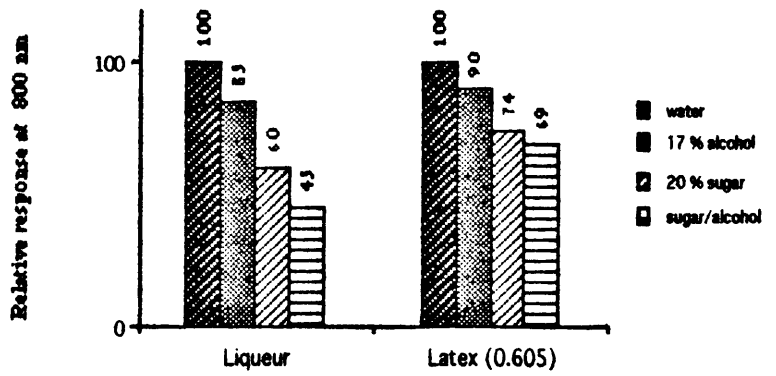


Fig. 3.1.10 Effect of different diluents on the relative turbidity values of a commercial cream liqueur sample (Emmetts) and a latex suspension (0.605 m). Diluents were 17 % v/v alcohol; 20 % w/v sugar + 17 % v/v alcohol with 20 % w/v sugar. The response with water was assigned a value of 100. (The R value was relatively unchanged, data not shown).

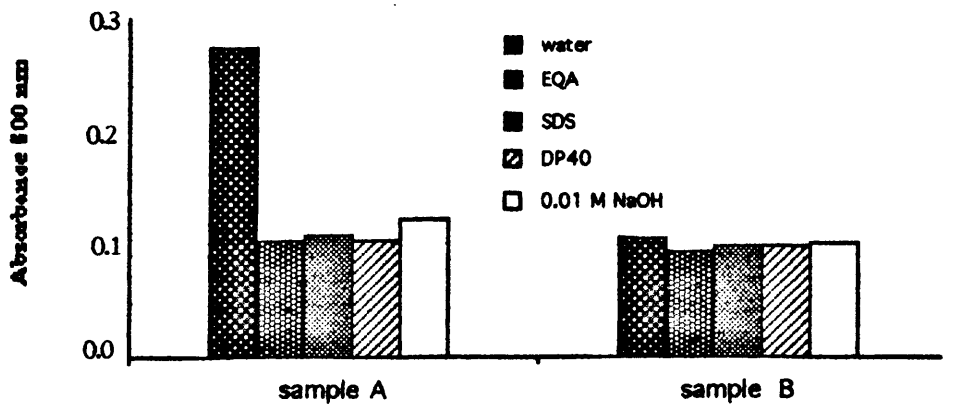


Fig. 3.1.11 Effect of different diluents (see legend), with similar refractive indices as water on the turbidity values of two liqueurs. Sample A was thickened and clustered while samples B was of normal appearance and viscosity. The R value of the thickened sample A was 0.370 as compared to 0.175 for sample B.

DISCUSSION

Before considering the two simple and relatively inexpensive ways to access the particle size of emulsions, i.e microscopy and spectroturbidimetry, it is useful to discuss the significance of different average particle diameters/size distributions. There is often vagueness and ambiguity in the literature on the application and meaning of these terms.

It is obvious e.g. see Tables 3.1.1,3.1.2, that it is possible to characterise an emulsion by different single average particle diameters. Each of these diameters seeks to describe the population of particles in terms of a single so called "equivalent sphere" (Rawle, 1993). None of these different diameter description is either right or wrong, rather each describes a different property of the particle distribution being measured. For example, the d_{32} is the equivalent surface area mean diameter and is of interest since it may be used to determine the amount of interfacial area that is covered by an emulsifier. Thus, an emulsion which has half the d_{32} of another similar type system probably has twice as much emulsifier at the interface. The d_{43}, or equivalent volume(weight) diameter is useful since it indicates the mean around which most of the mass lies. It is not as biased, as is the number-volume mean (d_V), by the large number of smaller particles in an emulsion which do not contribute to the volume.

The diameters as discussed above, give no indication of the actual distribution of particles populations. However, there are as other widely used and useful terms, which can be derived from particle distribution data obtained by certain instrumental methods. The *median* diameter is the value that divides a population into two equal halves and the *mode* is the most common value in a distribution. In a normal or Gausinan distribution, the mean, median or mode will be similar. However, with bimodal systems, e.g. as produced with the latex suspensions in Tables 3.1.1 and 3.1.2, they will be different. An obvious advantage of the more advanced, but expensive, instruments like the Coulter LS190 is that they generate such frequency curves e.g. Fig. 3.1.9. However, the LS190, as with most current laser-diffraction machines only measures particle sizes greater than 0.1 μm . This is of some importance since a significant proportion of the fat of some cream liqueurs is below 0.1 μm . In contrast, some photon correlation instruments e.g. the Coulter N4, can resolve particles down to 4 nm.

Microscopy

In general, phase contrast light microscopy was found to be a useful technique since it enables the operator to directly view an emulsion without staining ; the difference in refractive indices between the continuous and disperse phases ensures visualisation.

When sizing particles by microscopy it is desirable to dilute the sample to a fat content of about 1-2 % for counting since very high fat emulsions like mayonnaise appear very indistinct otherwise. A large number of globules should be sized and counted for accurate results e.g. > 300. This is very tedious and prone to error, however, an advantage is that different types of particles can be differentiated e.g clustered particles, undissolved matter etc.

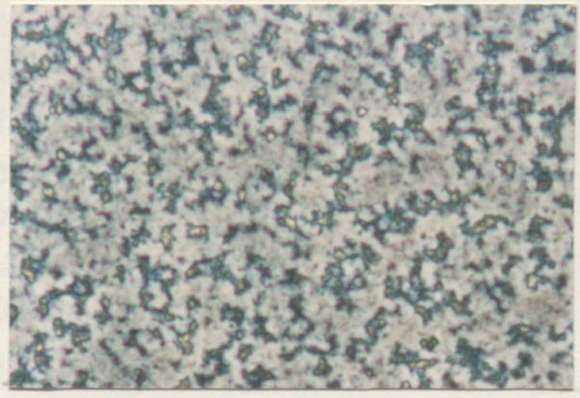
While one cannot size very fine emulsions, e.g. highly homogenised milk or cream liqueurs with the light microscope, a proportion of the fat dispersion in cream liqueurs can be visualised quite successfully. Thus, it is possible to get an "impression" of the fineness of the sample and see individual particles > than ca 0.2 μm , especially in the diluted samples (x400 magnification was preferred in this study simply because oil-immersion is not required, but of course x1000 will give slightly better resolution). Larger globules e.g. 1-5 μm , which may cause creaming problems, are immediately obvious. However it is hard to establish whether all the globules are distinct or separate or whether some particles are large casein micelles.

Cream liqueurs diluted to ca 2 % fat usually look similar to undiluted liqueurs (Fig. 3.1.12*a*). However, some undiluted samples change significantly after a couple of minutes under the cover-slip. The individual particles start to cluster together to form non- uniform views (Fig.3.1.12*b*). This phenomena also occurred under hanging drop slides, so it does not appear to be related to forces set up under the cover-slip. This leads to the question; are the globules in a clustered state in situ ? If so it would appear that these clusters are very easily broken up e.g. by squashing into a very thin film with a cover-slip but they reform almost immediately. In general, the thickness of the cream layer under the cover slip is important for visualisation and this should be as controlled as much as possible. In this study 5 μl was spread evenly under the cover-slip. Even with care, e.g. use of gelatine in diluted samples, there were variations in thickness, especially at the sides of the cover-slip, due to uneven flow of sample.

While the interpretation of fine emulsions, such as cream liqueurs, by microscopy could be considered highly empirical, operators can become very experienced with practice and training. Photographs should be taken to record images, since it is



a



b

Fig. 3.1.12. a) Photomicroscopic appearance of a *freshly* prepared undiluted or diluted cream liqueur b) sometimes the fine appearance changed to a clustered type appearance. Magnification x 400. Bar indicates scale.

2·5 µm.

almost impossible to remember differences when looking at a number of samples, however resolution is not often reproduced in photographs. For these reasons it was decided not to use microscopy as a routine method in this study.

Spectroturbidimetry

Effect of particle size and refractive index on TV.

The light scattering results generated using the latex particles indicated that standard unmodified laboratory spectrophotometers can be useful in assessing particles size. The TV (A 800) of particles with diameters in the range 0.1-1 μm were largely linear. Sizes below 0.1 μm would show very low TVs, with associated increase in error. More particle sizes > ca 1.0 μm should be tested to establish an upper limit for linearity .

The method is very sensitive to small changes in particle size e.g. the TV(A_{800}) increased ca. 8 fold for the ca. 3 fold increase in latex particle diameter (0.114 to 0.303 μm).

The turbidity response is strongly dependent on the refractive index (n_D) difference between the disperse and continuous phase. Thus, fat particles (n_D = 1.462) will show significantly smaller TVs than the polystyrene latices (n_D = ca 1.6) of equivalent average size when dispersed in water (n_D = ca 1.333). Similarly, if the refractive difference of the continuous phase is increased towards that of the fat, e.g by adding alcohol (n_D = ca 1.361) or sugar, then the TV also decreases dramatically (Fig. 3.1.10). For example, the TV (A800) of a liqueur sample, dispersed in water or 60 % m/m sucrose (n = 1.449) was 0.040 or 0.003 , respectively.

Relationship between TV and average particle diameters,

There are two main disadvantages of simple light scattering data. The first is that the readings are only empirical indices of particle sizes in the approximate range 0.2-2.0 μm and the second is that no indication of frequency distribution is obtained (even the full spectroturbidimetric method of Walstra (1968), only gives a value for the standard deviation of the volume-surface weighed distribution). However, as the results in this study have shown, TV's of cream liqueur or homogenised milk emulsions can be correlated to average mean diameters found by other more sophisticated instruments e.g. Fig. 3.1.7/8. Turbidity values are very sensitive to very small changes in

diameters, due to, for example, changes in homogenisation pressure or possible malfunctioning of the homogeniser.

Comparison of experimentally found diameters and literature values.

The d_{32}/ d_{43} values obtained in this study for cream liqueurs (ca. 0.2 -0.3 μm), are of similar magnitude to those of Paquin & Giasson (1989) who measured the particle distribution of cream liqueurs using dynamic light scattering. The average particle size reported, (d_{43}), was 0.21 μm , for a sample homogenised at 30 MPa with a high pressure valve homogeniser. Bucheim & Dejmek (1990), in a review, quote an average particle size (d_{32}) of 0.15 μm for cream liqueurs, but it is not clear whether these authors calculated this from their own electronmicroscopic studies. The results reported by Muir et al (1991), d_{43} ca. 0.45 μm , using a laser diffraction instrument, seem unusually large. The use of 0.9 % sodium chloride as the dispersing medium was unlikely to be the cause of the high result.

In general, the accuracy of the particle distributions generated by laser diffraction methods depends on the correct values for the refractive indices of the continuous and disperse phase , a value accounting for any light absorption of the particles and a suitable mathematical model to analyse the raw data. For photon correlation techniques, the viscosity of the dispersing medium is required. The inclusion of substances to eliminate the light scattering due to aggregation of fat particles and/or protein particles is also importance with both methods (Robin & Paquin, 1991).

There are large discrepancies in the d_{32} and d_{43} of milks homogenised at 20.7 MPa (3000 psi) in pilot -plant Rannie LAB homogenisers equipped with liquid whirl valves. Walstra (1975), using his full spectrophotometric method, reported a d_{32} value of 0.22 μm, in contrast to value of 0.50 μm reported by McCrae (1994) using forward lobe laser light scattering. A value of 0.4 μm was found by Coulter Nanosizer in this experiment (Fig. 3.1.7). Note, Coulter Nanonsizer readings are taken to approximate to d_{32} readings (Tables 3.1.1/3.1.2).

Walstra's (1975) d_{43} results, ca. 0.44 μm, are twice that of the corresponding d_{32} results , whereas in this study (Fig. 3.1.7) the d_{43} results, 0.6 μm (EQA method) are about 50 % greater than d32. Goulden & Phipps (1964) report an average diameter, which is the d_{43} according to Walstra (1975), of 0.55 μm using similar process and equipment.

Wavelength ratio value (R)

Since latices (Fig.3.1.1) or emulsions (Fig. 3.1.5/6) of widely different average particle size had similar TVs at any one wavelength, some indicator of the wavelength dependence should be noted. The R value , calculated using turbidity values at the arbitrary wavelengths of 400 and 800nm, as suggested by Kaufman & Garti (1981), was simple to calculate and effectively distinguished such dissimilar emulsions. The full spectrophotometric method of Walstra (1968), which calculates d_{32} and d_{43} values for emulsions with average d_{32} in the range 0.2-2.0 μm, also measures light scattering at different wavelengths.However, this method involves much more complex calculations including knowledge of the refractive indices of dispersed and continuous phases at the different wavelengths.

Aggregation index (AI)

Another index that may prove useful for cream liqueur systems is one relating the TVs of diluted emulsions using water and water with a protein dissociating agent as diluents. Only small quantities of dissociating (detergents, salts, NaOH) are added such that any change in turbidity was due to the break-up of protein-linked clusters and not to changes in the refractive index of the dispersant. Very small emulsion particles, which individually show little TV, can show large increases in TV if they form clusters.

(Note; if a wavelength of 800 nm is chosen for turbidity readings for cream liqueurs using distilled water as diluent, the contribution of the free proteins particles to the turbidity value was found to be negligible; i.e . > 98% of all the turbidity is due to the dispersed fat phase. This is not the case for say homogenised milk, where the casein micelles would contribute significantly to turbidity if water only were used as a diluent.

We will define an *aggregation index,(AI) as*

$$AI = [(TV_W/TV_{DS}) - 1] * 100$$

where TV_W and TV_{DS} are the turbidity values at A800 of the samples measured in water and in dissociating solution, respectively. Thus, it can be calculated that the AI of the clustered sample in Fig. 3.1.11 is 164 while that of the normal sample is 12. A high value indicates protein bridging as the cause of aggregation; these bridges are broken down by the detergents. A low AI could mean a low protein bridging or alternatively very strong bridging of particles.

Recently, Tomas *et al.* (1994*b*), using laser light scattering, quantified aggregation in milkfat/skim-milk emulsions of various protein/fat ratios by measuring particle size (d_{32}) in the presence and absence of 1 % SDS. For the emulsions of high protein/fat ratios (0.5 - 1), both measurements coincided indicating no aggregation, while at low ratios e.g. 0.1, there was a three-fold difference between the apparent diameters in water and diameters in dissociating media, indicating significant aggregation.

A disadvantage of the AI and of methods such as that reported by Tomas *et al.* (1994) is that the dilution of the emulsions in itself will break up many of the aggregated bodies. Measurement of aggregation in undiluted system is more desirable. Darling (1982) measured the decrease in viscosity over time (0-60 secs) in homogenised creams. The magnitude of the decrease was related to the disruption of the polymer-bridged aggregates, which had increased the effective phase volume (and thus the viscosity) of the system. The specialised rheological apparatus necessary to attempt similar type experiments was not available for the Brookfield RVT used in this study.

CONCLUSIONS

Spectroturbidimetry is a simple and inexpensive way to monitor average particle size, homogenisation efficiency, degree of clustering etc. in cream liqueurs. However, it is desirable to "calibrate" ordinary laboratory spectrophotometers using latex particles and/or more specialised particle sizing equipment.

Phase contrast microscopy is an invaluable technique in the study of emulsions including cream liqueurs, since one can quickly assess the fineness of an emulsion and detect large globules that may cause creaming. The use of light microscopy in research on cream liqueurs is limited since particles cannot be sized and the techniques are highly empirical. Microscopy would be very suitable in the quality control of liqueurs.

Experiment 3.2 *Compositional and physico-chemical evaluation of commercial cream liqueurs*

INTRODUCTION AND OBJECTIVE.

The production and marketing of Irish cream liqueurs is of commercial importance, accounting for the annual utilisation of approximately 8,000 tonnes of butterfat, 1,000 tonnes of m.s.n.f. and 1,700 tonnes of added protein, mostly in the form of sodium caseinate. Despite this fact, there is virtually no published data on the basic properties of these home produced products.

The objective of this section was to study characteristics of the three main brands of Irish cream liqueurs. Two of these brands, Baileys (B) and Emmetts (E) are owned by Gilbeys of Ireland and the other, Carolans (C), is owned by Cantrell & Cockrell. The analysis and behaviour of these products should provide a basis for comparison with products manufactured by pilot-plant equipment.

MATERIAL AND METHODS

Four samples of each of the 3 brands were obtained during the course of the 3 year study. They were labelled E or B or C ; 1,2,3,4. Samples were received ex-factory or from supermarket shelves.

Samples of cream liqueurs containing either 0 or 0.5 % sodium stearoly lactylate (SSL) or monodiglycerides (MDG) were manufactured according to the scheme outlined in the General Materials and Methods section.

The test methods used for fat, protein, ash, calcium, phosphorus, total solids, light microscopy, pH, viscosity and incubation measurements were as previously described (see General Methods and Materials; section 2.3). Turbidity values and size analysis were as mentioned in the previous section (3.1).

One sample of each brand was tested for *sucrose* by the polarimetric method for sweetened condensed milk (IDF 35A;1992) and *alcohol* by distilling 100 ml of liqueur at 20 °C (plus ca 100 ml of water, used to wash out the volumetric) into a 100 ml volumetric. The distillate (ca 95 ml) was brought to volume at 20 °C and the alcohol content of same was calculated by measuring its density using a pycnometer. Densities can be converted to % alcohol using tables e.g. AOAC (1984).

RESULTS

Chemical analysis

Table 3.2.1 (overleaf) summarises the data obtained from the analysis of the cream liqueurs samples. There were not major difference between the four different bottles of each of the 3 brands; thus the results are reported as averages with the range of results also presented. All data is reported in % m/m unless otherwise stated.

Samples B and C contain significantly higher levels of fat than E, i.e. ca. 16-16.5 versus 14.5-15 % fat, respectively. The Gerber method was not as precise (+/- 0.2 %) as the Rose-Gottlieb method (+/-0.1 %), but not withstanding this, the results obtained by the two methods were slightly different. To further investigate this, samples of known composition were manufactured and analysed for fat content using the two methods (Table 3.2.2). The Gerber and Rose-Gottlieb results agreed in the case of controls (no SSL or MDG) and the MDG containing samples, but the Gerber results were always higher (ca 0.3%) for the SSL-containing samples.

Table 3.2.2. The effect of two different methods of fat analysis (Gerber and Rose- Gottlieb) on the analysis of cream liqueurs containing 0.5 % sodium stearoyl lactyate (SSL) or monodiglycerides (MDG). The control samples contained 0.5% extra sucrose.. The Gerber results are estimated to the nearest 0.3% while the Rose -Gottlieb results are to the nearest 0.1 %. Analyses were performed in triplicate.

	% fat (w/w)	
Product	Rose -Gottlieb	Gerber
No LMWS	15.5	15.4
SSL containing	15.7	16.0
MDG containing	16.0	16.0

Table 3.2.1 Summary of the analysis of commericial cream liqueurs. Results (with ranges shown in brackets) are the average values of 4 samples . For some analyses only 2 samples were tested and the ranges are not reported.

Analysis	Baileys B		Carolans C		Emmetts E	
Proximate % w/w						
Fat- Gerber method	16.0	(15.7-16.3)	16.5	(16.0-16.7)	14.7	(14.0-15.0)
-Rose Gotllieb "	15.9	(15.8-16.2)	16.3	(15.8-16.5)	14.8	(14.2-150)
Protein	3.2	(3.2-3.3)	2.9	(2.7-3.2)	2.8	(2.7-3.0)
Total solids	39.6	(39.5-39.8)	39.5	(39.5-40.0)	39.6	(39.2-39.8)
Ash	0.28		0.40		0.30	
Carbohydrate [2]	20.0		19.6			21.8
Trisodium citrate.[3] .$2H_2O$	0.22		0.32		0.16	
Others[1]						
Calcium (mg/kg)	230		205		290	
Phos phorus "	381		350		370	
Viscosity (mPa.s)	26	(24-28)	27	(24-30)	29	(28-31)
pH	6.70	(6.69-6.72)	6.65	(665-6.66)	6.90	
Turbidity Value (A800)	0.055	(0.050-0.065)	0.040	(0.035-0.050)	0.130	(0.125-0.138)
R value	0.145	(0.135-0.158)	0.132	(0.129-0.137)	0.198	(0.190-0.210)
Average diameter [4](nm)	200		185		284	
Conductivity (mho)	400		470		350	

1. Alcohol taken to be 17 % v/v; (from labels) 2. by difference 3. citrate expressed as this salt 4. by Coulter Nanosizer.

The crude protein of the samples ranged from 2.7 to 3.2 %, with the protein concentrations in the four B samples remarkably similar at 3.2 %. The protein content of the C samples (2.7 to 3.2%) varied the most, while E samples, at less than 3.0 % protein, were consistently lower than the other two products.

The ash levels of product C (ca 0.4%) were significantly higher than those of either B or E (ca 0.3%). In contrast, calcium levels of sample E (290 mg/kg) were greater than both the C (205 mg/kg) and B products (230 mg/kg). Phosphorus levels of samples B, C and E were similar (ca. 350 mg/kg).

The total solids contents and the pHs of all the products were consistently around 39.5-39.8% and 6.7- 6.9, respectively. The " total carbohydrate" content, calculated by difference using protein, ash, fat and total solids measurements were all similar at about 20-22 % m/m. The sucrose contents of one of each of the samples B, C and E were 18.6, 18.5 and 19.5 %, respectively.

One sample of each of the brands was also tested for alcohol content. The products were found to contain the stated level (17.0 % v/v) of alcohol. This corresponds to 12.7 % m/m, assuming the average densities of cream liqueurs and pure alcohol are 1.058 and 0.7892 g/ml at 20 °C, respectively.

Two samples from each brand were tested for citrate. However, firstly, in order to validate the enzymatic procedure, we analysed laboratory cream liqueurs of known added-trisodium citrate dihydrate (0.20 %) content and these tested at 0.21 %. Samples containing no added citrate consistently tested at 0.05-0.06 %; this corresponded to the expected small citrate contribution from the milk serum portion of the liqueur. One sample that contained no serum gave a reading of 0.01%.

Citrate was present in all the liqueurs but at markedly varying levels. The trisodium citrate concentration of C was also greatest, being twice (0.32%) that of samples E. Samples B had an intermediate level. (The results were expressed as trisodium citrate dihydrate. To convert to citrate, multiply by 0.65). Conductivity measurements corresponded to these relative amounts of citrate.

Physical analysis

The viscosities of the fresh products ranged from 24 to 33 mPa.s (shear rate 122.3 sec^{-1}). In particular, samples of E never gave readings less than 28 mPa.s, while one sample each from the other two brands had the low value of 24 mPa.s.

The TV(A800) values of sample E (av. 0.130) were significantly higher than either samples B (av. 0.055) or especially samples C (av. 0.040) ; R values showed similar trends i.e average values of 0.198, 0.145 and 0.132 were obtained for samples E, B and C, respectively. These results indicate that sample E had a larger average particle size and this was confirmed by Coulter Nanosizer results; sample E gave readings (0.28 μm), which was ca 50 % higher than the other two brands. Similar trends and d_{32} values were obtained when some samples were analysed by a Coulter LS130 laser light diffraction instrument. The samples were diluted in EQA solution and the FRH model was used to analyse the data. Many samples showed bimodal particle populations ; see Fig 3.2.1

Phase contrast microscopy was used to examine the different samples and the resultant photomicrographs are presented in Fig. 3.2.2. This technique also showed up important differences between sample E and the other two brands. In undiluted samples, all E samples showed extensive clustering (Fig 3.2.2*e*) , while the other two samples were of a similar very fine appearance (Fig 3.2.2*a,c*). In addition large bodies, which showed polarisation behaviour, were obvious in samples E (Fig 3.2.3*b*); they were visible to the naked eye if a thin film of liqueur was allowed to drain on the wall of a glass container. These were considered to be crystalline fat or emulsifers. Other large bodies were thought to be complexes of particles and these had a distinctive whirl-type appearance (not shown) . The large bodies in the other two samples were presumably salt crystals (Fig 3.2.3*a*).

When liqueurs where diluted down to ca 2 % fat for visualisation, the E samples appeared slightly coarser than B or C, (Fig. 3.2.2. *b,d,f*). This is in agreement with particle size results. Occasionally large globules (1-5 μm) were visible in liqueurs. It is not known whether these were the result of the slow coalescence of smaller globules.

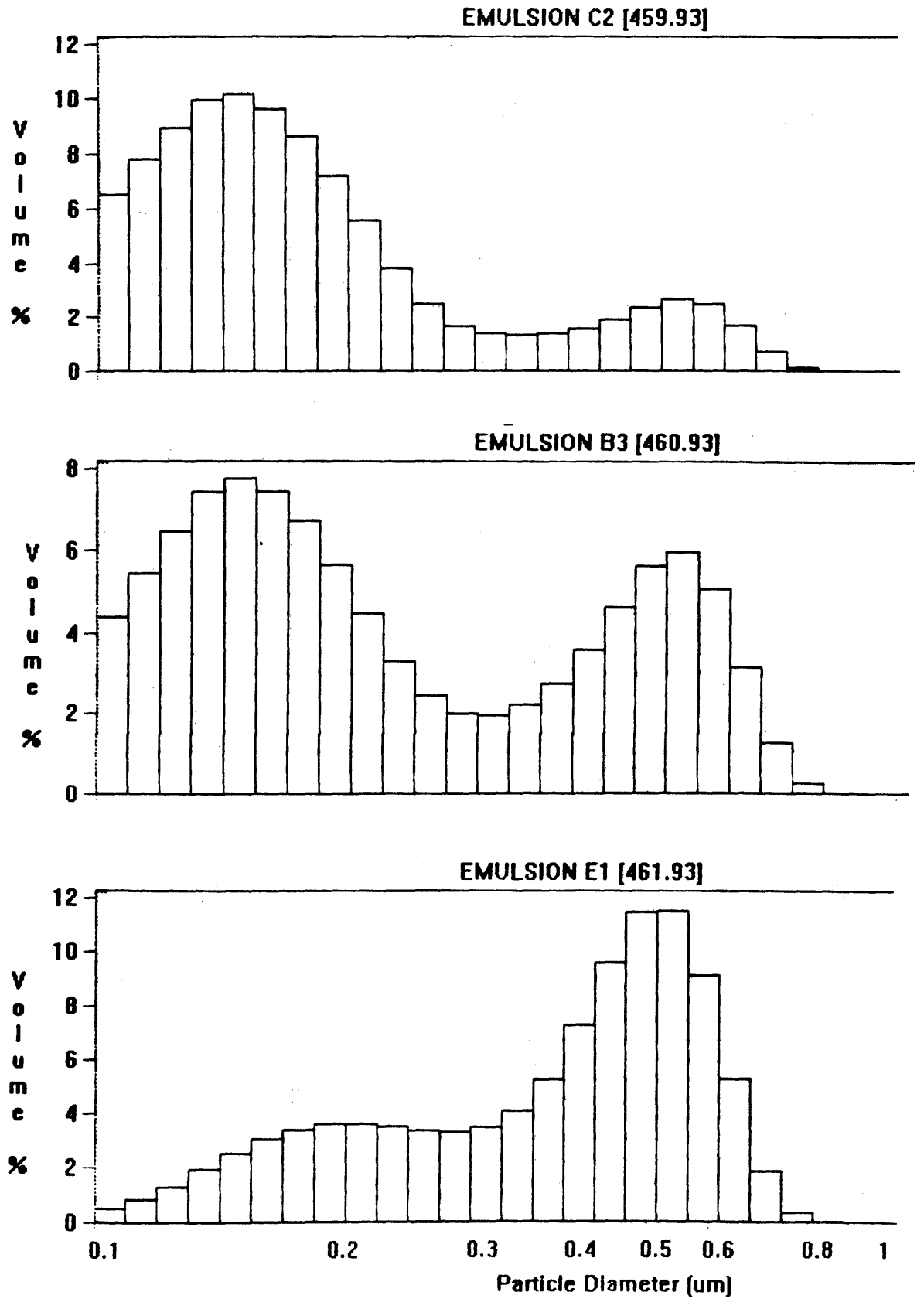


Fig. 3.2.1. Particle diameter /volume frequency distributions of commercial cream liqueurs C, B and E obtained with the Coulter LS190 laser diffraction particle sizer. See Materials and Methods section for more details ; note the optical model was Fraunhofer with PIDS. The reported d_{32} values (in the range 0.1- 1.4 μm) were 0.17, 0.21 and 0.32 μ m for C, B and E respectively.

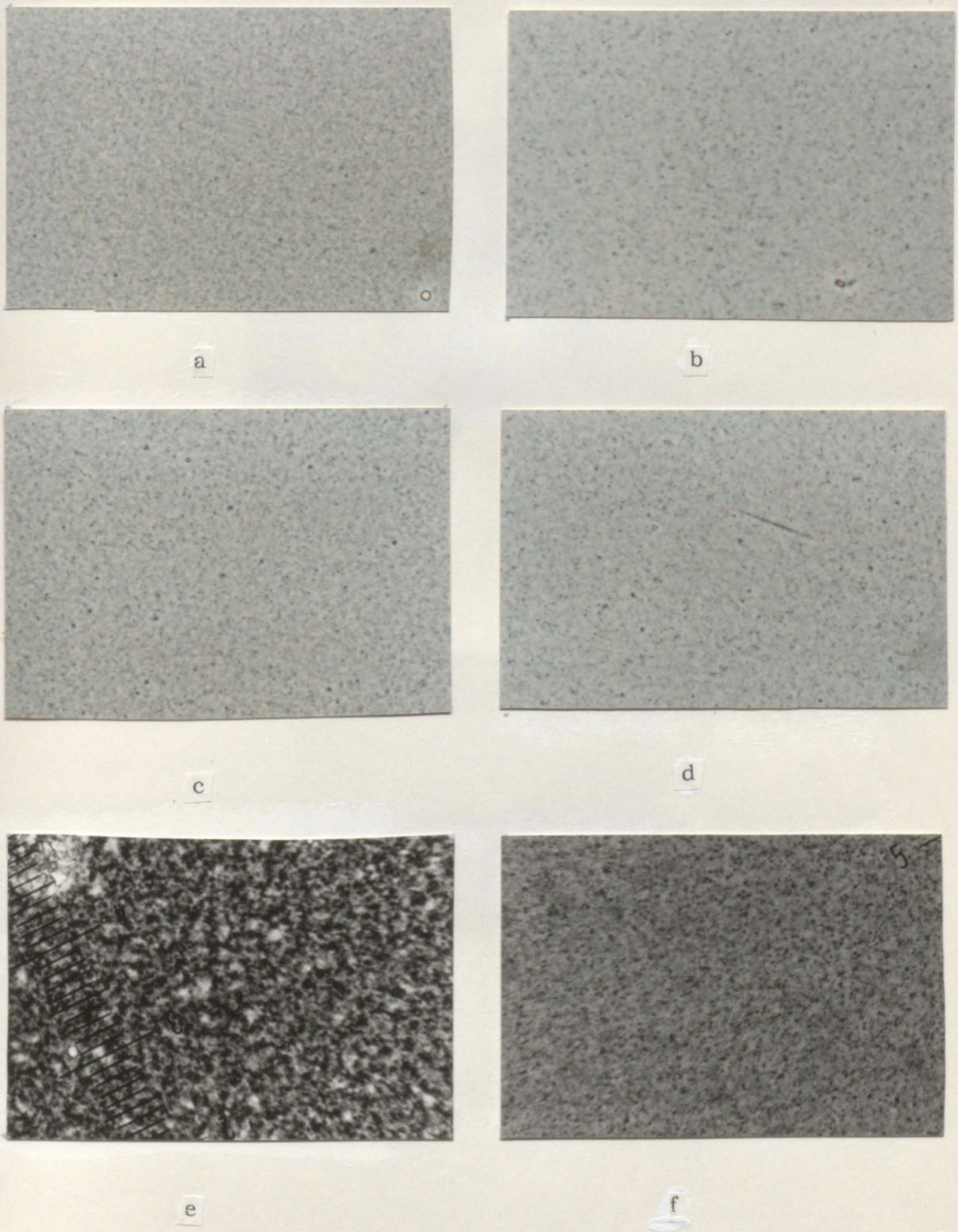
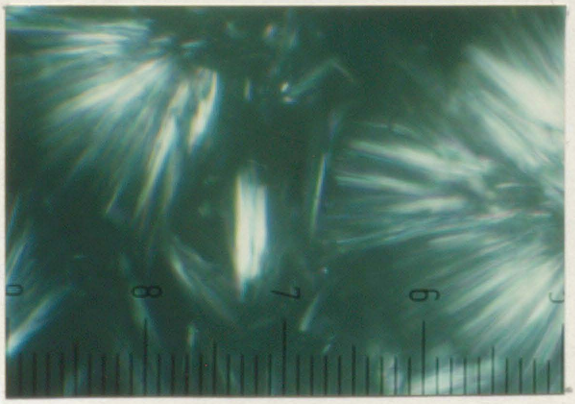


Fig. 3.2.2 Photomicroscopic appearance of undiluted commercial cream liqueurs a; Carolans c Baileys and e; Emmetts (which had a clustered type appearance). The corresponding appearance of diluted cream liqueurs (ca 2 % fat) are shown in b (Carolans),d (Baileys) and f (Emmets). Magnification x 400. Bar indicates scale.

a



b

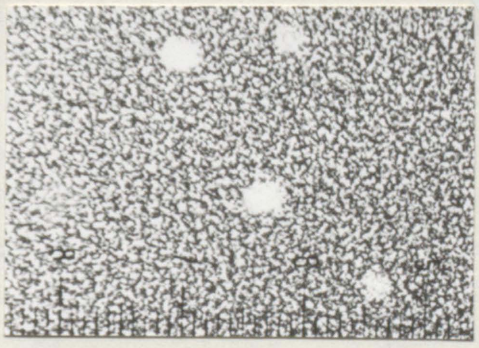


Fig. 3.2.3 Photomicroscopic appearance of a) crystalline material, often found in commercial liqueur C and b) of large granule, commonly found in commercial liqueur E.; this latter photograph was taken under polarised light. Magnification x 1000. Bar indicates scale.

1μm.

Incubation and storage tests

Samples B and C appeared perfectly normal after six months of undisturbed storage at ambient temperature i.e. there was no visible creaming or serum separation. The fat contents from the top, middle and bottom portions of 150 ml storage bottles were 17.0, 16.5, 16.0 % and 16.5,16.0, 15.0 % for C and B, respectively. In contrast, samples E showed marked serum separation and redispersible creaming after storage periods as little as three weeks. These creaming observations were confirmed by centrifuging the samples (neat or diluted in half with water) at 3000 ***g*** x 60 min ; samples of B and C showed no cream layers while E samples exhibited marked visible separation.

The three brands each show distinctive behaviour when incubated at 45 °C for 28 days (Fig.3.2.4). Sample C showed little or no increase in viscosity even after two months (results shown to 1 month), while sample E increased from 28 to 77 mPa.s over the 28 days. Samples B were the most notable; all examples showed remarkable thickening, especially during the first few days. The thickening was very temperature dependent (Fig.3.2.5).

Despite the very thick nature of the incubated B product (a thick custard type consistency), it showed no tendency to gel or to show serum separation. One such thickened B product had an AI of 68 as compared to a value of 8 for the unincubated sample. However, the EQA diluted thickened sample still had a TV 70 % higher than the corresponding non-thickened liqueur , indicating some aggregation that is not dissociated by the EQA diluent.

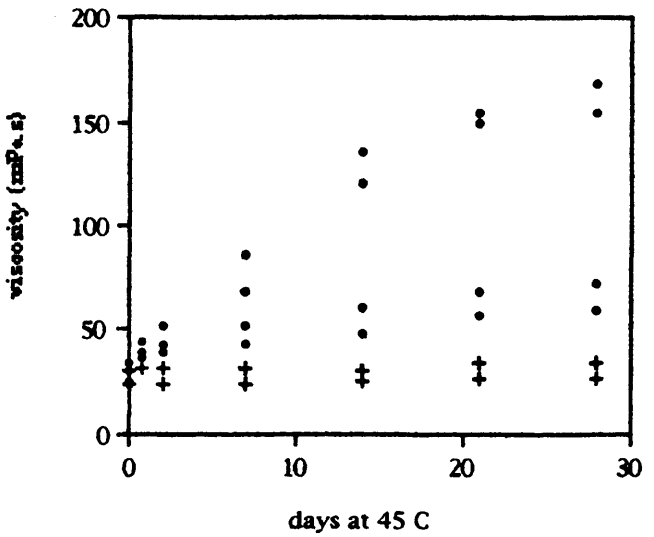


Fig 3.2.4 The effect of incubation time on the increase in viscosity of commercial cream liqueurs at 45 °C + Caroalns • Emmetts o Baileys . Graph shows the results of two separate sets of samples for each type of liqueur. Initial viscosities were measured at a shear rate of 122.3 sec-1,

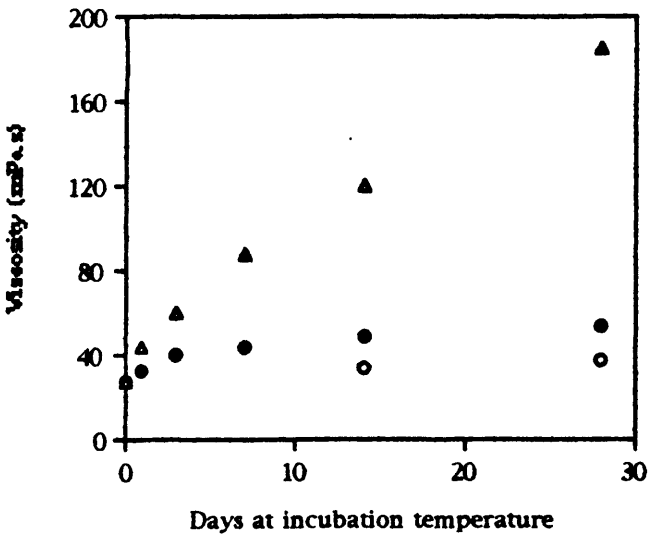


Fig 3.2.5 The effect of different incubation temperatures on the viscosity increase of a "B" liqueur when stored over 28 days at ; Δ 45 °C. , • 37 °C. and o ambient temperature.. Initial viscosities were measured at a shear rate of 122.3 sec-1, while all viscosities greater than 60 mPa.s were measured at lower rates.

DISCUSSION

Ingredient listing

Canadian legislation requires that an ingredient listing appears on bottles of cream liqueurs sold in that country. This affords a useful opportunity to ascertain the ingredients used in the manufacture of cream liqueurs B, C and E. Table 3.2.3 lists the declared ingredients; this data was obtained in 1991 and it is presumed the products have not changed. As expected, all the manufacturers list cream, sugar and alcohol as the main ingredients (water is not mentioned, although this is a major component). Sodium caseinate is also present in all three, presumably as the main emulsifier and thickener. All the samples contain synthetic emulsifiers, which we will refer to as low molecular weight surfactants, LMWS. Product C contained sodium stearoyl lactylate (SSL) , sample B contained monodiglyceride (MDG; see note below) while sample E contained a mixture of the two emulsifiers .

