ULTRAFILTERED AND DIAFILTERED SKIM MILK RETENTATES IN YOGURT-MAKING: COMPOSITION, PHYSICAL PROPERTIES AND SENSORY EVALUATION.

by

Muhammad Mahabub A. Khan

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MUHAMMAD MAHABUB A. KHAN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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DEDICATION

To

My Parents,

Bonnie, Samira & Najiyah

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ABSTRACT

Pasteurized skim milk for yogurt making was studied for its physical and chemical properties after reconstitution (control and MSNF), ultrafiltration (UF) and diafiltration (DF) with concentration brought to 12, 14 and 16% total solids. The flux rate decreased with increase in total solids or protein in the ultrafiltered or diafiltered retentates. The composition of permeate was not affected markedly during concentration of skim milk. Protein was increased from 3.25 to 13.91 by the UF method and from 3.12 to 15.4% in the DF retentates. Lactose was reduced from 4.90 to 4.24% in the UF and from 1.52 to 0.75% in the DF. Ash was increased from 0.54 to 1.11% and also from 0.25 to 1.08% in the retentates of UF and DF, respectively. The mineral composition, especially calcium and phosphorus contents, was influenced considerably by both methods of UF and DF. No major differences were observed for magnesium in any type of milk sample. Buffering capacity of milks rose exponentially with increasing solids level. Skim milk retentates concentrated to 16% displayed the highest buffering properties. The requirement for HCL and time to attain pH 4.4 was greater for both UF/DF retentates than those for control (SM) or MSNF. Within the shear rate range of 116-924 sec^{-1} most samples exhibited Newtonian fluid properties. However, a non-Newtonian flow (shear thining) behavior was observed with retentates of higher solids level (16%). The rheological properties of coagulated milk gels were also studied by small amplitude shear stress testing. Using this technique, the dynamic viscosity and viscoelastic properties

of milk gels produced by a mixed starter culture of S. thermophilus and L. bulgaricus (1:1) were assessed without affecting the coagulation process. Increased amounts of starter culture caused faster onset of gelation during acid-coagulation of milks as determined by their rigidity modulus (G*) values. The transition from sol ----> gel during fermentation was accompanied by a sudden decrease in phase angle (δ). The parameter was very sensitive to alterations in the viscous and elastic character of the gel network during fermentation. Kinetic analysis of the rheological data indicated that the process can be described by a first-order reaction model. Gel structure development was a multistage process as reflected by subtle changes in δ- time profiles. Higher amounts of acetaldehyde were found in UF yogurts. Decreases in acetaldehyde content during storage were more pronounced in SM milk yogurt than those of yogurts made from UF retentates at any solids level. A simple isocratic high performance liquid chromatographic (HPLC) technique was adapted for estimation of organic acids (orotic, citric, pyruvic, lactic and propionic acids) during fermentation and storage (4^0 C, 1-14 days) of yogurts. Overall, consumption of orotic and citric, and production of pyruvic, lactic, acetic and propionic acids were observed during fermentation. Very little changes in organic acid composition were observed during storage for both SM and UF yogurts except for acetic acid which increased. Citric acid also seemed to be completely consumed in both control and UF yogurt samples (16%) after 14 days. Perceived sensory attributes of yogurts (control and UF) at different solids level (12, 14 and 16%) were evaluated using quantitative descriptive analysis (QDA) during a 2 week storage period. Yogurts from UF milk had higher scores in thickness and chalkiness than SM samples of equiva-

lent solids level. Overall, the yogurt samples made from UF retentates (12% solids) were the most acceptable (scored in the middle range of the rating scale) in terms of thickness, chalkiness and sourness characteristics.

Chapter I

INTRODUCTION

The term "yogurt", as known in many countries, has been derived from the Turkish word 'jugurt'. The manufacture of fermented milk products, especially yogurts, from ultrafiltered milk has been growing rapidly with the attendant advantages of undenatured protein-rich products and greater overall economy. The projection Figures quoted for 1990 in yogurt consumption for UK alone amount 3.7 kg/head/year, while for Finland the reported Figure for fermented milk products reached 39 kg/head/year (Marshall, 1983). The increase in consumption of yogurt is also evident from the International Statistics which indicate a steady increase in total sales of yogurt world-wide (Rasic and Kurmann, 1978). Although the popularity of yogurt is increasing throughout the world, including Canada and the United States, very few studies on this product have been conducted in these countries, particularly for yogurt made with ultrafiltered skim milk.

Ultrafiltration has been widely used in the production of whey protein concentrates with varying protein/lactose ratios in Australia, New Zealand and some European countries. As a result of a growing demand for the consumption of skim milk yogurt, the dairy industry has been interested in using milk concentration methods in order to increase total solids in skim milk. Ultrafiltration appears as an alternative approach to strengthen yogurt coagulum and prevent syneresis which is

usually observed in yogurts made from skim milk fortified with non-fat milk solids (MSNF). It has been reported that milk concentrated by ultrafiltration to 18-20% total solids level produces a good quality yogurt (smooth, creamy and with typical acid flavor) that does not require homogenization (Chapman et al., 1974; Tamime and Deeth, 1980). Skim milk could, therefore, be concentrated to an optimum level and yet provide a product of good texture following ultrafiltration (Marshall 1983). In addition, a reduction in skim milk volume results not only in savings in refrigeration and transport costs but also in processing costs at the factory of the dairy processor. The problem of efficient collection, distribution and constant processing of large quantities of cooled fresh milk to finished products can be minimized by employing ultrafiltration processes. The process of skim milk concentration by ultrafiltration procedures, however, results in milk products e.g. cream cheese with high viscosity and texture (Covacevich and Kosikowski, 1977a). Thus it is important to understand all aspects of membrane processing of skim milk and the physico-chemical properties of membrane retentates during fermentation and storage at refrigeration temperatures.

The production and maintenance of yogurt products with optimum consistency and stability is one of the major concerns facing the yogurt manufacturing industry today. Factors which influence yogurt consistency include milk composition (proteins and salts), acidity, homogenization, type of culture, pH and heat treatment of milk (Heertje et al., 1985; Rasic and Kurmann, 1978). These factors also affect the final physical properties of the product, such as body, texture or firmness

(Flinger et al., 1988). The total solids level is essential for quality yogurt of adequate firmness without syneresis. The solids content in milk is commonly increased by addition of dried skim-milk solids or by vacuum evaporation which would require expensive and complex equipment. Membrane technology is another viable alternative procedure that has been developed for concentration of milk using various ultrafiltration and reverse osmosis systems.

Several researchers have claimed a superior quality of cow's milk yogurt made from ultrafiltered retentates (Abrahamsen and Holmen, 1981). It is, therefore, of interest to compare the characteristics of yogurt made from milk concentrated by various methods by determining and comparing the effect of processing and characterization of processed milk samples. It is also important to study the changes in chemical and physical properties of processed milk during fermentation with a mixed starter culture of Streptococcus thermophilus and Lactobacillus bulgaricus (1:1). The changes in chemical and sensory attributes of yogurt during storage is another important aspect to be considered from a manufacturer's viewpoint.

With these in mind, the objectives of this investigation were:

1) To produce concentrated milk samples (between 12-16% T.S.) using four different concentration methods (addition of non-fat dry milk powder into skim milk, ultrafiltration, diafiltration and reconstituted non-fat milk solids (MSNF)) and evaluate the chemical and physical properties of these materials.

2) Examine the effectiveness of a mixed starter culture of S. thermophilus and L. bulgaricus (1:1) to coagulate concentrated milk samples under various conditions (temperature, culture level) as probed by dynamic rheological testing and chemical analysis of the fermented products (pH / acidity, organic acids).

3) Follow the changes in pH, acetaldehyde content, organic acid profile and sensory attributes of the yogurt samples during storage at 4^0 C for two weeks.

Chapter II

REVIEW OF LITERATURE

2.1 Fundamentals of Chemistry and Physical Properties of Milk

2.1.1 Composition of Milk & Methods of Determination

Milk is defined as the lacteal secretion of cows which consists of water, lipids, protein, carbohydrates, salts and miscellaneous constituents of as many as 105 different molecular species. It contains not less than 8.25% of non-fat milk solids and not less than 3.25% milk fat (Jenness, 1988).

The fat in milk is composed of about 98% triglycerides, while the remainder consists of smaller amounts of free fatty acids, mono-and diglycerides, phospholipids, sterols and hydrocarbons. Milk fat can be analyzed by the Roese-Gottlieb extraction procedure and gravimetric determination of fat (Walstra and Mulder, 1964), by volumetric methods such as that of Babcock and Gerber (Ling, 1956; Horwitz, 1980) or by near infrared spectroscopy (3.4 or 5.7 μm; Goulden, 1964 and Horwitz, 1980).

The primary constituent of carbohydrates in bovine milk is the disaccharide lactose: 4-O-β-D-galactopyranosyl-D-glucopyranose. Several methods have been reported for quantitation of lactose in milk. Among the most commonly used methods are an enzymatic assay involving β-galactosidase and galactose dehydrogenase (Kurz and Wallenfels, 1974), chro-

matographic analysis (Reineccius et al., 1970; Beebe and Gilpin, 1983; Brons and Olieman, 1983), oxidation of the aldehyde function of the glucose residue (Horwitz, 1980) and infrared spectroscopy (9.6 μm; Goulden, 1964 and Horwitz, 1980).

Cows' milk consists of approximately 3.5% protein which is divided into casein and whey protein fractions. The proteins fall into several groups of polypeptide chains. The primary group occurs in the form of 'caseins' which consist of αs_1 -, αs_2 -, β-, and κ-casein with substantial genetic variation and post translational modification. It has been reported that the isoelectric points for all these sub-units range from about 4.9 to 6.0 and their molecular weights range from about 11,500 to 24,000. Each of these four polypeptide casein fractions possess a unique primary structure and thereby distinct physico-chemical properties. Almost all of the caseins are associated with calcium and phosphate in micelles of 20-300 μm in diameter (Farrell, 1988). Whey proteins are the second major milk protein group which includes α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins and components of the proteose-peptone fraction (Eigel et al., 1984). According to Horwitz (1980), the protein content can be determined by Kjeldahl analysis of nitrogen (N). Several other methods, reviewed by Booy et al. (1962), include colorimetric determination of ammonia and peptide linkages by the biuret method, tyrosyl group analysis, binding of anionic dyes to cationic protein groups, and infrared spectroscopy (6.46 μm; Goulden, 1964; Horwitz, 1980).

Milk salts are reported as both cationic and anionic ionizable substances having molecular weight of 300 or less. The principal cations are

Na^+, K^+, Ca^{++} and Mg^{++} and the anionic components are phosphate, citrate, chloride, carbonate and sulfate (Jenness, 1988). According to Murthy and Rhea (1967), the method of absorption or emission of radiation at specific wavelengths can be used for quantitative analysis of minerals (especially metals such as Na^+ and K^+). Calcium and magnesium can also be analyzed by atomic absorption methods. For phosphate determination, the most common method involves reaction of phosphate with molybdate to form phosphomolybdate complex. This is then reduced to a blue compound that absorbs at various wavelengths, usually between 640 and 820 nm, and can be thus quantified colorimetrically (Meun and Smith, 1968).

2.1.2 Milk Proteins

2.1.2.1 Protein Components of the Casein Micelle

Milk is considered to be a bicolloidal system which consists of the dispersed phase (the casein-protein complex) and the dispersion medium (the milk serum). The term "micelle" has been applied to the dispersed phase of milk, the casein-protein complex.

In dealing with skim milk, the retention of the unique properties of the milk proteins during processing is of the utmost importance. For example, the stability of the casein-protein complex is the underlying causes of many problems encountered in dairy technology. The forces which hold the casein micelle together as well as the forces which result in disruption of the casein-protein complex are, therefore, of primary importance.

2.1.2.2 Properties and Native State of Milk Proteins

A number of studies on the characterization of casein have concluded that there are four major components of the casein-protein complex, namely, αs_1 -, αs_2 -, β-, and κ-casein. Quantitative analyses of these fractions indicated that the best estimate is: αs_1 - 38%, αs_2 - 10%, β-36% and k-casein 13% (Davies and Law, 1980). Several researchers have provided detailed information about the individual caseins as reviewed recently by Farrell (1988) and Whitney (1988).

It has been stated that the αs_1 -casein, the major component of milk proteins, is insoluble under the conditions of temperature, pH and ionic strength of milk. It has eight or nine phosphate groups, depending on the genetic variant.

The second most abundant milk protein is β-casein which is a linear amphiphilic molecule; the N-terminal portion of the molecule contains essentially all the charged groups, while the C-terminal consists of apolar residues. This protein contains four or five phosphate groups (as phosphoserine residue), depending on the genetic variant (Swaisgood, 1982), and is often linked with other casein micelle proteins and serum proteins of milk. Its self-association in solution is temperature dependent. Like αs_1 -casein, β-casein is soluble at room temperature in the presence of Ca^{++} , while calcium-caseinate is soluble at 1^0 C.