(Note; it is assumed that the glycerol monostearate or GMS, listed for product B and E is in fact a mixture of mono-diglycerides. For example, Dickinson & Narhan (1989c) referred to a product containing 60 % monoglycerides as "monoglyceride", when if fact it is a mixture of mono and diglycerides. Pure "GMS", ca 90 % monoglycerides, is more likely to be used in the ice-cream manufacture).

Table 3.2.3 Ingredient listings found on labels of cream liqueur bottles sold in Canada.

Baileys (B)	cream, sugar, alcohol, whiskey, sodium caseinate, glycerol monostearate, caramel, natural flavourings .
Carolans (C)	Cream, sugar, alcohol, sodium caseinate, sodium stearoyl lactylate, trisodium citrate, caramel, annatto, flavourings
Emmetts (E)	cream , sugar, alcohol, sodium caseinate, flavourings, caramel, glycerol monostearate, sodium stearoyl lactylate, whiskey, disodium phosphate, butylated hydroxyanisole.

Chemical Analyses

Analytically, the three brands of liqueur examined were fairly similar and corresponded to those systems mentioned in the literature i.e. 15-16 % fat products with total-solids of ca 40%. There were small but significant differences between the products and these are discussed below, along with comments on the suitability of the analytical methods used. There are no official published methods for the analysis of cream liqueurs.

Elsewhere on the market there are products with fat levels ranging from as low as 4 % to 12-13 % (data not shown). Most of these products still have total solids ca 40 % and their viscosity may be maintained by extra protein. However, we are only concerned with the higher quality high fat products, since they represent most of the Irish production.

Fat

Products B (16.0%) and especially products C (16.5%) contained higher total fat levels than product E (14.7%). Overall, the modified Gerber method, being fast and reasonably accurate, is a suitable method for the quality control of these products. The Rose-Gottlieb method, which is more time consuming, is less suitable for quality control nor does it appear to measure all of LMWS. These substances are lipid-like and thus can be expected to contribute to the fat result. The SSL appeared only partially extracted by the Rose-Gottlieb procedure (Table 3.2.2). Presumably the alkaline treatment in the latter technique hydrolyses SSL into water soluble lactate and a fat soluble (analysable) stearoyl component. In contrast, the acid conditions of the Gerber tests do not hydrolyse the SSL or MDG and hence they contribute to the fat result. In order to quantify the percentage of lipid material originating from these emulsifiers or butterfat, one could perform a fatty acid profile on the total extracted "Gerber-fat".

Protein

The macro/semi-macro Kjeldahl method is a suitable procedure for analysis of protein in cream liqueurs. Assuming that the fat in a 16 % butterfat product (samples B, C) originated from 48 % fat cream (ca 1.8% protein), then ca 0.6 % of the protein in the liqueurs can be calculated to be serum protein. Thus, the remaining 2.6% originates from sodium caseinate, and assuming an average protein of 90 % for this ingredient, this corresponds to a 2.9 % addition of sodium caseinate. This is in line with, or a little less than, figures quoted in the literature. The protein content of the C samples, at 2.7-3.2%, varied the most, possibly indicating a non-standard formula. Increasing

or decreasing the caseinate level could control the product viscosity within a specified range.

Salts and Ash

The enzymatic test used for citrate analysis proved successful. Although the results were not an extensive validation of the technique, they did show that most, if not all, of the citrate was extracted and measured. The most accurate results were obtained using a citrate standard (ca 0.35g/l) made up from trisodium citrate used in experiments. Inexplicably, neither the citric acid standard supplied by the kit manufacturer, nor an equation for calculation provided with the kit (Boehringer Mannheim, 1992), proved satisfactory. Results calculated from UV data at 340 nm gave more accurate answers than those using readings at 365 nm. No reports could be found in the literature utilizing the enzymatic test for liquid emulsions, including milk. It is an attractive test since it utilizes non-toxic reagents; the classical test for citrate in milk utilizes pyridine.

The amount of citrate in 15-16 % fat liqueurs originating from the 48 % fat cream can be calculated to be ca. 0.05 %. Taking this and the total citrate figures into account, the concentrations of added trisodium citrate.$2H_2O$ in brands B, C and E were calculated to be ca 0.17, 0.27 and 0.11%, respectively. Thus, although sample B has no listed salt, the results clearly show that citrate was added at concentrations recommended in the literature. Sample E contains only 0.11% and this may be because, as stated in the label, it also contains another stabilizing salt, disodium phosphate. All of the phosphorus e.g 360 mg/kg in sample B, can be accounted for by assuming the presence of 17 % serum at 1000 mg/kg and 3% sodium caseinate at ca 6500 mg/kg. From the limited results obtained in this study it would be impossible to ascertain the quantities, if any, of added phosphate salts in sample E. This was because the total phosphor us levels are dependent on the proportions of casein/caseinate in the total protein figures, and this was not known with certainty.

Given that pure trisodium citrate. $2H_2O$ was found to give an ash value of ca 50 % under the test conditions used here, it was not surprising that the ash levels of the liqueurs (0.28-0.40%) correlated with the citrate levels. The ash level of a sample without any added salts can be calculated to be ca 0.2 %, assuming serum and caseinate have ash contents of 0.6 and 3.5 % respectively.

The calcium levels measured in some of the above ashes, were ca 210-230 mg/kg for samples B and C ; this was in line with the contribution from ca 17 % serum. However, despite having a lower fat content, the calcium concentration in sample E

was higher, indicating higher levels of milk-solids non-fat inclusion; this may have been from the use of a lower fat cream or addition of some milk powder in the formulation.

Others

Although it took two hours to perform, the total solids test was very reproducible (0.1-0.2 g/100g). Nevertheless, large amounts of samples can be processed together. It is essential to dilute the weighed amount of cream liqueur with water before hot plate evaporation since this prevents crust formation. The total solids contents of all the products were remarkably consistent and these results were used to estimate the carbohydrate content of the sample (ca 19-20%). Most of this can be assumed to be sucrose; however lactose, originating from the serum portion, can be calculated to be about 0.8 %.

Measurement of the sucrose content of liqueurs is of importance, not only from a quality control perspective but also since an export subsidy is available for this ingredient. The sucrose contents of three liqueurs were successfully performed using the polarimetric method. The sensitivity and accuracy of the procedure was confirmed using a sample of known sucrose concentration. However, disadvantages of this method include; long duration; use of lead and zinc acetates; specialised instrumentation and operator technique. Enzymatic or HPLC methods could be more suitable methods ; however these were not investigated in this study.

Physical analyses and incubation studies

Initial results

Significant differences in particle size/particle size distribution between sample E and B/C were confirmed by microscopy, light-scattering and instrumental data.

Organoleptic evaluation (own observations) also indicated that sample E has different particle properties than the other two emulsions. For example, sample E, whilst having a similar viscosity and 6 % less fat than C or B, is significantly more "creamy" than the other 2 samples. As mentioned in the literature review, an effective particle size distribution of solid-type particles can contribute just as much to the creamy sensation as viscosity or fat content. This would imply that, in the undiluted state (i.e. as perceived by the tongue), sample E has a completely different particle size distribution than samples B and C. It is probable that the clustered nature of globules (also apparent under light microscopy) in sample E gave rise to both its different texture and propensity for creaming. The manufacturer must consider that the resultant amount of creaming in this brand is "commercially acceptable".

(Note; The bimodal nature of the frequency/volume curves generated by Coulter LS 190 have not been reported previously for cream liqueur emulsions. Normally, these are depicted with very narrow distribution curves around an average of diameter of ca 200 nm. It is not known whether the bimodality of the LS 190 results are artefacts; more detailed research is necessary to ascertain whether the mathematical modelling conditions were correct.)

Storage and incubation studies

In the literature (e.g. Banks & Muir, 1981,1982), cream liqueurs were incubated at 45 °C for up to 2 months in order to predict their stability. The authors stated that the samples should not show serum separation or excessive thickening or gelation.

The extensive thickening of product B at 45 °C (Fig. 3.2.3) , albeit without any sign of gelation or syneresis, would be considered, by the above criteria, as a sign of instability. The literature indicated that viscosities should only double and not increase 8-10 fold over a month, as found with sample B at 45 °C. (Even at lower temperatures e.g. e.g. 30 -35 °C, product B showed significant thickening). In contrast, sample C was extremely stable to viscosity increase at 45 °C. (Note: The manufacturer of product B may not be too concerned about thickening since he

may argue that the product should never have reached a high temperature anyhow, and the thickened nature is proof that "mistreatment' of the product occurred).

However, none of the commercial products examined showed gelation, which is the more serious instability problem. Thus, the measurement of viscosity increase at 45 °C is not a very reliable test for commercial cream liqueurs.

Light microscopy of thickened emulsions indicated that marked coalescence did not take place, thus confirming that aggregation and the formation of a weak network caused the massive increase in viscosity. The dramatic decrease in turbidity values on addition of dissociating agents (e.g 0.1 % detergents) to highly diluted thickened liqueurs indicated that protein aggregation was an important cause of thickening. Other stronger reagents e.g. urea, β-mercaptoethanol or urea (Pouliet *et al.* , 1990) could be added to diluents in order to probe the nature of the linkages. The increase in the number of the larger particles/aggregated particles that occurred during thickening was measured by Banks & Muir, (1985) with the Coulter Counter and by Muir *et al.* (1991) by a laser light diffraction. With the latter method, there was a very good correlation between the average particle size of the uppermost decile ($d_{v,0.9}$) and viscosity. The authors suggested that particle size estimation can be substituted for rheological measurements as a means of estimating the extent of age gelation during accelerated shelf-life tests of cream. The laser light scattering instrument was simpler to operate than the Coulter Counter and a result was obtained in 5 min.

CONCLUSIONS

While three commercial cream liqueurs showed similar proximate analysis, they differed markedly in their concentrations of citrate and (stated) presence of low-molecular weight surfactants.

Indices of particle size/particle size distribution (light scattering, microscopy etc) indicated that commercial product E was the coarsest and most clustered emulsion, and this probably accounted for its creaming behaviour on standing at ambient temperature. The other two emulsions had simil ar particle size and did not show creaming during long term storage at ambient temperature.

The products showed distinctive viscosity patterns when shelf-life tested at 45 °C for 28 days. Sample B showed extensive viscosity increase while sample C showed no increase. Sample E showed intermediate behaviour.

The differences in behaviour between products B and C may be caused by formulation and/or process details; it can be guessed that inclusion of SSL and/or high citrate decreased the rate of thickening at 45 °C. The role of different processing methods may be important however, no commercial details were available to support this theory.

None of the products gelled at 45 °C.

Experiment 3.3 *Cream liqueur manufacture and storage stability.*

INTRODUCTION AND OBJECTIVE.

Twelve batches of pilot-plant manufactured cream liqueurs were manufactured using various formulations/processes. The objective of this work, which produced 93 cream liqueur samples of known composition and processing history, was to establ ish-

- *a satisfactory processing technique for the manufacture of experimental cream liqueurs . This was important since the literature in this area is vague and there was no history of cream liqueur processing in the laboratory. There are also no reports on the manufacture of cream liqueurs using Irish cream, caseinate etc.*

- *useful and reliable long term stability testing schemes for cream liqueurs. Testing methods outlined in the literature, e.g. use of viscosity increase at 45 °C for stability testing, may not always be relevant , as was shown in the Experiment 3.2.*

MATERIALS AND METHODS

Cream was obtained from University suppliers except batch 1 which was a frozen cream received from Imokilly Co-Op, Cork. Sufficient caseinate ("K1") was purchased to last throughout the study and this was used in all samples, except batches 1 and 2. The caseinate used for batches 1 and 2 was a sample taken from the departmental dry store and was of unknown origin. It was necessary to add ca 3.5 % K1 caseinate to achieve viscosities comparable to commercial products; ca 26/27 mPa.s. The trisodium citrate level used was 0.18% (0.16 % m/m as anhydrous trisodium citrate or 7 mM citrate). A few products were manufactured without citrate.

The formulation, processing and testing methods (largely developed in this experiment) have been previously outlined in detail (see General Methods and Materials).

All of the samples were homogenised twice, except products in batches 8 and 13 (1 pass only). With twice-homogenised liqueurs, small amounts (150 ml) of the first pass samples were normally collected for subsequent testing. Most of the samples were processed at 55 °C.

For shelf-life stability, 12-18 hour-old samples were placed in an incubator at 45 °C, for a period of at least 28 days. The visual appearance was noted at regular intervals. Occasionally, the viscosity, at different storage times, was measured with some samples,. At later stages, e.g. when the ambient temperature samples were 6 -12 months of age, their storage stability was re-evaluated by the following - further visual observation, 45 °C incubation and heating at 65 °C for up to 60 min.

Table 3.3.1. Summary data for 13 experimental batches of cream liqueurs., consisting of 101 different formulation/combinations , 36 of which were performed in duplicate . All samples were homogenised twice except batches. 2, 5 and 13 (once) . See Appendix 3 for further details ; for information on abbreviations ,etc. see footnotes at end of tables.

Batch No.	Cream delivery date	No.of tests	Principal object of the experiment.	Process Details			Analytical Details (average)					Incubation details
				Press. (MPa)	Temp. (°C)	Homo geniser	% t.s. (w/w)	% fat (w/w)	Vi (mPa.s)	TV (A800)	pH	
1	20 05 91	14x 1	General process development; use of frozen cream; use of LMWS; 1-S and 2-S processes	20.7; 17.2/ 3.5	55-65	Gaulin	ns	ns	22-34	ns	7.35. MDG 7.00; SSL	SSL samples didn't thicken; MDG and control samples doubled in viscosity;after 28 days at 45°C
2	20 10 91	16x 1	Effect single/double homogenisation, LMWS and 1-S and 2-S process	20.7; 17.2/ 3.5	65	Gaulin	39.8	ns	22-29	ns	7.30. MDG 7.11; SSL	As above
3	05 08 92	6x 2	Effect of 1-S, 2-S processes and different homogenizers 3.3% sod. caseinate used.	25.9	55	Gaulin, Rannie	39.3		19 (2-S) 23 (1-S)	45(1-S); 145(2-S)	6.80	No sample gelled at ambient temp. after 1 year; 2-S samples gelled at 45 °C after 1 year
4	11 08 92	6x 2	Effect of 3 temperatures with 1-S and 2-S processes; 3.4 % sod.caseinate use	24.1	45 65 85	Rannie	39.3	14.8	19(2-S) 22(1-S)	54(1-S) 150 (2-S)	6.73	5 of 12 samples gelled at ambient temp. after 1 year; 1 sample stable if samples incubated at 45 °Cafter 1 year

Table 3.3.1 cont

Batch No.	Cream delivery date	No.of tests	Principal object of the experiment.	Process Details			Analytical Details (average)					Incubation details
				Press. (MPa)	Temp. (°C)	Homo geniser	% t.s. (w/w)	% fat (w/w)	Vi (mPa.s)	TV (A800)	pH	
5	25 08 92	6x 2	Effect of double or single homogenization on 1 and 2-S processes ; 3.5 % caseinate used.	20.7; 17.2/ 3.5	55	Gaulin	39.7	15.8	24-27	70(1-S) 180 (2-S)	6.75	3 of 12 samples gelled at ambient after 1 year; 1 sample stable if samples incubated at 45 °C after 1 year
6	02 09 92	6x 2	Effect of 3 pressures on 1 and 2 S processes. 3.6 % caseinate used	13.8-31.0	55	Rannie	40.2	16.2	25-28	60-140	6.78	4 of12 samples gelled at ambient after 1 year. 2/12 stable after imcubation at 45°C after 1 year
7	20 10 92	6x 2	Effect of different prehomo - genisation mixing technique 1-S process; 3.6 % caseinate	20.7	55	Rannie	40.1	16.0	28-30	60-70	6.73	5 of 12 gelled after 1 year at ambient. 12/12 gelled after incubation at 45°C after 1 year.
8	28 10 92	6x 1	Effect of two homogenisers , single/double stage and presence of citrate; 1-S process, 3.1 % caseinate	20.7; 17.2/ 3.5	55	Rannie Gaulin	39.8	16.0	24-26	100-142	6.67- no citrate 6.79 no citrate	Non citrate samples gelled at 45°C after 1 month ,from fresh. Citrate samples gelled at 45 C when put in oven at 2 months

Table 3.3.1 cont.

Batch No.	Cream delivery date	No.of tests	Principal object of the experiment.	Process Details			Analytical Details (average)					Incubation details
				Press. (MPa)	Temp. (°C)	Homo geniser	% t.s. (w/w)	% fat (w/w)	VI (mPa.s)	TV (A800)	pH	
9	10 11 92	6x 2	Effect of extra heat before and after homogenisation. 1 and 2- S process used. 3.5 % caseinate. used.;	20.7; 1S 27.6; 2S	55	Rannie	40.0	15.8	20-23 (1-S) 25-30 (2-S)	70 ;1-S 145;2-S	6.77	8 of 12 gelled at ambient after 1 year.. 11/12 gelled at 45 C after 1 year.
10	09 12 92	4x 1	Use of normal and sour late lactation creams. 1-S process ;3.5 % caseinate used.	20.7	55	Rannie	40.2	16.5	24-33	70-90	6.74; norm 6.40 sour.	2 of4 gelled at ambient after 1 year; all gelled at 45 °C when incubated after 1 year.
11	09 12 92	2x 1	Use of sour cream ;different source from above; 1S process. 3.5 % caseinate	20.7	55	Rannie	40.1	16.4	31	88	6.50	2 of 2 gelled at ambient after 1 year.
12	26 01 93	15x 1	Miscellaneous; ; effect of pressure ; of LMWS addition of citrate ; 1 and 2-S processes; +/- alcohol; 3.5 % caseinate ysed.	13.8-27.6	65 (1-S) 75 (2-S)	Rannie	39.8	16.0	22-30	45-150	6.44-6.65	3 of 12 gelled at ambient after 1 year. 6/12 gelled at 45 C after placing in oven 1 year

Table 3.3.1 cont.

Abbreviations	1-S and 2-S	single and two step processes
	Press.	pressure
	Temp.	temperature
	% t.s.	total solids
	Vi;	initial viscosity
	TV	turbidity value i.e. A800 of 16 mg fat / 100ml of diluent
	ns	no sample measured
	-C,+ C (or citr.)	minus or plus the presence of citrate. in the sample
	SSL, MDG	sodium stearoyl lactylate , monodiglycerides
	fat	measured by Rose Gottlieb test.

Batch no.;	Liqueur batch number used for a given set of samples which used the same cream
Process date;	The date of processing the liqueur samples is given in days/month/year. The age of the milk/cream at this stage was normally 3-4 days
No. of tests	the number of different formulations processed; x 1 or 2 means single or duplicate respectively.
Homogeniser	two different makes of homogeniser.
Incubation details	The appearance ,especially with regard to gelation, of the 1 year old , ambient temperature samples is reported . The effects of incubation of the 1 year samples at 45 C are also reported.

RESULTS

The experimental results are summarised in Table 3.3.1. The main findings are considered under the headings (a) turbidity values (b) viscosity and (c) storage/incubation tests. Unless otherwise stated, all data refers to 2 pass citrate-containing samples.

Turbidity values of final products.

Effect of processing conditions

The following processing factors were found to influence the turbidity value (and hence the average particle size)- pressure, type of process, number of passes, type of homogeniser and to a certain extent temperature of processing:

As expected an increase in pressure decreased the TV (A800) readings (Fig. 3.3.1) The *rate* of decrease was very slightly greater for the 1-S process as compared to the 2-S process. The 1-S process produced average particle sizes lower than the 2-S process even at considerably lower pressures e.g the TV values of the 1-S process at 13.8 MPa (0.088) was lower than the value of the 2-S process at 31.0 MPa (0.112). Most of the samples in these experiments were 2 pass samples but, where the corresponding 1 pass samples were tested, the TVs were ca 25 and 17% less for 1-S process and 2-S processes, respectively.

There was no difference in the TV between 1-S products manufactured with the Rannie or Gaulin homogenisers, however the Gaulin gave results slightly lower (15%) than the Rannie for the 2-S process (batch 3). The processing conditions were 2 x 25.9 MPa at 55 °C in both cases. In contrast, the Rannie gave significantly lower values (0.100) than the Gaulin (0.140), if only 1 pass at 20.7 MPa at 65 °C was used (experiment 8, 1-S product).

Increasing the temperature of processing from 45-85 °C had no effect on TV of 1-S samples but resulted in a 15 % decrease in 2-S samples (e.g. experiment 4; data not shown).

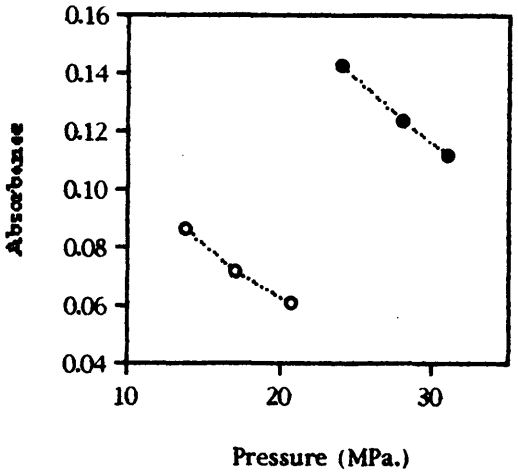


Fig. 3.3.1 Effect of increasing the homogenisation pressure on the turbidity values (absorbance at 800 nm) of cream liqueurs processed by the o 1-S or • 2-S processes.. Data from batch 6.

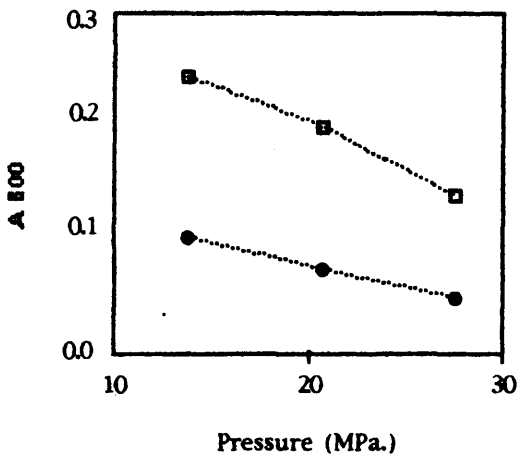


Fig. 3.3.2 Effect of substituting alcohol with an equal volume of water on the turbidity value (A 800) of 1-S processed liqueurs . Three differents pressures at 65 °C were tested. (batch 12) ▯ water containing sample, • alcohol containing sample.

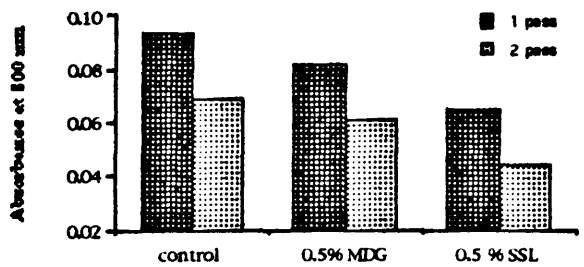


Fig. 3.3.3 Effect of added LMWS (0.5 % w/w) on the turbidity values of cream liqueurs.. Both 1 pass and 2 pass samples were assessed.. MDG; monodiglycerides , SSL ; sodium stearoyl lactylate (Data from batch 12).

Effect of ingredients

The replacement of the *alcohol* by a similar volume of water, before homogenisation, led to the dramatic changes in TV (Fig 3.3.2). Water containing samples (batch 12) had values 2.4-2.8 times higher than the corresponding alcohol containing samples; the differences depending on the homogenisation pressure used. A log/log plot showed that the rate of decrease in TV with pressure is slightly higher for the I-S product (data not shown).

Omission of *citrate* from the formulation increased the TVs by 0-40 % in most of the samples of batches 8 and 13. The biggest difference was in samples from experiment 13 where the prehomogenisation mix was held at 65 °C for 30 min before homogenisation. However, one citrate containing sample had a greater TV than a corresponding non-citrate sample; this was a sample homogenised at 17.2/3.5 MPa in the Gaulin homogeniser (batch 8).

The effect of including 0.5 % SSL or MDG on the TV values of 1-S processed liqueurs is shown in Fig. 3.3.3. Addition of MDG had a minor effect, while addition of SSL lead to a significant reduction in TVs in both one and two pass samples.

viscosity of final product

Effect of processing conditions

Samples manufactured by the 2-S process had lower viscosities than the corresponding 1-S process ; typical values were 22 and 26 mPa.s , respectively (batch 9). The viscosity of the 2-S process seemed independent of heating. (This was also found in batch 4). In contrast , the viscosity of the 1-S products increased somewhat with increasing heat / time of processing. For example, homogenising a mix previously held at 55 °C x 1 min or 65 °C x 30 mins, resulted in products with viscosities of 22.5 or 26.5 mPa.s, respectively.

Effect of ingredients

The results from three batches (8,12,13) quantified the effects on product viscosity of excluding citrate from mixes that were subsequently homogenized. The results were conflicting. In batch 12 (2 passes at 20.7 MPa , 65 °C, 3.5 % caseinate), there was a large increases in initial viscosity (i.e. 25 to 35 mPa.s) on omitting 0.16 % trisodium citrate, while addition of 0.16 % trisodium citrate to the non-citrate liqueur *after homogenisation* did not have any effect. No such difference was evident for experiment 13 (1 pass at 27.6 MPa , 65 °C, 3.1% caseinate). In experiment 8, (1 x 20.7 MPa, 65 °C x 20 mins holding before) citrate samples had slightly higher viscosities than the non-citrate samples i.e. 26 versus 24 mPa.s, respectively.

If an equal volume of water replaced the alcohol solution in the formulation of 1-S liqueurs (2 passes, 55°C, 13.8-27.7 MPa), the viscosity decreased from 22.5 to 12.5 mPa.s.

The inclusion of LMWS, in addition to the existing amount of sodium caseinate, increased the viscosity. Batches 1 and 12 , both processed at 65 °C and with 0.5% of SSL or MDG , showed that there LMWS directly replaced sodium caseinate on a weight for weight basis.

Storage /incubation tests

Ambient temperature storage; visual appearance in the first month.

Samples of 1-S products, homogenised *once* through the Rannie at 20.7 MPa, showed very little creaming. In contrast, those processed through the Gaulin showed extensive (up to 10 % in batches 1,3,8) visible creaming after ambient temperature storage. Differences were not obvious if the products were homogenised *twice* at pressure greater than 20.7 MPa. The 2-S products showed more creaming and serum separation than 1-S products.

The samples containing water instead of alcohol, showed very extensive creaming on standing at ambient temperature. The cream layer reached a constant level of ca 10-20 % after 2-5 days.

Ambient temperature storage; visual appearance after storage for up to 12 months

After extended storage at ambient temperature, the most dramatic feature in samples was extensive thickening and gelation. The extent of visible gelation at ambient temperature after 1 year varied widely ; see summary Table 3.3.1. To take the extremes ; in batch 3, none of the 12 samples showed gelation while in batch 9, 8 of 12 samples showed gelation. In the latter example, all the samples were normal looking after 1 month and only 3 of 12 showed visible gelation after 6 months. All of these samples were 1-S samples, although the formulation and processing conditions varied slightly. The earliest gelation time for a citrate containing sample, ca. 14 days, occurred with a 2-S sample in batch 5 (2 x 24.1 MPa at 55 °C, Rannie). Other samples gelled after 1 month and 6 months. One pass samples were invariably more stable than the corresponding 2 pass ones (about 27 samples) to gelation at ambient temperature, except for a single sample that gelled while the corresponding 2 pass samples did not.

Another interesting aspect of the gelation behaviour at ambient temperature was the behaviour of replicated process samples. Despite similar analytical parameters, in at least half of the cases, one replicate showed gelation while the other was normal. Duplicate samples were normally processed the day after the first sample; cream and caseinate syrup being stored overnight in a cold room. However there was no pattern as to whether the earlier-produced sample showed the gelation or vice versa.

On ageing, the gels exhibited a gradual syneresis of a clear dark coloured liquid. After about 6-12 months of age, the serum often constituted about two thirds of the volume of the sample and the rest was a solid plug. Samples incubated at 45 °C (see below) never showed such extensive syneresis.

45 °C accelerated storage test; viscosity increase during the first 28 days

When fresh samples were incubated at 45 °C, the high pH samples (pH ca 7.3) obtained in batches 1 and 2 showed relatively low increases in viscosity compared with the other "normal pH" samples (pH ca. 6.8), Fig. 3.3.4. and in addition there were no obvious visual defects in these samples. The viscosity of "normal-pH" samples (all those sample processed with K1 caseinate) increased dramatically during storage at 45 °C and samples often showed signs of instability e.g. appearance of slight serum and particulates on the glass walls. Relatively few samples showed actual full gelation during this initial incubation; exceptions were samples in batch 9, where a sample gelled after 1 week.

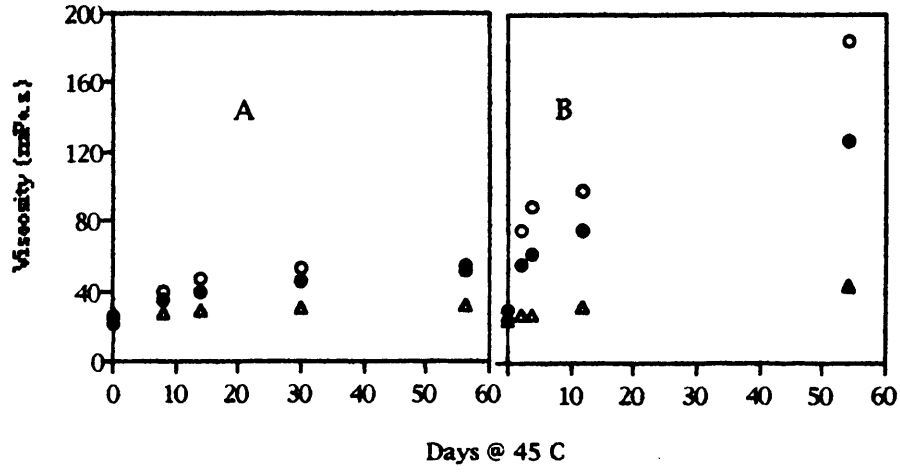


Fig. 3.3.4 The effect of LMWS on the viscosity increase at 45 °C in different cream liqueur formulations . o, control (no LMWS), • MDG containing sample Δ, SSL containing samples. Graph A are the high pH samples (batch 2) while graph B is the normal pH samples. e.g batch 12.

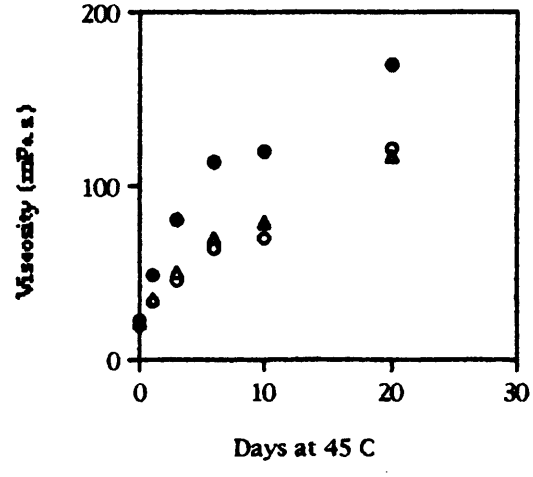


Fig 3.3.5 Effect of citrate addition on the viscosity increase at 45 °C of cream liqueur incubated when fresh ; • product containing citrate before homogenisation. Δ citrate added to product after homogenisation; to the level of the previous sample o samples contained no citrate . These liqueurs were 1- S samples homogenised at 65 °C (part of batch 12 experiments)

The inclusion of 0.5% w/w SSL markedly decreased the viscosity increase at 45°C; this was the case for both the normal (batch 12) and high pH samples (batches 1,2): MDG had little effect on viscosity increase (Fig 3.3.4).

Samples produced without citrate (batches 8,12 and 13 ; 1-S process , 1 x 20.7 MPa, 65 °C) gelled during the first 28 days of incubation. In batch 12, addition of citrate after homogenisation prevented gelation. This sample thickened less than the sample with citrate added before homogenisation (Fig. 3.3.5); a similar situation was observed with a 2-S sample produced in this experiment (data not shown).

45 °C and 65 °C accelerated storage tests; viscosity increase in older ambient temperature or fridge stored samples.

While freshly made samples incubated@ 45C did not always show gelation, the same samples could demonstrate extensive thickening or gelation if they were re-incubated when older. One month old samples were incubated at 45°C for periods of 1 day or 28 days or at 65 °C for 1 hour (a "heat test"). These tests were an attempt to accelerate or predict the gelation behaviour that might occur over very long periods at ambient temperature.

Table 3.3.2 summarises such incubation and viscosity data for liqueurs produced for batch 7. None of the samples gelled at 45 °C if put in fresh, although the viscosities increased significantly over 14 days. All 1 year old samples eventually showed gelation, if incubated at 45 °C for 28 days, despite the fact that 6 of 12 of the unincubated samples had viscosities equal to or less their original value. Note that in 10 of 12 of the one year old ambient temperature samples, the results of the 1 hour 65°C heat test predicted those of the longer 45 °C test. The data in Table 3.3.2 also demonstrated the lack of reproducibility between the behaviour of replicate samples.

As found with the ambient temperature gelation results, the one pass samples were more stable to gelation than the two pass samples upon accelerated storage. The effect varied with batch. For example, while all one year old two pass samples gelled when incubated at 45 °C (batches 10 & 11, 6 samples each), only 1 of 6 of the 1 pass samples did so. In batch 12, one and two pass samples received identical heat-treatments and were analytically similar, yet only the two pass sample gelled. In contrast, 11 of 12 two pass and 9 of 12 one pass samples gelled in experiment 9. The use of LMWS may also prevent gelation. This was indicated by reincubating the

samples shown previously in Fig. 3.3.4. The control sample, which appeared normal when incubated fresh, gelled when incubated at 12 months of age. The corresponding LMWS containing samples did not. Unfortunately, the high pH LMWS liqueurs from batch 1 and 2, were discarded after their initial examination.

Table 3.3.2. The effect of sample age on the results of various test parameters was measured e.g. the viscosities of ambient temperature samples were read after various intervals. These samples were also placed at 45 or 65 °C for various periods. See footnotes for more details. (Data from Batch .These 1-S samples were homogenised twice at 20.7 MPa at 55 C on the Rannie) .

	Ambient temperature viscosity data[1]			45°C incubation[2]		Appearance of sample after 1 hour at 65 °C		
Product	V_i (mPa.s)	% V_6 (%)	% V_{12} (%)	$V_{0\text{-}0.5}$ (mPa.s)	$V_{12\text{-}13}$ (mPa.s)	6 m fridge	6 m amb.	12 m amb
7.1a	29	-3	-2	227	G	ok	ok	ok
b	30	-10	+65	262	G	ok	G	G
7.2a	28	-8	+25	250	G	ok	G	G
b	29	-4	-4	234	G	ok	ok	ok
7.3a	29	+11	G	214	G	ok	G	G
b	29	-8	-3	237	G	ok	ok	G
7.4a	29	-6	G	224	G	ok	G	G
b	28	G	G	378	G	G	G	G
7.5a	29	G	G	288	G	G	G	G
b	30	-3	-3	314	VT	ok	ok	ok
7.6a	30	-5	0	275	G	ok	ok	G
b	30	-2	-2	304	VT	ok	ok	ok

1. The initial viscosity (Vi) was recorded and the % change in viscosity (% V) calculated after 6 and 12 months at ambient temperature. Viscosity measurements were taken at 20.0 C

2. ($V_{0\text{-}0.5}$); The viscosity of products that were put into the incubator when fresh , was measured after 0.5 months (14 days) at 45 C.
($V_{12\text{-}13}$) The appearance of 1 year old samples that had been incubated for up to 1 month at 45 C is noted; G; gelation VT; very thick..

3. Ambient temperature (amb.) and fridge samples were heated to 65 C for 1 hour and cooled. The appearance of the sample was noted G; gel ok; no gel. The samples were six months (6m) and twelve months old (12m)

DISCUSSION

These preliminary studies investigated and established; formulation and manufacturing procedures for small-scale manufacture of cream liqueurs (throughput; ca 150 l/min). Details are outlined in the General Materials and Methods section, together with some procedures for initial testing and shelf-life testing procedures. General comments are included below.

Some initial work was also performed on the influence of ingredients and processing parameters on the properties of the final products. Since most of the latter work was repeated and more extensively investigated in the next section, only a brief discussion is presented here.

Formulation and manufacturing technology

All the products were formulated to contain ca 40.0% total solids and 16.0 % fat. Initial experiments showed that alcohol loses, which would increase the total solids and decrease the alcohol levels, could be prevented if the processing container was covered. Water contamination, due to collection of the sample before all the water was flushed through the homogeniser, was also a potential problem. This was because relatively small samples were being processed. When water contamination did occur, it was usually only 1-2 % , which led to a drop in 0.4-0.8% in solids readings; this amount was easily detected since duplicate samples in the total solids test usually agree by 0.1% (0.2 % max).

For production of liqueurs with 1 homogenisation pass, a starting quantity of 2500 g mix is sufficient to obtain a final sample of 300-500 ml. For a two pass operation, a starting quantity of 4750 g (ca. 4500 ml) is recommended, of which 2250-2500 ml should be collected for production of the second pass. The first pass product is then put through the homogeniser and ca 500 ml of 2-pass product collected. (Note- to obtain a representative sample of 1-pass product, it is best to take a sample from the pooled product after all the product has passed through, rather than from the outlet *during* the processing).

The Rannie homogeniser, where single-pass liqueurs produced at 27.6 MPa were similar to those produced after 2 passes at 20.7 MPa, proved more efficient and easier

to operate than the Gaulin model. (One pass products were not satisfactory on the Gaulin). The above pressures and conditions were much less than those recommended (2 x 31.1 MPa) in the work of Banks *et al.* (1982), where a poppet ("flat") valve construction, similar to the Gaulin, was used. The efficiency of the corrugated ("liquid whirling valve") on the Rannie is as a result of the "multiple homogenisation" effect (Stistrup & Andreasen, 1966; Walstra, 1975).

Only thin cream lines formed, if any, after storage at ambient temperature. This "ordinary creaming", due to large globules etc, was distinguished from very thick clustered cream caps which formed much later on as the product was thickening towards gelation. These lines or rings were not obvious at 45 °C, probably due to the increased viscosity of the continuous phase which would lead to reduced creaming.

Initial and shelf-life testing of products

Since the total solids/fat ratio will be the same for any given set of liqueurs, only one sample per set was chosen for fat analysis (Rose-Gottlieb). The fat content of the other samples could be calculated by multiplying their total solids contents by this ratio.

In freshly produced emulsions, turbidity tests proved simple, rapid and sensitive for characterising homogenisation efficiency/particle size (see section 3.1). The initial creaming /visual appearance data was confirmed by TVs. It was possible to reproduce average particle size values comparable to commercial samples using the small scale pilot plant. For example, TVs comparable with commercial sample B were obtained by homogenising samples at 2 x 20.7 MPa on the Rannie. Samples resembling sample C i.e. TV ca 0.045 were attained by 1-S samples homogenised twice at 25.9 MPa or twice at 20.7 if 0.5 % SSL was used. The high TVs of the E samples were comparable to TV obtained for 2-S samples i.e. ca 150. This evidence would suggest that while samples B and C are 1-S products, commercial sample E is produced by the 2-S process. It is, of course, impossible to tell what pressures or how many passes were used by the commercial manufactures.

During shelf-life testing of emulsions, visual observation at ambient temperature best characterised creaming. The presence of cream rings or collars at the top of bottles or extensive watery serum at the bottom of bottles are signs of creaming and may be unacceptable in certain circumstances. This creaming occured relatively quickly (in the first week) and was due to inadequate homogenisation and /or clustering. It was more obvious at ambient temperature since incubation at 45 °C thickened the liqueur and thus inhibited creaming.

The extent of thickening in experimental samples, incubated at 45 °C , was significantly greater than that reported in the work of Banks and coworkers. However, it was comparable to that exhibited by commercial sample B i.e. while very thick after 1 month at 45 °C, it never gelled. All the above samples had a "normal" pH i.e. ca 6.7. Interestingly, the high-pH samples of experiments 1 and 2 (pH ca 7.3), showed relatively little thickening, suggesting that high pH could limit this phenomenon.

Prediction of age thickening /gelation in cream liqueurs during incubation at 45 °C was dependent on the age of the sample when put into the incubator. This has not been previously reported for cream liqueurs or other long-life samples. Furthermore it was difficult to predict gelation behaviour from initial analytical and incubation data. It would appear that the best way to predict gelation is to incubate aged samples. Incubation at 65 °C mostly mirrored that at 45 °C.Therefore,it is a useful test for quickly exposing most of the potentially gellable samples.

No attempt was made in this study to predict the behaviour of the liqueurs by measuring the alcohol stability of the original milk/creams although this information would have been useful. However in the absence of this information , we can only speculate that it is unlikely that there would have been any definite correlation between the two systems. For example, the ratio of milk salts to protein and the type of protein in milk and cream liqueur systems are very different. In addition, Roche (1993) made a low fat liqueur system containing ca 12% milk-solids non fat (about ten times the level in 16 % fat cream liqueurs) and could find no correlation between the alcohol stability of the original milks and powders and instability in the product. Rather, the instability of the product seemed to be due to an (unknown) complex balance of various constituents.

Even if unsuitable batches of creams could always be positively identified by an alcohol test, a manufacturer , for economic or logistical reasons, may seek to manufacture a stable cream liqueur from it by the use of additives or process variations. These practical aspects are of interest in this study.

Influence of processing and ingredients on cream liqueurs

Viscosity is an important quality control factor in the organoleptic assessment of cream liqueurs. This can be simply controlled by varying the amount of added sodium caseinate in the formulation. However, considerable savings in caseinate usage (ca. 15 %) can be attained if

(a) the 1-S process is used instead the 2-S one

(b) the temperature /times of processing are increased from 55 °C to 65 °C with longer holding times

(c) LMWS replace caseinate on a one to one basis up to a level of 0.5%.

In that situation a level of about 3.0 % added sodium caseinate will suffice and this will give total protein levels in the order of that of the commercial samples. Otherwise much higher protein values were necessary to obtain viscosities close to commercial products e.g. 3.5 % added caseinate.

In agreement with the work of Banks & Muir (1982) and Muir (1987), products manufactured by a two- step process (alcohol/sugar added after homogenisation) were less stable than one-step products (alcohol added before homogenisation). The 2-S products had about twice the TV and exhibited much more creaming than the 1-S samples and they resembled commercial product E in this respect. While the addition of 7.2 mM (0.18 %w/w) trisodium citrate improved the stability of products it did not always prevent age-gelation. This was unexpected since the literature would suggest that a product should be stable once it contains sodium citrate at al level of ca 0.18 % and is homogenised sufficiently to prevent creaming. The citrate is considered to chelate free calcium ion and thus prevent protein aggregation leading to gelation (e.g. Banks *et al.* , 1983).

The viscosity increases on incubation of the LMWS-containing experimental samples can be related to the results obtained with the (LMWS containing) commercial samples. The SSL containing samples showed little or no increase in viscosity at 45 °C and this is in agreement with the results of sample C, which also contained SSL. Similarly, the MDG containing sample behaved as sample B, which contained MDG and the samples produced by the 2-S process behaved like the E commercial samples. These results confirm the earlier indications that samples B and C are 1-S samples and sample E is a 2-S product. More importantly, the addition of low molecular weight surfactants seemed to prevent the phenomenon of age-gelation.

CONCLUSIONS

It was possible to reproduce the characteristics of commercial cream liqueurs using pilot-plant production and batch sizes as little as 2.5 kg.

Age-gelation of experimentally-produced citrate-containing liqueurs, stored at ambient temperature or incubated at 45 °C, was the main defect observed. This phenomenon did not seem to correlate to viscosity increases at 45 °C but was prevented by addition of low molecular weight surfactants, a high pH or replacement of the alcohol with water.

The single sample of caseinate and/or "unstable" batches of cream utilised in this experiment may have been sources of instability.

Chapter 4

Emulsion and Shelf-life Characteristics of Cream Liqueurs as Influenced by Ingredients

Experiment 4.1 *Effect of alcohol on the properties and stability of cream liqueurs.*

INTRODUCTION AND OBJECTIVE.

Preliminary research (Experiment 3.3) had indicated that, at the levels in cream liqueurs, alcohol promoted age-gelation and prevented creaming.

Hence, the objective of this experiment was to elucidate the role of alcohol in the above defects by producing liqueurs of various alcohol contents using one or more different processes. The study was divided into three parts:

- *Part 1. Effect of alcohol added after homogenisation.*
- *Part 2. Effect of alcohol added before homogenisation.*
- *Part 3. Use of alcohol -treated caseinate in non-alcoholic emulsions*

MATERIALS AND METHODS.

The normal 16 % fat / 40 % t.s. formulation was used to manufacture liqueurs. The K1 caseinate was used in this study, except for part 3 where MD product was also used.

The alcohol contents of the final liqueurs were varied by adding alcohol solutions of various strengths. (Note that alcohol concentrations used in this experiment were quoted as % v/v rather than as % m/m basis. This was because liqueurs of different alcohol contents have different densities).

Fig. 4.1.1 outlines the manufacturing processes for the three parts of this experiment. All samples were homogenised with 1 pass of the Rannie homogeniser at a pressure of 27.6 MPa (4000 psi); other rele vant details are given with the results.

The details of the general testing methods were as outlined in previous sections. However, for the turbidity tests it was not accurate to weight out samples for further dilution in the turbidity tests. This was because the emulsions could have slightly different densities. Hence an equal volume (calculated to be 94 µl) of liqueur was taken to ensure that an equal quantity of fat (16mg) was taken for each test.

Part 1; Effect of alcohol level added to a homogenised cream emulsion

A 2-Sb process technique (see Fig 2.2.1, earlier) was used for batches 20/21 and 26;

Cream bases (cream, water, caseinate, citrate) were homogenised at 70 °C at 27.6 MPa with one 1 homogenisation pass (batches 20/21). Water/sugar or alcohol/water/sugar solutions were added to the cooled homogenised bases to produce samples with alcohol contents of 0 and 17 % v/v alcohol. A preservative (0.05 % sodium azide), dissolved in the water/sugar or sugar/water/alcohol solutions, was used in the water (0% alcohol) samples. All the water/alcohol /sugar mixtures were brought to the same volume before stirring into the homogenised cream bases. In this way all the resultant emulsions had the same composition on a m/v basis. This is

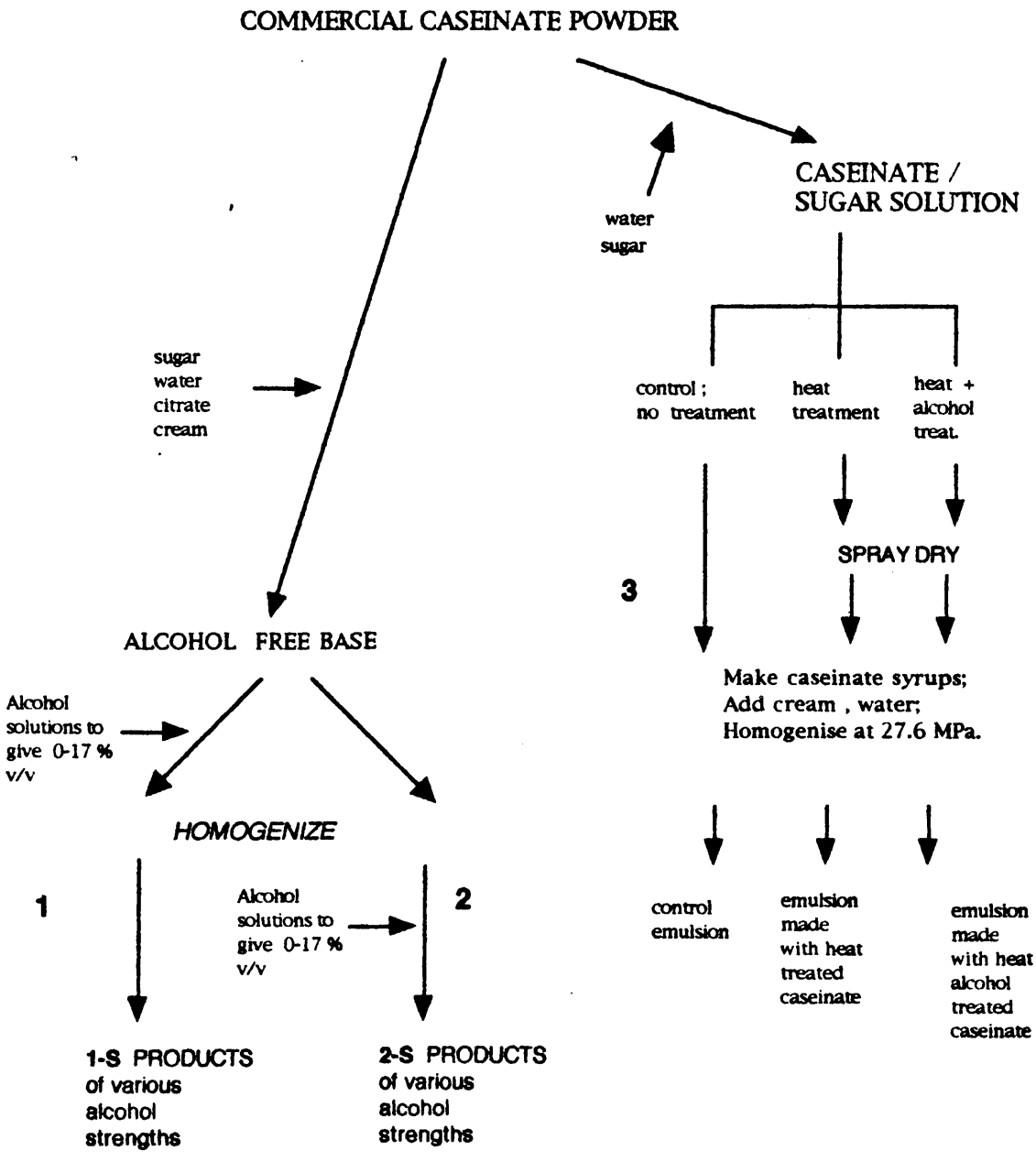


Fig. 4.1.1 Outline of experiments performed during the investigation of the effect of alcohol on emulsions. Parts 1 and 2 studied the effect of different levels of alcohol added before and after homogenization, respectively. Part 3 examined the effect of a mild heat and / or alcohol treatment of sodium caseinate on properties of non-alcoholic emulsions. . See text for further details .

the important criteria when measuring such phase-volume dependent measurements as viscosity and creaming.

The experiment was also repeated with a 2-S process (cream batch 25) using a processing temperature of 55°C. A sample containing a final alcohol level of 8.0 % v/v was also produced.

Creaming was measured either by centrifuging at 3000 ***g*** x 60 min or by allowing 100 ml of the sample stand at ambient temperature for 7 days. The cream layer was calculated as the % volume of the emulsion. Turbidity values (see note previously) were also monitored. These samples were kept for 1 week.

Part 2. Effect of alcohol added before homogenisation

A 1-S process was used for these experiments i.e. all the alcohol/water solutions were added (ca 20 % of the final liqueur volume) before homogenisation. The final alcohol levels were 0, 8 and 17% v/v alcohol (batch 19). Samples were processed at 65 °C, with 30 mins holding before homogenisation at 27.6 MPa.

For batch 26, extra alcohol levels were evaluated (0, 2.8, 5.6, 8.5 and 17.0 % v/v). The samples were processed, without citrate, at 27.6 MPa at 55 °C. These samples were not kept for long term stability results.

Testing, as described in the previous section, was continued up to 180 days of age

Part 3. Preparation and use of mild heat/alcohol treated sodium caseinates (MD and K1) in emulsions.

Sodium caseinate containing solutions were prepared as follows (per 50 litres) :

1.75 kg caseinate (K1 and MD) and 12.5 kg of sugar were dissolved in enough hot distilled/deionised water to bring the syrup solution to ca 38 litres. Twelve and a half litres of an alcohol solution (80 % v/v) was added, with efficient stirring, to the cooled syrup, to give a final alcohol strength of ca. 21 % v/v . The analysis of this caseinate/sugar/ alcoholic solution corresponded to that found in the continuous phase of a cream liqueur. The final volume was kept in a milk churn. A non-alcoholic solution was produced using 12.5 litres of water instead of the alcohol solution.

Solutions, in the churn, were then heated to 65 °C in a large water bath and held there for 30 min. This treatment corresponded to typical conditions used in cream liqueur manufacture.

After heat treatment, the solutions were spray dried in a Niro Atomiser Spray drier (Copenhagen, Denmark), using an inlet temperature of 180 °C and an outlet temperature of 85-90 °C. The resultant sugar / caseinate powders were stored in plastic bag liners until use.

The moisture contents were determined and the sodium caseinate and sucrose contents were estimated by knowing the original ratio of these ingredients . The final product was ca 12.3 % caseinate and 87.7 % sucrose (m/m dry-basis).

Caseinate syrups were made with these two mildly heat-treated powders ("alcohol treated" - KI H + A or MD H + A and "non-alcohol treated" caseinates- K1 H or MD†H), as well as with standard caseinate and sugar (the "control" caseinates- KI or MD), by adding the required amount of water and caramel.

The above caseinate syrups were incorporated into non-alcoholic "liqueurs" i.e. water replaced the alcoholic solution in the formulation of these emulsions. They were processed at a pressure of 27.6 MPa at 55 °C (no holding before hand) by the 1-S process. Trisodium citrate, where used, was added to samples before processing . The non-citrate samples were adjusted to a similar pH with NaOH, to eliminate pH as a factor in any differences. Cream batch no. 29 was used and the experiment was duplicated.

Samples were tested for % gravity creaming (ambient temperature), TV and viscosity. The samples were kept for 2 weeks.

RESULTS

Part 1; Effect of alcohol addition to emulsions *after* homogenisation (a 2-S process).

Table 4.1.1 summarises the changes brought about by substituting all of the alcohol with water in a 2-S produced liqueur (batch 20). Similar results were obtained with samples from batches 21/26. There were significant differences between alcohol and water containing samples.

Results from fresh emulsions

Viscosity at ambient temperature Addition of water rather than alcohol to the alcohol-free base reduced the viscosity of the emulsions by half (batch 20). In batch 25 samples, the same pattern was observed e.g. the viscosities of the 0, 8 and 17 % v/v alcohol samples were 12, 16 and 21 mPa.s, respectively.

For comparison purposes, the effect of alcohol addition on the viscosities of a variety of other solutions were examined (Table 4.1.2). Addition of alcohol to solutions containing high solids concentrations, whether sugar only (e.g. sucrose syrup), milk solids only (e.g. a 40 % skim milk solution) or milk solids/sugar (e.g. condensed milk) containing systems, led to relatively large increases in viscosity. In contrast, the viscosities of 10 % m/m caseinate solutions, when diluted with equal amounts of water or alcohol solution (to 21 % v/v alcohol final concentration) were similar.

Turbidity value / aggregation index If a homogenised alcohol-free emulsion was used as the basis for the water only and 17 % v/v alcohol products, then as expected, the TVs of these 1/1000 *diluted* samples were the same. The values were 0.165 in batch 20 and 0.210 for batch 26. The aggregation indices of samples were very low i.e. ca 5 % (batch 26). Microscopic examination of the emulsions showed that the water containing samples became very clustered as compared to alcoholic samples.

Creaming Cream layers were measured after centrifugation of batch 20 samples. The results were 13% and 2 % of the volume of the emulsion for the 0 and 17 % v/v alcohol products, respectively. In contrast, the creaming value of *both* of these emulsions when diluted by half with water was 5 %.

Table 4.1.1 The effect of the addition of equal volumes of (i) an alcohol solution or (ii) water, to a homogenised alcohol-free base. (Batch 20): average of duplicate samples

	Alcohol content of sample (% v/v)	
Test	0	17.0
Initial results		
pH	6.50	6.76
Viscosity (mPa.s)	13.2	23.2
TV (A800)	0.163	0.165
Creaming %	13	2
45 °C incubation results		
DVI -3day (%)	-3	+70
28 day incubation of 180 day old sample;	Stable	Gel

Table 4.1.2 The effect of adding alcohol or a similar volume of water (control) on the viscosity of various solutions. Alcohol (of various strenghts) was added to achieve a final concentration of 21 % v/v alcohol .

	Viscosity (mPa.s) after dilution		
Starting system	with water (control)	with alcohol	Comments
50 % w/v * or 75% w/v ** sugar	2.1 21.1	4.2 60.0	Alcohol added as 42 * or 100 % ** v/v solutions, repectively to achieve the 21 %v/v alcohol
10 % w/w sodium caseinate (K1)	16.5	16.5	Alcohol added as a 80 v/v solution to achieve 21 % v/v alcohol
Caseinate syrup	18.5	27.0	80% v/v alcohol or water added to achieve 21 % v/v alcohol and 3.5 % sodium caseinate (K1) 23 % sugar w/v .
Sweetened condensed milk	15.2	31.3	Commercial product diluted with 1:1 with 42 % v/v alcohol or water.
ca 40 % w/w skim milk solution	13.9	20.8	Product reconstituted from powder. 80 % v/v alcohol added to achieve 21% v/v alcohol .

Creaming in batch 26 (2-S process), was measured after 7 days undisturbed storage at ambient temperature; the values were 22, 15 and 5 % for the 0, 8 and 17 % v/v alcohol samples, respectively. Most of the creaming was completed after 3-4 days and during that time separate cream lines were often seen at the top and bottom half of the containers. These eventually merged to create the final single cream line.

pH There was a significant increase in pH (0.26 unit) due to the inclusion of the 17 % alcohol in the formulation. Adjustment of the pH of the water only samples with NaOH, did not influence any of the results reported above (batch 25: data not shown).

Results after incubation and storage of emulsions

Fresh samples from batch 20/21 were incubated at 45 °C for 3 days . The 0 % alcohol samples decreased in viscosity when incubated i.e. a % DVI of - 3, in marked contrast to the figure of + 70 for the alcohol containing sample. The viscosity of non-alcoholic and alcoholic samples decreased ca 20 % and 7 %, respectively, when held for 6 months at ambient temperature

The 180 day old 17 % alcohol samples gelled when incubated at 45 °C for 28 days. The water containing samples showed little if any increase in viscosity. The calcium sensitivities of these two samples (increase in turbidity due to addition of 1mM Ca^{++}) were similar when diluted 1/1000 in alcoholic or aqueous diluents (data not shown).

Part 2; Addition of various levels of alcohol to emulsions *before* homogenisation

Testing of fresh emulsions

Initial viscosities / creaming Initial viscosities and the creaming values obtained for the two different batches (26, 19) are shown in Fig. 4.1.2. The results were qualitatively similar to the 2-S samples mentioned previously, i.e. viscosity and creaming were significantly influenced by the presence of alcohol. However there were important quantitative differences. Creaming was especially sensitive to presence of alcohol during processing. For example, 2.8 v/v alcohol reduced the gravity creaming from 27 to 20 % while the viscosity (10 mPa.s), was not effected. Samples at an alcohol level of 8.5 v/v showed only 4 % creaming even though their viscosities (12 mPa.s) were not much greater than that of the 2.8 % sample. One very high level of alcohol (23% v/v) was tested but the liqueur was unstable (data not shown).

TVs / particle size In contrast to the 2-S samples (see Part 1, above), the TVs (A800) of the 1-S samples decreased linearly with increasing alcohol content . In batch 26, values decreased from 0.250 to 0.084 with increasing alcohol content (0-17 % v/v) ; Fig 4.1.3. Use of high levels e.g. 20 % v/v led to thickened liqueurs and very high TVs e.g. 0.405. The aggregation indices (< 6%), were low for all alcohol levels except the 17 % and the thickened 20% v/v liqueurs (AI ca 25%).

Coulter nanonsizer readings obtained from batch 19 samples indicated that the average particle size (d_{32}) decreased slightly i.e. 0.290 to 0.205 μm on addition of the 17 % alcohol (the corresponding TVs decreased from 0.207 to 0.082). However, samples analysed by the Coulter LS showed bigger differences in d_{32} i.e the values for the 0 and 17 % samples were 0.52 to 0.23 μm, respectively; Fig 4.1.4. illustrates typical size distributions for the above type samples.

More detailed investigations using these instruments (and other particles sizing methods) is necessary to decide whether the Nanosizer or LS 190 results are more accurate. The TV value data quoted above can be shown to correlate with the Nanosizer results (data extrapolated using Figure 3.1.8).

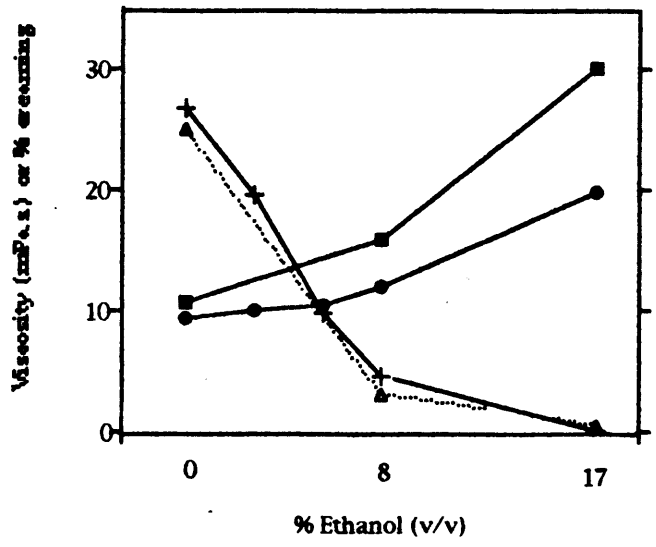


Fig. 4.1.2 Effect of ethanol content on the % creaming and the viscosity of cream liqueurs processed by the 1-S method. +; % creaming (centrifuge) in batch 19, Δ % creaming (7 day gravity test) in batch 26. Viscosity in batch 19 ◆ or batch 26● .

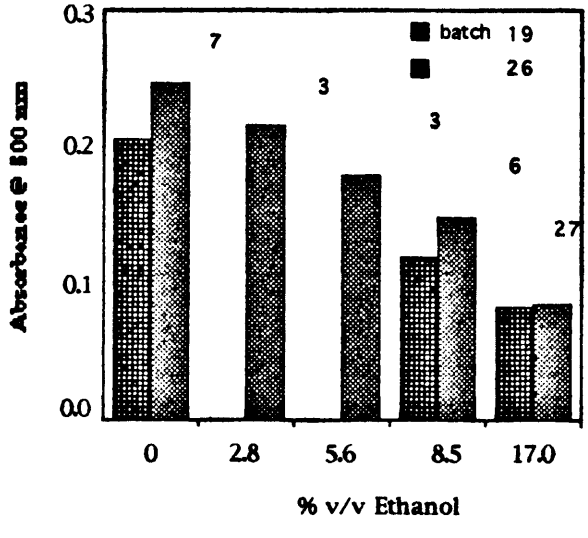


Fig. 4.1.3 Effect of ethanol content on the turbidity values (A 800 nm; diluent was EQA) of cream liqueurs manufactured by the 1-S process.. Results were from two different batches (19 amd 26) . The aggregation indices (batch 26 samples. only) are shown above the vertical bars .

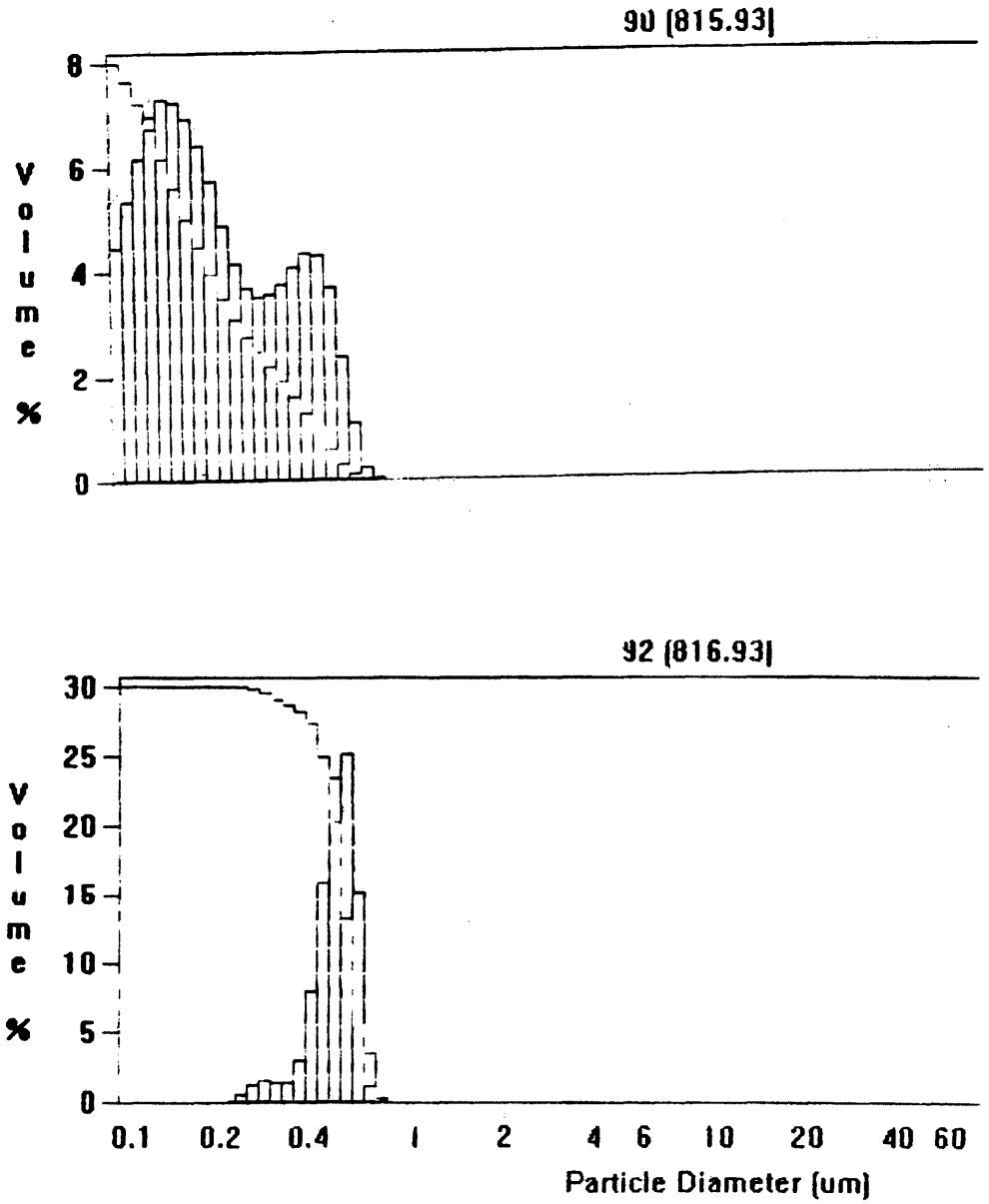


Fig. 4.1.4 Particle diameter /volume frequency distributions of samples processed with 17 % ($ample 90) and 0 % ($ample 92) alcohol See Materials and Methods section for more details ; note the optical model was Fraunhofer with PIDS. The reported d32 values (in the range 0.1- 1.4μm) were 0.48and 0.20 μm for 0 and 17 % v/v alcohol samples, respectively.

Incubation / storage.

Fresh samples from batch 19 (0, 8.5 and 17 % v/v alcohol) were incubated at 45 °C for 3 days. The % DVI data varied significantly with alcohol content, as was the case in the 2-S samples. The values are shown in Fig 4.1.5, together with the ambient temperature viscosity data at days 0 and 180. There was a dramatic increase in the rate of thickening of samples at alcohol levels between 6 and 12.7 % alcohol and this was not associated with changes in initial viscosity or TV (A800) . The 180 day samples of this batch were stable when incubated 28 days at 45 °C.

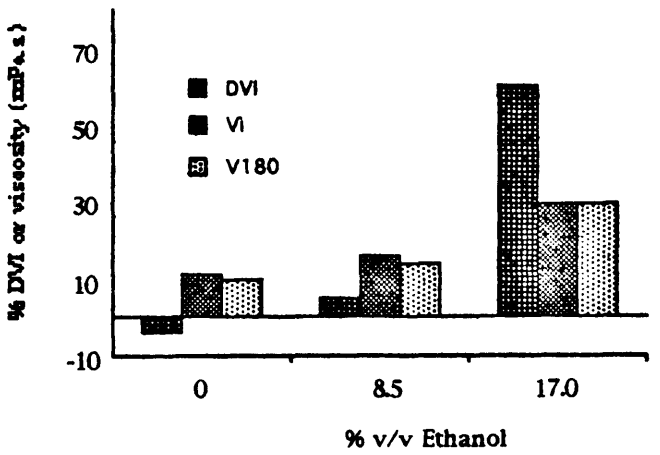


Fig. 4.1.5. Effect of increasing ethanol content on the initial rate of thickening at 45°C (expressed as % DVI, measured over 3 days). Cream liqueurs were processed by the 1-S method. The initial (V_i) and the 180 day (V_{180}) viscosities of ambient temperature stored samples are also shown. Results from batch 19, only.

Part 3; Effect of mild heat / alcohol modification of sodium caseinate on the creaming stability of non alcoholic emulsions.

In order to establish whether alcohol treatment has any irreversible physico-chemical effects on sodium caseinate, sugar-containing solutions of the two caseinates, MD and K1, were subjected to a mild heat treatment both with (KI H + A, MD H + A) and without (KI H, MD H) alcohol present. Non-alcoholic emulsions produced using these products were characterised when fresh and after standing for 7 days at ambient temperature. The results were compared to those of similar emulsions manufactured with the "untreated caseinates (K1, MD). Batch 27 cream was used.

Viscosity

Viscosities of all the control samples were slightly higher than the treated samples i.e. 14 vs 11.5 mPa.s. The addition of citrate resulted in the slight decrease (ca. 3-4 %) in the viscosity of all the samples.

Changes in creaming patterns.

There were significant differences in creaming due to mild heat and/or alcohol treatment of the sodium caseinate solutions. These changes varied with the source of the caseinate; see Fig 4.1.6.

In both cases, the "control" samples (emulsions manufactured with untreated commercial powders gave significantly higher creaming than the "treated " caseinate containing samples. This was most obvious when citrate was included in the formulation. There was a large increase in creaming due to citrate addition with the K1 sample (only) ; in the other 5 samples, there were relatively small differences.

The non alcohol treated K1 H samples showed less creaming when compared to those treated with alcohol / heating and drying (K1H + A). The MD caseinate showed the opposite trend i.e. the MD H + A sample showed less creaming the MD H sample.

turbidity

Differences in turbidity values (A800 in water), although less dramatic, largely mirrored those of the creaming data, especially for MD-caseinate containing emulsions. Figure 4.1.7 demonstrates the relation between TV (A800) and creaming; the greatest correlation was with the + citrate samples.

Aggregation index values were very low for all samples (0-5 %) in agreement with results obtained in part B.

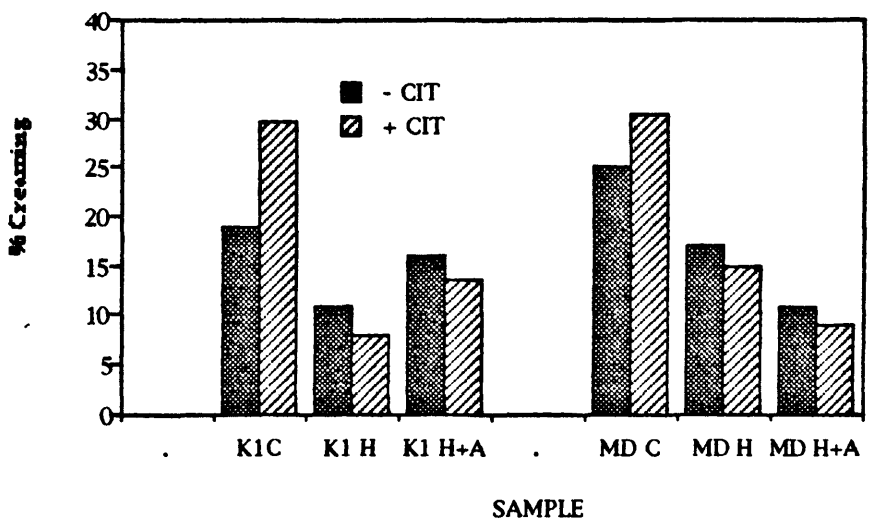


Fig. 4.1.6 Effect of caseinate modification on the creaming stability of emulsions (alcohol-free "cream liqueurs "). C ; control caseinate (untreated powder from bag); H; heated caseinate. , H+A; heat and alcohol treated. product. Creaming was reported as % visible gravity creaming. Two caseinates were used , K1 and MD. See text for further details .

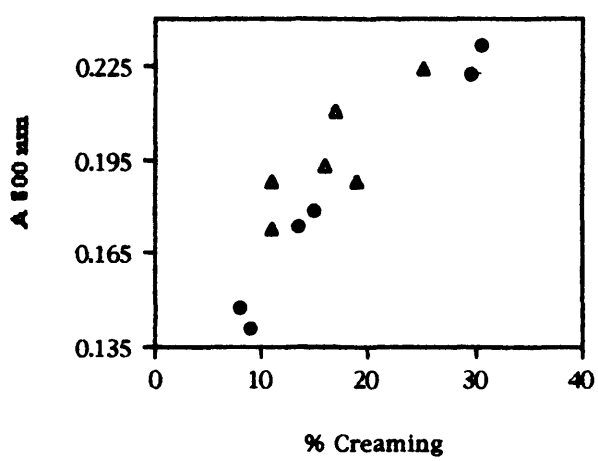


Fig. 4.1.7. The relationship between turbidity values (A800 ,measured in water) and the % gravity creaming of the above samples, Δ no citrate • + citrate.

DISCUSSION

The data, which confirmed and elaborated on results obtained in Experiment 3.3, indicated that alcohol had very significant effects on the creaming and thickening/gelation properties of emulsions. The most significant results were obtained in part A, where differences in the above phenomena were shown to be due to the presence of alcohol in the continuous phase, rather from any processing effect *per se*. However, the results from parts B and C, indicated that the alcohol could have some direct physico-chemical effects on the properties of caseinate. These results are discussed in more detail below.

Creaming

There are five possible explanations to explain the large differences in creaming between non-alcohol and alcohol containing samples; these are coalescence, an increase in continuous-phase viscosity, a decrease in average particle size , particle aggregation and a greater density difference between continuous and disperse phase in alcohol samples (see Conclusion).

Coalescence coalescence of the globules was ruled out as the cause of creaming in the water or low-alcohol containing samples; for example, the TVs (A800) and R values from alcohol and water containing emulsions were identical in 2-S processed samples (Part A).

Viscosity it is tempting to consider that the increased viscosity of the continuous phase (due mostly to the effect of alcohol on high concentrations of sugars) is the main cause of the large differences in creaming between the water-only and alcohol-containing samples. However, the evidence suggested that apparent viscosity is not the most important factor. For example

- there was a 6 fold difference in creaming, not a 2 fold one as predicted from Stokes Law, between 0 and 17 % samples (part A);

- a 6 fold difference in creaming was also found between 0 and 8.5 % alcohol samples (part B) despite minor viscosity differences;

- an alcohol containing liqueur was diluted down (with ca 10 % of an equivalent alcohol solution) to the viscosity of a water only sample, with no significant increase in creaming (data not shown) .

Decrease in average particle size this cannot be the main explanation since emulsions with the same initial particle size distribution (samples produced by the 2-S process; part A) showed very large differences in creaming. The decrease in average particle size that occurred with increasing alcohol content in the 1-S process (part B) probably had a minor effect in decreased creaming in those samples.

Aggregation The results indicated that it was the presence of alcohol that created the special physiochemical environment that prevented well homogenised emulsions from creaming via an aggregation or clustering mechanism. This type of clustering can be distinguished from clustering that takes place during thickening of liqueurs at 45 °C or clustering that can take place on omisson of citrate in most 1 -S samples (see later), since the clusters were very easily dispersed on dilution with water and shown normal aggregation indices (AI). The are three possible separate mechanisms, namely electrostatic repulsive, rheological and steric, involved in explaining this type of aggregation. Combinations of this effects may occur:

Electrostatic repulsion fat globules, are repulsed by one another (electrostatic repulsion) due to favourable changes in the charges on the interfacial proteins. It is known that the presence of alcohol influences the pKa of the ionisable amino acid groups through changes in the dielectric properties of the medium (Pierre, 1989). Note; It is interesting that alcohol-containing caseinate emulsions (> 5 % w/w; Bullin et al, 1988) were very susceptable to flocculation on 1:1 addition of 2 M sodium chloride. This may be due to a decrease in the magnitude of the repulsive electrostatic charge.

Rheological changes besides differences in apparent viscosity (see earlier), changes in the conformation of the proteins in the presence of alcohol, may contribute to the weak gel type network (higher yield values etc). This was not be measured by the high shear measurements in the present study. Dickinson & Woskett (1988) noted that just 1 % wt ethanol in the aqueous phase was sufficient to prevent formation of any surface viscosity in adsorbed caseinate films. They considered that ethanol disrupts the interactions, possibly of a hydrogen bonded nature, which lead to viscous film formation.

Steric stabilisation Another mechanism that could be involved in the observed particle aggregation is steric stabilization. Here, interparticle interactions, mediated via interfacial and/or non-adsorbed proteins, are reduced in the presence of alcohol. This may not involve κ-casein, since evidence from micellar casein environments (Horne & Davidson, 1986; Griffin *et al.* , 1989) indicates that alcohol collapses κ-casein, which is the steric stabilizing layer in micelles.