The αs_2 -casein in the most hydrophilic component of the caseins, and has two disulphide bonds which can interact with those of β-lactoglobulin upon severe heat treatment. This protein contains no known carbohydrate groups but has 10 to 13 phosphate groups which make the molecule

very sensitive to calcium ion concentration (Kinsella, 1984; Swaisgood, 1982). In contrast to other caseins, it is also the least susceptible to coagulation phenomena due to the alternating negatively charged and hydrophobic areas in its structure.

κ-casein, the fourth major component of the casein complex contains carbohydrate molecules and has two disulphide bonds at the N-terminal. This glycoprotein has only two phosphate groups and it is soluble over a very broad range of calcium concentration. Because of the calcium solubility, the κ-casein is thought to be responsible for the stability of casein micelle; i.e. it stabilizes the calcium-insoluble αs- and β-caseins (McMahon and Brown, 1984; Swaisgood, 1982). The N-terminal is mostly hydrophobic while the C-terminal of this molecule is hydrophilic (polar and charged). It is also the κ-casein fraction which is cleaved by rennin (chymosin) and triggers the coagulation of milk proteins in cheese manufacturing; the resulting products are termed "para-κ-casein" and "macropeptide".

2.1.2.3 Milk Serum Proteins

The milk serum phase is composed of about 20% of the milk protein that is soluble in the aqueous phase of milk. The serum or whey proteins are those which remain in solution after the micelles are removed. The major non-casein serum proteins are primarily a mixture of β-lactoglobulins, α-lactalbumin, bovine serum albumin and milk immunoglobulins (Eigel et al., 1984). As a group, these proteins are more heat sensitive and less calcium sensitive compared to caseins (Kinsella, 1984).

β-lactoglobulin is the major globular serum protein which consists of up to 50% of the non-casein protein of skim milk (Cerbulis and Farrell, 1975, 1976) and exhibits unique structural characteristics because of its amino acid sequence. About 47% of the molecule is unordered structure at the pH of fresh milk (Kinsella, 1984). Each 18,400-dalton monomer has two disulphide bridges and one free thiol groups. The latter can participate in disulfide interchange with other proteins, including κ-casein during heating. This protein has well-defined secondary, tertiary and quarternary structures which are susceptible to denaturation.

α-lactalbumin is the second major serum protein and accounts for up to 25% of the whey protein and about 4% of the total milk protein as shown in Table 1. It has a quite compact secondary structure, with a monomer weight of 14,174 and found to exhibit far more structural stability than β-lactoglobulin. The protein contains no free sulfhydryl groups and has four disulfide bonds. Recent studies also showed that α-lactalbumin is a Ca^{++} -binding protein and that upon removal of calcium the conformation of α-lactalbumin is altered substancially (Kronman et al., 1981). Similar conformational changes occur at low pH. In whey protein concentrates, both acid denaturation and calcium-binding may play an important role in the retention or loss of the functional characteristics of this protein.

Table 1. Average composition of Warm Skim Milk Protein.[a]

	Grams/100 g milk	% Total protein
Colloidal casein	2.36	74
Serum casein	0.26	8
β-Lactoglobulin	0.29	9
α-Lactalbumin	0.13	4
Bovine serum albumin	0.03	1
Total immunoglobulins	0.06	2
Other protein	0.06	2

[a] : All values normalized to 3.2 g total protein/100 g milk; Farrell and Thompson (1974).

Other serum proteins include serum albumin and immunoglobulin (IgG) which account for up to 1% and 2% of the total milk protein, respectively (Table 1). Serum albumin has 35 cystein residues which are found as 17 intrachain disulfide linkages and one free sulfhydryl group. IgG has the familiar immunoglobulin structure, with two heavy and light chains. All of these proteins possess a significant amount of native structure and are also sensitive to various types of denaturation (Brown, 1988; Farrell, 1988; Walstra and Jenness, 1984).

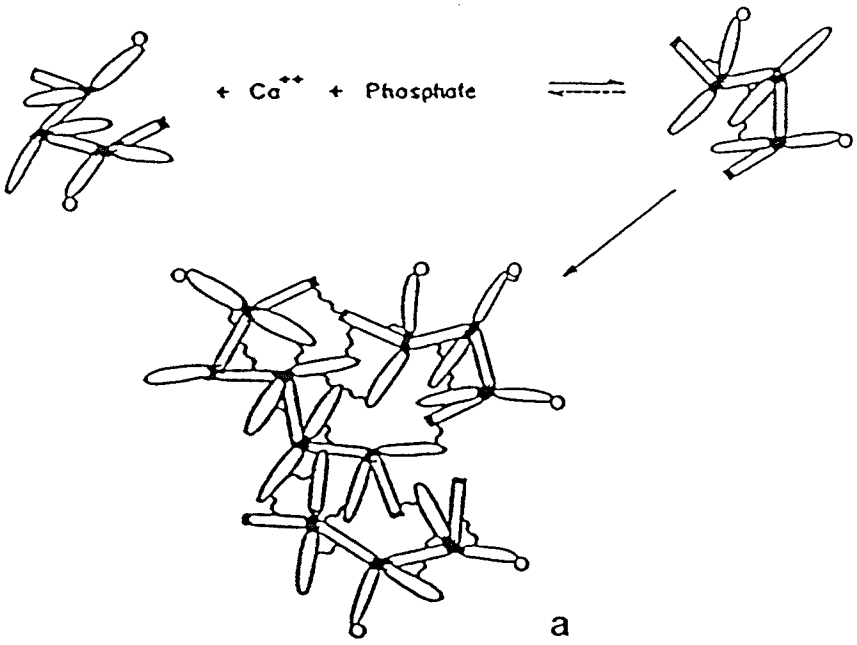
The stabilizing forces, which are responsible for the stability of the protein system in milk primarily involve hydrophobic and electrostatic interactions. Other forces such as hydrogen bond and disulfide bonds also contribute to the protein stability. The mode of action and characteristics of these forces as applied to isolated milk protein fractions or food systems (e.g milk) have been discussed in detail by Farrell (1988).

2.1.2.4 Casein Micelle Structure

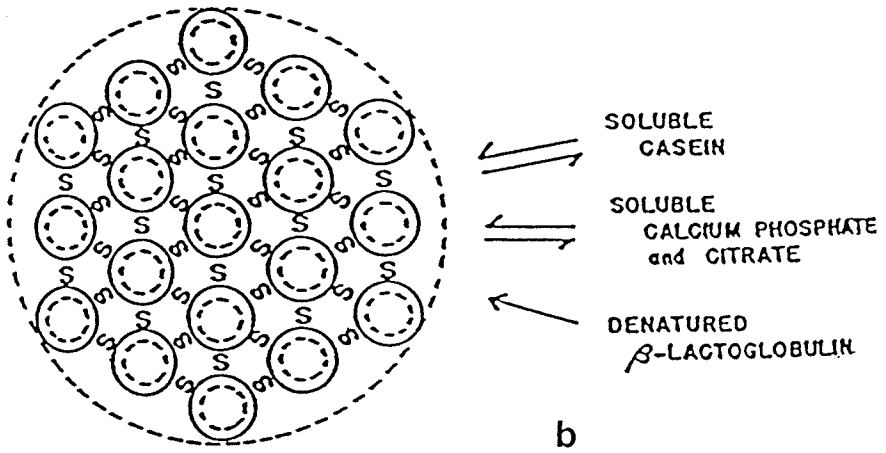
Various models have been proposed by researchers in order to develop an intimate knowledge and understanding of the association mechanism responsible for the unique structure of the casein micelle.

The formation of the casein micelle, as proposed by Rose (1969), is presented in Figure 1a. In this model, β-casein monomers self-associate into chain-like polymers to which αs_1 -monomer are attached and κ-casein interacts with the αs_1 -monomers. As the micelle is formed, Ca-phosphate

Figure 1. a) Schematic representation of the formation of a small casein micelle. The rods represent β-casein, the more eliptical rods represent αs_1 -casein, and the S-shaped lines depict apatite chain formation. The circles represent κ-casein (adapted from Rose, 1969); b) Structure of the casein micelle proposed by Morr (1967). The S-shaped lines represent calcium phosphate linkages between small spherical complexes of the αs_1 -, β-, and κ-casein.


+ Ca++ + Phosphate

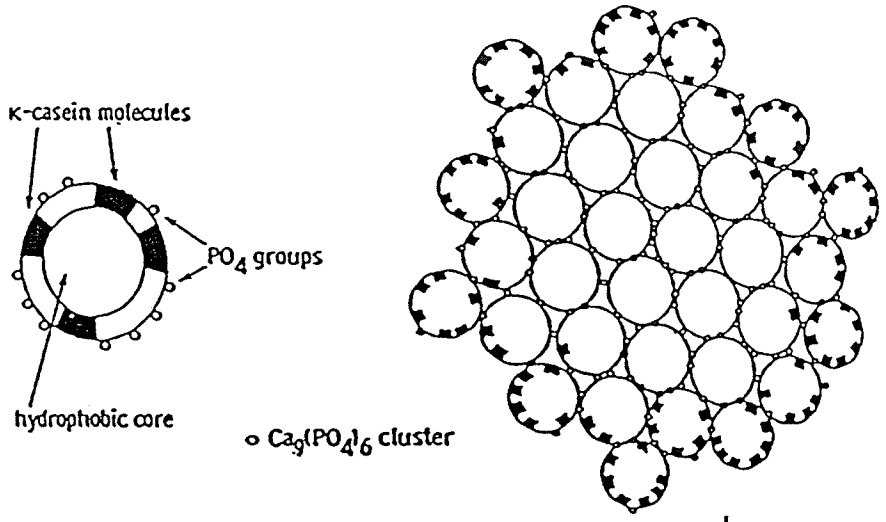
a


SOLUBLE
CASEIN
SOLUBLE
CALCIUM PHOSPHATE
and CITRATE
DENATURED
β-LACTOGLOBULIN

b

is incorporated into the network as a stabilizing agent. An alternative, submicellar structure, for the casein micelle has been suggested by Morr (1975), based on studies of disruption of casein micelles. It has been proposed that the αs_1 -, β-, κ-monomers form small uniform submicelles which are stabilized by hydrophobic bonding and calcium caseinate bridges and, in turn, are aggregated into a micellar structure by colloidal calcium phosphate (Figure 1b). According to Slattery and Evard (1973), casein monomers interact to form submicelles but their casein composition could vary. Submicelles which are rich in κ-casein are found predominantly on the surface of the casein micelle, contributing as a stabilizing force to the overall structure. This model also predicts that the submicelles which are poor in κ-casein would more likely be located internally. Finally, the submicellar model of Slattery and Evard (1973), as has been recently elaborated by Schmidt (1982), is depicted in Figure 2. From this figure, it is obvious that not all of the apolar chains would be buried because of the high incidence of apolar residues in all of the caseins and the large number of monomers present. Therefore, many hydrophobic patches probably exist on the surface of the micelle. In most cases, aggregation is prevented by the charged surface groups presented in all three casein components. κ-casein has been reported to be a highly significant component responsible for promoting stability of micelles against aggregation with its 'hairy' hydrophobic glycopeptide protrusions which give rise to entropic repulsion. The areas which contain esterified phosphate groups clearly lack of secondary order structure and have the capability to bind cations e.g. Ca^{++} (Clark and Ross-Murphy, 1987). Studies on the calcium content in skim milk have suggested that more than 90% of the calcium is

Figure 2. Casein micelle model proposed by Schmidt (1982). Schematic representation of a submicelle (a) and a casein micelle composed of submicelles (b).


κ-casein molecules
PO_4 groups
hydrophobic core
o $Ca_9(PO_4)_6$ cluster
a
b

either associated in some way with other ions or found within the casein micelles (Holt et al., 1981).

The casein micelle is a highly porous, well solvated, system that has two distinct forms of ions: an outer domain (perhaps in the form of a charged double layer) and an inner system that is not easily washed away. Several studies have suggested that complex formation between occluded salts (i.e., amorphous calcium phosphate) and casein could occur which indicates that colloidal calcium phosphate is involved in maintaining the structural integrity of the casein micelle. Colloidal calcium phosphate must be also involved in complexation with citrate ions. Although considerable progress has been made, the exact mechanism of casein stabilization and structure are not fully understood (Brown, 1988).

2.1.3 Coagulation of Milk Proteins

The milk caseinate system is unique among other protein systems because of its ability to withstand high temperatures. However, when milk is heated at 100^0 C for 20 to 30 min at a pH of less than 6.5, it coagulates to form a gel.

The term "denaturation" is also used to indicate molecular unfolding and aggregation or coagulation phenomena of milk protein systems. Dairy products, including yogurt, cheese etc., are subjected to a variety of changes in temperature, pH and concentration from milk collection to finished products which result in destabilization of the casein colloidal system. The formation of coagulum or gel is basically due to the

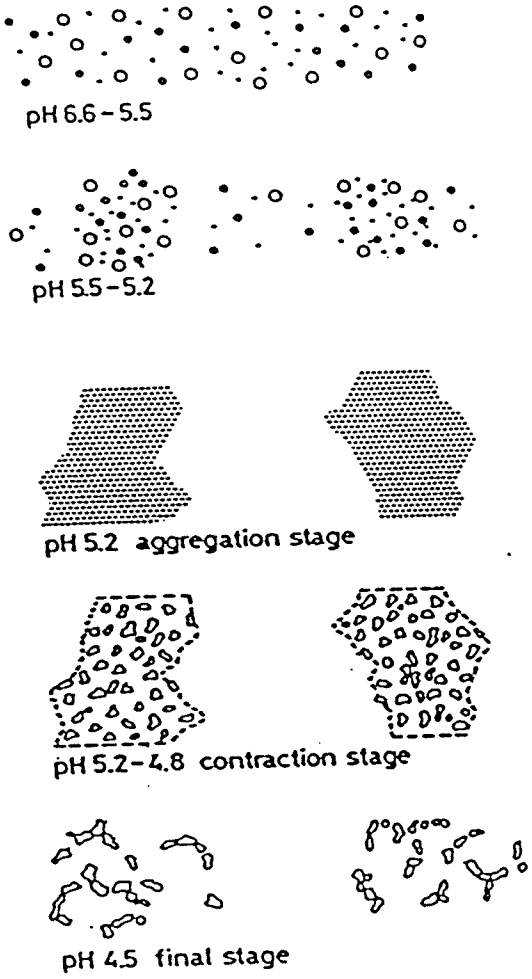
destabilization of the casein complex and is irreversible in nature. These types of gels are divided into three different groups (Tamime and Robinson 1985): gels formed by enzymes (e.g. cheese); heat induced gels and acid gels formed by adding acid in milk (e.g. yogurt). The discussion below is primarily limited to bacterial fermentation of milk for yogurt making which mainly involves coagulation of milk proteins as a result of lowering the pH during the fermentation process.

From the discussion above, it is apparent that casein particles (calcium caseinate-phosphate complex) in milk as well as inorganic calcium and phosphorus (8%) and a little magnesium and citrate exist in the form of a stable colloidal system. In fresh milk, the electrical charge carried by the casein-phosphate particles play an important role in maintaining a stable equilibrium between micelles and the liquid phase of milk. Therefore, factors which can markedly affect the stability of casein micelles are ion composition as well as changes in the hydrogen ion concentration.

2.1.4 Acid-Mediated Coagulation of Milk Proteins

Several mechanisms or methods of inducing casein solutions to aggregate or gel are known. These include the removal of the 'hairy' regions of κ-casein by proteolytic enzymes which causes micelle aggregation and gelation, as it is known in the cheese-making process. Alternatively, lowering the pH, as it occurs during production of yogurt, also results in gelation due to destabilization of the milk casein colloidal system. This method of casein aggregation in an acidic environment produced by bacterial action has been considered to be the basis of yogurt-making (Heertje et al., 1985) and is shown schematically in Figure 3.

Figure 3. Schematic drawing of the structure formation in acid-coagulated milk gel. Source: Heertje et al. (1985).


pH 6.6 – 5.5
pH 5.5 – 5.2
pH 5.2 aggregation stage
pH 5.2 – 4.8 contraction stage
pH 4.5 final stage

According to Clark and Ross-Murphy (1987), aggregation of casein under acidic conditions, produced by microbial fermentation, is a phenomenon that involves micelle association due to their decreased charge. It is important to note that the isoelectric point of casein is around pH 4.6. Low pHs also induce the dissociation of the colloidal calcium phosphate from the calcium caseinate-calcium phosphate complex, resulting in greater amounts of Ca^{2+} and $H_2PO_4^-$ ions in solution and a product with lower ash content. Acidification to isolelectric conditions, eg., pH 4.5 to 5.0, completely dissipates the colloidal calcium phosphate structure as well as releasing other inorganic ions from the casein precipitate into the aqueous phase (Pyne and McGann, 1960). Initially, lowering the pH of milk causes solubilization of some protein components from the casein structure. With the further reduction in pH several processes take place ending in reaggregation of the proteins (Heertje et al., 1985). A structure is finally build-up by protein molecules which are integrated into a coarse-stranded type of particulate network.

Protein denaturation is dependent on pH (Brown, 1988). At low pH, serum proteins denature onto micelles and join them together (Creamer and Matheson, 1980). Therefore, serum proteins when denatured do not precipitate separately in milk but rather coprecipitate with caseins upon acidification (Brown, 1988). In yogurt manufacture, it is also important to note that serum protein stability decreases significantly in the acidic zone (pH 4.6-4.7). Upon acidification, obtained by bacter-

ial growth, calcium and phosphorus (bound with casein as a tricalcium phosphate) are gradually removed from the caseinate molecules and are converted to a soluble state. The mean size of casein particles remains fairly constant in the pH range 6.6-5.3 (Rasic and Kurmann, 1978). Calcium removal from native micelles initially dissociates the weakly bound β- and κ-caseins, while leaving relatively intact a micellar network of αs-caseins. This phase is followed by extensive aggregation (indicating interactions between casein molecules) and formation of larger particles (Heertje et al., 1985; Figure 3). The aggregation process is then followed by contraction and finally association/rearrangement of aggregated particles into bigger structures to yield the milk protein network. Precipitation or aggregation is completed at pH 4.6-4.7 which represents the isoelectric point. At this point, the calcium is separated and bound with lactic acid as calcium lactate and thus make casein free of bound salts (Rasic and Kurmann, 1978).

The serum proteins are also affected by heating of yogurt milk, usually at 80-85^0 C for 30 min or at 90^0 C for 5 min. Most serum proteins are denatured, thereby favouring associations between protein molecules. Furthermore, a specific interaction between κ-casein and β-lactoglobulin can take place which is considered important for heat-treated milk in yogurt manufacture. Hence, in yogurt, protein coagulation occurs with the coprecipitation of the casein and denatured serum proteins. The remaining serum contains the uncoagulated proteose-peptone fraction, amino acids (non protein nitrogen compound), urea, creatine, creatinine and other dissolved components. On the other hand, in yogurt made from HTST pasteurized milk or upon spontaneous acidification of milk, coagu-

lated proteins represent mainly casein precipitates (Rasic and Kurmann, 1978); the uncoagulated portion contains proteins along with the proteose-peptone and other dissolved constituents.

Studies on the effect of concentration of milk proteins suggest that concentration retards heat denaturation of proteins in solution. Apparently, denaturation decreased as the total solids level of milk increased (Whitney, 1977).

2.1.5 Viscosity of Fluid Milk

The viscosity of skim milk is affected by changes in pH, primarily because of alterations in casein-micelle structure (Jelen, 1979). It is, therefore, necessary to know the flow characteristics of processed skim milk as it may undergo some physico-chemical changes during various concentration procedures. To measure the flow behavior of low viscosity fluids, such as milk, several types of viscometers are employed. However, not many studies have dealt with the rheological properties of commercially processed fluid milk. Traditionally, capillary viscometers have been used as a reasonably sensitive instrument for measuring low viscosity fluids. Yamamoto et al. (1986) have indicated that both human's and cow's milks were found to exhibit non-Newtonian fluid behavior. Early reports using a modified Ostwald Viscometer, without a pressure head, to characterize processed milks, have also shown non-Newtonian flow patterns (Whitnah and Rutz, 1956).

In recent years, rotational viscometers, assisted with computerized data acquisition systems, have been found to be more appropriate for

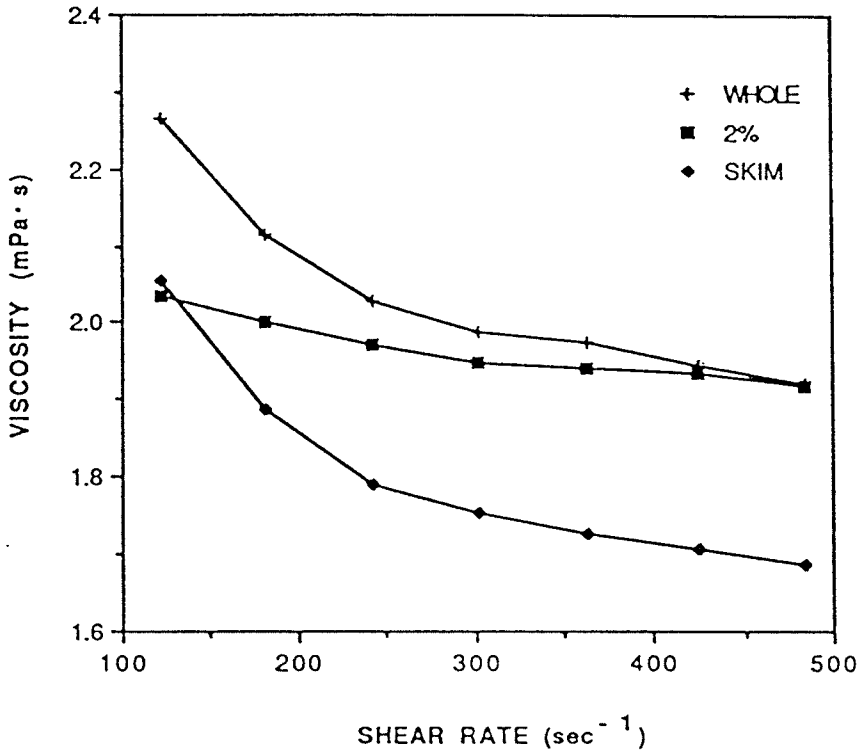
these kinds of measurements. Wayne and Shoemaker (1988) have used a computerized rotational steady shear viscometer with cone and plate fixtures to measure the rheological properties of various types of fluid milk. They have observed rheological differences among various types of fluid milks where shear rate plots demonstrated a slight shear thinning dependence of viscosity (Figure 4). From the findings of this study, the relationships between composition and flow behavior of these types of fluid products could be established.

2.1.6 Buffering Properties

According to Covacevich and Kosikowski (1977a), the manufacture of cream cheese directly from standardized skim milk retentate evidences difficulties in reaching the required final pH in a normal ripening time. It has also been reported that these difficulties are mainly due to the high levels of acid required to minimize the strong buffering properties of the standardized precheese mixture (Covacevich and Kosikowski, 1979).

Researchers have studied the relevance of buffering capacity of milk in standard cheese milk (Breene et al., 1964; Maubois and Mocquot, 1975). A study on ultrafiltered skim milk, concentrated to maximum and used for cheese making, indicated that the buffering capacity of direct ultrafiltration retentates of skim milk rose exponentially with increasing total solids (Covacevich and Kosikowski, 1979). Mistry and Kosikowski (1985) have also observed that the time to reach pH 4.6 for ultrafiltered skim milk retentate was a function of bacterial culture population and the total protein level of the retentate. It has been observed that an average of 0.48% lactic acid was required to reduce the

Figure 4. Apparent viscosity versus shear rate for three fluid milk types. Source: Wayne and Shoemaker (1988).


2.4
2.2
2.0
1.8
1.6
VISCOSITY (mPa · s)
100
200
300
400
500
SHEAR RATE (sec⁻¹)
WHOLE
2%
SKIM

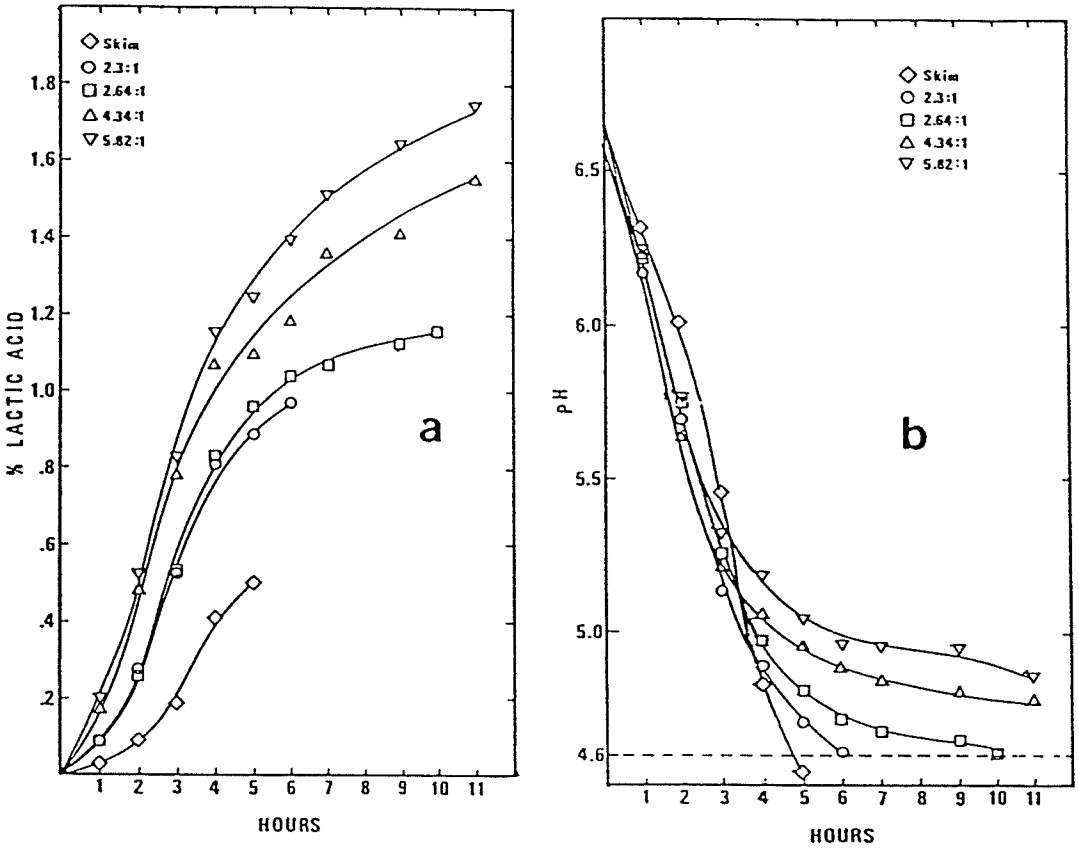
pH of plain skim milk to 4.6, compared with 1.01% for skim milk retentate concentrated 2.3 fold and 1.14% for skim milk retentate concentrated 2.6 fold (Figure 5a). In the same study, it has been found that skim milk retentates concentrated to 4.3:1 and 5.8:1 were unable to reach a pH of 4.6, even when the titraTable acid was greater than 1.8% (Figure 5b). In this context, Sutherland and Jameson (1981) have reported that the pH of freshly pressed cheddar cheese made from direct ultrafiltered and diafiltered retentates was higher than the optimum 5.2, possibly due to the higher buffering capacity of the milk.