Thickening and gelation

While the addition of alcohol increased the initial stability of the products (e.g. less creaming), it had the opposite effect on the storage stability of the products. There was increased susceptibility to thickening and gelation, in the presence of alcohol even with samples of the same initial particles size distribution (part A). This may be because the particles are arranged differently in the three dimensional space of the emulsion: In alcoholic samples, an interlocking network of well spaced out particles is formed which can immobilize water leading to an increase in the effective discontinuous phase, thus increasing viscosity and possib ly leading to gelation. As mentioned in the section above, these particles may be more susceptible to this macromolecular interlinking by nature of the effects of alcohol on the interfacial macromolecules. In water containing samples, the particles may aggregate together in clusters e.g. grape- bunch type effect, which, while leading to creaming, does not form extensive interlinking networks. (Note: gelation of emulsions made with 0 % alcohol, even those made with the highly unstable cream in batch 19 -see later experiments- has never being observed. However, more long term experiments need

to be performed before on could say that water-only samples never gel during incubations or storage).

Calcium binding may also play a role in explaining the differences in thickening behaviour in alcoholic/non alcoholic systems. It has been found that caseinate dissolved in alcoholic sucrose solutions is about 10 times more susceptable to "aggregation", as measured by turbidity increase, than caseinate in aqueous sugar solutions (see next experiment for more details on calcium sensitivity of caseinate solutions).

Effects of alcohol/processing on average particle size.

Processing in the presence of alcohol decreased the average particle size (Part B), and this factor may have contributed somewhat to the decrease in creaming in alcohol-containing samples. These results are in agreement with research with model systems containing n-tetradecane (15 or 10 %) and sodium caseinate (0.25, 1 or 3%) in 0.005 M phosphate buffer (Bullin *et al.*, 1988; Burgaud & Dickinson, 1990). For example, the volume median diameter of the ca 20 % w/w alcohol (in continuous phase) sample (ca 0.8 μm) was half that of the corresponding water-only sample (Burgaud & Dickinson, 1990).

Bullin *et al.* (1998) considered that the superior emulsification efficiency of caseinate, in the presence of alcohol, can be explained in terms of the substantial lowering of the interfacial tension between oil and continuous phases. The authors stated that another contributory factor could be the change in state of aggregation of the protein; the existence of larger casein particles in the alcohol-containing samples would mean faster mass transport of protein to the interface and therefore more efficient homogenisation (Dickinson & Woskett, 1988). The creaming, turbidity and viscosity results in part C the current experiment, also indicate that heat/alcohol treatment can modify caseinate to produce a more surface active product, which when used in non-alcoholic emulsions, results in a finer average particle size emulsion. This accounts for some at least of the decreased creaming values of the treated samples.

It was surprising that such a mild treatment, such as solubilization/heating at 65 °C for 30 min and spray drying, decreased the extent of creaming in emulsions prepared

with these caseinates. Sodium caseinate is normally considered a very heat stable protein. However, these results are in agreement with results found in preliminary experiments, where increased heating / processing temperatures decreased the TVs of 2-S cream liqueurs.

The concentration of alcohol is critical to the decrease in average particle size ; the amount present in cream liqueurs 17 % v/v (i.e. 23 % v/v of the continuous phase) is almost optimum. Levels > 19 % v/v lead to production of highly unstable liqueurs, presumably due to aggregation of the caseinate and these results are similar to those found by Banks & Muir (1985).

CONCLUSIONS

Increasing the alcohol content of liqueurs, in the range 0-17 % v/v, increased (i) the initial viscosity (ii) the rate of viscosity increase at 45 °C and (iii) the stability to creaming. The stability to gelation was decreased in the same circumstances.

Aggregation of fat globules* was considered to be the principle cause of creaming, although rheological and/or, in the case of 1-S processed products, decreases in average particle size could also play roles.

The increased emulsification efficiency of caseinate homogenised with alcohol is due mainly to the presence of alcohol in the continuous phase, although previously heat and/or alcohol-treated caseinate sometimes showed superior emulsification properties (decreased average particle size) in non-alcoholic systems.

The differences in viscosity increases and gelation behaviour, on incubation of the samples at 45 °C may be due to differences in the three-dimensional relationships between the globules. This leads, in the case of 17 % v/v alcohol products to interactions between interfacial and/or unadsorbed proteins with resultant immobilisation of water (gelation).

* The exact contribution of the greater density difference between continuous and disperse phases in the alcohol containing samples should be further investigated. For example, the (lower) densities of the continuous phases in alcohol-samples could be increased to that of the water-only samples by adding extra sugar.

Experiment 4.2 *Different sodium caseinates:- properties in solution and in cream liqueurs.*

INTRODUCTION AND OBJECTIVE.

The results of preliminary experiments, reported in Chapter 2, demonstrated an unexpected and serious gelation problem in many experimental samples. This happened despite the fact that liqueurs contained the recommended amounts of citrate and were adequately homogenised.

Since all of the defective cream liqueurs were produced with the same caseinate (K1), it may be considered that this caseinate was one of main factors responsible for the instability. The objective of this trial was to ;

- establish if the use of different caseinates resulted in cream liqueurs of different initial characteristics (e.g. pH, viscosity, etc.) and storage stabilities.

- provide further information on the shelf-life testing methods and defects already mentioned in Experiment 3.3.

MATERIALS AND METHODS

Sodium caseinates (powders)

Eight different samples of commercially and laboratory-produced sodium caseinates, as well as two heat treated samples, were utilised in the experiment to characterise different properties in various non-emulsified model systems (aqueous solutions and fat-free bases) and in standard cream liqueurs. These are outlined in Table 4.2.1. Protein contents of caseinates were measured by macro-Kjeldahl (Kjeltech System) and moisture contents by drying to a constant weight in an oven at 102 °C. In addition the following tests were performed on two caseinates (K1, MD); ash (after treatment at 550 °C for 4 hours), fat content (Gerber method as described by Eisses *et al.*, 1972), whey protein content (method as described by deKoning *et al.*, 1976), calcium contents by atomic adsorption spectroscopy (IDF, 1992*b*)and phosphorus (IDF, 1990) . On one occasion samples were also examined by urea -polyacrylamide gel electrophoresis (alkaline urea -PAGE) by the method of Andrews (1983). Gels were stained by the method of Blakesley & Boezi (1977).

Manufacture of "heat-modified" caseinates using sample K1.

In an attempt to see whether post-production heat treatment would modify the properties of a commercially produced caseinate, a sample of K1, in solution,was subjected to two different treatments i.e. roller drying or autoclaving. The caseinate was dissolved (10 % m/m) with hot distilled water, cooled to ambient temperature and left overnight in a 5 °C cold room. The following procedures were then used;

- *Roller drying* of the above solution was performed on a Simon Richard pilot-plant roller dryer. The drum speed was 1 revolution/50 seconds and the steam pressure of 275 kPa was used. It was calculated that a film of casein solution was in contact with the hot (130°C) drum for about 40 sec. The dried product had a very low unsettled bulk-density and its water content was reduced to 0.1 % m/m.

- For *autoclaving*, 5 kg of the caseinate solution was autoclaved for 20 min (not including heat-up and cool down time) at 115 °C. On cooling, the solutions were frozen on shallow trays and freeze dried (Unitop 800 L, Virtis Co., New York). The final water content of the product was 0.1 % m/m.

Table 4.2.1. Details of the various sodium caseinate samples used in the study. Further details are presented in the results section.

Code	Samples details	Comments
K1-K4	Four samples of "Kerrynor" product from Kerry Group, Tralee.	K1 was chosen as the standard caseinate at the start of the study. The other 3 K samples were from different production codes
MD	Sample of Mirprodan 31 product from MD Foods , Denmark.	Supplied by Signer Hegner, London. This product is marketed as a low calcium product and is recommentded for cream liqueur usage.
NZ	Sample of Alinate 180; New Zealand Dairy Board.	Recommended for cream liqueur usage. Gift from the Irish Dairy Board, Moorepark
AV	Sample from Avonmore Foods, Ballyraggart	Commercial pilot-plant product
SP	Scottish Pride, Scottish Milk Marketing Board.	This sample was in the Department store for many years
RDL	A UCC pilot roller dried sample	No details of product or formulation

Non-emulsion systems

Formulation

In order to characterise differences between samples caseinates were incorporated into three different types of solution:

- *Aqueous solutions ("caseinate solutions").* Caseinate solutions were prepared, usually at 1 -6 % m/m protein in distilled water, using a Cenco Solubility Index Mixer (Cenco Ltd., Netherlands). To produce a solution of x % protein, (x/p) grams of caseinate were added to 100-(x/p) grams of hot (55-60 °C) distilled water in the special glass jar, where p= % protein of the caseinate divided by 100. Automatic mixing (90 s) was started, after which the sample was allowed to deaerate and cool for at least 1 hour. Where necessary, solutions were diluted down to working concentrations.

- *Caseinate syrups* Uncoloured caseinate syrups, of similar composition to those coloured ones used in cream liqueur manufacture, were prepared with (m/m) ; 5.7% protein (from the caseinate), 39 % sucrose, 0.37 % w/w trisodium citrate and ca 54 % boiling distilled water. After mixing (see above method), the samples were placed in a water bath at 50 °C for 10 minutes. They were allowed to cool and placed in a cold room overnight.

- *Fat Free Mixes (FFMs)* A fat-free mix (FFM) is an unhomogenised solution that contains all the ingredients of a cream liqueur except the fat and phospholipid constituents. Small quantities can be produced in an attempt to simulate aspects of the real cream liqueur system. When formulating a FFB, all the ingredients are added except the butterfat globules of the cream. Hence all the ingredients of the standard 16 % fat product are concentrated (on a weight basis) by a factor of 100/84. Skim milk is added at a level corresponding to its presence in the continuous phase of the cream liqueur. Table 4.2.2 shows an example of a formulation. Citrate or other salts can be added when required.

Table 4.2.2 Basic formulation of a fat free base (FFB). Additives e.g. citrate were included as desired.

Ingredient	w/w
Separated milk	20.0
Sugar	22.5
Sodium caseinate	3.6
Alcohol (94 % m/m)	16.1
Citrate etc	x
Water	to 100

Tests

The solutions mentioned above were characterised using the following tests; pH, turbidity, particle size (photon correlation spectroscopy), calcium sensitivity, incubation tests and solubility. Most of these were performed as described in previous sections. The following extra tests are described.

Solubility indices; the solubility indices of freshly made 1 % protein solutions were estimated by expressing the A_{280} of centrifuged supernatants (an index of protein concentration) as a percentage of that of the original solution. Supernatants were produced by centrifuging 20 mls samples at 30, 000 ***g*** x 60 min or 50 ml samples at 1000 ***g*** x 20 min. Before readings, the protein solutions/supernatants were diluted by 1/50 with 0.1M NaOH.

Calcium sensitivity changes induced in caseinate solutions on exposure to Ca^{++} in a simulated liqueur continuous phase systems, were measured according to basic methods;

Turbidity method Caseinate solutions where diluted with various alcohol/sugar/Ca^{++} solutions, to give a final concentration of 0.5 % or 1.3 % protein, 0 -1.33 mM Ca^{++} , 22 % v/v alcohol and 26 % w/v sugar: For example; 2 ml of a solution containing 32.8 % v/v alcohol, 38 % w/v sugar and 2 mM Ca^{++} were added to 1ml of a 4 % protein solution in a disposable cuvette. (All solutions were

at 20 °C and were adjusted to pH 7.0 with 0.1 M HCl or NaOH). After capping the cuvette, the solutions were mixed immediately by ten inversions. The absorbancies at 800, 600 or 400 nm were read after 30 mins to monitor the appearance of very fine milky precipitate. The calcium sensitivities of a batch of 10 month old FFBs were assessed by adding 0.1 ml into 3 ml diluent so that the final concentration of alcohol/sugar was as above; the Ca^{++} was 1 mM.

Precipitation method the amount of protein precipitated by centrifugation, after addition of Ca^{++} to alcoholic/sugar solutions, was measured essentially by the method of Arima *et al.* (1964), e.g.; 4 ml of either 0, 20, 40 or 60 % v/v alcohol were added, with stirring, to a 6 ml solution which contained; 2 mls of 1.5 % caseinate, 0.3 mls of 0.1 M $CaCl_2$, 1.7 ml distilled water and 2 ml of 0.2 M borate buffer (pH 6.8). The solution was centrifuged at 1000 ***g*** x 10 min, after which the protein content of the supernatant was compared to the original solution by the method described earlier for "Solubility ". However, the test was carried out at ambient temperature and not at 0-2 °C, as described by Arima *et al.* (1964).

"Filterability" the filterability of caseinate solutions (either before or after calcium addition) was assessed by pushing ca 2 ml samples through a 13 mm 0.22 μm syringe type membrane filter (Gelman Acrodisc, no. 4454). A single sample of K1 (produced after dilution of a 6 % sample) was also assessed by measuring the amount of protein passing through 0.05 μm (Millipore type VM) and 0.22 μm (Millipore type GS) filters in a vacuum filtration type unit. Filters were ca 4 cm in diameters and only 20 ml samples were used to avoid possible clogging of the filters. The protein concentrations and absorbancies of filtrates were estimated as described previously (see "Solubility indices", above).

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Cream liqueur systems

Manufacture

Table 4.2.3 gives details on the 4 cream liqueur batches used in this experiment. Cream from a commercial source was used in three of the batches (15-17) ; the remaining experiment (18) used cream from a single herd. The sodium caseinate samples used were as outlined previously (Table 4.2.1). Based on previous experiences, the following formulation and processing schemes were used.

Homogeniser, batch size and number of passes; it was decided to use only one pass of the more efficient Rannie homogeniser to process samples. Previous results had shown that these 1 pass samples were just as, if not more, stable than 2 pass samples. This reduced the size of the batch size in half to 2750 g and thus saved in the cost of expensive ingredients i.e. alcohol and cream.

Homogenisation pressure a pressure of 27.6 MPa ,with the one pass procedure mentioned above, is sufficient to produce a product stable to creaming.

Time /temperature, protein addition Previous experiments had shown that it is possible to produce liqueurs with a viscosity and protein content similar to that of commercial products by using an added caseinate level of ca. 3 % and by using a process temperature of 65 °C with holding at this temperature for a period of time.

Table 4.2.3 Details of 4 different batches of samples processed to study the effect of different caseinates on the properties of cream liqueurs.. Most of the samples were duplicated (x2). Batches marked * used a commercial cream source , while batch 18 used cream from an individual herd (see Appendix 3). Details of the different caseinates used were given in Tables 4.2.5 and 4.2.6 (see later) .

Batch no.	Process date	no. of samples	Object of experiment	Caseinates used	Process conditions
15*	31 03 93	12x 2	The use of 6 different caseinates in the 1-S and the 2-S processes.	K1, K2, MD, NZ, RDL ,AV	27.7 MPa; 1 pass at 65 C with 30 mins holding before. homogenisation
16*	20 07 93	8x 2	The use of six different caseinates and 2 heat treated K1 samples; 1-S process only.	K1, K1R, KIA, K2, K4, NZ, RDL, SP	27.7 MPa; 1 pass at 65 C with 30 mins holding before. homogenisation
17*	18 05 93	8x 2	The use of 2 different caseinates processed with and without citrate (1-S process only). Citrate was added to some samples afterwards..	KI and MD	27.7 MPa; 1 pass at 65 C with 1 min or 30 mins holding before. homogenisation
18	23 07 93	4x 1	The use of two different caseinates processed with 2 pass	K1 and SP	27.7 MPs; 1 and 2 pass at 55°C x 1 & 65°C x 30 mins holding before homogenisation.

tests

The following tests were performed on fresh samples ; TV (water diluent only), viscosity, pH, total solids and fat (selected samples). In order to evaluate creaming, some samples were observed under the microscope or were centrifuged.

Samples were incubated at ambient temperature, 45 and 65 °C in an effort to characterise the viscosity increases and in order to predict, as soon as possible after manufacture, any tendency to gelation (Table 4.2.4). Details of these tests have been outlined in experiment 3.3.

Calcium sensitivity tests on cream liqueur samples were performed by diluting cream liqueurs 1/1000 in Ca^{++} containing alcoholic/sugar solutions. The final composition of solution was; alcohol (22 % v/v), sucrose (26 % w/v), Ca^{++} (0, 1mM) and pH 7.0. Changes in the A800 and /or R value were noted. Calcium sensitivities on a 6 month old batch (batch 16) of the FFBs, were also performed at the same time as the corresponding liqueurs; in this case, 0.1 ml of FFB was added to 3 ml of diluent.

Table 4.2.4 Details of incubation testing schemes used for the four series of samples produced in this experiment.. Ambient temperature samples were tested when they were at various ages.:

Batch	Age of ambient sample (days) when incubated by:		
	45 °C incubabation tests		65 °C test
	3 day test	28 day test	1 hour
15	0,14,42,92,180	180, 340	1,6,13,26,42,56, 84, 180
16	not performed	0, 28, 180,	0,1, 14, 28, 56,84, 180
17	0, 28	180	0,1, 14, 28, 56,84, 180
18	not performed	0, 28, 180,	0,1, 14, 28, 56,84, 180

RESULTS

Analysis of powders

The analysis of the caseinate powders and their 1 % protein solutions are summarised in Table 4.2.5.

An obvious visible difference between the powders was their relative bulk densities. All the K samples and the NZ sample had values ca 50 % higher than the AV, MD and SP samples. These high bulk density products were similar in consistency to dried skim milk powder i.e. finely granular. The low values are similar to "fluffy" dried powder products produced in this department by using a disc atomizer. Roller drying the K1 product produced a very low bulk density product since the speed of the rollers was set such that a very dry powdery, rather than a flaky, product was obtained. Freeze-drying the autoclaved K1 product produced a hard foamy type structure which was ground down for use. The colour of the this product was a very distinctive salmon pink . This was due to reactions, probably of the Maillard type, which occurred during the autoclaving process and before the actual freeze-drying.

All the caseinates contained ca 95 % m/m protein (dry basis), while gel electrophoresis of the samples revealed differences in the upper and middle regions of the lanes (Fig 4.2.1) All of the K samples had clearer looking upper areas and contained two small bands between α and β casein Heat-treatment of K1 by roller drying and especially autoclaving, caused a significantly darkening / blurring of the upper region possibly indicating that non-K samples received significantly more heat-treatment during processing. i.e. samples MD and SP had dark upper regions and sample SP also contained less obvious bands in general The α-casein component almost disappeared in the autoclaved samples and this was not noted in other samples. However, none of the other samples showed evidence of Maillard reactions (off-colour or smell)

The calcium content of four of these samples indicated large differences: The SP product was very low in calcium (0.01%) while the MD and the K products contained 0.05 and 0.07 %, respectively.

The ash, fat and % whey protein contents of two caseinates (K1 and MD) were analysed and were found to be basically similar (Table 4.2.5).

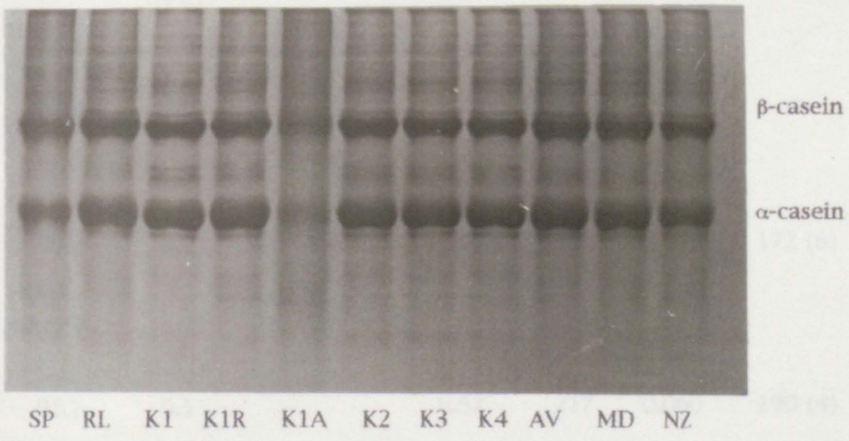


Fig. 4.2.1 Ge;l electophoresis (alkaline urea type) of different caseinate samples. SP; Scottish Pride, RL; Departmental roller-dried caseinate, K; various Kerry caseinates-A; autoclaved and R- roller dried treated K1, Av; Avonmore, MD (Danish), NZ; New Zealand Dairy Board..

Table 4.2.5 Analysis of the sodium caseinate samples used in the study. Results are presented for the powders and 1 % protein solutions (uncentrifuged) prepared from them. Particle size was read with the Coulter Nanosizer, using ca 0.3 % protein solutions. A sample of skim milk was included for comparison. See Table 4.2.1 for details of codes; samples K1R and K1A were produced from K1 by autoclaving and roller drying treatments, respectively. .

	Powder analysis			Analysis on 1 % protein solutions				
Code	Protein (% w/w)	Moisture (% w/w)	others * (% w/w)	pH	Conductivity (mho)	Turbidity (A660)	Particle diameter (nm) **	Solubility index (%) ***
K2	87.5	6.7		6.65	242	0.079	-	-
K3	88.5	7.0		6.66	272	0.059	-	-
K4	88.0	7.0		6.71	247	0.080	-	-
K1	90.2 (0 % whey)	6.2	4.0 Ash 0.07 Ca 0.07 P 0.9 fat	6.59	217	0.046	172 (6)	96
K1R	95.7	0.5		6.52	217	0.060	190 (4)	100
K1A	95.7	0.5		6.36	252	0.147	310 (4)	98
MD	88.5 (0% whey)	6.0	3.5 Ash 0.05 Ca 0.08 P 0.9 fat	6.96	292	0.300	350 (3)	93
SP	85.0	9.6	0.01 Ca	6.61	302	0.061	225 (3)	100
NZ	91.5	4.3		6.56	212	0.062	ns	ns
AV	87.5	9.6		6.73	232	0.082	ns	ns
RDL	88.8	8.0		6.76	252	0.100	ns	ns
Skim milk							180 (1)	35

* Ca; % m/m total calcium; P; % m/m total phosphorus.
** The polydispersibilty index is included in brackets.
ns; no sample measured
*** 30000 g x 60 min

characteristics of aqueous caseinate solutions (as 1% m/m protein)

The results of tests on these solutions are also reported in Table 4.2.5

pH, conductivity

The pHs of the 1% protein solutions varied from 6.52 - 6.96, although most of the samples were around 6.7 +/- 0.1. The MD sample was an exception; this sample had the highest pH; 6.96. Severe heat treatment of the K1 sample significantly reduced its pH in solution from 6.59 to 6.36. Conductivity values, which would reflect the amount of free ions e.g. Na^+, H^+ etc. did not correlate well with pH values e.g. sample SP and MD had similar conductivities despite having different pHs (6.61 versus 6.92). Heat treatment of the K1 sample increased its conductivity by 16 %.

Size indices

The appearance of the solutions varied significantly and this was reflected in the absorbance readings at 600 nm (A 600) , particle size and solubility index readings :-

- The high A 600 readings of the MD sample was related to the large amount of insoluble protein particles (ca 7 % by weight) sedimented after centrifugation at 30 000 g x 1 hour. The A600 readings were decreased four-fold during this operation while ca 90 % of the turbidity could be removed by filtering through a 0.45 μm syringe filter.

- K samples showed a significant amount of very large insoluble particles (ca 4-5 % of the protein), when prepared as a 1 % solution in the standardised mixing fashion. Some of these particles sedimented out quickly, while the others remain in suspension. They were visible to the naked eye were be centrifuged out at low centrifugation values e.g. *g* x 1000. Paradoxically, it was found that if 1 % solutions were prepared from 6 % m/m stock suspensions, little or no such sedimentation was found, despite that fact that the turbidity of these diluted-down 1% solutions was greater than the customised 1% solutions.

- The average particle sizes of the caseinate suspensions, as indicated by the Nanosizer were very large i.e. 200-300 nm; (Table 4.2.6). The Coulter N4 instrument, which has a broader sizing range (4-4000 nm) than the Coulter Nanosizer (40-3000 nm), gave very different results when used to assess the KI sample. The N4 gave an average particle size (differential weight) of 49 nm for the K1 sample (Fig. 4.2.2) while the Nanosizer result was 240 nm.

  Average particle sizes for the MD caseinate solution and a skim milk sample were similar on both instruments, thus the large particle sizes of the MD sample, which were indicated by centrifugation and filtration results (above) were confirmed. In addition, size distributions generated by the N4 and these indicated bimodal distributions, especially for the MD sample where two peaks with maxima at 95 and 533 nm, were obvious.

  The average size in a skim milk suspension increased on addition of TSC (Coulter N4 and Nanosizer); Table 4.2.6. Addition of 10mM citrate decreased the Nanosizer average particle size for the K1 and MD samples. Spectrophotometric data indicated that the R value for skim milk, ca 0.150 (diluted in water) increased to 0.180 on standing for 1 hour. However, the value stayed the same if 5 mM Ca^{++} ion were added to the diluent (data not shown).

Calcium sensitivity

The calcium sensitivity of the solutions, which depended on the method of measurement and on formulation techniques, varied widely (Figs. 4.2.3, 4.2.4).

When assessed by the relative increases in A 600 in alcoholic solutions, the K1 samples showed highest calcium sensitivity (Fig. 4.2.3), the MD sample the least sensitivity, while the SP samples showed an intermediate response. The heat treatments of K1 did not seem to have a major effect on the calcium sensitivity.

In contrast, the K1 sample was the most "stable" and the SP sample the most unstable to Ca^{++} if the amount of sedimentable protein in 24 % v/v alcohol solutions was

Table 4.2.6 The average particle size of aqueous caseinate solutions as measured by Coulter Nanosizer, Coulter N4 (both photon correlation spectroscopy instruments) or optical spectroscopy. Both unimodal and differential weight mean diameters are reported for the N4. The caseinate solutions were prepared from 5 % m/m protein stocks that had been centrifuged at 1000 x *g* for 20 min .

	Average particle size (nm)			Spectrophotometric data	
		Coulter N4			
Sample	Coulter Nanosizer	uni modal	diff wgt	A800	R index
Caseinates					
KI	240	234	49	0.038 $(0.015)_f$	0.202, $(0.178)_f$
KI +TSC	190			0.038	0.188
MD	220	299	293	0.229 $(0.029)_f$	0.237, $(0.156)_f$
MD + TSC	200			0.257	0.241
Skim milks					
skim milk	155	178	125	ns	0.150
skim + TSC	188	192	189	ns	ns

TSC; 10 mM trisodium citrate
ns not tested
f; a sample (ca 3 ml) was filtered through a 0.45 um membrane syringe f ilter (Gellman, Acrodisc).

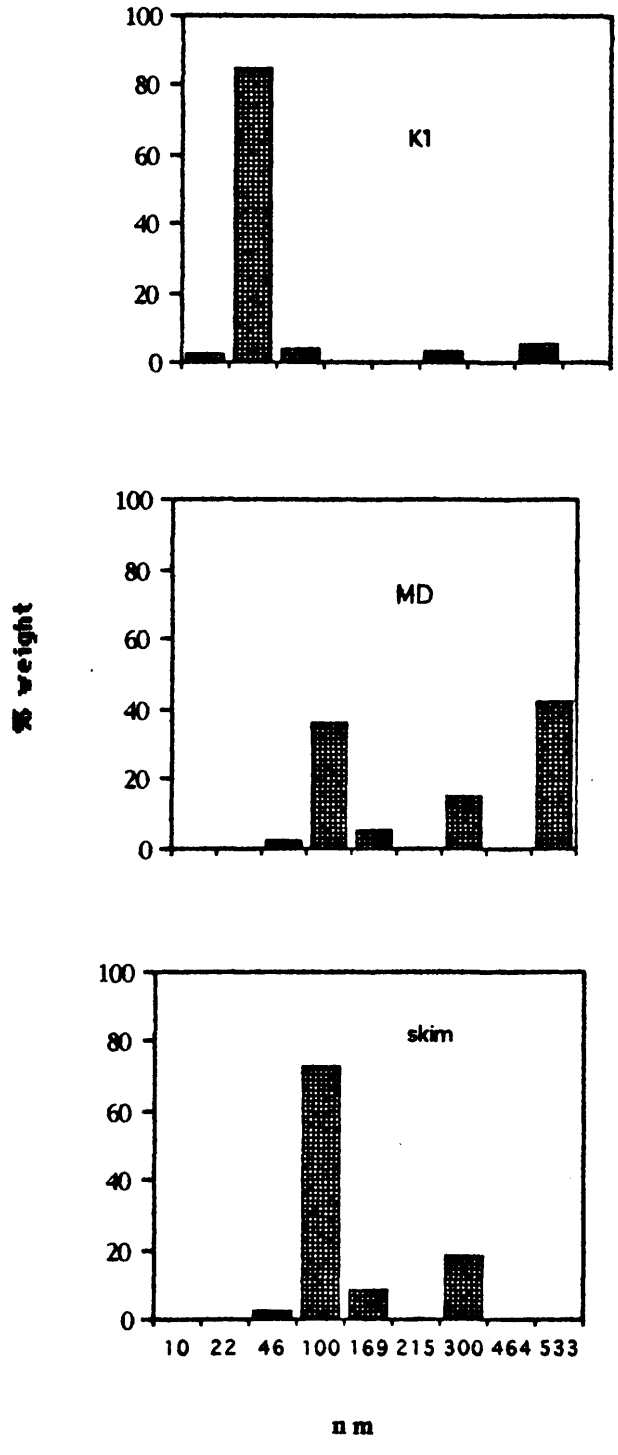


Fig. 4.2.2. Size frequency distribution (by weight) of two different sodium caseinate suspensions , (K1 and MD) and skim milk, as estimated by photon correlation spectroscopy (Coulter N4). The diluent was water at 20 °C.

measured (Fig. 4.2.4). Note that the ratio of Ca^{++} to protein was ten times higher in this precipitation test as opposed to that in the light scattering method).

The method of preparation of the reaction mixtures in the turbidimetric method influenced the results of the K1 samples. Preparing a 1 % protein for reaction using a 6% stock led to ca 45 % higher turbidities in the presence of Ca^{++} than if the protein was supplied from a " dedicated" 1 % solution. Turbidities were reduced to half if a smaller volume of stock protein was added to the reaction mixture (mixing ratios 1/9) as opposed to the 1/1 method. Mixing ratios/methods had little effect on the final turbidities of the MD and SP samples.

Despite the large increase in A600 as a result of calcium addition in alcoholic solutions, the R value decreased by ca 30% as compared to the non-calcium containing samples (Fig. 4.2.5). The (A 600) or R values were also relatively unaffected by filtration through membrane filters. Further experiments, which measured the Ca^{++} induced turbidity in non-alcoholic systems , demonstrated that alcohol sensitised the caseinate to calcium induced increase in A 600 readings (Fig 4.2.6). For example, addition of 1 mM Ca^{++} in alcoholic solution leads to a 11 fold increase in A 600 readings as opposed to a 4 fold increase after incorporation of 5 mM Ca^{++} in aqueous systems. Again, despite the large increases in A 600 values of the Ca^{++} containing solutions, (resulting in milky appearances) , the R value values were smaller than the non-calcium containing controls (Fig. 4.2.6). This is especially true of the non-alcohol containing system; here the R value decreased by more than 100% on addition of 5 mM Ca^{++}. This indicated a significant decrease in average particle size of the suspension, and this finding was supported by the fact that twice as much of this more turbid Ca^{++} containing solution could be passed through a 0.2 μm filter without blockage as compared to the non-calcium containing control. However, the alcohol containing solutions, where addition of 1 mM of Ca^{++} also resulted in a decrease in R (only % 20), were actually slightly harder to filter through the membranes than the non-calcium controls .

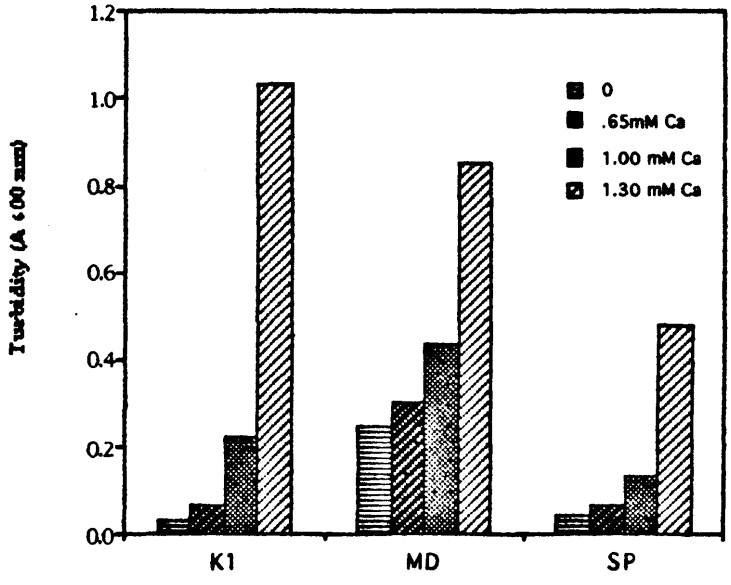


Fig. 4.2.3. The effect of addition of various levels of calcium ion on the turbidity of 3 sodium caseinate solutions . The calcium was dissolved in an alcohol/sugar mixture such that , when added to the caseinate , the final concentrations were ; 0 - 1.3 mM Ca^{++}, 1.3 % caseinate, 22 % v/v alcohol and 26 % m/v sucrose; the pH was 7.0.

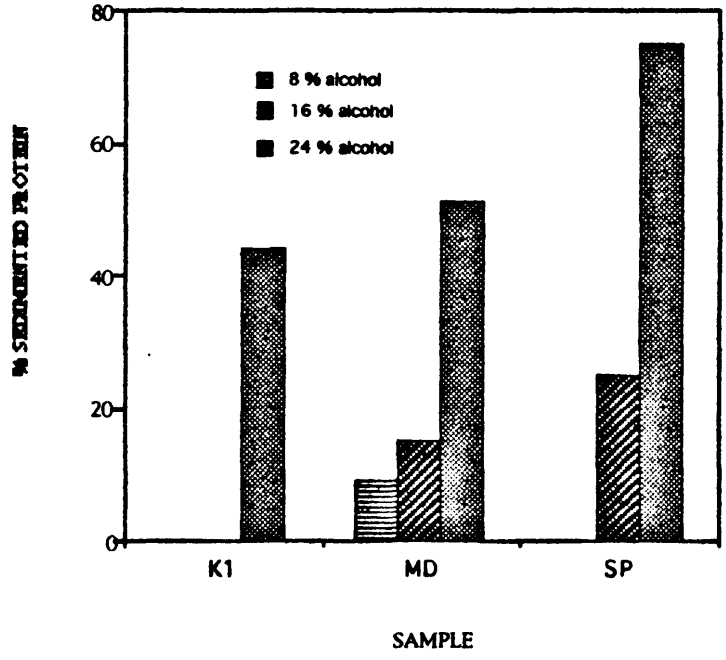


Fig. 4.2.4. The effect of addition of different alcohol concentrations. on the sedimentable protein (1000 x *g* for 20 min) in 0.3 % protein in solutions containing 3 mM calcium ion. The system contained 0.04 M borax buffer, pH 6.8. All figures quoted are concentrations in final reaction mixtures. For details of caseinates, see Table 4.2.1.

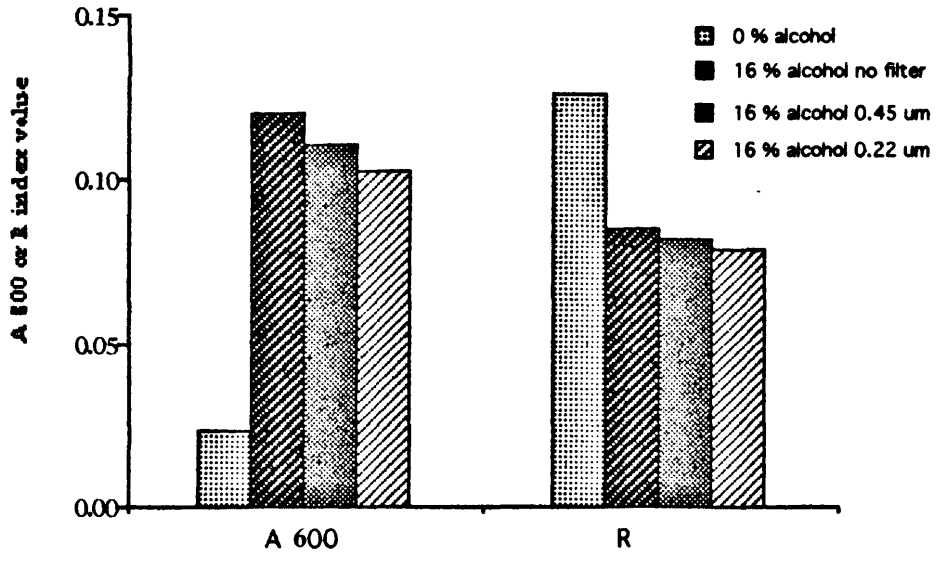


Fig. 4.2.5 The effect of addition of 16 % alcohol on the A600 and R values of K1 caseinate solution (see Fig 4.2.4 for details of composition). The turbidity and R values of 0.45 and 0.22 μm filtrates (after syringe membrane filtration), were also measured. The values for an unfiltered water sample ("0 % alcohol") are also shown.

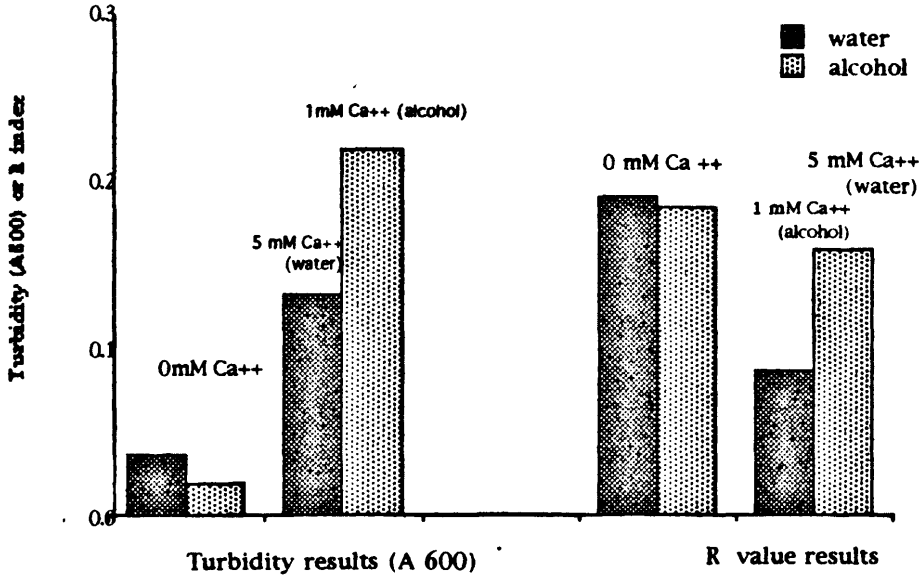


Fig. 4.2.6 The effect of addition of calcium ion on the turbidities (A600) and R index values of K1 caseinate suspensions. Two systems were used; the alcoholic/sugar diluent ("alcohol") as described previously (Table 4.2.3)or a water only system.("water"). The final concentration of calcium ions was 1 mM in the case of the alcohol and 5 mM in the water systems, repectively.