The buffering capacity of ultrafiltered skim milk retentates is greater than plain skim milk, presumably beacuse of their higher protein content (Covacevich and Kosikowski, 1979). A milk of higher buffering capacity would require more starter culture to produce the necessary amount of lactic acid for reducing the pH (Mistry and Kosikowski, 1985).

2.1.7 Flavour Components and Organic Acids of Yogurt

In addition to lactic acid produced from carbohydrates, mixed starter culture (e.g. S. thermophilus and L. bulgaricus) fermentation of milk yields numerous flavor carbonyl containing compounds. The most important flavor components are: lactic acid, acetic acid, acetaldehyde, acetone, acetoin and diacetyl. L. bulgaricus possesses the enzyme threonine aldolase, which may contribute to yogurt flavor through the production of acetaldehyde from threonine (Hickey and Jago, 1983).

Figure 5. a) Lactic acid production in skim milk and skim milk retentates by direct-set, frozen concentrated lactic starter at 32^{0} C; b) Changes in pH in skim milk and skim milk retentates by direct-set, frozen concentrated lactic starters at 32^{0} C; Mistry and Kosikowski (1985).


Skim
2.3:1
2.64:1
4.34:1
5.82:1
% LACTIC ACID
HOURS
a
pH
HOURS
b

Although L. bulgaricus plays the most important role, it has been observed that the level of acetadehyde is much greater in mixed cultures of S. thermophilus and L. bulgaricus, presumably due to synergistic growth (Table 2). Acetaldehyde has been claimed to be the most important flavor compound responsible for the characteristic aroma of yogurt (Rasic and Kurmann, 1978; Tamime and Robinson, 1985).

During fermentation of milk, streptococci lower the substrate pH to about 5.0, thus preventing an excess of acetaldehyde production by L. bulgaricus. Finally, the lactobacilli further decrease the pH to 4.0-4.4. Studies have indicated that during fermentation the production of acetaldehyde becomes evident at pH 5.0 and reaches a maximum at 4.2. The pH eventually stabilizes at 4.0 (Tamime and Robinson, 1985).

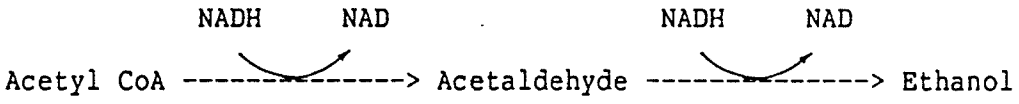
The influence of storage on acetaldehyde content in yogurt made from different types of milk has been reported by Tamime and Deeth (1980). Using the findings of previous studies, they indicated that the losses of acetaldehyde from yogurt during storage depend on the type of milk used for processing. Following 24 hours of storage "full fat or whole milk" demonstrated little change in acetaldehyde content, while in "skim milk" yogurt the level decreased. The effect of storage at refrigeration temperature on acetaldehyde concentration in yogurt with or without addition of sucrose has been reported by Bills et al. (1972).

Table 2. The production of carbonyl compounds (ppm) by yogurt starter culture.[a]

Organism	Acetaldehyde	Acetone	Acetoin	Diacetyl
S. thermophilus	1.0-8.3	0.2-5.2	1.5-7.0	0.1-13.0
L. bulgaricus	1.4-12.2	0.3-3.2	Trace-2.0	0.5-13.0
Mixed cultures	2.0-41.0	1.3-4.0	2.2-5.7	0.4-0.9

[a] : Tamime and Robinson (1985).

The data presented in Table 3 indicated that in both cases the level of acetaldehyde continued to decrease during refrigerated storage for 14 days. Decline in acetaldehyde content with time is not surprising because of the ability of lactic organisms to reduce acetaldehyde to ethanol. This is supported by the pathway of fermentation reported by Marshall (1987), according to which the reactions at the end of the heterolactate fermentation pathway proceed as follows:



The production of acetaldehyde and other flavor compounds by the mixed starter culture in yogurt takes place during the fermentation process. The final levels are dependent or influenced by the presence of specific enzymes which are able to catalyse the formation of carbonyl compounds from various milk components such as lactose, amino acids and nucleic acids (Tamime and Robinson, 1985). Table 4 summarizes the possible metabolic pathways and reactions initiated by the two starter organisms which ultimately lead to formation of the flavoring compounds in this product.

Upon storage at 4°C, many metabolic end products, including lactic acid and acetaldehyde, further develop, providing the characteristic yogurt flavor. Lactic acid production is the most important chemical process which takes place during yogurt manufacture. In this regard, Tamime and Deeth (1980) reported that lactic acid helps to destabilize the casein micelle, resulting in coagulation of the milk protein and formation of a gel structure.

Table 3. Acetaldehyde content of yogurt during refrigerated storage at 5° C.

Refrigerated storage (days)	0% Sucrose	8% Sucrose
	Acetaldehyde (ppm)	Acetaldehyde (ppm)
1	25	22
2	22	20
4	19	17
7	18	14
10	15	10
14	13	9

Source: Bills et al. (1972).

Table 4. Possible origin of flavour compounds produced by *S. thermophilus* and *L. bulgaricus* during the manufacture of yogurt.[a]

Milk constituent	Reaction involving acetaldehyde formation
Lactose	Via pyruvate during glycolytic cycle Via acetyl phosphate or pyruvate Direct decarboxylation of pyruvate
Amino acid	Valine ---> acetaldehyde + (possibly) alanine Threonine ---> acetaldehyde + glycine Cleavage of threonine to glycine & acetaldehyde Conversion of methionine ---> threonine ---> acetaldehyde + glycine
Nucleic acid	Thymidine ---> acetaldehyde + glyceraldehyde-3-phosphate

[a] : Data compiled by Tamime and Robinson (1985).

Several organic acids, including orotic, citric, pyruvic, lactic and propionic, occur in dairy products, including yogurt, as a result of bacterial growth (Table 5, Marsili et al., 1981). Quantitative analysis of these acids in yogurt is, therefore, essential for determining bacterial culture activity, as well as for flavor assessment and nutritional evaluation of the product. Marsili (1981) has also studied the organic acid composition of buttermilk during fermentation. Changes in the profiles of organic acids are shown in Figure 6.

2.2 Chemistry and Production of Yogurt

2.2.1 Starter Culture

Yogurt is a fermented dairy product, resulting from the growth of S. thermophilus and L. bulgaricus in warm milk, and characterized by a smooth, viscous gel like properties with delicate walnutty flavor (Tamime and Deeth, 1980). These two bacterial species are members of a large group of lactic acid producing microorganisms which are gram positive, nonsporeforming, microaerophilic, and have an optimum growth temperature between 37-45^{0} C (Matalon and Sandine, 1986).

The total number of these organisms initially inoculated into each litre of milk is 90-100 x 10^{8} (Abrahamsen and Holmen, 1981). The two bacterial cultures, introduced at 1:1 ratio, remain viable in the yogurt until it is consumed. Even after the normal self life, i.e. 20-40 days, both of these fermenting microflora still survive in large numbers. Greig and Harris (1983) reported that the total number of lactic

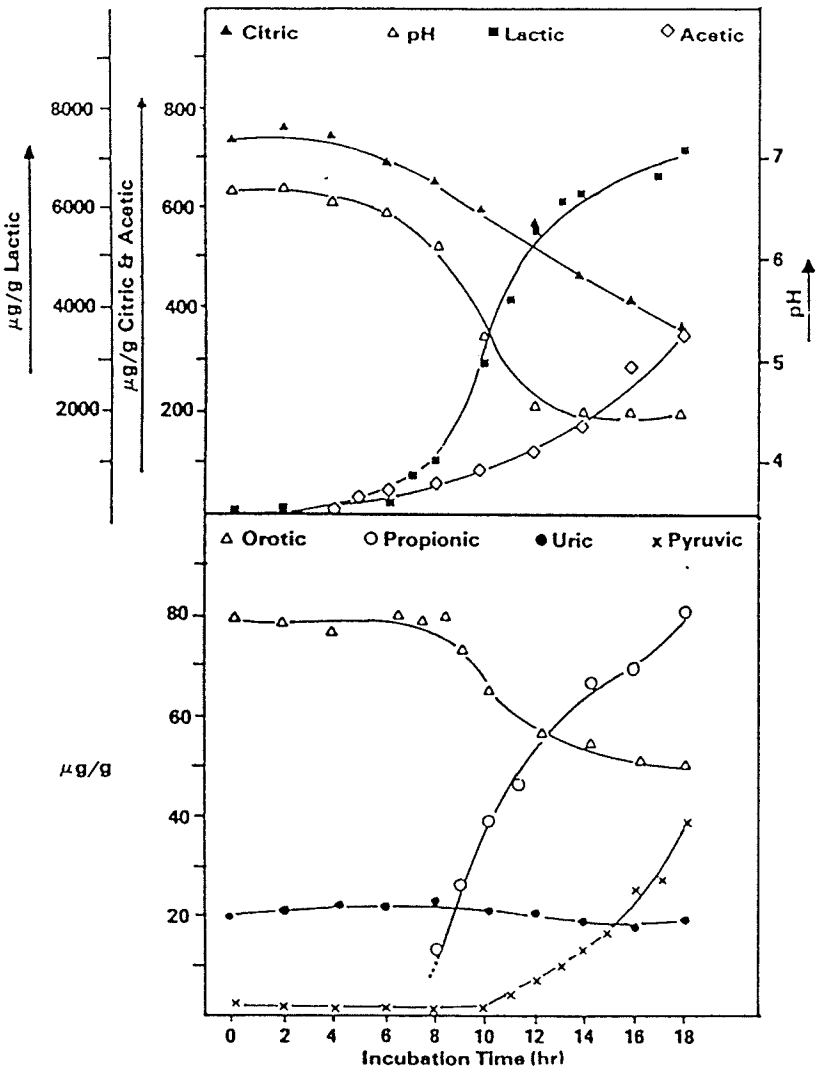
Table 5. Organic acid composition of typical commercial dairy products.[a]

Products	Concentration, μg/g[b]					
	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
Whole milk	83.6 (1.0)	940 (40)	<4	<60	<100	<120
Butter-milk (cultured)	39.3 (1.2)	60 (1)	<2	5890 (10)	850 (10)	<60
Sour cream	48.6 (2.4)	120 (0)	<4	8410 (160)	900 (30)	180 (20)
Yogurt (plain)	72.5 (2.5)	710 (30)	24 (0)	14550 (150)	120 (20)	<120
Sharp Cheddar cheese	4.9	25	26	5140	600	(-)

[a] : Marsili et al. (1981).

[b] : Uncertainties represent average deviations of complete duplicate determinations; number in parenthesis refers to standard deviation.

Figure 6. Changes in the chemical composition of cultured buttermilk during a typical fermentation as determined by HPLC. Source: Marsili (1981).


▲ Citric
△ pH
■ Lactic
◇ Acetic
μg/g Lactic
μg/g Citric & Acetic
pH
8000
6000
4000
2000
800
600
400
200
7
6
5
4
△ Orotic
○ Propionic
● Uric
x Pyruvic
μg/g
80
60
40
20
0
2
4
6
8
10
12
14
16
18
Incubation Time (hr)

acid bacteria in yogurt could not be measured adequately due to many factors involved which may affect the final numbers. The factors include: initial inoculation level, ratio of S. thermophilus to L. bulgaricus and even competition from other organisms such as coliforms, yeasts and moulds.