Caseinate solutions; effect of sucrose and heat

The effect of sugar on the viscosity of caseinate solutions was examined by comparing solutions of an equal w/v specification. For example, a (m/v) 7.6.% protein, 50 % sucrose containing solution was, at ca 50 mPa.s, about five times more viscous than a 7.6 % m/v aqueous sample.

The viscosities of unheated caseinate syrup solutions , made from the different caseinates, varied between 31.7 (NZ) and 45.9 (K3) mPa.s. The viscosities of the other five samples (K1,K2,MD,RD and SP) were similar; ca 37-42 mPa.s. Heating of these made-up caseinate syrups at 65 °C for 1 hour resulted in small viscosity decreases (0-6 %) except for the K1 samples which showed an 6 % increase .

Use of autoclaved K1 caseinate (K1A) but not roller dried caseinate (K1R), in the formulation of a separate batch of syrups , resulted in significantly lower viscosities of compared to the K1 control (Fig 4.2.7).

The pH and light scattering of these uncoloured syrups varied in accordance with trends noted earlier for the 1 % casein solutions. The subsequent heating of caseinate syrups (65 °C for 1 hour) had only a minor effect (-2 to +4 % change) on A 600 values except in the case of the K1 sample where they increased by ca 45 %. The use of KIH caseinate in syrups reduced the pH by ca 0.09 unit as compared to the untreated K1 syrup (Fig.4.2.8).

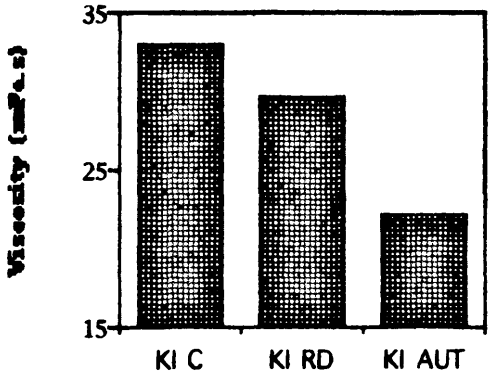


Fig 4.2.7 The viscosity of caseinate syrups (5.7 % protein and 39 % sugar) made from K1 caseinate , roller dried K1 (K1 R) and autoclaved K1 (K1 A),

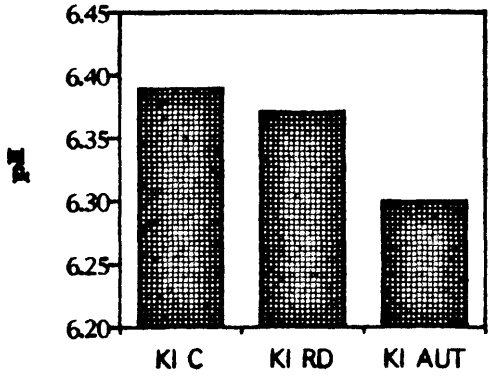


Fig 4.2.8 The pH of caseinate syrups (5.7 % protein and 39 % sugar) made from K1 caseinate, roller dried K1 (K1 R) and autoclaved K1 (K1 A) .

Cream liqueurs; results from freshly made liqueurs

The results from cream liqueurs manufactured using 10 different caseinates are summarised in Tables 4.2.9-4.2.11. Four different batches of cream were used during these experiments. There were large differences in all the variables measured, as a result of the use of different caseinates, cream batches and methods of processing:

Viscosity

The use of the 1-S process resulted in products with higher viscosities than the corresponding liqueurs manufactured by the 2-S process, but the relative amount varied with the different caseinates (Table 4.2.9). For example, the K1 and K2 samples showed differences of about 33 % between the processes, while the others had only about half this increase.

The viscosities of the different caseinate samples also varied within the 1 or 2-S processes; e.g. the viscosities of samples K1 and NZ (same protein content) were 27.2 and 23.0 mPa.s, respectively. The trends were the same for a repeat batch (Table 4.2.10) where all liqueurs had the same added protein level. Here, the viscosity values ranged from 20.8 (NZ) to 25.4 (K4) mPa.s. "Heat modification" of the K1 samples lead to a marked reduction (25%) in the viscosity of the final liqueurs, especially for the autoclaved samples.

The previous two sets of data related to liqueurs processed at 65 °C with ca 30 min holding before homogenisation, however selected samples in batches 17/18 (K1, MD, SP) were processed at 55 or 65 °C with different holding times; (Table 4.2.11). The viscosity of the K1 samples could be increased from ca 24/25 to 28 mPa.s if the temperature treatment was increased from 1 to 30 min before homogenisation. However, increased heat treatment of the MD product had little effect on the viscosity.

Turbidity values

The turbidity values of 2-S samples, at ca. 0.180, were about 3 times that of the corresponding 1-S processed samples, thus indicating a larger average particle or effective particle size (Table 4.2.9). Within the process types, the TV of the different samples were remarkably similar (Tables 4.2.9, 4.2.10). The 1-S turbidity values obtained in batch 16 were slightly higher than in the batch 15 ; 0.074 compared to

Table 4.2.9 The initial viscosity, absorbance and pH values of Batch 15 cream liqueurs which were stabilized by different caseinates and which used the two different methods of processing. The values are the average of duplicate samples. All samples contained 3.0 % sodium caseinate. Average total solids and fat were 40.0 and 16.0, respectively.

Sample	Single step process (1-S)			Two step process (2-S)		
	Viscosity (mPa.s)	T.V. (A800)	pH	Viscosity (mPa.s)	T.V. (A800)	pH
15.K1	27.2	0.067	6.75	20.5	0.178	6.76
15.K2	27.0	0.067	6.84	19.8	0.177	6.83
15.MD	23.1	0.065	6.94	20.5	0.180	6.95
15.NZ	23.0	0.064	6.77	19.8	0.182	6.77
15.RDL	23.6	0.065	6.88	20.1	0.175	6.88
15.AV	22.2	0.067	6.87	19.6	0.182	6.87

Table 4.2.10 Initial viscosity, pH and absorbance values of cream liqueurs which used different caseinates and heat treated K1 caseinates. for manufacture (Batch 16). Values are the average of duplicate samples, These samples were formulated to contain 2.7 % protein, added as sodium caseinate . Average total solids and fat were 39.3 and 15.8 %, respectively.

Sample	Viscosity (mPa.s)	T.V. (A800)	pH
16.K1	24.6	0.074	6.74
16.KIR	20.1	0.075	6.74
16.K1A	18.1	0.075	6.71
16.K2	23.8	0.075	6.83
16.K4	25.4	0.075	6.86
16.MD	22.2	0.074	6.94
16.NZ	20.8	0.070	6.76
16.RDL	22.8	0.072	6.88
16.SP	21.2	0.070	6.79

Table 4.2.11 The initial viscosity , pH and absorbances of samples from Batches 17 and 18 . Different prehomogenisation heat treatments were used. Data from 17 is the average of duplicates while batch 18 data are results from single productions only.

Sample	Viscosity (mPa.s)	T.V. (A800)	pH
65 °C x 30 min			
17.MD	23.6	0.071	6.91
18.SP	22.0	0.065	6.78
17.KI	27.7	0.080	6.74
18.KI	28.3	0.073	6.73
65 °C x 1 min			
17MD	23.8	0.070	6.93
17K1	25.4	0.077	6.72
55 °C x 1 min			
18 SP	22.0	0.069	6.79
18 K1	24.8	0.076	6.74

0.065 and this could be related to different cream batches or homogeniser efficiency. Increased prehomogenisation heating did not significantly effect the TV (Table 4.2.11).

pH

Neither the method of manufacture or heating differences before homogenisation, had any effect on the final liqueur pH (Tables 4.2.10/4.2.11). However, in agreement with the results obtained with caseinate solutions in the previous experiment, the pHs varied significantly between different caseinate samples ; e.g. there was a 0.20 pH difference between the K1 and MD based liqueur samples. Autoclaved K1 containing samples (KIH) showed a slight reduction of 0.03 units over the control (K!); corresponding caseinates syrups had showed a 0.09 difference.

Cream liqueurs; results from stored samples

Previous experiments showed two factors, creaming and thickening/gelation were of importance in the stability of products. Results obtained in this section also showed that coalescence of certain samples occurred on incubated storage. Shelf-life stability are summarized in Tables 4.2.12, 4.2.13 and 4.2.14 and they are discussed in more detail below;

Creaming

Creaming, which would occur relatively quickly at ambient temperature, was not visible in the 1-S samples after 14 or 28 days. Thin, ca 1 mm, cream lines were occasionally present (Batches 15/16) while Batch 18 liqueurs were very clean on the glass bottles. In contrast, the 2-S samples (produced in batch 15) showed significant cream layers after 28 days; centrifugation of these neat and 1:1 diluted samples produced cream layers of 3 and 6 mm, respectively, while the corresponding figures for the 1-S samples were < 1 mm.

Viscosity and gelation after 180 days storage at ambient temperature

After 6 months at ambient temperature storage, there were large differences in the extent of gelation between the different 1-S processed liqueurs.; i.e. 4/12 (four out of twelve), 0/18, 7/8 and 0/4 samples in batches 15, 16, 17 and 18, respectively, showed gelation (Tables 4.2.12-14). Caseinate K1 was used in all of these four batches; two which exhibited gelation. Of the 10 common samples (including duplicates) between batches 15 and 16, three showed gelation in batch 15 only, indicating that this batch was more unstable than 16. Batch 17 was the most unstable batch where 3/4 MD containing samples exhibiting gelation; corresponding samples in batches 15/16 were stable.

The effect of processing method was briefly examined. Equivalent samples were produced by the 1-S and 2-S procedure in batch 15 (Table 4.2.12). In total, 5/12 in the 2-S as opposed 4/12 for the 1-S samples, exhibited gelation. However, the caseinate-types that gelled in the 2-S procedure were not necessarily the ones that gelled in the corresponding 1-S samples and vice-versa. For example, two 1-S processed NZ samples gelled at ambient temperature while the corresponding 2-S produced samples did not. In addition, one sample each of K2 and RDL gelled in the 2-S process without any of these being so in the 1-S process.

Table 4.2.12 The effect of different incubation treatments on the viscosities of samples from Batch 15. Six different caseinates were used in two different processes., 1-S and 2-S. For details of caseinate codes see Table 4.2.2

	Viscosity (mPa.s) of ambient temperature sample at day;		Viscosity (mPa.s) after incubation at 45 °C for ;					
			28 days starting on day ;	3 days starting at day ;				
Sample	0	180	180	0	14	42	92	180
One step process (1-S)								
15.K1a	27.4	G	G	83	86	G	G	G
b	27.1	33.7	G	82	77	83	182	G
15.K2a	27.7	32.3	201	69	68	68	70	69
b	26.4	30.0	191	67	69	66	68	66
15.MDa	23.8	26.4	99	FF	34	36	40	44
b	22.4	23.8	86	FF	33	35	38	38
15.NZa	23.1	G	G	51	G	G	G	G
b	22.8	G	G	44	69	G	G	G
15.RDLa	24.1	26.4	152	30	44	36	46	46
b	23.1	24.4	79	28	30	33	38	38
15.AVa	22.4	(224) G	G	38	90	121	G	G
b	22.1	24.1	201	37	48	50	52	52
Two step process (2-S)								
15.K1a	20.5	G	G	69	124	G	G	G
b	20.5	19.8	G	61	63	68	168	G
15.K2a	20.1	19.8	G	71.3	55	55	92	G
b	19.5	G	G	73	82	G	G	G
15.MDa	20.5	19.1	G	FF	36	33	38	74
b	20.5	19.7	140	FF	32	33	34	36
15.NZa	19.8	18.2	G	42	41	40	49	65
b	19.8	18.5	G	46	45	46	99	99
15.RDLa	19.8	18.1	G	35	40	41	50	95
b	20.5	G	G	FF	54	G	G	G
15.AVa	19.8	G	G	57	G	G	G	G
b	19.5	G	G	54	G	G	G	G

G; gelation , FF free fat.

Table 4.2.13 The effect of different incubation treatments on the viscosities of samples from Batch 16. Nine caseinates, including 2 heat treated samples were used. For details of caseinate codes see Tables 4.2.2/3

Sample		Viscosity (mPa.s) of ambient sample at day;		Viscosity (mPa.s) after incubation at 45 °C for 28 days starting at day		
		0	180	0	30	180
16.K1.	a	22.4	25.7	195	215	221
	b	24.8	*	198	198	162
16.K1R	a	21.1	22.4	178	191	178
	b	19.1	*	130	163	137
16. K1A	a	17.5	18.5	131	135	69
	b	18.8	*	149	170	83
16.K2	a	24.1	25.4	185	178	149
	b	23.4	*	170	170	135
16.K4	a	25.4	27.4	198	185	168
	b	25.4	*	196	175	129
16.MD	a	22.4	22.4	FF	69	69
	b	22.1	*	FF	69	59
16.NZ.	a	20.5	21.1	125	185	G
	b	21.1	*	64	99	66
16.RDL	a	23.1	23.1	FF	144	G
	b	22.4	*	64	99	66
16.SP.	a	21.4	22.4	FF	96	99
	b	21.1	*	FF	92	96

* no samples read , but normal appearance ;
G gelation, FF; free fat.

Table 4.2.14 Viscosity and incubation data for batch 17 and 18 experiments, which each used K1 and one other caseinate.

Sample	Viscosity (mPa.s) of ambient sample at day; 0	180	45 °C sample after 28 days starting day 180	Process detail
17.MD.1m.a	23.8	G	G	mix held for 65 °C x 1 min.before
b	15.5	G	G	homogenisation
17.MD.30m -a	24.1	24.1	250	mix held for 65 °C x 30 min before
b	23.1	G	G	homogenisation
17.K1.1m -a	25.4	G	G	mix held for 65 °C x 1 min before
b	20.1	G	G	homogenisation
17.K1.30m- a	27.7	G	G	mix held for 65 °C x 30 min before
b	27.7	G	G	homogenisation
18.SP.55 °C	22.0	23.0	83	held at 55 °C x 1 min
18.SP.65 °C	22.0	22.4	78	held at 65C x 30min
18.K1.55 °C	24.8	26.0	211	held at 55 °C x 1 min
18.K1.65 °C	28.3	30.0	168	held at 65C x 30min

G ; gelation

The initial results from batches 17/18 (Table 4.2.14) indicated that increasing the holding time at 55/65 °C before homogenisation had little effect on viscosity/gelation. We cannot predict from the results in batch 16 (Table 4.2.13) whether the autoclaving and roller drying heat-treatments given to the K1 caseinate would protect against gelation, since the control liqueur made with "unmodified" K1 did not gel.

Interestingly, the six month viscosities of the stable, ambient temperature stored 2-S samples (above) were slightly lower than their initial viscosities (ca 3-10 %) while the reverse (ca 10 % higher results) was the case for the 1- S samples (Table 4.2.12). There was a similar trend for the 1-S samples in batches 16 and 18 (Tables 4.2.13; 4.2.14) . Samples KI,b and Av,a in batch 15 had much higher viscosities than their starting values and they were considered to be in the initial stages of the thickening that would eventually lead to gelation.

Viscosity increase and gelation during 45 °C storage tests.

The extent of thickening at 45 °C was quantified by measuring the viscosity increases over 3 days (batch 15; Table 4.2.12) or 28 days (batch16/17/18; Tables 4.2.13; 4.2.14). In general all the K samples (1, 2, 4) showed the greatest amount of thickening (2-3 times the starting viscosity), while the MD caseinate samples exhibited the lowest increases. For the stable samples (see below) , the extent of thickening was generally independent of the age of the sample when incubated. An exception was the liqueurs made with the autoclaved caseinate (K1A) where the viscosity increase at 45°C measured after 6 months was about half of that of the fresh sample (Table 4.2.13).

The rate of increase in viscosity of the liqueurs at 45 °C was basically a reflection of the behaviour of the caseinates, since similar differences were found if the corresponding fresh batch 16 non-emulsified systems (i.e. fat-free bases) were incubated. The % DVI values (28 day test) of MD, SP and KAv fat-free bases were only one third of the K1 results, in agreement with trends for the liqueurs. In general rates of thickening were about 10 times more in the liqueurs than in the FFB's, although there was a good correlation between the % DVI results of the FFBs (1-28 days) and the results generated from 180 days old liqueurs (Fig. 4.2.9).

It is obvious from the results, especially those in Tables 4.2.12, 4.2.13, that many 6 month old samples that appeared normal at ambient temperature subsequently gelled when incubated. These results are in agreement with similar observations recorded in Experiment 3.3. Hence, the appearance of the 180 day (6 month) old samples if

incubated for 28 days at 45 °C can be considered the most severe test for overall liqueur stability.

The gelation trends noted between cream batches and different caseinates for the ambient temperature results (above), also hold true for the same samples incubated at 45 °C. However, the results of the 180 day 45 °C test showed that 2-S samples were more unstable than 1-S samples; for example 11/12 (eleven out of twelve) and 5/12 liqueurs gelled in the 2-S and 1-S processes, respectively (batch 15; Table 4.2.12). An interesting feature of gelation results is that replicate samples (usually produced a day apart but using the same ingredients) do not always agree i.e in 3/4 cases only one of each duplicate liqueur gelled at 45 °C.

Calcium sensitivity tests on liqueurs or fat free bases.

Viscosity/ gelation data was also compared to the calcium sensitivities of the samples mentioned above (Fig. 4.2.10). There was a good relationship between the calcium sensitivity of the cold stored cream liqueur samples and the ambient temperature-stored FFBs (batch 16 samples used). In contrast there was a poor relationship between the calcium sensitivity of the cold stored liqueurs and the %DVI calculated from the ungelled 6 month old ambient temperature samples (Unfortunately calcium sensitivities were not measured on fresh products, since the test was not developed at that time).

Calcium sensitivity was measured in selected 10 month-old 5 °C samples from batches 15/16 (Table 4.2.15). All these cold stored products were of normal viscosity. In general, samples that would eventually show gelation (15.K1b, 16.NZ.a, 16.RDL.a), had very high calcium sensitivities. The exception was sample 15.K2 which had a very high value but was shown to be stable. The calcium sensitivities for K1 and MD liqueurs from batch 15 were 29 and 75 % higher, respectively than those of batch 16 readings, indicating that factors in the cream portion of the cream liqueurs influence this response.

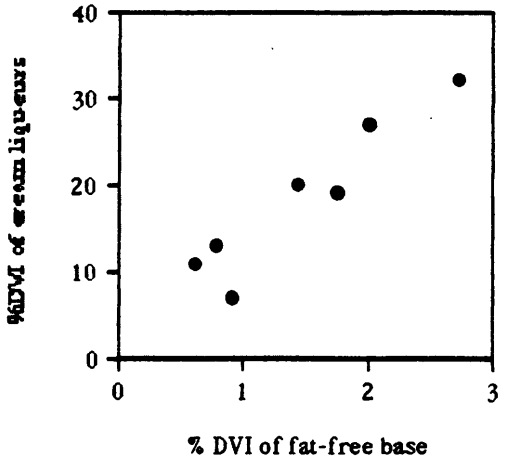


Fig.4.2.9 The % daily viscosity increase (%DVI) of a freshly incubated fat free base (FFB) was compared to % DVI of the corresponding liqueurs. The liqueurs were tested when 180 days old

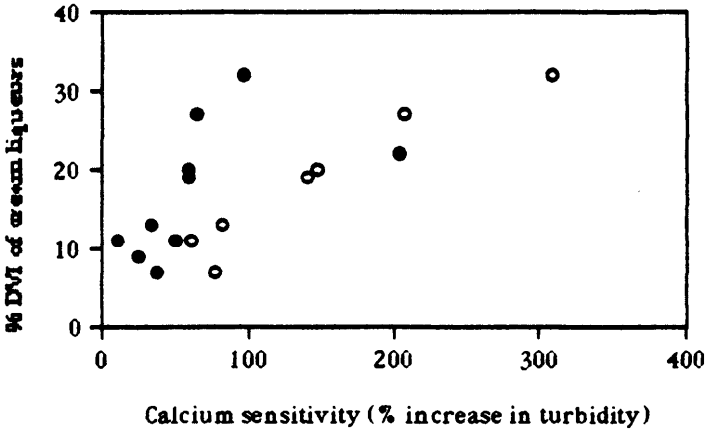


Fig. 4.2.10 The calcium sensitivities of the six month old FFBs (o) or six month old fridge cream liqueurs (•) are plotted against the % DVI value from the six month old ambient sample. (non gelled samples from batch 16). Data taken from Table 4.2.15

Table 4.2.15 The calcium sensitivities of selected samples from batches 15/16.are given below. The cream liqueurs were 6 month old fridge samples and the fat-free bases were six month old ambient temperature samples. Tests were performed on diluted samples as outlined in the text. % DVIs for 180 day old cream liqueurs are given for comparison. purposes.

Sample	Calcium sensitivity (% increase in A 800 on addition of 1 mM Ca++		% DVI of 180 day old liqueurs (45°C) for 28 days)
	Cream liqueur	Fat Free base	
15.K1 b	246		G
15.K2 b	204		22
15.MD b	51		11
15.RDL b	25		9
16.K1.a	98	309	32
16.K1R.a	64	208	27
16.K1A.a	11	62	11
16.K2.a	59	141	19
16.K4.a	59	148	20
16.MD.a	38	77	7
16.NZ.a	222	117	G
16.RDL.a	149	80	G
16.SP.a	35	82	13

65 °C storage tests; coalescence.

Cream liqueur samples were also incubated for up to one hour at 65 °C and the stability results generally mirrored those obtained over longer periods (3- 28 days) at 45 °C . However, as with the 3 day 45 °C test, therewas a significant proportion of false negatives.

Interestingly, the incubation of samples at 65 °C (and occasionally at 45 °C) resulted in a marked oiling off (appearance of free-fat) and/or creaming in certain liqueurs, due tocoalescenceof the fat globules (Table 4.2.16 and Fig. 4.2.11). Coalescence was dependent on process, caseinate type and age of sample when tested:-

The K samples were stable to coalescence. However, autoclaving (K1A), but not roller drying (K R) changed the properties of K1 to that of a sample demonstrating coalescence (batch 16). The SP sample, tested for the first time here, also showed marked coalescence on heating of the freshly produced emulsions. Free fat/cream layer was still very evident in the heated 1 month old samples, although it was not present when tested at two months of age. The most susceptible sample, MD, eventually showed no visible creaming/fat when tested after 2 months. The results in Table 4.2.15 also indicated that coalescence occurred more extensively in the 1-S process; e.g. after 14 days, none of the four non-K 2-S samples showed coalescence, while all four corresponding 1-S processed liqueurs did. In addition, as shown previously, the phenomenon depends on the age of the sample; the older the sample the more resistant to coalescence.

Table 4.2.16 The effect of caseinate type, different manufacture processes and sample age on the extent of fat globule coalesence after incubation for 1 hour at 65 °C. Results shown below are for batch 15a samples .

	Appearance of sample after incubation at 65 °Cx 1 hour starting at day;					
	One step process (1-S)			Two step process (2-S)		
Sample	0	14	28	0	14	28
15.K1	ok	ok	ok	ok	ok	G
15.K2	ok	ok	ok	ok	ok	ok
15.MD	FF	FF	FF	FF	ok	ok
15.NZ	ff	G	G	ok	ok	ok
15.RDL	ff	ff	ok	FF	ok	ok
15.AV	ff	ff	ok	ff	ok	G

ok; non gellled but thicker than unheated;
ff; ca 10 % free fat/cream layer visible
F; ca 20 % free fat/cream layer visible.
G; sample gelled.

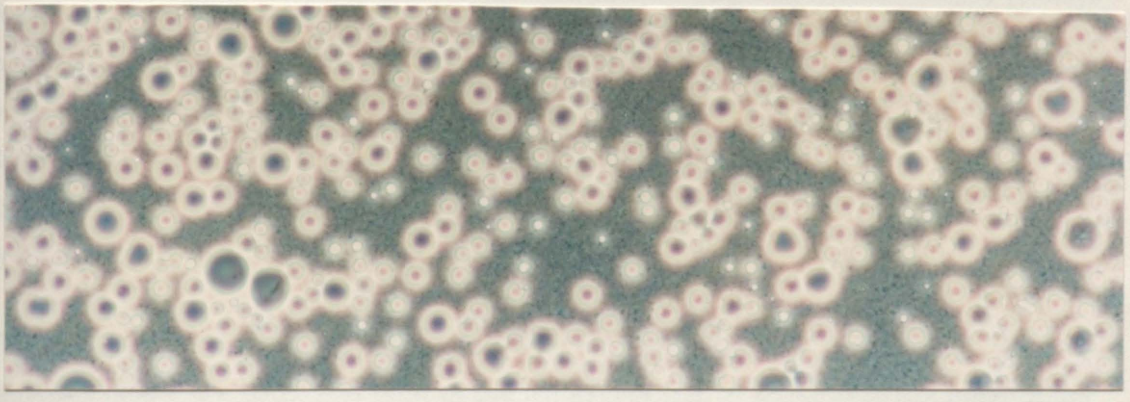


Fig. 4.2.11 The microsopic appearance of an MD stabilised liqueur sample after incubation at 65 °C for 10 min. Magnification x 100. Bar indicates scale.

DISCUSSION.

Muir & Dalgleish (1987) and Dalgleish & Law (1988) found that various commercial sodium caseinates behaved differently to (i) incubation in simulated cream liqueur systems (ii) addition of calcium ions and (iii) casein analysis. However, the properties of different sodium caseinates in different cream liqueurs, or indeed in other dairy emulsions, have not been reported.

Characteristics of different caseinates in solutions

Solubility and size indices

Besides the large average particle diameters generated by the Nanosizer, three other unusual phenomena were noted in the study of the various caseinate solutions. These were (i) the appearance of the MD solutions (ii) the apparently high solubility of caseinate in the presence of sucrose and (iii) the protein insolubilities of (uncentrifuged) K samples on standing. These are discussed below:-

Interpretation of different average particle diameters The very high average particle diameters reported by the Nanosizer (for all samples other than MD) or the "unimodal" diameters (N4), are probably incorrect. However, average weight diameters, obtained from N4 weight distribution data, were significantly smaller. For example, the N4 indicated that most of the particles in the K1 solution (ca 85 % m/m) occurred in the 22 nm range. In contrast, the average particle diameter calculated by Nanosizer for K1 was 190 nm. Famelart & Sureh (1994), who also used a N4 instrument, also reported small particle sizes using their caseinate; 80 % of the protein was in particles ca 11 nm.

The apparently high Nanosizer result may be explained if we consider that all particles below the detection limit of the Nanosizer (i.e. < ca 40 nm) are considered "solvent molecules" and are therefore not counted. Thus, the value of 190 nm is the "average particle size" of that (relatively small) sub-population of particles above the ca 40 nm point (e.g large protein / lipoprotein particles, small fat globules). Famelart & Sureh (1994) attributed the high unimodal results for the N4 to the presence of large lipid particles.

The colloidal nature of caseinate has been investigated by relatively few authors; most studies utilise laboratory produced material and the most commonly used method of analysis is gel chromatography. For example, Pepper (1973) eluted a solution of

* A similar type explanation can be given to explain the high R values (indicating a high average particle size) most of the caseinate samples i.e. most of the caseinate particles are not scattering light and the result reflects only an average for the very large particles.

laboratory caseinate through an Agarose column at 30 °C. Two peaks were characterised; the first minor one corresponding to the position of pure κ-casein and the main peak corresponding to a mixture of α–s and β–caseins. A sample of calcium-free casein micelles gave a peak corresponding to the main peak above. In another study, Creamer & Berry (1975) found that both a laboratory caseinate sample and extensively dialysed casein micelles, gave very similar gel filtration patterns at 37 °C. The authors concluded that the casein micelle subunits are not of fixed dimensions but are casein aggregates/polymers of varying sizes that are in equilibrium with each other, resulting in continued interchange between casein monomers and aggregates. Recently Haque *et al.* (1993) applied laboratory produced caseinate to a gel permeation column. Some of the protein was considered to have self associated and was eluted in the void volume, but most of the response eluted in a broad band indicating a wide range of these (smaller) aggregate sizes. This peak corresponded to the retention volume of bovine serum albumin (m.w. 67,000). The same authors also obtained results from dynamic light scattering and the results indicated a mean hydrodynamic diameter of ca 180 nm.

Results from electron microscopy of non emulsified and emulsified samples containing sodium caseinate (Bucheim & Dejmek, 1990; Foley & O' Connell, 1990) and from solubility data obtained from ultracentrifugtion of solutions of caseinate (Mulvihill & Murphy, 1991; Famelart & Surel, 1994), indicate that sodium caseinate is in a "soluble" state with small particle sizes. However, ultracentrifugal methods depend on density differences between the particle and the continuous phase for resolution and, as noted previously for refractive index differences, highly hydrated caseinate particles may not behave like less hydrated particles (e.g. casein micelles).

Visual appearance of MD caseinate solutions compared to the other relatively translucent samples, the very turbid appearance of the MD solutions was exceptional. This correlated with the proportion of large particles reported these suspensions by the N4 i.e. ca 36 and 42 % m/m of particles were reported in the size ranges of 95 and 533 nm, respectively. The sample also clogged up 0.22 μm membrane filters very quickly. However, there was no obvious analytical differences (e.g fat, whey protein contents) to account for the exceptional appearance of the MD sample as compared to the K1 caseinate; manufacturing procedure could be important. Dalgleish & Law (1988) noted that certain roller caseinate solutions would not pass through filters and they considered that this was due to the presence of non-dispersible protein, which was sedimented by centrifugation at 65,000 *g* x 60 min . However, the roller dried

K1R sample produced for this experiment was more soluble than the original K1 caseinate.

Given the particle size distribution obtained with the 3 % MD protein, the suspension should, like casein micelles in solution (e.g skim milk), appear milky-white. This was not the case and possible explanations are (a) that the effective refractive index differences between of caseinate particles and water are much less (due to highly hydrated nature and absence of mineral matter) than those between casein micelles and water and/or (b) the large MD caseinate particles may be loose, highly-hydrated aggregates of smaller fragments which act optically (light scattering) as very small particles but functionally (Brownian motion) as large particles. In addition, the particle size distribution of casein micelles in skim and the above caseinate are probably different.

Solubility of caseinate in concentrated sucrose solutions Surprisingly, it is possible to dissolve relatively high concentrations of caseinate in sucrose solutions. To take an example ; it is possible to produce a syrup of 9 g protein dissolved in 45 g sugar and 46 g water. Under normal circumstances, 45 g of sugar would require ca 19 g of water for dissolution at room temperature. This would only leave 27 g of water to dissolve 9 g of caseinate, which would result in a 33 % m/m caseinate solution. Normally, it is only possible to have a solution of ca 15 % m/m sodium caseinate at ambient temperature, and even this has a very high viscosity. Even if we express the above caseinate syrup on a w/v basis i.e ca 19 % w/v, (i.e. including the volume of sugar), it has a much lower viscosity than a 19 % w/v aqueous solution. These results may indicate that the sugar is preventing the full hydration (swelling) of the caseinate. In this scenario, the partially hydrated particles appear clear in suspension because they have a similar refractive index as the syrup.

The results of Cornell *et al.* (1994) indicate that there are no interactions between sucrose and caseinate and alcohol in solutions. Femelart (1994), using photon correlation spectroscopy, showed that 30% sucrose in reconstituted milk, increased the casein micelle diameter by ca 20%.

protein insolubility of K samples the protein insolubility of all of the K solutions (i.e. sedimentation of very large particles on standing or to low speed centrifugation) may be due to method of manufacture. It is known that this product is often produced by re-dissolving already-dried acid casein, rather than production from fresh curd. One can only speculate as to the greater solubility of 6 % as opposed to 1

% solutions; possib ly some form of beneficial interactions occur between protein molecules/associations at the higher concentrations. Despite these results in solution, there was never any insoluble protein obvious when the K samples were incorporated into cream liqueurs. The findings reinforce the view that tests for functionality, such as solubility tests, are highly empirical.

Calcium sensitivity in non-emulsion systems

Different caseinates showed varied responses to added Ca^{++} in the non-emulsion alcoholic systems. The amounts of calcium needed to cause increases in absorbance is much less in these alcohol containing systems than it aqueous systems. This demonstrates that caseinate in an alcohol system has an increased affinity or decreased stability to Ca^{++} ion. This is probably due to differences in the amino acid ionisation values (pKas) of casein in alcohol solution. Experiments indicated that it was necessary to add ca 10 times more calcium to produce an equal amount of turbidity change than if a water only system was used. This may provide an explanation as to why water-only containing emulsions are more stable to thickening/gelation than corresponding alcohol containing ones (see Experiment 4.1, previously)

In this experiment,calcium sensitivity were measured mainly by the light-scattering method, where the increases in A 600 readings, in alcoholic media, showed that the K, NZ, AV samples were most , while the SP, and especially the MD sample, were least, sensitive to added Ca^{++}. Light-scattering results from Dalgleish & Law (1988), in *aqueous* systems, showed that roller caseinates, which had a higher heat treatment, resulted in very low sensitivities (increase in turbidity) as compared to spray dried or laboratory sources. The laboratory caseinate started to aggregate at the lowest concentration of added Ca^{++} (5 mM), followed in sensitivity by the spray dried samples; the six spray samples tested showed significant variations in sensitivity. The roller caseinates, showed virtually no increase in turbidity until a Ca^{++} concentration of 18 mM was attained. These results indicated that heat treatment of a caseinate may influence increases in turbidity. However, in the present experiment, the autoclaving and roller drying of a caseinate sample (K1) did not have any significant effect on sensitivities. (It should be borne in mind that the caseinates used by Dalgleish & Law, 1988, were from different sources). Recently, Famelart & Surel (1994) studied the effect of adding calcium (0-75 mM) to 2 % w/v solutions of commercial sodium caseinate. The amount of colloidal calcium increased from about 0.02 to 0.18 g/kg

protein during the addition of 12.5 mM of Ca^{++} and this was due to the formation of ultracentrifugable particles, which could be sedimented by successive centrifugations. As calcium was added, the pH decreased ; most of the decrease occurred when the first 12.5 mM was added; the pH reached 5.79 on addition of 75mM. Addition of this amount of Ca^{++} led to solubilization of all the sodium in the caseinate solutions ; 50% was soluble (i.e non ultrafiltrable) in the control samples. Solubilization occurred even when the calcium containing solution was readjusted to the pH of the control.

Dalgleish & Law (1988) also measured the particle sizes of the caseinates in 15 mM Ca^{++} by laser light scattering (no other details given) and the particle readings for the laboratory, spray and roller samples were ca 645, 190 and 110 nm, respectively. Unfortunately, no particle sizes were reported for solutions with no added Ca^{++}. The milky suspensions, in either aqueous or alcoholic/sugar solutions, are presumably either "insoluble" calcium caseinate formed after the saturation of binding sites by excess calcium or micelle type bodies. The possible binding sites are monoester phosphate groups, contributed by serine and threonine residues and carboxyl groups of aspartic acid and glutamic acid (Dickson & Perkins, 1971). Zittle (1956) reported that these opaque suspensions were lower in viscosity than suspension with no added Ca^{++} that this may be due the formation of more compact and symmetrical aggregates. That this is so is indicated by the fact that aqueous milky suspensions in this experiment were easier to filter and had smaller R values than the non-milky suspensions without any added calcium . Cloudy suspensions in alcoholic solutions while having smaller R values were harder to filter indicating the nature of the calcium complexed particles (e.g. shapes etc) may be dependent of dispersant. Results of the R value of the cloudy solutions and centrifugation results would indicate that what is happening is not a precipitation per se, rather a formation of structures more like native casein micelles. Calcium, together (with phosphate; not added here) is an essential component in the formation of synthetic type casein micelles. (Schmidt & Koops, 1977; Schmidt *et al.*, 1979) .

However, the light-scattering results reported above for this experiment did not correlate with those obtained using the centrifugation method of Arima *et al.* (1964). In fact, results showed the opposite trends i.e. the MD protein was the most unstable in the protein precipitation test while being the most stable in the light-scattering test. The centrifugation/precipitation test could be considered a more "real" measure of protein reaction with Ca^{++} .

Zittle *et al.* (1956) produced useful basic information on calcium induced "precipitation", though again in *aqueous* sodium caseinate solutions. Relatively large concentrations of calcium chloride were required to precipitate (as measured by centrifugation at 3000 *g*) sodium caseinate without heat, and the extent was pH dependent e.g 20 % and 8% precipitated on addition of 20 mM to 2 % sodium caseinates at pHs 6.6 and 7.6 respectively. Precipitation was also increased by heating at 90 °C, although much of this was reversible if allowed to cool. Zittle (1961) showed that κ-casein protected other caseins from calcium precipitation and that treatment with chymotrypsin decreased this stabilisation, especially if these samples were subsequently heated.

Characteristics of fresh cream liqueurs that were stabilised with different caseinates.

Viscosity

The K1 containing samples had a significantly higher viscosity (1-S process) than other proteins and this is in agreement with results from solution studies. The higher viscosity of all caseinates in the 1-S process over the 2-S process may be due to the effect of heat/alcohol/homogenisation on whey protein since trial liqueurs manufactured by including 1-2 % added whey protein showed very high viscosities e.g see Tunney (1992).

Assuming that these differences as perceptible, there are obvious cost advantages in using the higher viscosity caseinates/process techniques; e.g reduced inclusion level etc. The lowering the protein level, whilst achieving a minimum desirable viscosity, may reduce the instability potential of the system, if we assume that protein is responsible for gelation.

homogenisation efficiency

The processing conditions used in this experiment (1 pass of the Rannie at 27.6 MPa), produced samples that showed no significant creaming. It was calculated that increasing the pressure to 31.0 MPa would reduce the average particle size (as indicated by TV) to that of the B and C commercial liqueurs.

Despite showing different characteristics in solution, all the different caseinate samples displayed the same emulsifying ability (as indicated by similar average particle sizes) when processed into final product. It is possible that use of lower protein/fat ratios e.g. 0.05-0.10 may show up differences in emulsifying potential between the different caseinates; the protein:fat ratio of typical liqueurs was relatively large (0.2).

Calcium sensitivities of emulsions.

The calcium sensitivity of liqueurs was measured in highly diluted samples by monitoring the increase in absorbancies due to addition of Ca^{++}. In theory, this calcium sensitivity test differs significantly from similar tests on caseinate solutions due to lower caseinate concentrations and the presence of emulsified caseinate (the concentration of Ca^{++} was similar with both systems). For example, the final concentration of (free) caseinate in the solution systems was 1.3 % protein (e.g. Fig. 4.2.5) while the corresponding level on free caseinate in 1/1000 diluted emulsion systems was only ca. 0.002 (see note below); however if we consider that protein-covered droplets act as "protein", the effective protein concentration in diluted emulsions is higher e.g ca 0.02.

Despite the above differences, the trends in calcium sensitivities shown by the different caseinates in the emulsions were similar to those found previously in solution. (For example, samples stabilised with MD or SP showed significantly smaller increases in absorbancies that K samples.) This was not surprising since at least half of the protein in a liqueur will be free. However, one cannot tell whether the interfacial protein/caseinate would show different responses to added calcium as compared to the free protein. Experiments which would isolate the fat globules (without disrupting the interfacial layer) would be necessary to establish this.

(Note; on one occasion, the amount of unemulsified protein was calculated by performing a mass balance on a totally gelled and synerised product ; 67.3 % of the volume of a container was free serum and 22.7 % was a hard gel, which in turn consisted of 44.5 % v/v serum and 54.5 % v/v of protein-coated fat globules. It was calculated that about half of the nitrogen (protein) was unemulsified (i.e present in the serum). Dickinson *et al.* 1989c, who used ultracentrifugal techniques, found that ca. one third of the caseinate was unemulsified in their experimental cream liqueurs).

Other researchers have measured effect of Ca^{++} in non-alcoholic laboratory-produced caseinate-stabilised emulsions. Dickinson *et al.* (1987*b*) produced tetradecane emulsions (o = 0.56, p/f ratio; 0.014-0.020 at pH 7.5, 20 mM imadizole ; P = 30 MPa) with sodium caseinate and also α_{s1} , β and κ caseins. The stability of the concentrate emulsions was monitored by diluting the emulsions by half to a final electrolyte concentration of 5, 10 or 15 mM $CaCl_2$ or 2 M NaCl, and measuring droplet aggregation by Coulter Counter. When monomeric caseins-coated emulsions were flocculated by salt addition, they were readil y deflocculated by dilution with a non salt containing buffer. This contrasted to the behaviour of a commercial sodium caseinate, whose deflocculation was very slow. This suggested that there was stronger calcium induced association between the adsorbed protein layers when all the casein components are present together. Certain batches of κ-casein, isolated from milk, were not able to form stable emulsions. Dissociation of the disulphide linkages with 2-mercaptoethanol lead to the formation of more stable emulsion (smaller particle size) that were stable to calcium ions.. Earlier, Chesworth *et al.* (1984) studied phosphate-buffered butterfat emulsions of low protein:fat ratios (0.0025-0.005) i.e low protein load. In that study, it was shown that, at low protein:fat ratios, the oil droplets are more easily flocculated in the presence of sodium chloride. The results also showed that two Dutch caseinates were more susceptible to flocculation than the standard Scottish caseinate used. The Dutch caseinates contained 0.49 and 0.84 g /kg calcium as opposed to the 0.14 g/kg for the Scottish sample. Hence the salt stability of caseinate stabilized emulsions may be sensitive to the composition of the caseinate emulsifier (ionic and protein composition) and its state of aggregation.

Recently, Agboola & Dalgleish (1995), investigated the calcium induced aggregation of caseinate stabilized oil-in-water emulsions (20 % soya ϕ_{oil} 0.3-2.0% protein, 0-100 mM NaCl, buffered at pH 7). Destabilization in this experiment was monitored by an increase in apparent average particle diameter (d_{43}) of the emulsions on addition of various amounts of buffered 50 mM $CaCl_2$ solution (final Ca^{++}, 2-16 mM). Stability increased with the amount of protein in the emulsion and decreased with the concentration on added Ca^{++}. Sodium chloride (added here before emulsification) stabilized the emulsions to added calcium, for example, addition of Ca^{++} to a final concentration of 10 mM in the 2 % protein emulsions had no effect on the average particle size of the 100 mM NaCl containing emulsions, while a similar addition with the 0 and 50 mM NaCl increased "particle size" ca 6 fold. "Increased " particle size was due to clustering of the individual droplets.

Results of incubation tests on cream liqueurs made with different caseinates.

Note: Some care is necessary in interpretingthe results of the cream liqueur samples since different batches of cream were used in the four batches mentioned in this experiment. (One the other hand, the use of different batches of cream provided useful information on raw material variation).

Coalescence

Coalescence of the fat globules 65 °C was very extensive for all the non-K samples, in particular the MD and SP containing liqueurs. None of the K samples, either in this experiment or in the preliminary section, showed this phenomenon, indicating some fundamental difference in the properties and behaviour of these proteins. This behaviour was not seen at room temperature nor in corresponding non-alcoholic liqueurs, indicating that the presence of alcohol /heat was essential for the mechanism.

The extent of coalescence, as with the extent of thickening, was dependent on the age of sample when incubated. This can be explained by considering that the composition of the interfacial layer/continuous phase is probably in a state of change e.g. the original interfacial membrane is slowly being replaced by more surface active proteins/ peptides etc.

During homogenisation, larger protein particles rather than more surface active proteins molecules or surfactants may be preferentially adsorbed (Walstra, 1988; Singh *et al.* ,1993) at the interface. Once the membrane is formed a process of displacement / adsorption begins which will eventually form a layer of different composition. This process may take a few months at ambient temperature and the rates of the different reactions are such that coalescence is never seen. However, if the temperature is raised, as in the 65 °C accelerated stability test, then the displacement rate is favoured over the replacement rate and this, together with the increased collision rate of the particles, favours coalescence. It is well known that the combination of highly surface active materials and very high temperatures can cause massive coalescence of fat e.g the BDI test for liberating fat from milk (Driessen *et al.*, 1977) ; these reagents were also used by O' Neill (1991), in this laboratory, to extract fat from cream liqueurs.

The fact that K samples, of any age, do not show coalescence may indicate that the initial layer formed with these caseinates is already at equilibrium and/or it is so

"strong" that it is resistant to coalescence. The decreased coalescence behaviour of all non- K samples over a period of 2-3 months also indicates a change ("strengthening") of the interfacial layer. Such changes at the interface, due to physical or physiochemical action could be investigated by electron microscopy or compositional studies of interfacial material.

thickening

There were significant differences in the rates of thickening of liqueurs made with different caseinates during high temperature storage of liqueurs. The samples which showed the largest and smallest initial viscosity increases (e.g. as measured by 3-day % DVI) were those that showed similar patterns in fat-free systems. Muir & Dalgleish (1987) found large differences in the rate of viscosity increase between commercial caseinates in fat free systems and they postulated that this was due to variation in factors during production of caseinates (note that the FFB used by the above authors did not contain any serum portion). For example, Muir & Dalgleish (1987) found that commercial roller dried samples showed less viscosity increases than spray-dried ones, and this could have been a consequence of changes in protein structure due to heat. The use of severely heat-treated caseinate in this experiment (K1A) led to significant decreases in the % DVI, as compared to results for K1, especially if measured after six months.

There appeared to be some correlation between viscosity increase at 45 °C and calcium sensitivity, e.g. the K samples always had high indices while MD and SP proteins had correspondingly low ones. Similar type results were also indicated from the results of Muir & Dalgleish (1987) and Dalgleish & Law (1988). These correlations may be co-incidental (i.e calcium has no role in thickening mechanism) or alternatively the different responses to calcium may indicate different protein conformations which behave differently to incubation. For example, severe heat-processing could lead to a structure which has increased calcium binding activity.

Gelation in general

Gelation was observed in liqueurs made with different samples of commercial caseinates. This defect is of most interest as it is highly undesirable and was never observed in commercial samples. While there may be some indications to show that some caseinates are more prone to gelation e.g. K1, it would appear that there are other causes of the observed differences. This is most likely to be the cream since this is the main variable in most of the studies reported above. Cream used in this experiment was mostly processed from a commercial liquid milk silo (average 10

herds). These seemed to be no obvious seasonal pattern to explain differences in gelation; e.g. the least and most extensive cases of gelation were from samples in July and May, respectively.

There are relatively few detailed studies on the effect of milk composition on gelation of other dairy products. Newstead *et al.* (1978) showed that there was a distinctive seasonal effect on the initial viscosity and more particularly, on the incubated viscosity (56 days at 30 °C) of recombined sweetened condensed milk (RSCM). Selection of skim milk powder was the most important variable with regard to viscosity and age thickening of RSCM. Early season RSCM (Aug.-Dec. in Australia) increased ca 2.1 fold on incubated storage, while mid/late season milk showed little change. Overall, factors such as stage of lactation/seasonality and region of production were considered more important than the heat-treatment of the powder. Muir & Sweetsur (1992 *a,b*) studied the effects of season on the extent of age-thickening in-can concentrated milks (31 and 39% solids); however these products contained additives (see later).

Correlations between stabilityand other tests.

Results from the various accelerated incubation tests indicated that not only are initial viscosity increases and gelation not related (as claimed by Muir and co-workers) but that the age of sample when incubated is critical. This latter fact has not been commented in studies with other long shelf life products; however various authors have noted unusual results in the use of different shelf-life storage temperatures. For example Snoren *et al.* (*1971a*), who examined indirectly processed UHT milk (138 °C x 2-5 s.), noted that gelation occurred after 13 months at 4, 20 and 30 °C but not at 37 °C. Kocak & Zadow (1985c) reported that age gelation did not occur in UHT milk stored at 40 and 50 °C. Gelation occurred quickest at 20/25 °C and was retarded at low temperatures e.g. 2/10 °C. The authors cited an observation, made to them by another researcher, whereby a sample of UHT milk that had been inoculated with bacterial proteolytic enzymes, did not gel until it was first held for a short period at room temperature. Mittal *et al.* (1988) studied the behaviour of 3.5% fat UHT recombined milks (140 x 3 s direct, 15/3 MPa). Samples were stored at 30 °C and 5 °C. A sharp rise in viscosity and decrease in ethanol stability preceded visible gelation. All samples that were stored at 5 °C had gelled before the 32 nd week. Samples stored at 30 °C gelled at a later stage. An emulsifier (0.15%) was used in the formulations .

Newstead *et al.* (1978) compared the usefulness of an "accelerated" test, i.e. 40 °C x 7 days, in predicting the response to the 56 day 30 °C test for recombined sweetened

condensed milks. There was an good correlation (80 % of variance) when all the seasons data (see later) were taken into consideration, however 55 % of this variance was due to seasonal effects . It was more realistic to consider the two tests over periods where seasonal difference was not the major factor. Here, it was not possible to predict the viscosity after 30 °C storage from the 40 °C data ; only 32 % of the variance was accounted for. The authors questioned the value of the accelerated test as a basis for selecting skim milk powder for manufacture of the above product. Dickinson *et al.* (1989c) also noted that changes that occur at 45 °C in cream liqueurs may not occur at ambient temperature and vice-versa.

As mentioned previously, there were several incidences where replicate experiments produced different gelation results. Examination of the viscosities of three pairs of such products, after six months of storage at 5 °C, produced an interesting correlation:- The results showed that the samples that consequently gelled in this trial showed a slight decrease in viscosity (ca. 5-10 %) as compared to their original readings while the stable samples had the same or a greater viscosity (ca. 0-5 %) when measured after the six months. Thus a slight thinning of the samples could be indicative of subsequent gelation, while slight thickening is not an indicator of subsequent thickening and gelation. This is the opposite from that expected, and are unlikely to be coincidental, although it would be prefe.rable to have used a viscometer of higher sensitivity. It will be recalled that 2-S samples in batch 15 decreased in viscosity at ambient and these were very susceptible to gelation. Other long-life dairy systems also showed slight decreases during storage (Harwalker, 1993).

Mechanisms of gelation .

It is clear from the experiment that gelation can be a complex phenomenon and there may be many mechanisms involved. Thus , while in certain cases one main mechanism can be identified , it other cases, gelation may be the *net* result of opposing reactions (some promoting and some protecting against gelation). Mechanisms to explain the importance of alcohol in gelation were mentioned in the last experiment (4.2); here further points are put forward to explain gelation at ambient and elevated temperatures in 17 % v/v alcohol liqueurs:-

Gelation of 1-S containing samples homogenized with citrate.

It is proposed that the mechanism leading to gelation is a 2 phase process. The first phase involves sensitisation and /or aggregation, in some way, of the protein coated globules at the interface and the second phase involves the actual networking of the these particles into an immobilised gel system. The reactions can occur to varying degrees thus accounting for different expressions of the gelation process

The first phase can occur at room temperature or in the fridge. Many of the unstable samples are of normal viscosity at room temperature even for periods up to 18 months. Yet these aged samples gelled very quickly when incubated at 45 or 65 °C. The first phase may even occur preferentially at ambient temperature and this would explain why many unstable samples do not show instability when put into incubators when fresh i.e. the first phase reaction(s) are not favoured in fresh samples at high temperature and can never occur in such situations. The actual mechanism of the sensitisation reactions is unknown, but it could involve the interfacial proteins forming an aggregated layer at the interface. Homogenization is known to sensitise proteins to aggregation e.g no fat-free bases (FFB) , even those without citrate have shown gelation. This is an indication that it is the spreading and attachment of protein to the interface that sensitises the material to gelation. When proteins attach at the interface, hydrophobic parts tend to orientate towards the fat and hydrophilic parts to the continuous phase i.e the structure is changed.

The rate of the second phase , i.e. the actual aggregation, is facilitated possibly by ionic or other material , by heating and most importantly by the presence of alcohol. It can occur at ambient temperature, if the first phase has been extensive enough and / or if there is enough "bridging material". Samples can even gel in the fridge and here it is considered that the first phase reaction is very extensive.

possible role of enzymes in gelation .

The presence and effects of enzymes from serum portion may be significant in certain situations since the conditions in a cream liqueur are relatively non-denaturing and enzymes are likely to be active. These could cause reactions that sensitize the micelles during long term storage and thus play a role in gelation. It has been shown by many groups of researchers (e.g. Manji & Kakuda, 1988; Kohlmann *et al.*, 1991; Snoeren & Both, 1981; Richardson & Newstead, 1979) that proteolytic activity (native or bacterial origin) is the essential first step in the gelation in *unconcentrated* UHT milk. For

example, in good quality or aseptically drawn milk, the activity of plasmin is the main influence on these first stage reactions (Kohlmann *et al.*, 1991; Kohlmann *et al.*, 1988; Snoeren & Both, 1981; Snoeren *et al.*, 1979). Too much or too little activity does not appear to lead to gelation of the milk, and this is in line with the frequent observation that there is no correlation between gelation and the extent of proteolysis. In contrast, studies on UHT *concentrated* skim systems (de Koning *et al.* 1985; deKoning & Kaper, 1985), which included proteinase inhibitors (DFP and or aprotinin) have demonstrated that age gelation in concentrated systems can occur without any proteolysis.

The role of heat stable bacterial enzymes was demonstrated by adding either culture supernatants from *Pseudomonas* organisms (Richardson & Newstead, 1979) or a small amount of cold-stored raw milk (Snoeren *et al.*, 1979) to product before UHT processing. Manji *et al.* (1986) reported that lack of gelation at 37 °C may be due in part to high activity of the proteinases resulting in a high degree of breakdown of any gel-type network which may form.

The above enzymatic reactions can be considered the most important or primary stage in the gelation reactions of unconcentrated sterilised milks. However, a second physiochemical type stage is considered necessary for the aggregation of these enzymatically sensitised casein micelles/casein micelle-coated globules in a gel-network. The fact that "gellable" liqueurs of normal viscosity always showed large increases in calcium sensitivity may indicate that the proteins have been chemically altered, possibly be enzymatic action.

It is interesting to speculate whether the cream liqueur system resembles more a concentrated milk system or an unconcentrated system. The protein content of the system (ca 3-3.5 %) would indicate that cream liqueur should resemble an unconcentrated system. However, if we consider, as Walstra (1990) noted, that casein(ate) covered globules act in effect like protein particles, then the effective protein phase volume is raised dramatically, and the system will act as a concentrated protein system. In addition, we could consider that the conformation of casein in caseinate covered fat globules in some ways more like micellar casein than free caseinate, since the outer protein layer is "concentrated" on the oil interface, more rather like a casein micelle (ca. 25 % protein). Also the hydrophobic core of the fat globule may orient the protein into a more micelle-like conformation (hydrophobic core/hydrophilic shell) as opposed to the more soluble hydrophilic free caseinate aggregates.

CONCLUSIONS

Overall there were significant differences in the analysis and behaviour of different samples of caseinates in the non-emulsion systems. As a group, the K samples showed similar properties, while the other caseinates varied widely. In particular, K samples, and especially K1, showed large increases in absorbancies on addition of Ca^{++} and high increases in viscosities when incubated. Samples of SP and MD caseinate showed opposite trends, although the reasons for different responses is not known with certainty. The MD protein was unique because of the presence of relatively large particles in solution. The use of photon correlation spectroscopy would appear to be the best method for determining particle sizes in caseinate suspensions

When the different caseinates were incorporated into cream liqueurs and subsequently tested, the trends in calcium sensitivity and viscosity mostly agreed with results obtained with solutions. In addition, all fresh non-K samples exhibited coalescence when heated at 65 °C.

Marked gelation was also noted in many samples. This defect was difficult to predict, since the age at which the sample is tested is important. Incubation of fresh samples, even for as long a period as 28 days at 45 °C, can produce many false positive results although never any false negatives ones. No other test or indicator consistently correlated with the "benchmark" test i.e. incubation of a 180 day old sample at 45 °C for 28 days. Many different caseinates displayed gelation at ambient and elevated temperatures. While it was difficult to put statistical significance on the results, the K1-containing samples appeared to be the most susceptible.

Gelation/thickening were promoted by homogenisation, probably due to a change in the conformation of the protein and/or an increase in the effective reactive protein concentration. The actual mechanism may be a two-stage process of varying rates of reaction.

The incorporation of an autoclaved caseinate in liqueurs lowered the rates of viscosity increase at 45 °C but caused coalescence at 65 °C. Future research could study the effects of other casein modifications e.g. ; dephosphorylation, proteolysis (e.g. rennin, plasmin treatments) , calcium addition etc., in solutions, model systems and liqueurs, in order to further probe the mechanisms of instability.

Experiment 4.3 *Effect of addition of citrate and other additives on the properties of cream liqueurs.*

INTRODUCTION AND OBJECTIVE.

The organic salt, citrate, is the most extensively studied additive used in 16 % fat cream liqueur formulations. It is considered a vital component and its main effect is thought to be prevention of gelation through the chelation of calcium ions which originate from the serum phase of the cream (Muir & Banks, 1985). However, as results in this study have indicated, the case may not be that simple, i.e some products were unstable with citrate present at recommended concentrations. In addition, the stage of addition of citrate during processing effected certain properties of the liqueurs.

Low-molecular weight surfactants (LMWS) are the other main class of additives found in cream liqueurs (Chapter 3.2). Whilst their use and function in liqueurs are not well documented, preliminary results in this study, as well as their presence in all of the commercial samples, indicated that they perform some useful function.

The objective of this experiment was to further investigate the effects of added citrate and LMWS in liqueurs. Most of the experiments were performed using 1-S produced cream liqueurs which contained citrate and LMWS at 0.18 % and 0.35% m/m, respectively.

In addition, other additives were briefly evaluated and the role of the serum portion of the cream liqueur was also assessed by manufacturing butteroil liqueurs.

MATERIALS AND METHODS.

Non emulsion systems

Caseinate syrups Caseinate syrups, as used in the production of cream liqueurs, save for the omission of caramel, were prepared with (m/m) ; 5.7% protein (from the caseinate), 39 % sucrose, 0.37 % w/w trisodium citrate and ca 54 % boiling distilled water. After mixing (see above method), the samples were placed in a water bath at 50 °C for 10 minutes. They were allowed to cool and placed in a cold room overnight. The following day either 1.23 g of 30% m/m sugar (- citrate sample) or 30% m/m trisodium citrate (+ citrate sample) was added to 100 g of the syrups.

Cream liqueurs

Most of the samples were liqueurs manufactured by the 1-S process and all these samples contained K1 caseinate. The level of added trisodium citrate in the experiments was 0.18 %, except in one batch where two other levels of citrate were added. For the non-citrate samples, a 30 % sucrose solution was added instead of the 30 % TSC* solution. One batch also used other additives i.e. sodium hydroxide (NaOH), sodium hexametaphosphate (SHMP) and Tris (tris-hydroxy-methyl-amino-methane).+ All LMWS were melted before incorporation into the pre homogenisation mixture.

General formulation, processing and testing was as described previously. More particular conditions are mentioned along with the results. Additions of NaOH, Tris and HMP were as solutions; the amount being calculated by trial experimentations. The experiments are summarised in Fig. 4.3.1 (+/- citrate samples only) and Table 4.3.1 (+/- citrate /LMWS).

In previous experiments, the amount of caseinate was decreased *pro rata* when LMWS were incorporated. This was in order to keep cream liqueur viscosities at a similar level. However, in order to exclude the possibility that decreased protein levels could be responsible for the observed changes, this was not practised in these batches i.e caseinate inclusion levels were kept at the same level in all samples.

* Trisodium citrate

\+ "Tris" is an organic base, in contrast to e.g NaOH, which is inorganic and contains a metallic cation.

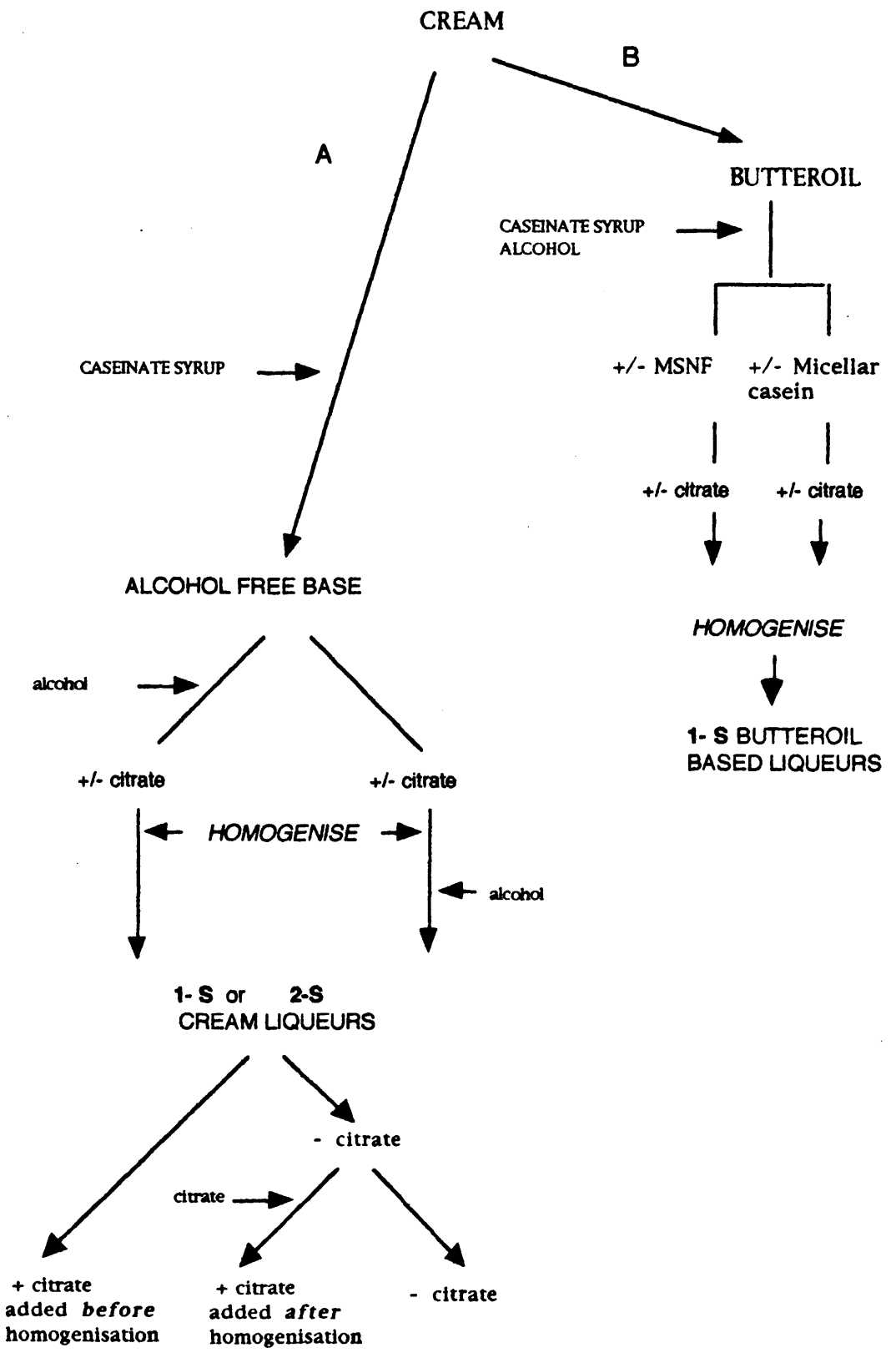


Fig. 4.3.1 Outline of experiments performed during the investigation of the effect of citrate addition to liqueurs. Most of the samples were cream liqueurs (A), produced mainly by the 1-S process. Citrate , 0.18 % , was added either before or after homogenisation. In one experiment , different levels of citrate and other salts were added before homogenization only. Two batches of butteroil based liqueurs (B) were produced to study the influence of milk solids non fat (MSNF) or micellar casein, with and without citrate. See text for further details of experiments.

Table 4.3.1 A summary of some details from 5 different K1-containing batches , processed to study the effect of low molecular weight surfactants (LMWS) on the properties of cream liqueurs. (or butter liqueurs batch 31).

Batch no	Process date	no. of tests	Variable examined	% ingredients used / other comments
30	01 09 93	12x 2	The use of LWMS , with and without TSC, in the 1-S and 2-S process	3.3 % caseinate , +/- 0.35 % SSL / MDG Processed at 65 °C with 30 mins holding before. homogenisation
31	24 03 94	7x 2	The influence of the serum phase / MDG on the properties of butteroil and cream liqueurs. The use of citric acid ester of a MDG.	3.1 % caseinate used and 0.35 % LMWS. 0.5 % citric acid ester used, with no TSC. Processed at 65 °C.
32	20 10 93	6x 2	The effect of increasing the quantity of SSL from 0.35 to 0.50 % m/m , with and without TSC.	3.3 % caseinate Processed at 65 °C
33	16 09 93	18x 2	Effect of 3 different pressures; 1 and 2 passes and +/- TSC in liqueurs processed by the 1-S method.	3.3 % caseinate +/- 0.35 % LMWS. Processed at 65 °C after 20 mins holding before homogenisation
34	06 10 93	12x 2	Effect of 4 different time /temperature treatments; 55 or 70 °C for 2 or 20 mins before processing	3.3 % caseinate +/- 0.35 % LMWS

The samples were processed on the Rannie homogeniser with 1 pass at 27.6 MPa unless otherwise stated.
SSL; sodium stearoyl lactylate; MDG; mono-diglycerides; TSC; trisodium citrate.

Butteroil liqueurs

Batches of butteroil liqueurs were made to investigate the effects of the serum portion (ca 1.5% w/w) of the cream liqueur.

Ingredients

For batch 24 samples, a concentrated butter (fat 98.7 %) was first prepared from the cream by the following method; the 48% fat cream was chilled overnight at 5 °C after which 5 kg of this material was beaten in a large whisk. When the grains were the size of peas, the buttermilk was drained and the grains collected in a stainless steel container. The container was placed in hot water and, after 1 hour, the resulting butteroil was siphoned off and tested for water content by drying to a constant weight in a vacuum oven (102 °C). The fat content was calculated by difference assuming that for every 1% water in the concentrated butter there is 0.09% MSNF present. A commercial sample of butteroil (99.9 % fat), purchased from Kerry Group PLC, Tralee, was also used in a separate batch.

Where required, separated milk or a sample of micellar casein were used as sources of serum constituents. The micellar casein was produced by spray drying a solution produced on an Alpha Laval MFS-19 microfiltration plant using an 0.1 μm ceramic membrane (see Gaynor, 1995, for details.) The composition of the product was ca 84% protein, 2.75% calcium, 1.76 % phosphorus and 8.55 % ash.

Formulation / processing

A butteroil / water "mixture", weighed out in the proportions 48/52 was substituted directly for 48 % cream in recipes. Butteroil formulations contained 3.5 % caseinate, thus achieving final protein and viscosity values close to that of cream containing samples. Corresponding cream liqueur samples were produced incorporating the cream used for butteroil production. These samples contained 3.0 % caseinate. All products were processed at 65 and 45 °C.

In micellar casein containing experiments, two casein(ate) syrups, of equal protein content, were manufactured; the control contained 3.5 % sodium caseinate and the experimental contained 3.0 caseinate and 0.6 % micellar caseinate. Micellar casein containing syrups required very vigorous and extensive high-shear mixing (40 mins) to ensure solubilization of the protein. A similar treatment was given to the normal caseinate syrup. The products were processed as normal, with the TSC being added

just before the heating-up stage; temperature of homogenisation was 55 °C with no holding before hand.

Butteroil based products were manufactured (batch 31). The butteroil was obtained from Kerry Group PLC, Tralee, and the buttermilk was produced in the Department by spray drying the buttermilk from churn-produced butter. (Milk from University suppliers was used to produce the cream) These liqueurs contained 3.1 % caseinate and were processed at 60 °C with no holding, at a homogenisation pressure of 27.6 MPa. As well as liqueurs containing no MSNF, some samples contained either added buttermilk solids (MSNF) and/or mono-diglycerides at 1.5 % or 0.35 % m/m, respectively, in the four possible combinations.

Testing.

Tests of liqueurs (pH, viscosity, incubation tests, visual observation, TVs and calcium sensitivity) were as outlined previously.

RESULTS

The results are presented in two main sections. The first mainly considered both the effect and the stage of addition of trisodium citrate on liqueurs. The effects of some other inorganic additives were mentioned for comparison. In the second section, the effects of added citrate and/or added low molecular weight surfactants (SSL and MDG) were examined. In both sections, the effects of serum constituents were considered by the manufacture of some butteroil based liqueurs.

Addition of 0 or 0.18 % trisodium citrate to liqueurs without LMWS.

Table 4.3.2 summaries initial viscosity and stability data from five 1-S and two 2-S produced products. A different batch of cream was used for each sample. All these products contained K1 caseinate and either 0 and 0.18 % trisodium citrate, which was added before homogenisation. These and other results are detailed below:-

Initial results

As expected the addition of citrate led to an increase in the pH. The pHs of the "control" cream liqueurs samples (no citrate added) ranged from 6.55-6.65 and the addition of TSC increased the pH by varying amounts e.g by ca 0.16 for the lower pH controls (6.55 to 6.71; batches 17,19,24) but by ca 0.12 for the higher pH controls (6.65 to 6.67 ; batches 22,23).

Viscosity

The initial viscosity of the non-citrate 1-S products varied from 25.4-36 mPa s. The corresponding figure for the 2-S process was ca 21 mPa.s (Table 4.3.2). This large difference was despite the fact that the 2-S products contained 0.3% more caseinate and were processed at a higher temperature . Similar trends were noted in the previous experiment (4.2). The addition of the TSC had varying effects on the viscosity of the five 1-S products i.e. it sometimes slightly increased (batch 23) , slightly decreased (batch 24) or had little effect (batch 22) on the results. The addition of citrate to the two 2-S samples increased their viscosities slightly ; from ca 21 to 23 mPa.s. In contrast, the incorporation of citrate to non-emulsion systems e.g. caseinate syrups, always led to a decrease in viscosity (Fig. 4.3.2). The magnitude of

Table 4.3.2 Effect of citrate addition (0 or 0.18 %), added before homogenisation, on viscosity and ultimate stability of samples of cream liqueurs made with different batches of cream.. Stability test was carried out at 45 °C for 28 days; All 1-S samples were processed at 65°C with 30 minutes holding before homogenisation.; samples contained 3.0 % sodium caseinate except 23,24 (3.1 % caseinate). The 2-S samples were processed at 75 °C and contained 3.3 % caseinate.. The K1 caseinate was used in all the experiments (a and b refer to duplicate samples , where performed).

	0 % Trisodium citrate			0 .18 % Trisodium citrate		
	Viscosity (mPa.s) of ambient temperature sampleat day;		Stability at 45°C at day	Viscosity (mPa.s) of ambient temperature sample at day;		Stability at 45°C at day
Batch	0	180	180	0	180	180
Single stage process (1S)						
17.a	25.4	25.1	G	27.7	G	G
b*						
19.a	32.3	31.7	G	30.4	30.4	ok
b	34.7	35.3	G	29.4	30.0	ok
22	26.4	26.4	ok	26.4	25.7	ok
23.a	27.4	178.0	G	28.7	29.0	ok
b	26.6	25.7	G	28.7	28.7	ok
24.a	32.7	32.3	G	28.3	28.4	ok
b	36.3	36.5	G	28.5	28.4	ok
Two step process (2S-b)						
20.a	20.8	18.5	G	23.1	21.8	G
b	21.1	18.5	G	23.1	21.1	G
21	20.8	18.8	G	22.4	21.1	G

*; ca 10 % water contamination.

G ;gelation

OK ; viscosity < 300 mPa.s and no defects..

this depended on the source, with the K1-caseinates syrups showing the largest relative decreases.

The viscosities of ambient temperature stored liqueurs, measured at six months , were basically unchanged as compared to their initial viscosities for the 1-S products; however the 2-S samples consistently showed a slight decrease over the six months.

Turbidity Values (A 800, diluted in water) and aggregation indices

The initial turbidity values for the citrate-containing 1-S cream liqueurs ranged from 0.070 to 0.085, which were slightly higher than those found (ca 0.065 to 0.075) with batches 15/16 in Experiment 4.2. If citrate were omitted from the formulae, the TV increased but by varying degrees (Fig 4.3.3). For the samples with an initial pH of ca 6.56 the value was approximately twice that which it would be if citrate is added, whereas with the samples with the high initial pHs,the difference was only 25-35% In the latter case (batches 22,23) both these cream samples were March samples and were from the same single-herd supplier. As expected (see previous experiment) all 2-S samples manufactured with citrate, had very high TVs when compared with the corresponding 1-S samples. Unlike the results of the 1-S samples, the TVs were not influenced by citrate.

The aggregation indices of fresh cream liqueur samples (batches 22,23) were calculated for the high pH samples only and the results were very similar ; the non-citrate samples had values of ca 35 % while the citrate samples were 9-15 %. Aggregation indices of batch 19 and 24 samples indicated larger differences between the citrate and non-citrate samples. Note that more detailed information on aggregation indices of citrate and non citrate containing samples are also reported later.

No aggregation indices from fresh 2-S samples were calculated, however later examination of these samples showed that the inclusion of citrate had no effect on this property.

Thickening at 45 °C.

Although non-citrate samples, when aged, are more unstable to 45 °C incubation (see later), the *initial* rate of thickening of non-citrate samples was always lower (Fig.4.3.4) than the citrate containing samples The differences were often by as much as 10 fold. Addition of citrate to FFBs also resulted in a larger increase in viscosity than the non-

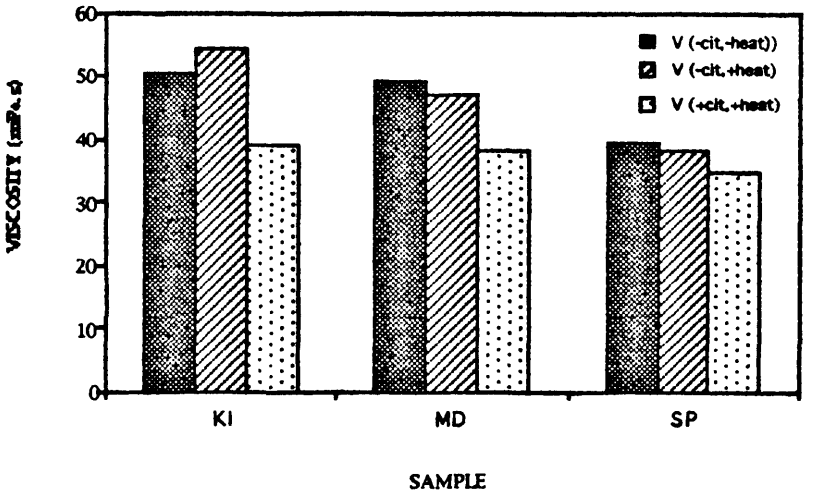


Fig 4.3.2 The effect of added citrate (0.36 % m/m) and/ or heat (65 C x 1 hour) on the viscosities of three different caseinate syrups. (formulation; 5.7 % protein; 39 % sugar, no caramel added). See Table 4.2.1 for details of samples

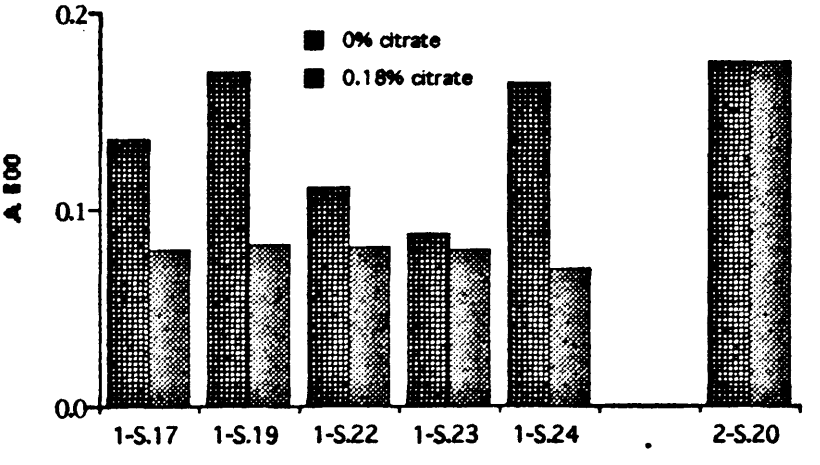


Fig.4.3.3. The effect of citrate on the turbidity values (A800) of cream liqueurs processed by the 1-S and 2-S methods Numbers refer to different batches. Samples were made with and without 0.18 % trisodium citrate.. Readings were taken on fresh samples.

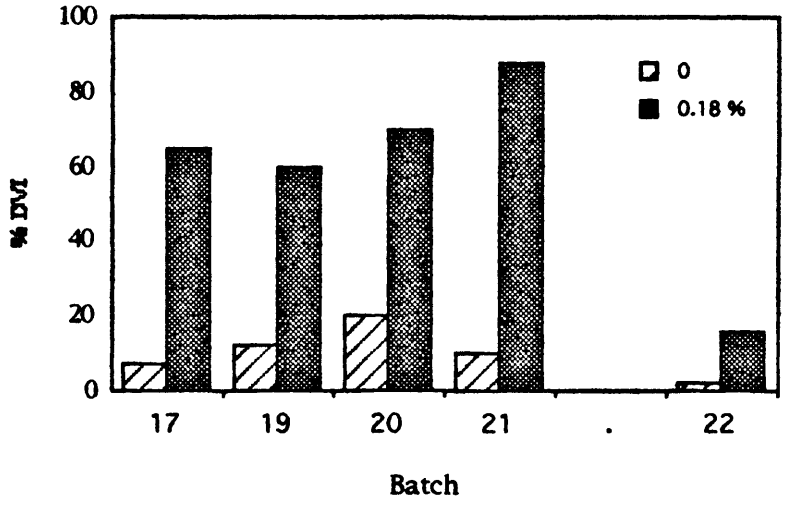


Fig.4.3.4. The effect of citrate addition on the % daily viscosity increase (% DVI) was measured in various fresh liqueurs, processed by the 1-S method. The DVI was calculated after 3 days incubation for samples 17-21 and after 28 days incubation at 45 °C for sample in batch 22.