Starter culture organisms also cause proteolysis of casein and thereby contribute to the development of flavor and texture in yogurt. S. thermophilus exhibits peptidase activity towards initial casein breakdown, while L. bulgaricus is more efficient in hydrolyzing native casein in yogurt milk. The synergistic growth of S. thermophilus and L. bulgaricus occurs in two phases. In the first phase, S. thermophilus is stimulated by L. bulgaricus liberating critical metabolites such as essential amino acids derived from degradation of the casein. In the second phase, the growth of S. thermophilus is slowed down due to the adverse effect of lactic acid, and with the reduced levels of oxygen, the growth rate of L. bulgaricus increases through the stimulative or synergistic action of S. thermophilus (Rasic and Kurmann, 1978; Tamime and Deeth, 1980).

Marshall and El-Bagoury (1986) studied the effect of increasing total solids in goats' milk by ultrafiltration, reverse osmosis and addition of goats' milk powder on S. thermophilus and L. bulgaricus activity by assessing acidity (pH) and aroma (acetaldehyde). The time (5 1/2 h) required to reach pH 4.3 to 4.6 for milk concentrated by reverse osmosis (15.25% total solids) was reported to be higher than for milk concentrated to 17.4% solids level by ultrafiltration which took only 4 1/2 h to reach pH 4.5. Similarly, acetaldehyde levels increased in milk con-

centrated by ultrafiltration when compared with reverse osmosis or when milk was fortified with goats' milk dried powder.

2.2.2 Rheological Properties of Yogurt

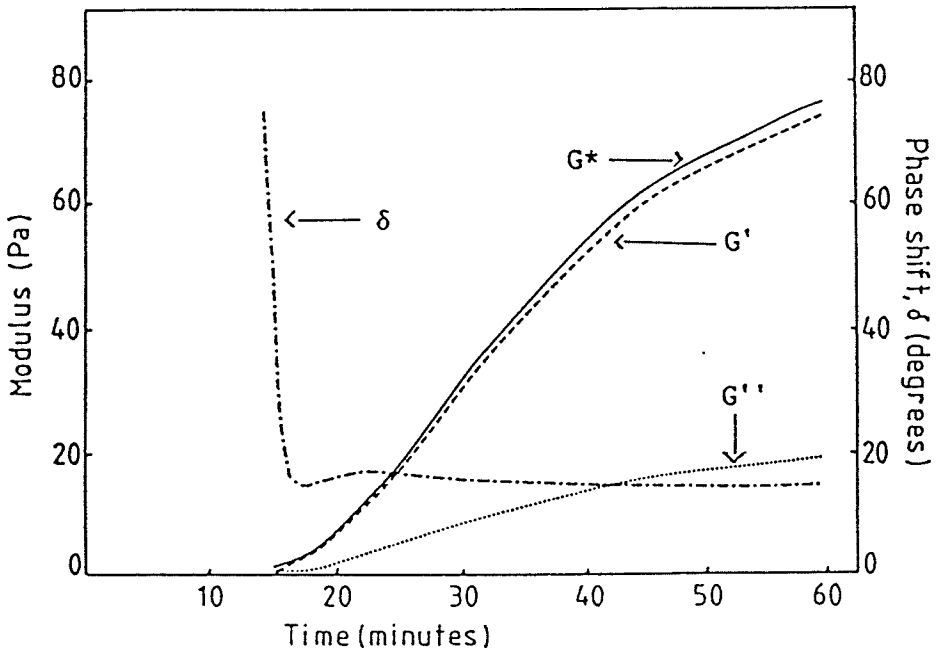
The main criterion of quality assessment of yogurt is the physical properties of the gel. The physical characteristics of yogurt are influenced by milk composition and manufacturing conditions. These include protein content, heat treatment applied to milk prior to fermentation, the process of homogenization, acidity levels, culture level, mechanical handling of coagulum and the presense of stabilisers (Parnell-Clunies et al., 1986; Rasic and Kurmann, 1978). The coagulation process of milk can be monitored using rheological techniques. In this respect, the gelation process has been followed by rotational viscometers (Green et al., 1978; Kopelman and Cogan, 1976; Kowalchyk and Olson, 1977; Scott Blair and Oosthuizen, 1961), while gel characterization has been reported by penetrometer measurements (Steinsholt, 1973). Flow behavior of yogurt, similar to many food materials, may be described as non-Newtonian. Yogurt products, therefore, show dependence of apparent viscosity on shear rate (Parnell-Clunies et al., 1986).

Bohlin et al. (1984) investigated the coagulation of renneted milk using a new dynamic rheological testing instrument. The primary advantage of this technique over other instrumental methods is that the gelation process is not affected during measurement; i.e. non-destructive small amplitute shear stress measurements over the whole coagulation process. Their findings also indicated that using such techniques the

milk coagulation process can be studied as a function of temperature and concentration of milk-clotting enzymes (rennet). Dynamic rheological testing is perhaps the most suitable method in probing time-dependent changes in structure of food materials. Mitchell (1980) in his review has provided details of the theory and applications of such methods in food systems. The rheological parameters one can monitor during small amplitude oscillatory testing are the complex modulus (G*), storage modulus (G'), loss modulus (G'') and phase shift angle (δ). In the studies of Bohlin et al. (1984), it has been found that the complex modulus started to increase 15 min after rennet addition, indicative of the secondary phase of milk coagulation (Figure 7). According to Ernstrom and Wong (1974), it must be noted that the primary phase of milk coagulation involves the enzymatic breakdown of κ-casein, whereas in the secondary phase milk eventually gels and coagulates. A sigmoid shaped curve of the complex modulus (G*) as a function of time is obtained, as shown in Figure 7, and the modulus reaches a constant value after the reaction. Prior to the rise in G* during milk coagulation, there is a rapid drop in the phase shift angle (δ) which is typical of a sol ---> gel transition (Figure 7). The slight local maximum for δ in the early stages of gelation has been reported to coincide with the maximum slope of the G* curve (Bohlin et al., 1984).

Kowalchyk and Olson (1977) reported that rennet-mediated coagulation of milk is affected by temperature. Bohlin et al. (1984) have observed that increased rennet concentration only causes a parallel displacement of (G*) whereas the higher temperature changes the kinetics of the coagulation process. In other studies, the relationship between rigidity

Figure 7. The secondary phase of milk coagulation at standard conditions: 31° C, .3 ml rennet/kg milk. G* = complex modulus, _____ ; G' = storage modulus, -----; G'' = loss modulus, ...; δ = phase shift, .-.-.-.-. Zero time indicates addition of rennet. Source: Bohlin et al. (1984).


80
60
40
20
0
Modulus (Pa)
δ
G*
G'
G''
10
20
30
40
50
60
Time (minutes)
Phase shift, δ (degrees)

modulus (G) of milk gels during clotting and time were studied (Scott Blair, 1960; Olson and Bottazzi, 1977). Using a thrombelastograph, Olson and Bottazzi (1977) have found that the concentration of milk-clotting enzyme affects the kinetics of the coagulation process. It has been also shown that a slower rate of increase in rigidity moduli (G) was accompanied by longer clotting times; an excellent linearity has been observed between these two factors for coagulated milks formed by immobilized and soluble pepsins. Furthermore, the rigidity modulus development in milk coagulum followed first-order reaction kinetics with respect to time.

The effect of ultrafiltration on curd tension of set yogurts has been studied by Abrahamsen and Holmen (1981). Using a Brookfield Synchro-Lectric Viscometer, these researchers observed higher curd tension for ultrafiltered yogurt samples than the control.

2.3 Concentration of Milk by Membrane Technologies

2.3.1 Ultrafiltration

The method of ultrafiltration is defined as the hydraulic pressure-activated process in which a fluid e.g. milk, is drawn from a reservoir (through a coarse prefilter, Figure 8) and then applied or pumped under pressure (and in a condition of turbulant flow) to one side of a semi-permeable membrane and recirculated to the reservoir. The turbulent flow is required to reduce concentration build up of polymeric constituents at the membrane surface (known as membrane polarisation) which otherwise adversely affect the rate of flux or flow through the membrane (Anonymous, 1986). This process separates components on the basis of molecular size and shape through the semi-permeable membrane which are used to pass or reject molecules selectively. Thus, ultrafiltration is basically a fractionating process. The low molecular weight solutes (salts and lactose) in the case of milk, far below the membrane retention level, pass through the fiber walls and emerge as permeate. The high molecular weight material, (e.g. milk proteins), is concentrated on the feed side of the membrane. The retentate is progressively concentrated in the reservoir until the desired concentration is attained.

Covacevich and Kosikowski (1977b) studied the effect of direct ultrafiltration of skim milk on the permeation rate, total solids and protein in the retentate (Figure 9). It has been observed that the permeation rate gradually decreased with the increase in total solids but the total solids (TS) in the permeate remain constant. The protein content in the retentate also increased with removal of permeate. They have further studied the effect of ultrafiltration and diafiltration on

Figure 8. Schematic diagram for ultrafiltration concentration of skim milk. Source: Anonymous (1986).

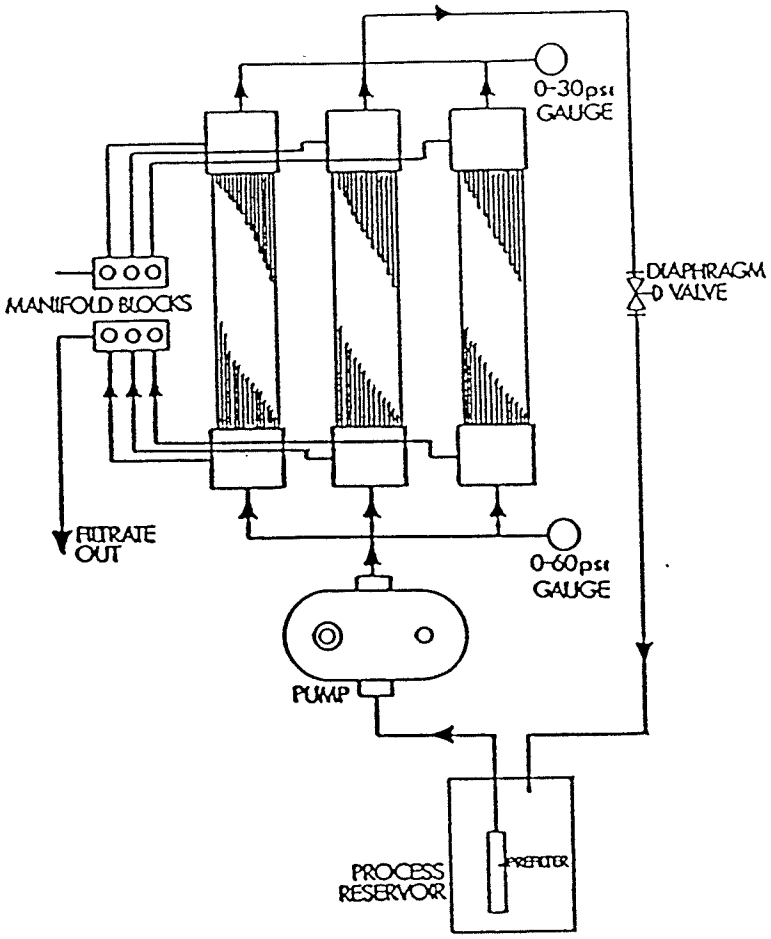
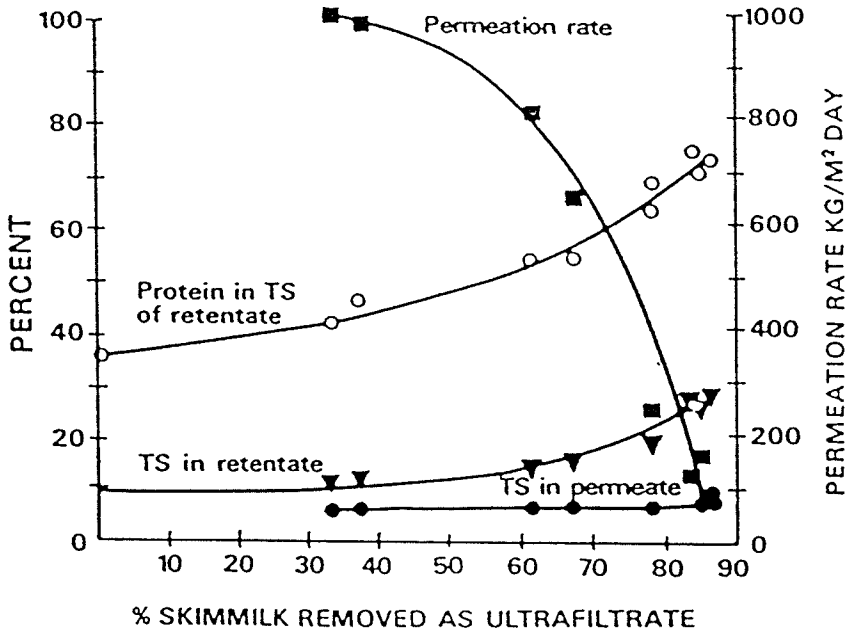

0-30psi
GAUGE
DIAPHRAGM
VALVE
MANIFOLD BLOCKS
FILTRATE
OUT
0-60psi
GAUGE
PUMP
PROCESS
RESERVOIR
HEATER

Figure 9. Effect of direct ultrafiltration concentration of skim milk on permeation rate, total solids and protein in the retentate and total solids in the permeate. Source: Covacevich and Kosikowski (1977b).


Permeation rate
Protein in TS
of retentate
TS in retentate
TS in permeate
PERCENT
PERMEATION RATE KG/M² DAY
% SKIMMILK REMOVED AS ULTRAFILTRATE
100
80
60
40
20
0
1000
800
600
400
200
0
10
20
30
40
50
60
70
80
90

composition of skim milk retentates and reported that the amount of protein (%) in the dry matter increases while percent lactose and ash content decrease by membrane processing of milk procedures (Table 6). The permeation rate also decreases upon ultrafiltration of whole milk along with concomitant increases in protein and fat content (Ernstrom et al., 1980).

The effect of ultrafiltration on elemental changes of milk has been studied by Covacevich and Kosikowski (1977a) and the distribution of the major minerals such as sodium, potassium, phosphorus and calcium in the basic materials and cream cheese samples are shown in Table 7. The data for skim milk and retentate have indicated that the amount of all elements have increased many folds in the retentate; phosphorus about 4 x and calcium upto 5 x, compared to skim milk. Other studies have been conducted on the effect of ultrafiltration on the calcium and phosphorus content of retentates. The results showed that there was a marked increase in both calcium and phosphorus content (Ernstrom et al., 1980).

Ultrafiltration of milk also results in retentates of higher buffering properties. As cited by Rash and Kosikowski (1982) and Covacevich (1975) for cheese manufacturing from ultrafiltered retentate, there is a resistance to change in pH, particularly during the critical hours of the process because of a strong buffering system. As a result, ultrafiltered milk "inhibits" starter activity, thus delaying or preventing decrease in pH, as compared to ordinary milk. As the buffering capacity is found to be increased because of increases in protein and insoluble salts by ultrafiltration (Covacevich and Kosikowski, 1979), the amount of lactic acid needed by starter culture to deliver a unit change in pH

Table 6. Dry matter composition of skim milk retentates obtained by various ultrafiltration procedures.[a]

Experiment[b]	Protein (%)	Lactose (%)	Ash (%)
1	72.2	19.7	8.2
2	83.2	8.9	7.9
3	87.6	4.7	7.7

[a] : Covacevich and Kosikowski (1977b).

[b] : 1 = direct ultrafiltration; 2 = single diafiltration; 3 = double diafiltration.

Table 7. Sodium, potassium, phosphorus and calcium of basic materials and cream cheese samples.[a]

Samples	Sodium (%)	Potassium (%)	Phosphorus (%)	Calcium (%)
Skim milk	0.03	0.16	0.07	0.10
Retentate	0.05	0.22	0.38	0.53
Permeate	0.03	0.14	0.04	0.02
Conventional cream cheese	0.20	0.16	0.09	0.06

[a] : Covacevich and Kosikowski (1977a)

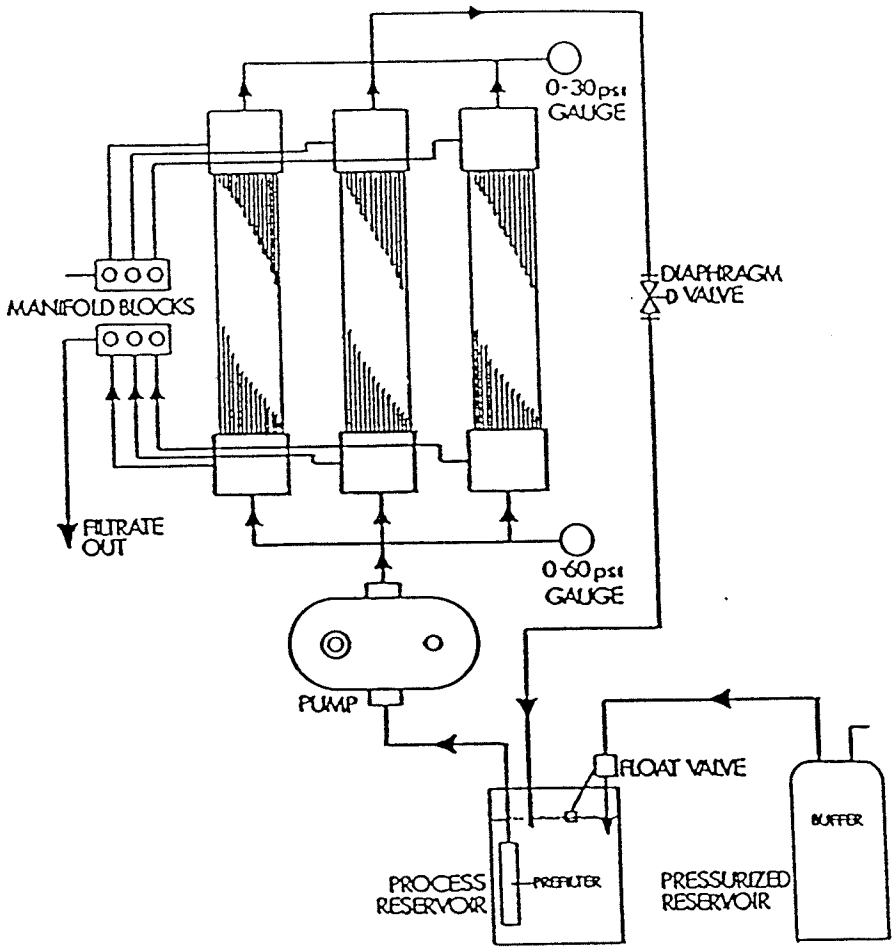
pH is considerably increased (Mistry and Kosikowski, 1983; 1985). This effect is greater with higher degree of ultrafiltration (Mistry and Kosikoski, 1985); the influence of higher buffering on cheese manufacturing could be minimized by adopting diafiltration techniques (Mistry and Kosikowski, 1986).

2.3.2 Diafiltration

Diafiltration is the most commonly used technique to obtain an appropriate lactose and mineral content in the concentrate. It consists of adding water at the latter stages of the ultrafiltration process thus reducing the concentration of all constituents. The fat and protein are re-concentrated, while the majority of the salts and lactose pass with the water through the membrane (Anonymous, 1986). During diafiltration, the permeation rates increased in both unacidified and acidified whole milk samples as lactose content decreased (Ernstrom et al., 1980).

In this mode, desalting proceeds as in the concentration mode. Ultrafiltration volumes are replaced by intermittent addition of deionized water from a separate reservoir after the desired solids level is reached in the direct ultrafiltration process. The mechanism involves a pressure gradient which transports salt convectively through the membrane with the wash fluid. The amount of protein in the concentrate by UF may be varied depending on the degree of 'washings' upon addition of fresh water. This, in effect, 'washes out' more of the non-protein solids leaving a higher percentage of protein in the retentate. The above technique is known as diafiltration process and is schematically shown in Figure 10.

Figure 10. Schematic diagram for diafiltration concentration of skim milk. Source: Anonymous (1986).


0-30psi
GAUGE
DIAPHRAGM
VALVE
MANIFOLD BLOCKS
FILTRATE
OUT
0-60psi
GAUGE
PUMP
FLOAT VALVE
BUFFER
PROCESS
RESERVOIR
PREFILTER
PRESSURIZED
RESERVOIR

Ernstrom et al. (1980) studied the effect of diafiltration on the calcium and phosphorus content of both acidified and unacidified whole milk. A little change in the calcium and a slight decrease in phosphorus concentration in unacidified retentates were reported, while marked decreases in both calcium and phosphorus in the acidified samples have been observed. Rash and Kosikowski (1982) have found that the diafiltration process reduced the level of lactose and ash, particularly calcium and phosphorus, in the precheese mix and led to differences in pH when compared with direct ultrafiltration Camembert pre-cheese mixes (Table 8). They have also observed that the double diafiltration technique removed a substantial amount of undissociated complex buffering constituents i.e. colloidal calcium phosphate from the casein micelles of the recirculating skim milk retentates.

Elemental analyses for calcium, phosphorus, sodium and potassium in the dry matter of retentates obtained by various ultrafiltration procedures have been studied by Covacevich and Kosikoski (1977b). They have indicated that the level of calcium in the dry matter increased with protein concentration in the single diafiltration experiments. On the contrary, all other minerals were found lower in the single diafiltration process compared with direct ultrafiltration (Table 9).

2.3.3 Membrane Fouling and Cleaning

Several researchers have worked on membrane processing of milk and other fluids (e.g. soy protein extracts). It has been observed that reduced flux rates in UF systems occur primarily due to membrane fouling. The rate and degree of fouling depends upon the nature of fluid and

Table 8. Composition and pH of ultrafiltration Camembert pre-cheeses made from ultafiltered partially fermented double diafiltered retentates.[a]

Component	Ultrafiltration[b] (%)	Diafiltration[b] (%)
Total solids	40.4	41.7
Total protein	14.6	15.3
Lactose	5.2	4.7
Calcium	0.36	0.33
Phosphorus	0.29	0.14
pH	6.7	6.2

[a] : Rash and Kosikowski (1982).
[b] : Averages of duplicate values on single trial.

Table 9. Calcium, phosphorus, sodium and potassium content in the dry matter of retentates obtained by various membrane concentration procedures.[a]

Experiment[b]	Calcium (%)	Phosphorus (%)	Sodium (%)	Potassium (%)
1	1.93	1.39	0.16	0.81
2	2.43	1.34	0.13	0.44
3	2.15	1.43	0.08	0.26

[a] : Covacevich and Kosikowski (1977b).

[b] : 1 = direct ultrafiltration; 2 = single diafiltration; 3 = double diafiltration.

the type of membrane (material and pore size). For example, severe fouling occured during concentration of proteins from soy extracts using polysulfone and acrylic-vinyl copolymer hollow fiber membranes. It was also found that the degree of fouling is higher for the copolymer type than the polysulfone membrane due to protein adsorption on the acrylic-vinyl copolymer (Nichols and Cheryan, 1981). During ultrafiltration of whole milk, Garontte et al. (1982) observed a decline in flux rate to almost 0 l/h before reaching a 5 x concentration factor, presumably due to deposition of a macromolecular layer on the membrane surface of hollow fibers. Studies by Tong et al. (1988) indicated that the decline in the flux rate is associated with irreversible protein adsorption on the surface of membrane.

Fouling of ultrafiltration membranes by protein and salts is a serious problem in the dairy industry (Richter, 1983), despite favorable hydrodynamic conditions (Kun-Pei and Cheryan, 1983; Tong et al., 1988). In addition to protein, calcium plays a vital role in interactions and processing of milk and milk products. It is reported that about two-thirds of the milk calcium at pH 6.6 is in the form of colloidal calcium phosphate, which is associated in some way with the casein micelles. The size of calcium-caseinate complex apparently affects their fouling tendencies. Even soluble calcium (e.g present in whey streams) can interact with and bind to negatively charged groups on the membrane by electrostatic or charge effects thus forming in a "salt bridge" between the membrane and proteins. This type of phenomenon could lead to even faster protein fouling. The problem can be minimized by adding calcium-sequestering reagents such as EDTA or citrates. For proteinaceous

feeds, it is also recommended to adjust the pH to values far removed from the isoelectric point of proteins (Cheryan, 1986) in order to improve flux rates.

Membrane fouling results in increased labor, shortened membrane life, higher energy costs, decreased retentate/permeate production and increase cleaning time/costs (Matthiasson and Sivik, 1980; Tong et al., 1988). Cleaning is required to eliminate the deposited foulants from the membrane which consist of organic and/or inorganic substances. Membrane fouling during ultrafiltration of milk is associated with the accumulation of organic material on the surface of membrane. Hedrick (1983) mentioned that the frequency of cleaning is important and varies from once every few hours to once a day for various food products. It has been shown that several factors affect the cleaning of membranes: composition, durability and configuration of membrane, nature and amount of deposits on membrane, pH and temperature of cleaning solutions, quality and purity of water (eg., hardness), and type of detergents (eg., acid, alkali, enzymes). A cleaning solution of sodium hydroxide (about pH 12) is recommended with protein foulants, and for severe fouling treatment, the use of enzyme-based detergents has been suggested (Cheryan, 1986).

2.3.4 General Description of Ultrafiltration Membrane Types

Ultrafiltration membrane technology has been developed in recent years for concentration, separation and purification of food products and their constituents. Several types of polymers and other materials have been used for the manufacture of selectively permeable membranes

for dairy applications. These include cellulose acetate, polyamide, polysulfone and other polymeric materials for munufacturing microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) membranes.