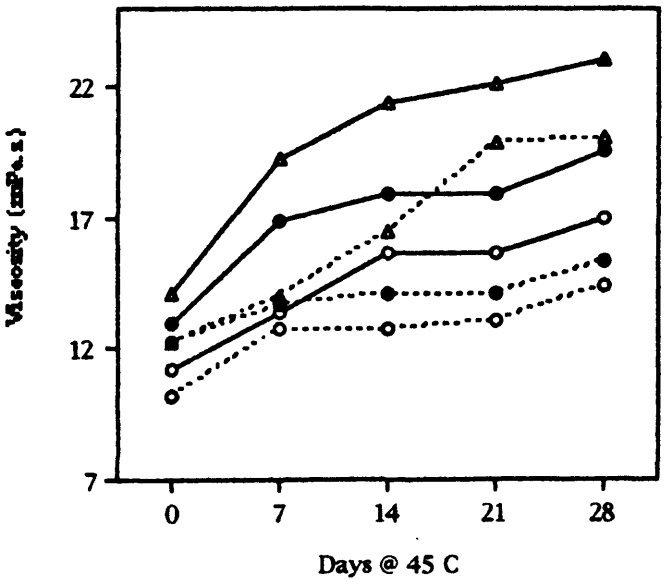


Fig. 4.3.5 The viscosities of fat free mixes during storage for 28 days at 45 °C. Δ; K1 •; MD o ; Rollerdry (lab). The effect of including citrate (solid lines) or ommitting citrate (dash lines) is illustrated.

citrate control samples (Fig. 4.3.5). Sample K1 increased at the greatest rate followed by the MD and RDL samples;

The addition of citrate to non-citrate containing liqueurs *after* homogenisation also produced interesting results. This method of citrate addition led to liqueurs which showed very low rates of thickening at 45 °C as compared to samples that contained the same amount citrate added *before* homogenisation. i.e. the rate of viscosity increase was about 8 times lower; Fig 4.3.6 . (This was observed for all K1 caseinate samples. There was not enough data to say if this behaviour would be common to all caseinates).

Long term storage / incubation results.

As expected, after 6 months the samples containing no added citrate were very unstable to 45 °C incubation (Table 4.3.2, previously). There was one exception however ; samples from batch 22 (1-S process) were the only non-citrate samples in the whole of this study not to gel and they can thus be considered exceptions. The reason was not clear, but was probably related to factors in the cream. Despite the instability of the samples at 45 °C, only one 1-S sample (sample 17b) gelled at ambient temperature. Addition of citrate before homogenisation in four out of five of these 1-S samples prevented ultimate gelation . The citrate-containing sample which

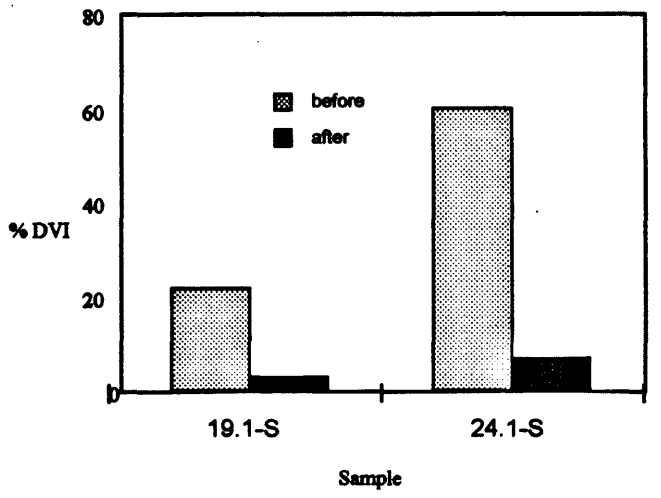


Fig.4.3.6 Effect of stage of addition of 0.18 % trisodium citrate on the initial rate of thickening in 1-S processed liqueurs at 45 C . Citrate .was added either before or after homogenisation The %DVI from Batch 19 was calculated from a three day incubation period, while that of batch 24 was from a 28 day incubation,

gelled was batch 17, which was previously mentioned in Experiment 4.2. Addition of citrate before homogenisation to the two 2-S samples had no effect on gelation at 45 °C, confirming earlier results that this method of manufacture is very unsuitable under the conditions operated here.

The inclusion of citrate before or after homogenisation was equally effective as adding it before hand in terms of ultimate stability. In a few cases (data from this experiment and the previous one), addition of citrate *after* homogenisation to 1-S samples prevented gelation,whereas the corresponding samples with citrate before hand showed gelation. Reverse results were also seen .

Coalescence stability of non citrate samples (K1 and MD samples)

In the previous experiment, fresh citrate containing liqueurs manufactured with MD caseinate showed extensive heat-induced coalescence when incubated at 65 °C. Here the MD based liqueurs, manufactured without citrate, do not show this effect. However, coalescence reoccurredwhen citrate was added after homogenisation. Samples of K1 based liqueurs do not show coalescence, either in the presence or absence of citrate.

Addition of various levels of citrate and other salts to cream liqueur batches.

Other citrate levels

The effect of adding different levels of citrate (0.00, 0.09, 0.18, 0.27% m/m; batches 19 and 23) was assessed in the 1-S process and in a 2-S process (0. 00, 0.18 and 0.36 %; batch 20). The effects of NaOH (with and without citrate), Tris and sodium hexametaphosphate (HMP) addition were evaluated in batch 23.; this experiment was performed in order to establish to what extent the beneficial effects of citrate are related to its ability to increase pH. The results of batch 23 samples are summarised in Table 4.3.3; reference is made to results from other batches.

As expected, the pH increased with TSC addition, although the *rate* of increase decreased noticeably with higher additive level. Conductivity measurements are also included for batch 23 and these increased with salt content.

The initial rate of thickening (over 3 days at 45 °C) was increased with increasing citrate (batch 19) for liqueurs were the citrate was added beforehand i.e. %DVIs were 12 , 31, 60 and 70 for the 0, 0.09, 0.18 and 0.36 % levels of TSC. In contrast, adding similar levels of TSC after homogenisation gave a %DVI of only 8 in all cases (data not illustrated ;these results from the 1-S processed Batch 19 samples.

In the 2-S process samples, if the amount of TSC was doubled to 0.36 % (either by addition before or after homogenisation) then the % DVI (3 day value) increased from about 70 to 95. As in all experiments to date, production of a 2-S sample with 0.18 % citrate did not prevent gelation. Doubling this amount to 0.36 % also had no effect, irrespective of whether the citrate was added before of after homogenisation.

The 1-S processed liqueurs in batches 19 and 23 (above) can be considered to have been produced from " good cream" since as little as 0.09 % citrate, added before homogenisation, prevented gelation. Unfortunately, of the many samples which gelled with 0.18 % citrate added before homogenisation , no corresponding samples were produced with higher levels (e.g. 0.27 or 0.36 % citrate added before homogenisation). Therefore we cannot say definitively whether a higher level, added *before* processing, would have prevented gelation. However, adding an extra 0.27 % TSC (trisodium citrate) after processing to four *unstable* 0.18 % citrate-containing samples (from previous experiments), prevented gelation in only one of the samples.

Table 4.3.3 The effect of the addition of different levels of trisodium citrate (TSC) and other additives - (NaOH; sodium hydroxide; Tris; tris-hydroxy-methyl-amino-methane , HMP; hexametaphosphate) on properties of batch 23 cream liqueurs. Results are the averages of duplicates. See footnotes for further details.

sample	pH	conduc-tivity (mho)	Vi (mPa.s)	Viscosity after 45℃	initial TV (A800)	Aggreg-ation index
% TSC (w/w)						
0	6.65	238	27.0	G	0.088	33
0.09	6.72	340	30.0	257	0.077	22
0.18	6.76	420	28.7	248	0.069	15
0.27	6.78	475	29.8	224	0.070	14
others (% w/w)						
0.03 NaOH	7.13	285	33.3	G	0.081	22
0.18 TSC + 0.03 NaOH	7.27	450	27.9	75	0.061	7
0.1 Tris	7.13	285	32.9	G	0.082	22
0.2 HMP	6.65	390	24.4	G	0.062	7

(V_i); Initial viscosity , TV ; initial turbidity value @ A 800 nm. and the aggregation index (% difference between turbidity measured in water and EQA solution).

The viscosity/appearance at 45 ℃ was noted after incubation of 180 day sample for 28 days;
G- gelation.

Table 4.3.4. Effect of the addition of NaOH to old liqueurs, of normal viscosity, that would gel if incubated at 45 ℃.

Sample from Batch;;	Amounts of NaOH (% m/m) ;					
	0	0.03	0.06	0	0.03	0.06
	pH			*Stability*		
10	6.35	6.78	7.25	G	OK	OK
17	6.61	7.21	8.14	G	G	OK
9	6.63	ns	7.8	G	ns	OK
24*	6.52	7.11	7.92	G	OK	OK
17*	6.58	ns	8.26	G	ns	OK

Stability refers to state after incubation for 28 days at 45 ℃.
* ; did not contain any citrate; the others contained 0.18 % TSC.
Batch number refers to different cream samples.
ns; no sample

Addition of other additives to cream liqueur batch 23.

In the preliminary section, the most stable samples (to thickening and gelation) were those that contained citrate and which had a high pH. Thus, the effects of increased pH, in the presence and absence of citrate were investigated further:-

The pH of a 0.18 % TSC sample increased from ca 6.8 to 7.3 on addition of 0.03 % NaOH before homogenisation; this lead to a marked decrease in the rate of thickening i.e. the viscosities after 28 days storage at 45 °C were 257 and 75 mPa.s, respectively. There was also in a reduction in the TV and the AI, as compared to the sample containing just 0.18 % TSC (see Table 4.3.3, previously). As both samples were stable to gelation, it was difficult to tell if the NaOH would have prevented this defect.

Instead, to further test the effect of increased pH, the pHs of the non-citrate samples were raised with NaOH and Tris before processing(Table 4.3.3). This had no effect in preventing gelation in subsequent liqueurs. However, there was a significant increase in the initial viscosity of products in this situation from 27 to 33 mPa.s, along with a 33% increase in TV.

In addition, sodium hydroxide was added to various samples (from previous experiments) that would gel if incubated but were at their original viscosity at ambient temperature (Table 4.3.4). Two of these sample did not contain any citrate. Addition of 0.06 % NaOH, which increased the pH dramatically, prevented gelation in all the samples. Increasing the pH of a sample from batch 24 (no citrate) from 6.52 to 7.1, with 0.03 % NaOH, resulted in a similar increase in stability. A sample from batch 17, containing citrate, was still unstable even when brought to pH 7.21 with the 0.03% NaOH. It will be recalled that this sample was from the most unstable batch seen in the study and thus may represent an exceptional case.

Sodium hexametaphosphate (HMP), at the 0.2 % inclusion level, significantly reduced the viscosity and the pH of the cream liqueurs, but it did not prevent gelation as compared to the normal 0.18 % TSC containing product. HMP was not tested in combination with TSC nor with addition of NaOH (Table 4.3.3)

The effects of addition of various salts, including HMP, at 0.2% m/m, on the viscosity increases in fat-free base systems were also examined (data not shown). There were

basically three types of response as compared to that of the citrate containing sample e.g. sodium hydrogen carbonate decreased the rate of thickening, while salts such as sodium hexamataphosphate and tetrasodium pyrophosphate dramatically increased the rates of thickening. Other salts showed responses close to the citrate samples eg. potassium citrate.

Butteroil liqueurs with 0.18 % citrate

Initial pH and viscosity

The addition of citrate to (non-citrate) butter or cream liqueurs increased the pH of the resultant products by 0.02 and 0.07 units, respectively.

Addition of citrate to butteroil based liqueurs had basically no effect on the initial viscosity of the products whether they were processed at either 45 °C (no holding) or 65 °C (30 min holding before) ; see Fig.4.3.7. Addition of citrate decreased the TV from 0.085 to 0.072, in this system. There was little differences in the TV's of any of the 45 °C processed samples. In contrast, addition of citrate to the corresponding 65 °C processed cream liqueurs in this batch decreased the viscosity from 35 to 28 mPa s and the TV from 0.0175 to 0.070. For both butter and cream liqueurs, increasing the temperature/time treatments significantly increased the viscosity e.g. the viscosity of non citrate and citrate containing butter liqueurs was increased from 25 to 31 and 23 to 31 mPa s, repectively, on processing at 45 and 65 °C.

Another set of butteroil liqueurs, this time incorporating 0.6% of micellar casein, was produced in a separate experiment (batch 25). The micellar casein containing samples had lower viscosities (ca 15%) than sodium caseinate samples (Fig. 4.3.8) and addition of citrate increased the viscosities of all products by about 7 %. The effect of the 0.6 % micellar casein on turbidity of 1-S butteroil based liqueurs is shown in Fig. 4.3.9. The presence of citrate had no significant effect on the TV in pure butteroil liqueurs (ca 0.060), but omission of citrate in micellar casein containing samples increased the TV to ca 0.100. The aggregation indices of the MC containing samples, especially the - citrate samples, were significantly greater than non-MC samples

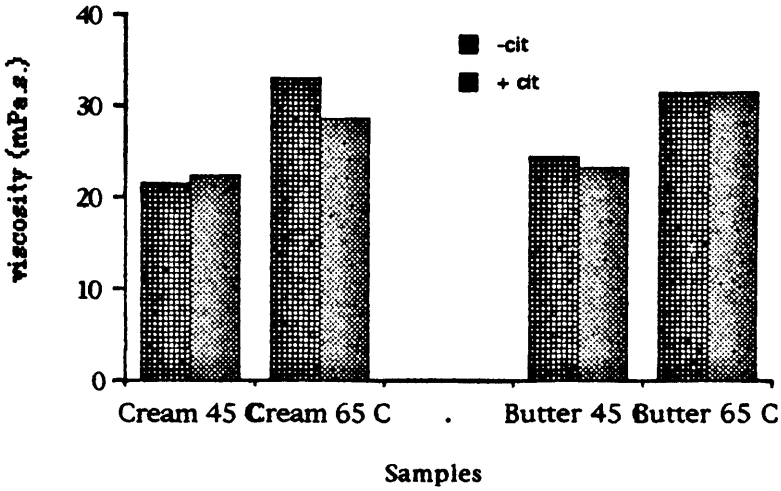


Fig. 4.3.7 The effect of citrate addition on the viscosity of butter and cream liqueurs homogenised by the 1-S process using two differernt process temperatures- 45°C (no holding) and 65 °C (30 min holding . Results are the averages of duplicate trials; . Butteroil was produced in the laboratory from the batch 24 cream. See text for further details..

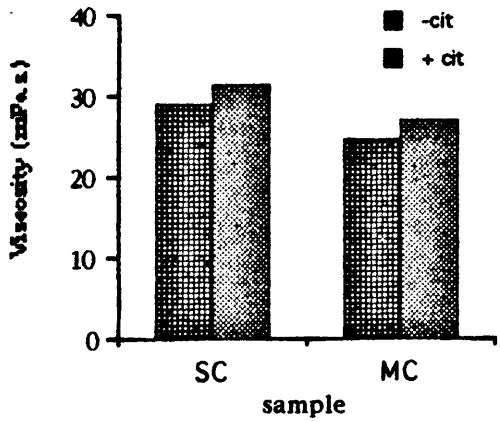


Fig. 4.3.8 The effect of added citrate on the viscosities of butter liqueurs containing sodium caseinate only (SC) or sodium caseinate /0.6 % micellar casein (MC). The final protein contents were 3.3 % protein. Samples were processed at 55 °C at 27.7 MPa., using the 1-S process.. A commercial butteroil from Dairygold. was used (Batch 25).

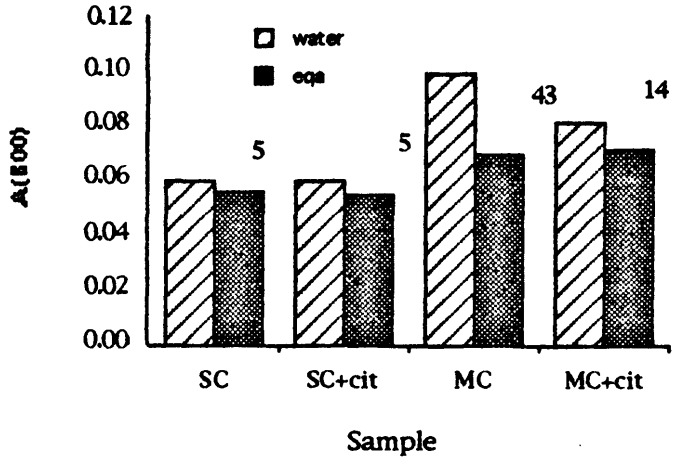


Fig.4.3.9. The effect of added citrate on the turbidities (in water and EQA) of butteroil based liqueurs containing sodium caseinate only (SC) or sodium caseseinte /0.6% micellar casein (MC). The aggregation indices were also calculated (data above bars). The final protein contents were 3.3 % protein. Samples were processed at 55 °C at 27.7 MPa , using the 1-S process. A commercial butteroil from Dairygold.was used Data from Batch 25.

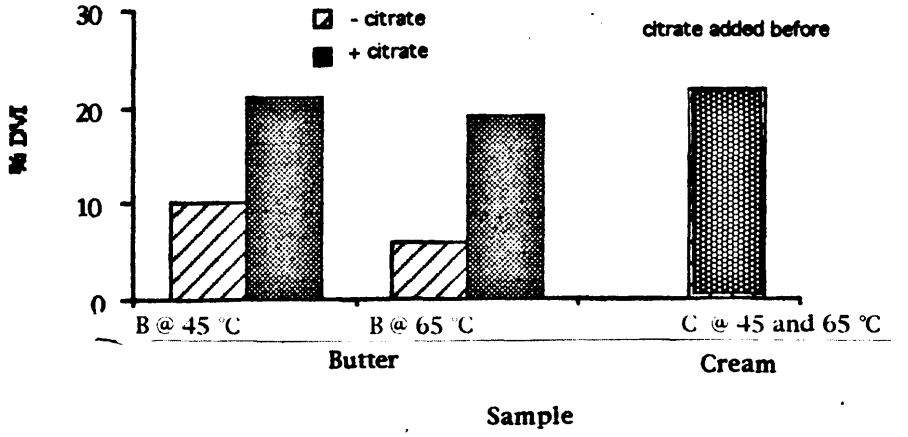


Fig. 4.3.10 The effect of citrate addditon on the 28 day % daily viscosity increase (%DVI)) of butter liqueurs (B) and the corresponding cream liqueurs (C), manufactured at two different temperatures (45 and 65°C) by the 1-S process Samples of cream liqueurs manufactured without citrate, gelled at 45 °C during the 28 day storage. Results are averages of duplicates.

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Thickening at 45 °C.

The addition of citrate to the butteroil samples led to a significant increase in the initial rate of thickening at 45 °C for batch 24 samples, see Fig 4.3.10. The % DVI (measured over 28 days from when the samples were put into the incubator on day 0) increased 2 or 3 fold on addition of 0.18 % TSC for the low and high heat treatments, respectively. Interestingly, the rates of thickening were similar in cream and butteroil samples when both contained TSC. The % DVI trends of 180 days old samples, if incubated at 45 °C for 28 days were similar to those reported above (data not shown).

In batch 25, butter liqueurs were produced which included either 0 or 0.6 % micellar casein (MC), with and without 0.18% citrate. All samples, except the 0.6 % MC/0% citrate sample (which gelled) showed relatively large increases in viscosity when incubated at 45 °C. The 0.6 % MC/+citrate samples showed a higher % DVI than the 0% MC/+cit. sample (32 and 25, respectively). In this batch there is only a slight increase in the %DVI values (22 to 24), on addition of citrate of the 0 % MC samples (i.e. only sodium caseinate used) . This was in contrast to the large increases reported above for similar samples in batch 24.

gelation

None of the pure butteroil liqueurs (in Batches 24,25), either with or without citrate, showed instability during long term incubation at 45 °C. In contrast, all the corresponding non-citrate cream liqueurs showed gelation. Gelation was observed in the duplicate non-citrate micellar casein containing butteroil samples and, as expected, addition of citrate prevented the occurrence. None of the corresponding non-citrate sodium caseinate /butteroil samples gelled.

Since Muir & Banks (1985) attributed the instability of non-citrate cream liqueurs to the presence of serum phase and in particular to the Ca^{++} ion, it was decided to add 2 mM Ca^{++} to samples of butter liqueurs mentioned above. Addition was made by carefully adding 10 % of a 20 mM solution in sugar /alcohol, to cold liqueurs. The samples, together with controls (no added Ca^{++}) were incubated for 28 days at 45 °C.

The results (data not shown) were conflicting i.e. addition of Ca^{++} to the non-citrate butter-liqueurs homogenised at 45 °C caused marked thickening /gelation as compared with controls but addition to the corresponding 65 °C sample did not lead to any difference. Addition of Ca^{++} to the citrate containing butter liqueurs did not have any effect.

Citrate and low-molecular weight surfactants (LMWS) in cream liqueurs

Four different batches of LMWS-containing cream liqueurs, processed with and without citrate (38 formulations in duplicate) were examined. Most of the formulations contained 0.35 % LMWS, and when added, 0.18 % trisodium citrate.

Initial pH

While citrate increased the pH of samples (see previous section), the inclusion of SSL decreased the pH of liqueurs relative to corresponding non-SSL products. The magnitude of the decrease, on addition of 0.35 % SSL, varied somewhat between different 1-S batches. For example in batch 30, the difference was 0.16 pH units (citrate containing samples) and in batch 34 it is only 0.10.units. Samples manufactured by the 2-S process had a slightly smaller decrease in pH when compared to the corresponding 1-S samples i.e. i.e. differences of 0.13 and 0.16 (batch 30 results). The addition of MDG to samples had no effect on the pH.

Initial viscosity

On omission of citrate from LMWS-containing samples, the most remarkable aspect was the very large viscosity increase which occurred in two out of three MDG containing 1-S samples; this increase did not occur in the corresponding 2-S sample for batch 30 (Figs.4.3.11 and 4.3.12). In contrast, the addition of SSL to non-citrate samples did not increase the viscosity anymore than the controls for either the 1-S or 2-S products.

In the citrate-containing 1-S liqueurs, addition of 0.35 % LMWS on top of the existing caseinate level, usually 3.3 %, did not result in any large increase (only ca 7-10 %) in viscosity. In contrast, there was an increase of ca 25 % in the viscosity of the 2-S samples due to the extra surfactant. The viscosities of the 2-S samples were lower than the corresponding 1-S samples (Fig. 4.3.11), in agreement with previous results, but the differences were less for the LMWS-containing samples.

Turbidity values (TV) and aggregation indices (AI)

Omission of citrate from MDG-containing samples, which gave rise to the large viscosity increases mentioned previously, was also accompanied by large increases in TVs (A800) (Fig 4.3.13); this was also found in the non-LMWS samples. The SSL

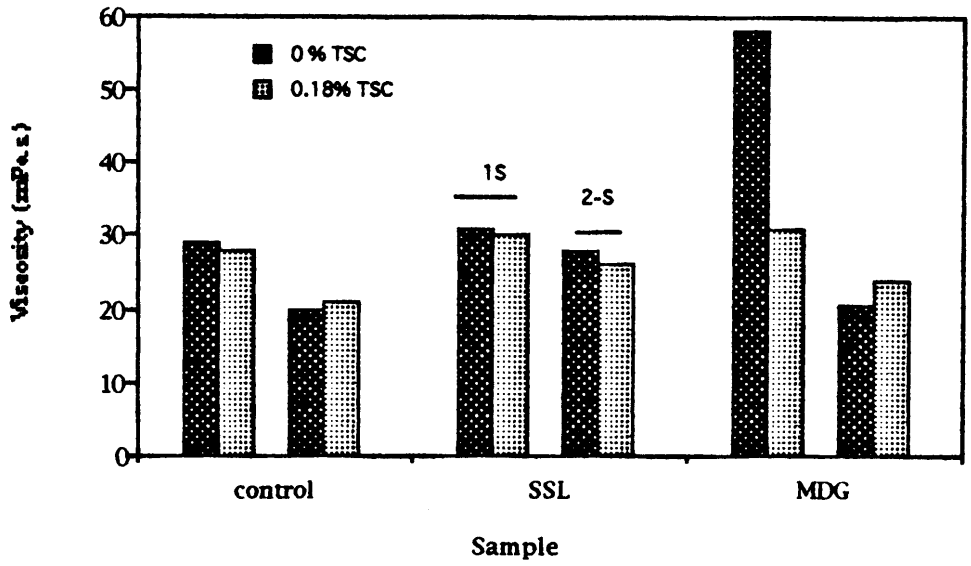


Fig. 4.3.11 The effect of addition of trisodium citrate (0 or 0.18 %) on the initial viscosities of liqueurs made with LMWS. Two different processes , 1-S and 2-S, were examined. The results are from batch 30.

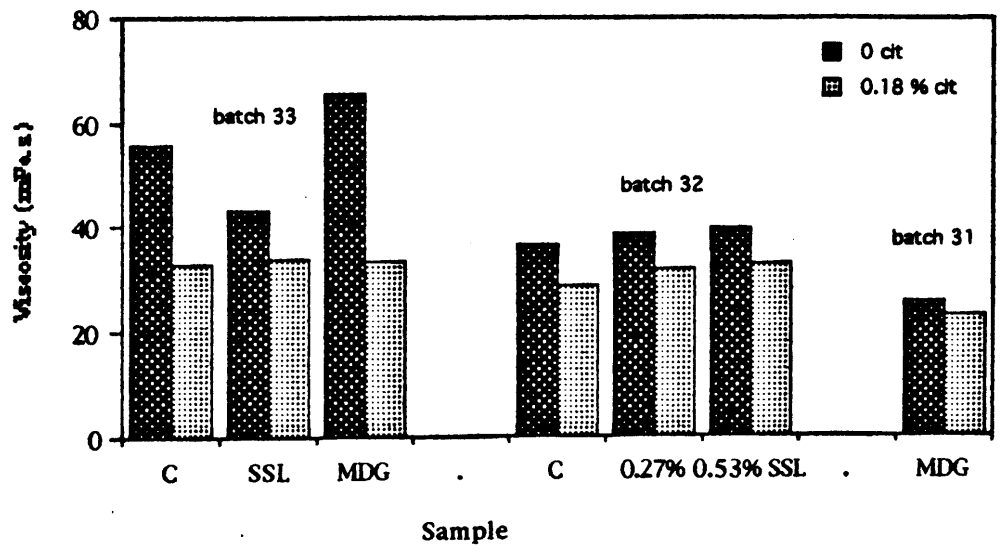


Fig.4.3.12 The effect of addtion of 0.18 % trisodium citrate (0 or 0.18 %) on the initial viscosities of 1-S liqueurs from batches 31-33.

containing liqueurs had significantly lower differences in turbidity values between citrate and non-citrate samples .

The inclusion of LMWS to citrate containing liqueurs reduced the TV (and hence the average particle size) in 2-S and especially in the 1-S processed products (Table 4.3.5). The response depended on pressure and number of homogenisation passes. From Fig. 4.3.14 one can deduce that the SSL-containing sample processed with 1 pass at 28 MPa produced a smaller average particle size than that a non-LMWS control liqueur homogenised with 2 passes at 13 MPa. There was an inverse relationship between TVs and increasing pressure, although the rate of decrease is slightly steeper for the two-pass samples (log/log plot not shown). Overall, for citrate samples, the SSL containing liqueurs consistently had the lowest actual turbidities (hence average particle sizes) followed, in order, by the MDG and control samples. Turbidity values (A800 water only) were measured for the 2-S samples in batch 30; there were no significant differences between +/- citrate samples for the control (ca 0.180) and LMWS samples (ca. 0.155).

Fig 4.3.15/16 shows the effect of added citrate, as well as increasing pressure and number of passes, on the AI index of the samples from batch 33. The citrate containing samples showed much smaller AI's than the non-citrate samples and in both cases the absolute values and patterns were different for SSL and MDG samples. In the non-citrate samples, the higher the homogenisation treatment (e.g. high pressure/2 passes) the higher the AI, and in general, the control samples (no LWMS) had the highest values. In the citrate containing samples, increasing pressure decreased the aggregation index for SSL samples, while it increased it for the MDG containing liqueurs. The control sample was very similar to the MDG sample.

While the non-citrate MDG sample had the largest initial TVs, it did not have the largest AI, indicating that there was no relationship between these readings, which also varied between batches e.g. in batch 30, the A800 (water) / aggregation index values were 0.220 / 110, but for batch 33 these readings were 0.234 / 44.

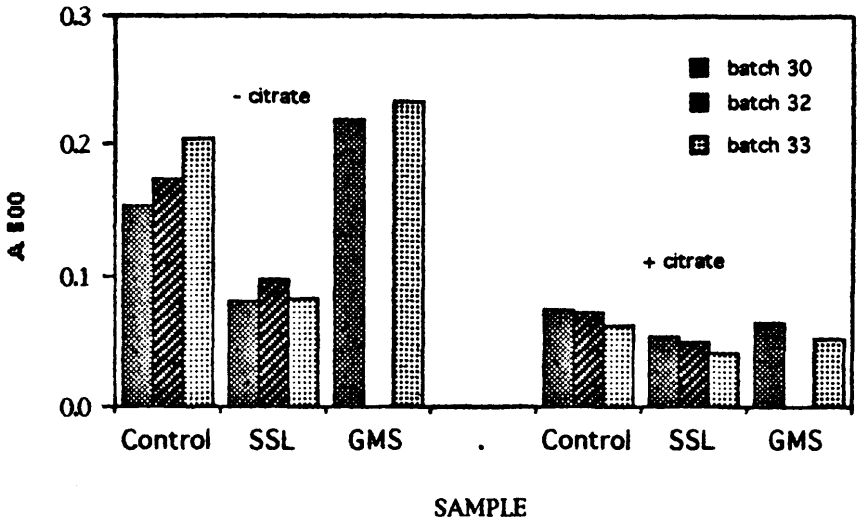


Fig. 4.3.13 The effect of trisodium citrate (TSC) addition on the turbidity values (A800 in water) of cream liqueurs from batches 30,32 and 33 (no MDG data for 32). All the samples were processed by the 1-S process with one homogenisation pass at 27.6 MPa.

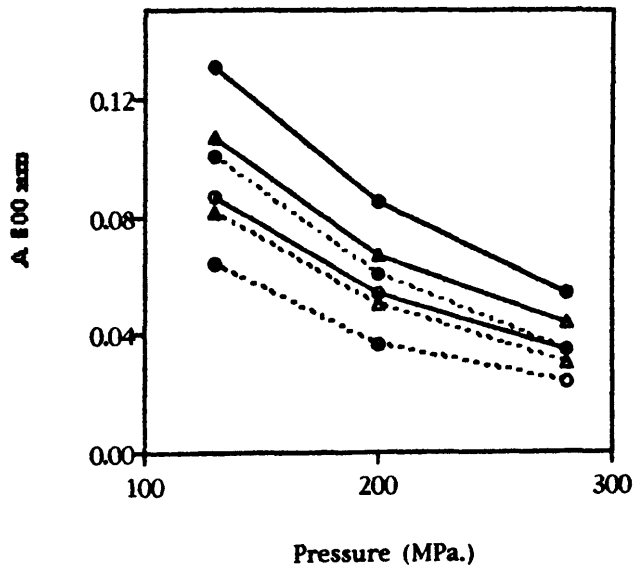


Fig. 4.3.14 Effect of increased pressure and number of passes on the turbidity values of citrate and LMWS containing liqueurs (1-S process). • control sample
o SSL containing sample Δ MDG sample. ; —— 1 pass samples, — 2 pass samples

Table 4.3.5 Effect of including LMWS on the analysis and stability of citrate containing liqueurs which were manufactured by the 1-S and 2 -S processes . Part A summarises details from batch 30 . Part B summaries the effects on three other 1-S batches.. All samples contained 0.35 % w/w LMWS unless otherwise stated and ontrol samples contained extra sugar instead of LMWS. All samples were processed at 65 C (except 34- 70 °C) and homogenised once at 27.6 MPa.). Data is the average of results from duplicate trials.

sample	pH	conduc-tivity (mho)	Vi (mPa.s)	V_{180} @ 45 °C (mPa.s.)	initial TV (A_{800})	Aggreg-ation index
Part A Batch 30						
One step process						
30. control	6.72	450	28.0	G	0.073	9
30 SSL	6.55	500	30.9	90	0.055	21
30 MDG	6.72	450	31.0	220	0.065	12
Two step process						
30. control	6.71	450	20.5	G	0.180	5
30. SSL	6.58	500	26.1	ser; 130	0.155	7
30. MDG	6.71	450	24.5	ser;149	0.155	8
Part B; Other 1 step batches						
33. control	6.70		33.0	240	0.063	17
33. SSL	6.64		34.3	125	0.041	17
33. MDG	6.70		34.3	211	0.051	16
34. control	6.70		31.4	VT	0.072	11
34 SSL	6.60		32.5	93	0.048	12
34 MDG	6.70		33.7	235	0.062	13
32. control	6.85		29.0	G	0.074	10
32. 0.27 % SSL	6.72		32.0	G	0.049	14
32 0.53 % SSL	6.65		33.0	OK; 86	0.043	19

(Vi) , initial turbidity value V180 is the viscosity after incubation of 180 day sample for 28 days.; G; gel ser ; evidence of serum separation, VT; very thick/lumpy >330 mPa.a. TV; initial turbidity value
AI is the aggregation index which is the % difference between turbidity measured in water and BQA solution.

Incubation data and stability.

The presence or absence of citrate effected the stability of LMWS containing cream liqueurs. In general, there was a synergistic stabilizing effect between citrate and LMWS; however LMWS destabilized products in the absence of citrate:

Omission of citrate Addition of LMWS, to non-citrate formulations induced gelation in all resultant liqueurs incubated at 45 °C. This occurred even when samples were incubated from day 0 and were of normal appearance and viscosity (non-citrate SSL samples) . In addition, the gelation occurred much quicker than in non-citrate non-LMWS samples.

In a few cases, citrate was added after processing to certain susceptible non-citrate SSL samples. Sometimes this protected them against gelation although, as noted in previous experiments, the result from replicates did not always agree. Also, the results of the 45 °C incubation and the 1 hour 65 °C incubation tests (described in Experiment 4.2) were remarkably different for these non-citrate SSL samples. Samples that did not gel at 65 °C often did so at 45 °C. This was the first time this type of behaviour (a false negative in an incubated test) was seen, and this test is obviously unsuitable for such samples.

In spite of the fact that all the previously mentioned samples were very unstable to incubation, most of the samples, including the very thickened clustered samples (e.g Fig. 4.3.11/12, previously) were still roughly the same viscosity if *unincubated* e.g. after 6 months at ambient temperature.

One batch of samples was processed (batch 31) which included a sample that contained 0.40 % of a citric acid ester of MDG (MDG-Cit). It can be calculated that this formulation contained the same amount of MDG as normal (0.35 %), albeit in a different form. Both products had approximately the same initial pH and viscosity but after 45 °C incubation for the first 28 days, the MDG-Cit containing product had a % DVI of 6 as opposed to 12 for the MDG product. More significantly, the MDG product gelled when incubated after 1 month but the MDG-Cit sample did not. A citrate analysis found that the esterified citrate in the emulsifier was not soluble (i.e. not analysed) in the continuous phase (data not shown).

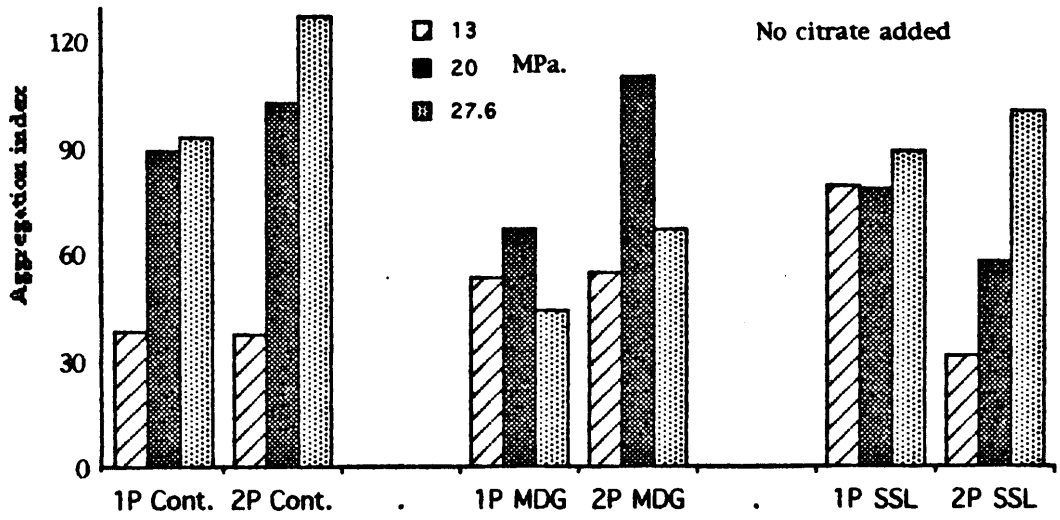


Fig. 4.3.15 Effect of 0.0 % citrate on the aggregation index of 1-S liqueurs. Samples were homogenised once (1P) or twice (2P) at different pressures (13,20, 27.6 MPa). Batch 33 samples.

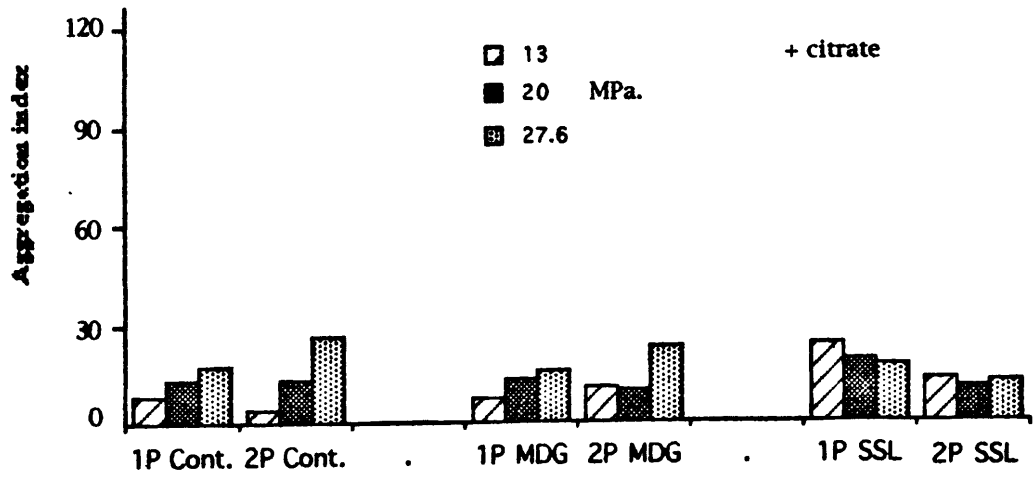


Fig. 4.3.16 Effect of 0.18 % citrate on the aggregation index of 1-S liqueurs. Samples were homogenised once (1P) or twice (2P) at different pressures (13,20, 27.6 MPa) Batch 33 samples.

Citrate containing samples The results of the incubation, at 45 °C, of 180 day 1-S and 2-S citrate-containing samples were included in Table 4.3.5. In general, the presence of SSL reduced the rate of thickening at 45 °C 2-3 fold, while MDG had only a slight effect. In batch 34, the rate of thickening decreased with increasing processing temperature for the SSL containing samples i.e. the final viscosities of the 180 day old samples after incubation at 45 °C for 28 days were ca. 190 and 100 mPa s for the 55 and 75 °C treatments respectively (holding unhomogenised samples for 2 or 20 minutes before homogenisation made little difference).