Cellulose acetate type of membranes consist of polymeric cellulose acetate derivatives. One important physical parameter that influences membrane properties is the degree of polymerization of cellulose. The use of cellulose acetate and its derivatives as membrane materials has several advantages (Cheryan, 1986):

a) high flux and high salt rejection properties
b) easy to manufacture
c) the raw material (cellulose) is a renewable resource.

The disadvatages are:

a) a fairly narrow temperature range, a maximum of 30^0 C
b) narrow pH range: mostly restricted to pH 2-8, preferably pH 3-6
c) poor resistance to chlorine
d) undergo the "creep" i.e., gradual loss of membrane properties (mainly flux) under pressure
e) highly biodegradable.

The raw materials used for the polyamide-type membranes have been characterized by having an amide bond in their structure (-CONH-). This class of membranes has been especially designed for reverse osmosis (hyperfiltration) applications, and has been found to have no major improvement over the cellulose acetate membranes. Moreover, the polyamide membranes appeared to be worse in some respect, particularly in regard to free chlorine tolerance in dairy applications.

Polysulfone membranes are polymers consisting of diphenylene sulfone repeating units. Polysulfone membranes are extensively used in ultrafiltration and thus are considered a breakthrough due principally to the following characteristics (Cheryan, 1986):

a) wide pH tolerances i.e., 1 to 13 and can be continuously exposed to this pH range which is definitely an advantage for cleaning purposes;
b) fairly good resistance to chlorine of up to 200 ppm for short-term sanitation and up to 50 ppm for long-term storage of polysulfone membrane;
c) membranes can be fabricated easily to provide a wide variety of configurations;
e) wide range of molecular weight cut-offs from 1000 up to 500,000 in commercial size modules.

The principal drawback, however, is the apparent low pressure limits; 25 psig/ 1.7 atm with polysulofone hollow fibers (Cheryan, 1986).

The appropriate choice of UF membrane together with diafiltration is essential to obtain a correct mineral (calcium) and lactose content in the concentrate (Anonymous, 1986). Thomas et al. (1986) have discussed inorganic type membranes (ceramic and sintered glass or metals) with regard to applications in the dairy industry. These type of membranes allow flexibility in the types of food products processed as well as sterilization and cleaning.

2.3.5 Applications of Ultrafiltration

Membrane technologies have been used commercially by the food industry for a variety of purposes. These include fruit juice concentration, sugar concentration, protein and enzyme recovery and waste water treatment (Parkinson, 1983). Concentration of skim milk by ultrafiltration and the parameters affecting the process were studied by Fenton-May et al. (1972). Similarly, the compositional changes of the retentate during the process were investigated by Bundgaard et al. (1972) and Maubois and Mocquot (1971). With respect to dairy applications, it has been established that milk retentates obtained from ultrafiltration can be used in the production of cheese, frozen desserts, yogurt and other dairy products (Bundgaard et al., 1972; Chapman et al., 1974; Covacevich and Kosikowski, 1977a,b; Kosikowski and Maters, 1983; Kosikowski et al., 1984). Ultrafiltration processes can be used in the concentration of milk constituents with or without diafiltration (Davies and White, 1960; Glover, 1971). The choice of methods is dependent on the objective of processing and the type of food systems being applied. Several studies have suggested that the ultrafiltration process can be applied to eliminate both the water and lactose fraction of milk in the manufacture of mozzarella and other semi-hard and soft cheeses.

According to Davies and White (1960) and Morr (1975), acidification of milk results in removal of larger quantities of calcium and phosphorus from the casein micelles. Similarly, Rash and Kosikowski (1982) observed that partial fermentation lowered the buffering capacity of diafiltered retentate probably due to the reduction of the casein-free dissociated calcium and phosphorus.

During the last decade researchers have been interested in the use of milk pretreated by ultrafiltration in order to increase the cheese yield and save in energy, milk-clotting enzymes, manufacturing time and whey disposal (Fenton-May et al., 1972; Mathews et al., 1976; Maubois and Mocquot, 1975). In addition, several other advantages have been reported which include optimum protein and fat standardization for cheese making, improved microbial and organoleptic quality of milk, specially by ultrafiltering milk on farms which reduces to about 50% the milk collection costs (Kosikowski, 1985). Another advantage of UF is that no heat is applied during membrane filtration and thus the protein is recovered in an undenatured state. The primary benefit of ultrafiltration, therefore, is that it produces an excellent, undenatured, soluble, protein-rich product with desirable functional and nutritional characteristics (Greig and Harris, 1983).

2.3.6 UF Retentate-based Dairy Products

Traditionally yogurt was made from sheep and buffalo milks and sometimes from goat's and cow's milk. The modern dairy industry, however, uses cow's milk for making yogurt (Abrahamsen and Holmen, 1981). Commercial yogurt is usually made from fortified milk containing 14-16% total solids. The fortification process is required to increase the total solids in order to strengthen the yogurt coagulum, increase the viscosity and prevent syneresis. Fortification can be achieved by several ways: (a) addition of skim milk powder, (b) by vacuum evaporation, (c) by ultrafiltration and d) by employing reverse osmosis (Tamime et al., 1984). Bundgaard et al. (1972) and Chapman et al. (1974) have

suggested ultrafiltration and reverse osmosis as methods of concentration for making cow's milk fermented products.

Other studies have shown that yogurts made from ultrafiltered milk have better flavor and viscosity compared to unconcentrated control milk (Abrahamsen and Holmen, 1981). It has been also found that the total solids and protein content in the ultrafiltered goat's milk increased while the amount of lactose decreased compared with other methods of concentration such as reverse osmosis, vacuum evaporation as well as the method of addition of milk powder. In the manufacture of Ricotta cheese, Maubois and Kosikowski (1978) found that acidification of cheese milk to an optimum pH 5.9 could be attained either before the ultrafiltration process, during ultrafiltration, or in the retentate containing 9.0 to 9.5% protein. The organoleptic qualities of the cheeses prepared before ultrafiltration were not significantly different from those derived from the ultrafiltered retentates.

Chapter III

MATERIALS AND METHODS

3.1 Processing of Milk Samples

3.1.1 Reconstitution of milk by Addition of Skim Milk Powder

Refrigerated pasteurized skim milk and skim milk powder were received from the Dairy Pilot Plant of Food Science Department, University of Manitoba, Winnipeg. The control milk, at 3 total solids level, was prepared by the addition of skim milk powder using the Pearson Square model (Appendix 1). Using the same method, another type of sample was prepared by direct addition of non-fat milk solids (MSNF) into distilled water. After adjustment of milk solids to ca 12, 14, and 16% T.S., the samples were packed in 300 ml sealed plastic bags and kept at -18° C until required.

3.1.2 Description & Principles of Operating Modes of the UF System

The UF laboratory scale equipment used in this study consisted of an electrically operated lobe-type pump, coupled with sanitary fittings to an Amicon Diaflo hollow fiber cartridges of a non-cellulosic polymer membrane. The cartridge (H5P10-43) had a 10,000 molecular weight cut-off, a surface area of 0.45 m^2 and an internal fiber diameter of 1.1 mm.

The maximum operating inlet pressure was 25 psi and a temperature of 50° C. The operating pressures and rate of recirculation were controlled

by the pump speed and a diaphragm-type back pressure valve. If the pressure exceeded 30 psi, a pressure limit microswitch automatically shut down the system.

3.1.3 Concentration of Skim Milk by Ultrafiltration

In the ultrafiltration (UF) experiments, 20 l lots of pasteurized skim milk were concentrated by an Amicon type diaflow hollow fiber membrane (H5P10-43). The milk was fed at 25° C and maintained at that temperature until the end of the concentration process. Inlet and outlet pressure were initially 20 and 14 psi, respectively. Since the inlet pressure increased during the experiment, a constant inlet pressure of 20 psi was maintained by gradually opening the diaphragm-type back pressure valve to release the pressure build up due to deposits on the membrane surface. Once the desired concentration of total solids, i.e. 12, 14 and 16, was attained, the retentate was placed in 300 ml sealed plastic freezer bags and kept at -18° C until needed.

3.1.4 Concentration by Diafiltration

This process involved two phases: first, a direct ultrafiltration experiment which was then followed by diafiltration by addition of deionized water to the UF retentate. The amount of water added was equivalent to the amount of permeate removed in the preceding ultrafiltration experiment carried out to reach the desired solid content level. The diafiltration process was performed under conditions identical to those of the ultrafiltration experiment until the diluted retentate reached similar total solids content level (12, 14, and 16%).

3.1.5 Membrane Cleaning Procedure

During the UF/DF experiments, low flux rates were encountered over time because of the deposition of organic substances on the membrane surface. In order to restore the normal performance of the membrane, a cleaning technique was applied based on the procedures suggested by Cheryan (1986) and Tong et al. (1988). Prior to and after each milk UF/DF trial, the system was flushed with water, using the hottest water (~ 50° C) possible that is compatible with the system. The system was then cleaned thoroughly with a commercial alkaline cleaning solution. The solution consisted of 20 l 0.1-0.4% sodium hydroxide (amount depends on the severity of fouling) plus 0.5% sodium lauryl sulfate (detergent) having a pH of about 11. The cleaning solution was recirculated for 20 min at 35-40° C. At this time the pump was shut off to allow a 15-20 minute soak before recycling the solution at high flow rates. After soaking, the cleaning solution was recirculated at a high flow rate (feed pressure 20 psig) for 30-60 minutes. The system was then flushed completely with 20 l or more of deionized water at 35-40° C in order to remove the detergent. The clean membrane water flux was determined using an additional 20 l of fresh 35-40° C deionized water. Permeate flux during ultrafiltration was analyzed by measuring permeate flow rates and dividing by the total membrane area of the system. Clean membrane water flux rate was measured with a little back pressure (retentate bleed valve pertially closed). Under these conditions, inlet and outlet pressure were 10 and 3.5 psi, respectively. The membrane system was finally sanitized with 200 ppm and stored in 50 ppm chlorine solution. If clean water flux was not up to expected values, the whole process was repeated.

3.1.6 Sampling and Analysis

In the ultrafiltration and diafiltration experiments, about 25 ml samples of both retentate and permeate were collected every 20 min and kept at refrigeration temperature of 4^0 C for further analysis. Similar aliquots for control milk samples prepared by supplementation or full reconstitution of skim milk powder were also collected in plastic bottles and kept at 4^0 C.

All analyses were carried out in triplicate. Total solids (% Brix) were determined by an Abbe' refractometer during both ultrafiltration and diafiltration methods. Flux rate measurements were carried out according to Tong et al. (1988) by expressing the permeate volume in liters per square meter per hour. Total solids (% Mojonnier) were determined by drying the samples in a 100^0 C vacuum oven for 30 min according to standard method (Marth, 1978). Fat, protein and lactose content in the milk samples and in the retentates and permeates were analyzed with a Milko-Scan 203 (A/S N. Foss Electric, Denmark) infra red analyzer (Marth, 1978). Some concentrated retentates were diluted with distilled water to 1:1 ratio prior to analysis. Ash content was determined according to the AOAC method (1980) and mineral analyses were performed by atomic absorption using a Perkin-Elmer 560 Atomic Absorption Spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). The phosphorous content in the ash was determined by the colorimetric method of Olsen and Dean (1965) using a Technicon Auto Analyzer. Titratable acidity (%) and pH were determined according to standard methods (AOAC, 1980).

3.2 Chemical and Physical Properties of Milk and Yogurt

3.2.1 Measurement of Buffering Capacity

Buffering capacity was determined using a slight modification of the method of Mistry and Kosikowski (1985) and Ernstrom et al. (1980) by employing a Titroprocessor 686 equipped with a Brinkman Dosimat 665 (Metrohm, Switzerland). Standardized diluted HCL (0.1N) of certified reagent grade (Fisher Scientific Co.) was added at a rate of 0.2 ml/min with the help of a microburette of Dosimat to a sample of 10 g continuously stirred by a magnetic bar. The equipment was designed to measure the pH continuously from an initial pH (>6.4) until a pH of 4.4 was attained. Some difficulties in keeping the solids in suspension, however, were apparent during pH measurements of diafiltered milk samples at all solids level (12, 14, and 16%). These samples were , therefore, diluted with distilled water prior to analysis and the pH changes were monitored. A plot of 0.1N HCL vs pH gave rise to titration curves for all samples and provided an index of buffering properties.

3.2.2 Viscosity Measurements

Flow profiles of processed non-fat milks with added solids (control), ultrafiltered (UF) and diafiltered (DF) samples as well as reconstituted non-fat milk solids (MSNF) adjusted to different solids level (12, 14, and 16%) were measured according to the method described by Wayne and Shoemaker (1988). All samples were tested in quadruplicate at 20° C. To ensure homogeneity of samples, a standard agitation procedure was followed, before each sample was drawn from a glass container to the rheometer's concentric cylinder fixture. A rotational Bohlin VOR rheom-

eter was used for the measurements of viscosity. Steady shear viscosity measurement (flow curves) were made over the range of 116-924 sec^{-1} . Viscosity measurements were carried out in the upward direction (i.e. increasing shear rate).