More importantly, the presence LMWS prevented the gelation of citrate containing liqueurs (Table 4.3.5, previously). This was most evident in the 1-S and 2-S samples from batch 30. All the 180 day old control samples gelled at 45 °C; control 2-S samples had even gelled after 1 month at ambient temperature. Inclusion of the LMWS prevented gelation in 1-S samples. For example in batch 34, where samples were processed at 55 or 70 °C with 2 or 20 min holding times, 4 of the 8 control samples showed full gelation or viscosities >330 mPa.s, while 3 out of 4 MDG-containing batches were stable. These stable MDG samples thickened (ca 250 mPa s) but did not gel when incubated at 180 days. The unstable samples were those processed at 55 °C with 2 mins holding before homogenisation, and this could be due to the fact that the melting point of the MDG is ca 65 °C.

None of the 0.35 % SSL containing samples which were given only 1 homogenisation pass showed gelation. However, one 0.35 % SSL sample in batch 33, when homogenised twice at 20 MPa, exhibited gelation. Surprisingly, the corresponding 1 pass sample, from which it was processed, did not gel. Both samples received the same temperature treatments (heating up and cooling down times etc.) and were of similar composition.

The effect of increasing the amount of SSL to 0.53 % to achieve an SSL/ caseinate ratio of 0.17 was examined in Batch 32. (A level of 0.27 % SSL was also used, but this sample, along with the 0 % SSL sample, gelled). All the samples had similar initial viscosities of 31-33 mPa s. The incubated viscosities of the 0.57 % sample is shown in Fig 4.3.17 and this was compared to data from other batches (30,33 and 34), which used the lower content of SSL. Increasing the level of SSL led to a large decrease in the rate of thickening, especially if measured from day 0. It should also be noted that, in all three of the 0.35 % SSL samples, the rate of thickening depended on the age of the sample when put into the incubator. The rate was approximately halved if 30 day

samples were incubated, but the rate had started to increase again at 6 when six month old samples were used (Fig. 4.3.17).

The % DVI's (28 day incubation period) of the six month samples from batch 34 were compared to the calcium sensitivities of the same samples (Fig. 4.3.18). There was a slight inverse relationship between the two variables ; for example, the SSL containing samples showed the highest calcium sensitivities, yet the lowest rate of increase in the incubator and the highest overall stabilities.

The stability results of some 1-S processed liqueurs, not directly part of this study , are briefly mentioned because of some interesting data All the samples contained citrate added beforehand and 0 or 0.35 % MDG. While the initial viscosities and TVs were similar for the 0 and 0.35 % MDG samples, in two of three different experiments, inclusion of MDG protected against gelation. Fig 4.3.19 illustrates data from one of these batches. Note it took ca 9 months before instability in the 0 % MDG sample to become obvious. In contrast, the 3 -day viscosity increase of the MDG sample decreased with age of sample. (The MD caseinate was used in these samples).

Most of the above data on the effect of LMWS in liqueurs relate to the 1-S product. Corresponding 2-S samples were only manufactured in batch 30. While they did not show gelation, LMWS containing 2-S samples exhibited some evidence of destabilizaion i.e. some serum separation. There was only limited data on thickening at 45 °C for the 2-S process (batch 30) and the amount of thickening depended on the age of the sample when incubated. When incubated from day 0 or day 30, the SSL containing sample thickened at half the rate of the MDG sample (the control gelled). However, if incubated from day 180, there were no great differences between the SSL and MDG samples ; this may have been because the samples were partially destabilised anyhow i.e. the incubated emulsions had a rough and serum type appearance.

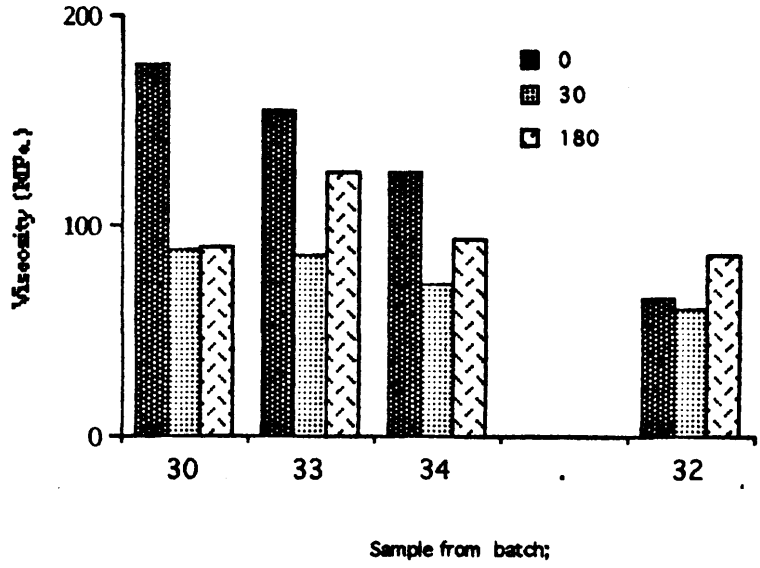


Fig. 4.3.17 The effect of different amounts of SSL on the resultant incubated viscosities of cream liqueurs. SSL concentrations ranged from 0.35% (batches 30-34) to 0.53% SSL (batch 32) and the liqueurs were incubated at 45 °C for 28 days , starting at different ages (day 0, 30, 180). Samples from batch 34, which contained 0 and 0.27 % SSL, gelled.

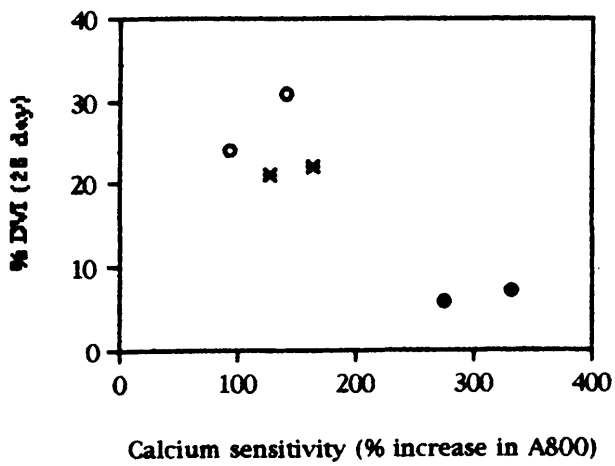


Fig. 4.3.18. The relationship between calcium sensitivities and % daily viscosity increase (%DVI) for batch 34 liqueurs o control * MDG containing , • SSL containing . Replicate results are shown.

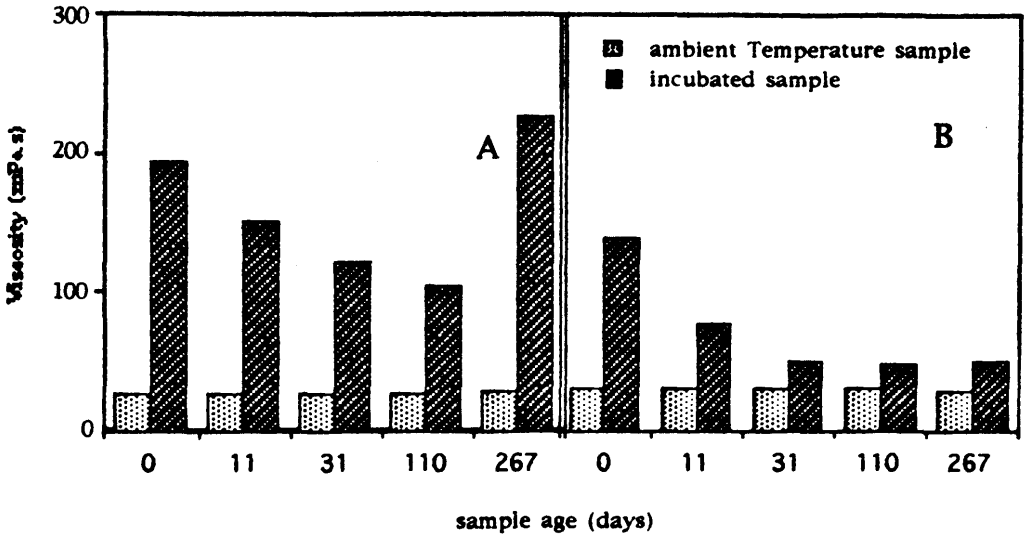


Fig. 4.3.19 The effect of 0% (A) or 0.35 % (B) added MDG on the ambient temperautre and 3 day incubated viscosities (45 °C) of citrate containg cream liqueurs. These samples, which were part of a separate study for an outside party, were processed (1-S) using 40 % fat cream and MD protein. Sample A proved unstable when incubated at day 300.

Mono-diglycerides (MDG) in butteroil based liqueurs

The clustering and subsequent gelation phenomenon that occurred on addition of MDG to non-citrate liqueurs was further investigated. We attempted to find out if this was due to some adverse interaction of the MDG with the caseinate or the serum (MSNF) portion of the liqueur. Non- citrate butteroil liqueurs were formulated with and without the MSNF portion; in this case present as buttermilk solids (1.5%). Mono-diglycerides at 0.35 % were also added to half of the samples. The results, which showed that MDG interacted adversely with the MSNF portion in non-citrate systems, are summarised in Table 4.3.6.

In the absence of the MSNF constituents (i.e. a "pure" butteroil liqueur) there were no adverse interactions in these non-citrate liqueurs ; the addition of MDG served only to slightly increase the initial and incubated viscosities. However, addition of MSNF to butter liqueurs before processing (forming in effect a reconstituted cream liqueur), especially in the presence of the MDG, led to the dramatic clustering, viscosity increases and gelation. For example, the inclusion of MSNF to a MDG containing sample increased the initial viscosity from 34.3 to 57.1 mPa s. The corresponding AIs increased from 13 to 95.

Table 4.3.6 The effect of the addition of MSNF (as buttermilk solids) and MDG to non-citrate liqueurs made with butteroil.

sample	pH	VI (mPa.s)	Stability V_{180} (mPa.s.)	initial TV (A800)	Aggreg-ation index
no MSNF					
31. control a	6.80	23.1	59	0.078	5
31. MDG	6.80	25.7	76	0.063	13
+ MSNF					
30.. control b	6.77	34.3	G	0.084	79
30 MDG	6.76	57.1	G	0.088	95

VI; initial viscosity,
(TV); initial turbidity value
Aggregation index % difference between turbidity measured in water and EQA solution).
Stability is the viscosity after incubation of 180 day sample for 28 days.;
G; gel
S; evidence of serum separation.
VT; very thick/lumpy >330 mPa.

DISCUSSION

The results of this section demonstrated that trisodium citrate (TSC) and the low molecular weight surfactants, sodium stearoly lactylate (SSL) and monodiglycerides (MDG) prevented the gelation of cream liqueurs. The effects of these additives on this and other properties e.g initial viscosity, thickening, together with some relevant work in non-alcoholic emulsions are discussed below. (Note; that, unless otherwise stated, the products produced in this experiment were homogenised with 1 pass at 65 °C using the 1-S process).

Initial properties (TV, initial viscosities etc)

In general, the inclusion of citrate in the non-LMWS stabilized alcoholic emulsions significantly decreased the average particles size in cream but not for the two batches of butteroil liqueurs. The results reported in Fig.4.3.9 suggest that citrate does not improve the surface activity of the 0.6% micellar casein present in cream liqueurs. Thus, added citrate, like alcohol (section 4.1), may enhance the surface activity (emulsification potential) of the caseinate system in cream liqueurs. Possible mechanisms for this improved emulsifying activity could be by :-

- the maintenance of caseinate in its original unaggregated colloidal state (e.g. interaction with Ca^{++} or other serum constituents. This could be termed an indirect effect.

- the direct modification of caseinate/serum protein into a more surface active emulsifier e.g. by the spreading and dissociation of colloidal aggregates.

- interaction with the cream fat globular membrane; it was noted that *unhomogenised* citrate-containing alcoholic mixes exhibited signs of emulsion breakdown (coalescence) if kept at 65 °C (data not shown). This indicated that the native cream interfacial material (milk globular membrane) was being replaced, in the presence of citrate, by caseinate even before homogenisation. This may make the caseinate a more effective emulsifier during subsequent homogenisation.

There are no comparable results for cream liqueurs reported in the literature, however, O' Connell (1986), who studied non-alcoholic caseinate/ milk-salt systems, found that addition of > 4 mM citrate before homogenisation halved the resultant average particle diameter(d_{43}) in the 18 % v/v vegetable oil/ 0.18% caseinate emulsion. The decrease in particle size was paralelled by a three-fold increase in ether extractable fat (indicating a thinner, more permeable membrane) and a three-fold decrease in viscosity (indicating less protein bridging). No results were reported for non-milk salt systems. In the cream liqueur experiments reported in this section, non-citrate liqueurs also showed large initial viscosities, presumably due to interaction/bridging of fat globules. At least part of this aggregation could be due to bridging interactions due to the occurrence of naturally present 0.6 % micellar casein for example, a MC-containing butteroil liqueur showed a higher TV and aggregation index than the corresponding caseinate-only sample.

Comparison between the aqueous system above and liqueurs should be treated with caution , not only because of the absence of alcohol, but because the casein fat ratio used by O' Connell (1986) is about 18 times less than that of a typical liqueur.

The MDG-containing samples showed the most dramatic increases in initial viscosities and AIs when citrate was omitted from formulations. Aggregation in pure monoglycerides (MG) and milk proteins stabilised *non- alcoholic* emulsions has been observed by other researchers. For example, Tomas *et al.* (1994*a*) noted that aggregation of recombined milk emulsions occurredwhen high concentrations of MG were incorporated. They considered that high levels of MG enhancedthe protein-protein interactions between the coated droplets and reduced protein-lipid interactions. Bridging flocculation could be via electrostatic interactions or calcium bonds. In this laboratory, Gaynor (personal communication), observed severe clustering of caseinate / monoglyceride stabilized non-alcoholic vegetable oil emulsions, which contained milk salts. The effect of adding extra citrate before homogenisation was not attempted.

In contrast to the MDG liqueurs, the non-citrate SSL liqueurs did not show high initial viscosities or aggregation indices, indicating that this ionic surfactant behaves differently than the non-ionic MDG. Tomas *et al.* (1994*a*) also noted similar differences in aggregation response between the ionic surfactant Tween 20 and the

non-ionic MG. The nature of the complexes formed between ionic and non-ionic surfactants and protein are obviously different ; for example, electrostatic repulsion is probably important with ionic surfactant-containing samples only.

Addition of citrate to MDG containing samples reduced the aggregation, probably due to similar effects as mentioned earlier for non-LMWS samples. There is an obvious beneficial synergistic effect where MDG and citrate are added together in these samples. The presence of surfactants, especially SSL, in citrate containing samples decreased the average particle size and this demonstrates that considerable time and energy input can be saved if emulsifiers are used to achieve a desired homogenisation efficiency. This is one of the primary functions and advantages of emulsifiers and is due to the lowering of the surface tensions of the system.

Incubated stabilities

Coalescence at 65 °C.

Citrate containing MD-caseinate stabilised liqueurs, manufactured in the previous experiment (4.2), showed extensive coalescence at 65 °C while samples without citrate present during heating never showed this phenomenon. This indicated that for MD (and possible other non-K samples) citrate is an essential component in the coalescence mechanism discussed previously (4.2). For example, in the presence of ca 17 % v/v alcohol and at high temperatures, citrate enables the (too) rapid interchange of interfacial material which results in coalescence. This could be because citrate ,under these conditions, "makes" unabsorbed protein more surface-active (causing competitive absorption) and/or citrate directly disrupts the interfacial proteins. The fact that K-caseinate liqueurs, made with citrate, did not coalesce suggested that their protein conformation/surface activity resembles those in the non-citrate MD liqueurs mentioned above.

Initial thickening at 45°C

Experiments measuring the % DVI of a butteroil liqueur and its corresponding cream liqueur indicated that most of the 45 °C induced increases in viscosity in the citrate-containing liqueurs in this experiment occurred due to the presence and nature of the caseinate.

Results with non-fat systems (FFBs) showed that rates of viscosity increase were ca 5-8 times less than for corresponding liqueurs (e.g. Fig 4.3.5 and other data not shown) indicating that the viscosity increases in liqueur systems were due to some effect of the homogenisation process on the caseinate covered fat globules and/ or free caseinate in the presence of alcohol. Donnelly (1987), using model systems, had also stated that homogenisation sensitises protein. Such results have also been found in commercial type products e.g. Pouliet *et al.*. (1990) found that increased homogeniser pressures increased aged thickening and gelation of a sterilised infant formula and the authors related this to the increased amount of interfacial protein which had more interaction (aggregation) potential.

The initial rate of thickening of non-citrate caseinate-only stabilised samples was much less than citrate-containing samples, despite the fact that the former are ultimately more unstable to gelation. This result, together with the finding that samples where citrate was added immediately after processing showed less thickening than samples with citrate present during processing, indicated that homogenisation in the presence of citrate/alcohol makes the proteins more likely to thicken (as opposed to forming a gel) when incubated. This is possib ly due to different conformations and amounts of protein present at the interfacial conformation and/or in the continuous phase. To further elucidate the relative contribution of the interface due to homogenisation in the presence of citrate or to the presence of citrate *per se*, it would be interesting to study the properties of a sample where all of the citrate had been removed after homogenisation e.g. by hydrolysis by the enzyme citrate lyase.

The inclusion of the ionic surfactant SSL and/or NaOH, in citrate containing 1-S processed samples, markedly decreased the rate of thickening at 45 °C; best results being obtained with high SSL/caseinate ratios and high pH (> 6.9). The common effect here could be the increase in the net negative charge and/or electrostatic repulsion in the protein molecules. In support of this, the inclusion of the non-ionic MDG did not have any effect on the rate of thickening.

Gelation

Gelation, including mechanisms to explain the influence of alcohol, different processing conditions and certain ingredients etc. has been discussed in the previous experiments (4.1, 4.2). Here further points relating to this defect in the presence of additives are included:-

In this experiment, the processing of 8 of 9 non-LMWS formulations without citrate led to gelation. This result, together with the fact that butteroil liqueurs made without citrate were stable, indicated that components in the serum solids (1.5 % m/m of the cream liqueur) contributed to gelation. Similar results were originally reported by Banks *et al.* (1981*a,b*), who assumed that as citrate is a known calcium ion chelator it prevents gelation by chelation of free calcium ion. However, no definitive evidence or mechanism was put forward to prove this. The following evidence indic ates the involvement of other, potentially more important, factors in the gelation of liqueurs:-

- on many occas ions in this study, it was observed that recommended level of TSC (e.g. 7 mM) , which should have chelated Ca^{++}, did not protect a significant amount of cream liqueurs from gelation .

- 0.6 % micellar casein caused gelation in a butteroil liqueur with no milk salts present.

- Dickinson *et al.* (1989*e*) found that addition of 0-10 mM Ca^{++} to MDG-containing emulsions containing 6 mM citrate, did not effect stability . Unfortunately, no data was reported for non-LMWS samples.

Hence both micellar / aggregated casein, as well as Ca^{++}, could have roles to play as agents that promote the massive aggregation that occurs during gelation. This dual involvement could help explain the complex and unpredictable nature of gelation. The effects of micellar casein (MC), which was briefly measured here, or any other separate component of the serum in cream liqueurs have not been previously studied. The role of other components e.g whey protein, milk salts, phospholipids, enzymes, caramel etc should be examined. After isolation, these fractions /compounds could be used in highly controlled experiments which measure the

effects of all these individual components either used individually or in combinations. The butter fat phase should not be considered to be an inert or non-functional constituent. In addition to causing conformational changes in the proteins at the interface, variations in triglyceride composition (due to diet etc) and in the natural levels of mono/diglycerides in the butterfat of the cream (due to lipolysis) may influence interfacial events.

Addition of SSL or MDG to 1-S liqueurs, processed without citrate, led to massive gelation on incubation at 45 C. This was hardly surprising in the case of the MDG samples, since they were very thick and clustered when fresh. However, the non-citrate SSL samples also showed rapid gelation, despite the fact that citrate and non citrate containing SSL samples had very low TV and similar viscosities. Addition of the HMP, an "emulsifying" salt that is known to interact with proteins (Vujicic & deMan, 1968; Vujicic *et al.*., 1968) and prevent gelation in micellar systems (see later), showed similar effects to SSL non-citrate system. It may be considered that these samples can show clustering aggregation and gelation even when no first-phase reactions (see discussion exp. 4.2) take place e.g samples manufactured without citrate (especially those also containing MDG). These samples will show gelation if incubated when fresh i.e they show large scale second phase reactions. This has the same net effect i.e. the formation of a gel-type network. First phase reactions may of course still occur.

However addition of citrate and LMWS to samples led to increased stability towards gelation i.e. there appeared to be a synergistic effect between these additives. (An increase in pH of citrate samples with NaOH also conferred extra stability, although this was not studied as extensively as the effects of LMWS). The stabilizing effects of anionic emulsifiers has not been previously reported in citrate (or non-citrate) cream liqueurs . However Banks *et al.* (1983), reported complex interactions between monoglycerides and citrate in from 2-S processed samples, where citrate was added after processing see Table 4.3.7. The method of addition and sequence of processing was critical, although no explanations or further studies on this phenomenon were reported. The authors also noted that one unstable sample showed massive oiling off in the incubator.

The results from one experiment in the current study (data not illustrated) where MDG-cit replaced MDG and citrate, indicated that the beneficial effect of citrate with

Table 4.3.7 Effect of the addition of 0.2 % emulsifiers (in final product) on the shelf-life of cream liqueurs manufactured by the 2-S process Citrate was added after processing to half of the samples. Data from Banks *et al* 1983.

	Shelf-life at 45 °C (days) using different emulsifiers		
Process used	no ne	GMS	GMO
A	8	8	8
A + citrate (10mM)	31	<1	<1
B	8	15	15
B + citrate (10mM)	31	75	66

GMS; glycerol monostearate; GMO; glycerol monoleate.; none; no emulsifier

Process A ; emulsifier was added at 80 °C to cream/caseinate/ sugar; the mixture was homogenised at 80 °C at 6.9 and then at 20.7 MPa.

Process B; Cream base homogenised at 80°C/6.9 MPa ; emulsifier was added and the mixture rehomogenised at 80 °C/20.8 MPa.

LMWS containing samples can be expressed either if the citrate ion is free or if it is bound. However, more research would be needed to establish if TSC and MDG could be replaced in all cases by the citric acid ester of monodiglyceride. Banks *et al.* (1983) had previously used this emulsifier at much higher addition levels and claimed that it gave a greasy taste to the liqueur.

Emulsifiers could prevent gelation in liqueurs by a mechanism whereby their presence at the o/w interface and/or their interaction with free proteins inhibits the interaction of any caseinate/casein covered droplets with serum components in forming a water-holding gel. If there are LMWS at the interface, a reactive part of the caseinate binds to the hydrophilic part of the LMWS. The more hydrophobic and non reactive sites of the caseinate are now exposed and these do not tend to form networks that will immobilize water. The presence of citrate is necessary for the above to happen. McClements *et al.* (1993) who studied the effects of LMWS on the gelation of whey protein stabilised emulsions also considered that non-ionic and especially ionic surfactants disrupted protein-protein interactions and gel network formation. The anionic surfactant, SDS, led to especially weak protein networks and this corresponds to the low thickening found with the anionic SSL in incubated storage tests in cream liqueurs in this project.

additives in other dairy products

Additives are commonly used to prevent age thickening/gelation in other long-life dairy products e.g. Buchheim *et al.* (1986) examined commercial UHT-treated creams (10-12 % fat) from 7 manufacturers and found that phosphates and citrates were commonly used and Alvarez de Felipe (1991), who studied commercial sweetened condensed milks, reported that one sample contained an (unnamed) additive. Whilst most of these systems are quite different than cream liqueurs (high levels of micellar casein, milk salts, high temperature treatment etc) it is useful to outline some results:

Amongst the earliest research data is that of Leviton *et al.* (1962), who demonstrated that sodium tetrapolyphosphate prevented age thickening of sweetened condensed milk and that the amount of additive was critical. For example, samples containing 0.03% salt (as a percentage of the liquid milk used) increased in viscosity from 2160 to 3000 mPa s. after 90 days at 27 °C as compared to a corresponding increase of

2000 to 13,200 mPa s for the control. Addition of 0.6 % salt promoted age thickening i.e. an increase from 2360 to 37,400 mPa.s.

Later, Wilson *et al.* (1963) and Leviton *et al.* (1963) showed that added polyphosphate protected against gelation in UHT sterilised concentrated whole milks. Addition of orthophosphates had the opposite effect. Snoeren *et al.*. (1979) also added sodium hexametaphosphate (0.05 %) and found a positive effect in delaying gelation but only in good quality milk. Carroll *et al.*. (1971) demonstrated the effectiveness of adding another polyphosphate (sodium tetraphosphate) to delay gelation in concentrated *skim* milk systems. However, Muir & Sweetsur (1992b), found that substitution of hexametaphosphate for disodium phosphate had a negative effect on the extent of age thickening at 45 °C conventionally-sterilised concentrated milk of high (39 %) solids. These samples also contained 0.3 % lecithin and trisodium citrate.

Kocak & Zadow (1985*b*) reported on the effects of various salts on the storage stability of UHT milk (direct steam injection, 140 °C for 4 sec). The salts were added to the milk before processing, as opposed to after processing as utilised by Snoeren *et al.*. (1979). Addition of sodium hexametaphosphate (SHMP) at a concentration of 0.05 or 0.1 % m/m resulted in at least a 6 fold extension of the shelf life. The addition of sodium citrate or EDTA, to this batch of cold stored raw milk, accelerated the onset of gelation. In a later study, Kocak & Zadow (1985*f*) demonstrated a considerable variation in the ability of various different SHMPs in the control of gelation of milk. McKenna & Singh (1990) demonstrated that the addition of sodium hexametaphosphate delayed the onset of gelation in various 20 m/m reconstituted concentrated *skim* milks (140 °C x 4 sec direct) .

Added phospholipids (purified or crude) have also stabilised against gelation (Leviton & Pallansch, 1962). This was especially obvious in modified concentrated milks containing a lowered (ca by 50 %) protein : fat ratio. Hardy *et al.*. (1985) added soya bean lecithin to conventional full cream evaporated milk (31 % and 39 % solids) and found an improvement in the age thickening characteristics of the products . Later, Muir & Sweetsur (1992*b*), working with similar systems, reported that there were differences in the efficacy between different commercial types of lecithin.

CONCLUSIONS

Citrate is an essential component in the production of 1-S processed liqueurs, although there were wide variations in the requirement for added citrate i.e for samples that "tend" toward stability, citrate levels as low as 0.09 % were adequate to prevent gelation while in other cases 0.18% did not protect against this defect. These results reinforce the view that variations in cream (see Experiment 3.3), as well as possibly in caseinate (see Experiment 4.2), were important in gelation of cream liqueurs produced in this study.

There appeared no differences, in terms of gelation (at ambient and incubated temperatures) whether citrate was added before or just after homogenisation. Such was not the case with the rate of thickening at 45°C, where addition before homogenisation enhanced viscosity increase, thus indicating different mechanisms of action of these two phenomena. Addition of citrate before or after homogenisation to the two 2-S samples had no effect on gelation at 45°C, confirming earlier results (in experiment 4.2) that this method of manufacture is very unsuitable, at least under the processing conditions operated here.

Addition of either of two dissimilar LMWS, in the absence of citrate, did not prevent and often enhanced gelation and thickening at 45 °C. In contrast, addition of these surfactants in the presence of citrate both enhanced stabilityto ambient temperature and 45 °C gelation and reduced average particle size.

Experiments with butteroil liqueurs showed that constituents other than Ca^{++} may be important in the instability of cream liqueurs e.g in one case micellar casein was shown to cause gelation. Other possible constituents that need to be evaluated are whey proteins, other milk salts and enzymes.

5. *Overall Conclusions.*

Cream liqueurs were manufactured successfully on pilot-plant equipment and the resultant liqueurs were similar to commercial products. Liqueurs exhibiting different characteristics were achieved by varying the method of manufacture, the processing conditions and the amounts of ingredients.

Routine dairy-chemistry test methods (e.g. protein, fat, total solids etc.) were used , and sometimes adapted, to characterise the above cream liqueurs. Some non-routine test methods were assessed for suitability and the results showed that an enzymatic test quantified citrate and a polarimetric method measured sucrose.

Phase contrast microscopy and turbidimetry (using a laboratory spectrophotometer) , proved complementary methods for characterising and quantifying the fresh emulsion. Simple turbidimetric indices, which can indicate homogenisation efficiency, can be calibrated against more sophisticated instruments to measure average particle diameter.

Examination of the two major commercial cream liqueur brands indicated that were produced using the one-step (1-S) procedure (alcohol added before manufacture); thus most of the subsequent work in the project concentrated on this method of manufacture. The 2-S manufactured liqueurs (alcohol added after homogenisation), produced under the same conditions as the 1-S liqueurs, were more prone to creaming. They also had a lower viscosity but despite this often tasted as "creamy" as 1-S products.

Gelation, either at ambient temperatures or during incubated storage was the most important and significant defect of some pilot plant cream liqueurs. It was never observed in commercial products. There was no definitive accelerated test that could, in all cases, predict gelation. The best procedure was to incubate product held at ambient temperature at 45 °C and to note progressively larger viscosity increases or gelation. For fresh products, especially those that contained LMWS, there was no correlation between the initial rate of viscosity increase or calcium sensitivity and gelation.

The following are some factors which effect gelation; these are based on the results of this study and may relate to some degree to the conditions used (e.g. homogeniser type, time/temperature treatments, ingredients etc.):

process type products manufactured by the 1-S process (alcohol added before homogenisation) were more stable than those manufactured by the 2-S process (alcohol added after homogenisation)

additives addition of low molecular weight surfactants (LMWS), in the presence of trisodium citrate (TSC, prevented gelation. LMWS should not be added without any TSC.

Adjustment of the pH to ca 7, with NaOH, is also beneficial in the presence of citrate.

temperature when using LMWS, a temperature greater than their melting point should be used. In general use of higher temperatures e.g. ca 65 °C for 1-S products and ca 75-80 °C for 2-S products is beneficial.

homogenisation products manufactured with 2 passes through a single stage homogeniser (corrugated valve) may be more unstable to gelation than one pass products.

raw materials the facts that, to date, butteroil liqueurs have never been observed to gel and there is considerable variation in gelation between batches, it is likely that factors in the cream influence gelation. Certain caseinates may also be more prone to producing liqueurs that gel.

There may be more than one mechanism of gelation in liqueurs but the following points are important;

Interfacial protein is more sensitive to gelation/thickening than unabsorbed protein. The degree/type of aggregation and physico-chemical modification of the proteins at the interface (e.g. by interaction with LMWS) are important. Variable coalescence behaviour of liqueurs at 65 °C, with and without added citrate, indicated that conformations at the interface can be different.

Micellar or aggregated proteins are important in bridging between globules; this is caused by a serum component of the liqueurs, which is disrupted by citrate. Interaction of this component(s) with LMWS, without citrate present, causes increased interactions when liqueur is incubated at 45 °C.

For citrate containing liqueurs (manufactured without LMWS), a change in the properties of the proteins, as indicated by an increase in calcium and/or ethanol sensitivities, indicated gelation would take place. However whether these changes were physico-chemical and/or enzymatic was not established. A net negative charge is important is decreasing interactions leading to gelation and thickening at 45 °C in the above system. This can be achieved by increasing the pH of the system with NaOH.

The use of LMWS, in the presence of citrate, decreased gel promoting protein- protein interactions, and in the case of SSL samples, the tendency to increase in viscosity at 45 °C. The omission of citrate from LMWS containing samples promoted aggregation/gel formation, especially in non-ionic emulsifier containing samples.

Appendices

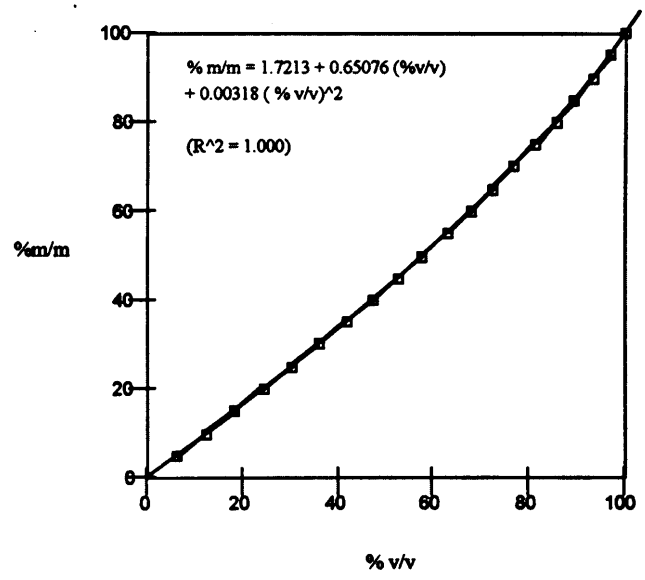
Appendix 1 List of some synthetic food emulsifiers and their legal and regulatory status

Chemical name	Abbre-viation	ADI value (mg/kg weight)	Regulation Status	EEC no.
Mono-diglycerides	MDG	Not limited	GRAS	E 471
Acetic acid esters of mono-diglycerides	AMDG	Not limited	GMP	E 472a
Lactic acid esters of mono-diglycerides	LMDG	Not limited	GMP	E 472b
Citric acid esters of mono-diglycerides	CMDG	Not limited	-	E 472c
Diacetyl tartaric acid esters of mono-diglycerides	DATEM	0-50	GRAS	E 472e
Salts of fatty acids	-	Not limited	-	E 470
Polyglycerol esters of fatty acids	PGE	0-25	GMP	E 475
Propolyene glycol esters of fatty acids	PGMS	0-25	GMP	E 477
Sodium stearoyl lactylate	SSL	0-20	Regulated	E 481
Calcium stearoyl lactylate	CSL	0-20	Regulated	E 482
Sucrose esters of fatty acids	-	?	?	E 473
Sorbitan monostearate	SMS	0-25	Regulated	491
Polysorbate 60	PS 60	0-25	Regulated	435
Polysorbate 65	PS 65	0-25	Regulated	436
Polysorbate 80	PS 80	0-25	Regulated	433

ADI ; is the acceptable daily intake ?; data not known

Regulation status; GRAS- generally regarded as safe , GMP- good manufacturing practise.

Appendix 2 The relationship between the % mass , volume and density of ethanol solutions at 20 C.; % m/m and % v/v data is displayed in the graph below. . An equation relating % m/m % v/v is also included.



The physical properties of pure anhydrous ethanol measured at 20 C.

Property	Units	Value
Molecular mass	-	46.07
Density	kg/l (vacuo)	0.7894
Boiling point	° C	78.3
Vapour pressure	mbar	58.1
Flash point	° C	12
Dielectric constant	-	25.7
Refractive index	n_D	1.3614
Viscosity	mPa.s.	1.22

Appendix 3A Details on creams used for products in Experiment 3.3 of the project; for details of the resultant products see Table 3.3.1 Note cream liqueurs manufactured from the creams below retained the same batch number as the first part of their code e.g. liqueur 7a (1-S) was one of two duplicate products manufactured by the 1-S process and with batch 7 cream..

Batch no.	Cream Supplier no. or source *	Delivery date	pH	Comments
1	Imokilly	28/5/91	-	frozen cream.
2	University supply	20/10/91	-	no further details of source.
3	6,17,72 in ratio 40:30:30	5/8/92	6.65	suppliers 6,17 and 72 had Spring calving herds
4	6,72 in ratio 70:30	11/8/92	6.61	"
5	6,17 in ratio 70:30	25/8/92	6.64	"
6	6,17 in ratio 70:30	2/9/92	6.65	"
7	304	20/10/92	6.61	supplier 304 had a liquid milk herd
8	Dawn Dairies, Ballinahina	21/10/93	6.71	
9	350	10/11/92	6.67	supplier 350 had a liquid milk herd
10	6	9/12/92 and 12/12/92	6.73	late lactation cream
			5.51	sour late lactation cream
11	Dawn Dairies, Ballinahina	9/12/92	5.14	sour cream from a commercial dairy
12	304	26/1/93	6.66	student projects

Appendix 3B Details on creams used for products in Experiments 4.1-4.3 of the project; for details of the resultant products see experimental sections.. Note cream liqueurs manufactured from the creams below retained the same batch number in the first part of their code e.g. liqueur 15.K1 a was one of two duplicate products manufactured with K1 caseinate and batch 15 cream.

15	Dawn Dairies, Ballinahina	27/3/93	6.63	see exp. 4.2
16	Dawn Dairies, Ballinahina	17/7/93	6.65	see exp. 4.2
17	Dawn Dairies, Ballinahina	15/5/93	6.64	see exp. 4.2
18	6	20/7/93	6.63	see exp. 4.2.
19	Dawn Dairies, Ballinahina	17/4/93	6.63	see exp. 4.1, 4.3.
20	Dawn Dairies, Ballinahina	1/5/93	6.64	see 4.1, 4.3
21	Dawn Dairies, Ballinahina	22/5/93	6.66	see 4.1, 4.3
22	350	9/3/94	6.65	see 4.3
23	350	16/3/94	6.65	see 4.3
24	6	17/8/93	6.61	see 4.3.
25	butteroil (Dairy gold)	18/5/94	-	see 4.3
26/27	350	10/11/94	-	4.1
28/29	Commercial dairy X	30/9/92 to 12/3/93	6.65-6.75	note ; these creams were used to make liqueurs for an outside party in a separate project .
30	6	31/8/93	6.61	see 4.3
31	350	23/3/94	6.65	see exp. 4.3.
32	Dawn Dairies, Ballinahina	16/10/93	6.71	see exp. 4.3
33	6/17 in 50:50 ratio	14/9/93	6.62	see 4.3
34	Dawn Dairies, Ballinahina	2/10/93	6.66	see exp. 4.3

APPENDIX 4 METHOD OF PREPARATION OF CREAM LIQUEUR FOR CITRATE ANALYSIS BY ENZYMATIC METHOD.

See Kit literature for detailed information (Boehringer Mannheim, 1992).

1. Weigh approx 5 g (a) of a well mixed sample into a glass stoppered test-tube.
2. Add 25 ml (b) of 1M perchloric acid and shake the tube vigorously
3. The mixture is allowed to settle and if then filtered into a 50 ml conical flask
4. 10 ml of supernatant (d) was pipetted into another container and the pH was adjusted to pH 8-10 using ca 1.95 mls of 5 M KOH (e)
5. This solution was placed in the refrigerator for 15 min after which the solution was refiltered (discarding the first few mls)
6. The concentration of the sample, C_S , in the supernatant , in g/litre, was ;

$$\frac{a * d * 100.}{(b + s) * (d + e)}$$

A portion of this solution (the diluted extracted liqueur) was used for citrate analysis (see details with the kit) by comparison to a trisodium citrate standard of ca 0.615 g/litre. This result and the C_S figure (above), were used to calculate the citrate content of the undiluted liqueur (g/kg) .

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