3.2.3 Starter Activity Tests

Changes in pH and titratable acidity (% TA) were determined (AOAC, 1980) using a 2% starter culture of S. thermophilus and L. bulgaricus (Hansen CH-3 strain; Hansen's Laboratory, Inc., Milwaukee, Wi) for all milk samples pasteurized at 62^0 C for 30 min. The pH and titratable acidity were determined during fermentation and were performed concurrently with the rheological testing of the inoculated samples using the Bohlin Rheometer. The pH measurements during the fermentation process were carried out at all three different solids level (12, 14, and 16%) tested in order to ensure proper starter activity. The pH was continuously monitored using a glass electrode (Radiometer pHM 28) at hourly intervals until a pH of about 4.4 was attained. Titratable acidity was determined using 0.1N NaOH and a few drops of phenolphthalein indicator (1% in ethanol) and results were reported as % lactic acid (AOAC, 1980).

3.2.4 Dynamic Rheological Testing

Rheological measurements were performed according to the method of Bohlin et al. (1984) using a Bohlin VOR Rheometer with a concentric cylinder geometry; cup and inner rotor diameters were 27.5 and 25 mm, respectively. Milk samples were first pasteurized at 62^0 C for 30 min and cooled to 40^0 C. A mixed starter culture (2, 3, and 4% level) of S.

thermophilus and L. bulgaricus (1:1) was used to inoculate portions of 100 ml milk. Approximately 11 ml of inoculated sample was pipetted immediately into the rheometer's sample holder (the outer cup of the concentric cylinder geometry). The desired incubation temperature (39, 42 and 45° C) was adjusted prior to each experiment. The inner cylinder was then lowered into the outer cup containing the milk sample. A few drops of liquid paraffin oil were added at the top of the solution to prevent evaporation during the 5-6 h fermentation process.

The dynamic rheological testing adopted in these studies provided a continuous monitoring of the viscoelastic properties of milk during fermentation without affecting the gel structure. Measurements were carried out at 1.0 Hz, and 1.8 % strain and data were collected at 5 min intervals. The gelation experiments were performed in duplicate for the 3% starter culture level and at all three temperatures of incubation tested.

The rheological parameters of storage and loss moduli (G' and G'' respectively) and tan δ were calculated using the data analysis program of the Bohlin rheometer. Coagulation kinetics were assessed by a similar method to that described by Olson and Bottazzi (1977) and apparent first-order rate constants, K, were calculated according to Avery (1974) using the expression:

$$K_r = \frac{2.303}{t} \log_{10} \left(\frac{G\infty - G_0}{G\infty - G_t} \right) \qquad (1)$$

or

$$\log_{10}(G\infty - G_t) = \log_{10}(G\infty - G_0) - \frac{K_r}{2.303} * t \qquad (2)$$

Where K_r is the rate constant
$G\infty$ = plateau modulus value upon completion of the reaction
G_0 = initial reaction modulus value (onset of gelation)
G_t = modulus at time t

3.2.5 Acetaldehyde and pH determination

A rapid quantitative method was employed for the determination of acetaldehyde in yogurt samples during storage. This method is an acetaldehyde-dye (3-methyl-2-benzothiazolone hydrazone) binding technique (Lindsay and Day, 1965) and tests were performed in duplicate after 1, 7, and 14 days of storage (4° C) using 3.0 g of control milk yogurt and 2.5 g of ultrafiltered milk yogurt samples. The amount of acetaldehyde was calculated using a standard curve obtained by adding various amounts of acetaldehyde directly to the collection reagent vessel and followed by the regular method for development of color. Absorbance readings of the acetaldehyde-dye complex were made at 666 nm. In the determination of acetaldehyde, it was observed that the oxidation time with ferric chloride (i.e. exactly 25 min) was very critical in obtaining reproducible data. The pH was also measured for all stored samples according to the AOAC (1980) standard method.

3.2.5.1 Chemical Analysis of Organic Acids

A High Performance Liquid Chromatographic (HPLC) method was employed for the quantitative determination of various organic acids present in yogurt samples. Using a slightly modified procedure to that reported by Marsili et al. (1981), organic acid analyses were carried out during fermentation of milk as well as for yogurt samples stored at 4° C for

1-14 days. The analysis was performed using a Water Associates Chromatograph (Milkford, MA) equiped with a M-45 solvent delivery system, a U6K injector and a model 440 UV detector operated at 214 nm. The system was interfaced to a VISTA data station (Varian 401) for data acquisition and peak area integration. All samples (20 μl injection volume) were run isocratically at a flow rate of 0.6 ml min^{-1} using filtered and degassed mobile phase (0.006N H_2SO_4 in distilled water) as an eluent through an Aminex HPX-87H (300 x 7.8 mm) column (Bio-Rad Labs., Richmond, CA) at 69^0 C in conjunction with a guard column. The column contained a strong cation exchange resin (8% crosslinked and 9 μm diameter) which separates organic acids by ion exchange and partition chromatography. Column calibration was carried out with standard solutions of known concentration (see Appendix 2) of orotic, citric, pyruvic, lactic, acetic and propionic acids; a typical chromatogram of the standard mixture of organic acids is shown in Appendix 3. The mobile phase was filtered through a 0.5 μ filter and degassed for 10 min under vacuum. Prior to sample injection, a standard cleanup method was performed by passing the sample solution through a 0.45 μ HV Millipore filter (Millipore Corp., Bedford, MA). Acetonitrile was used as the protein denaturant/solvent (see section 3.2.5.2).

3.2.5.2 Sample Preparation

Three types of milk samples were monitored for organic acid composition during fermentation and storage of yogurt: control, ultrafiltered and diafiltered milk samples at 14% solids. The organic acid profile was probed during fermentation for a 6 h period incubated with a mixed

starter culture of S. thermophilus and L. bulgaricus (1:1, 2%) at 42° C. A portion of these samples was collected at each hour during fermentation and after immediate freezing with liquid nitrogen it was kept at -18° C to cease the growth of bacteria. Sample preparation for organic acid analysis was carried out according to Marsili et al. (1981). The frozen samples of yogurt (5 g) were rapidly thawed by the addition of a mixture of acetonitrile/distilled water (4:1, 25 ml) and subsequently analyzed by HPLC, as described in section 3.2.5.1. The same procedure was employed in order to determine changes of organic acid content in control and ultrafiltered milk-based yogurts at 12, 14, and 16% solids level during various storage periods (1, 7, and 14 d at 4° C).

3.2.6 Sensory Analysis

Quantitative descriptive analysis (QDA), was chosen as the sensory method to assess yogurt samples made from different types of milk with various solids levels during two weeks of storage. Two types of experiments were carried out using a method developed by Stone et al. (1974) which uses a trained panel to produce data that can be quantified and provide a description of the products being developed. The purpose of the first type of evaluation was to determine any significant differences in thickness, chalkiness and sourness between control and ultrafiltered samples at a particular solid level. The second evaluation was designed to determine significant differences between the control and ultrafiltered samples at all solids level stored for 1, 7 and 14 days at 4° C.

3.2.6.1 Sample Preparation

In order to standardize the experimental yogurts, either non-fat milk powder or whey was used, wherever necessary, to yield to give 12, 14 and 16 per cent total solids in the final mix. Mixing was done using the Pearson square method (Appendix 1). The control and ultrafiltered milks were then pasteurized at 62° C for 30 min and, after cooling to 40° C, they were inoculated with the appropriate starter culture (S. thermophilus: L. bulgaricus 1:1) at a level of 2 per cent v/v. The mix was then dispensed into 28 ml plastic cups, capped and coded with three digit random numbers; the cups were incubated at 42° C for 5 to 6 h or until the pH reached at approximately 4.40. Samples were made about 30 hours prior to testing and held at 4° C.

3.2.6.2 Panel Training

Seven panelists were first trained for texture evaluation and then flavour during eleven sessions of approximately 45 minutes in duration. A 15-cm line scale with marks at each end and also in the middle for some parameters was used in this study and adapted from the original method of Stone et al. (1974); descriptors at these points were used. The panelists placed a vertical line across the horizontal line at the point at which they perceived the intensity of the parameter to be. Appendix 4 shows the ballot used in this study as emerged after training sessions. Panelists participated willingly in the evaluation of the yogurt samples. Four initial training sessions were carried out to familiarize the panelists with the yogurt products with respect to their basic tastes and the texture and flavour characteristics. Triangle

tests were used to determine the panelists' ability to detect differences in sourness levels of yogurt. In order to establish appropriate sensory parameters, experimental yogurt samples were tested and described by the panelists after a discussion with an administrator who was not involved in product testing. Both references and actual samples that were to be presented during the final test were used to evaluate their end-point descriptors and in some cases midpoints for assessing the range that would be encountered throughout the test of the samples. The panel also agreed to use a reference sample for sourness which was placed at "moderate sour" on the line so that scoring of test samples would be more consistent.

3.2.6.3 Final Tests

Six different yogurt samples were included in the experiment at each time: all samples were tested at least after 1 day of storage at which time they have reached a pH of about 4.2. Evaluation of one replicate was completed in the morning and a second replicate was completed in the afternoon of the same day. Two batches of yogurt treatments were made. Test dates were 1, 7 and 14 days of storage at 4^{0} C. Therefore, there were a total of six test days. The panel received six randomly coded yogurt samples served at refrigeration temperature in 28 ml plastic cups coded with three digit random numbers. Samples were presented to each panelist in a random order to prevent the panelists from being biased. The reference sample marked "S" was 90 g of a commercial Skim Milk Yogurt (0.05% BF) mixed with 60 g of 1% milk and corresponded to "moderate sour" on the line scale, as agreed upon by the panelists. All ref-

erence samples were made fresh before every replicate testing and had a pH of 4.25. Panelists were asked to evaluate the samples after having a cracker and a rinse with distilled water between samples. Evaluation of samples took place in individual booths in a sensory panel room equipped with white fluoresecent bulbs as a source of light.

3.2.6.4 Statistical Analysis

In order to analyze the data, a 60 point grid, i.e. a number from 1 to 60, was assigned to the panelists evaluations by superimposing a grid over the 15-cm line scale. Sensory data were analyzed statistically by analysis of variance, and differences among treatments and replicate means were determined by the Duncan's Multiple Comparison test. The effect of storage time was assessed by analyzing the differences between the treatment means at days 1, 7 and 14. Taste panel data were analyzed by the standard analysis of variance procedures using PROC GLM (General Linear Model) of the Statistical Analysis System (SAS, 1986).

Chapter IV

RESULTS AND DISCUSSION

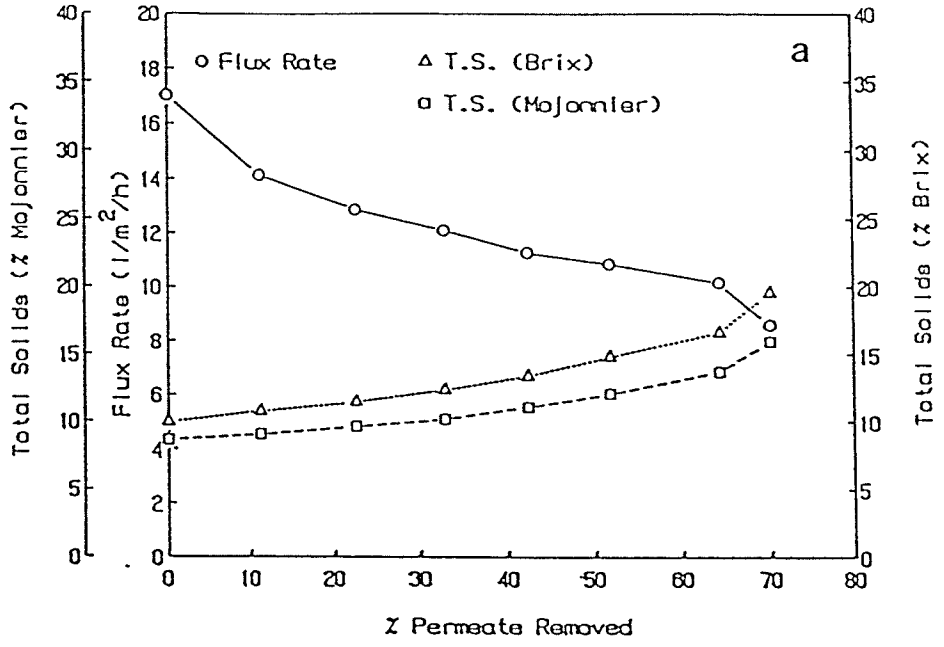
4.1 Processing Effects on Skim Milk Concentrated by UF and DF

4.1.1 Effects on solids level and flux rate

The effects of membrane processing of skim milk on the flux rate, total solids and composition of retentates are shown in Figures 11 and 12 for the ultrafiltration and diafiltration experiments respectively. The values are means of three replicates; the corresponding numerical data are summarized in Appendices 5-8. The composition of the permeates obtained during UF and DF is given in Table 10. The UF experiments were aimed at providing skim milk retentates with solids level of 12, 14 and 16% to be used for subsequent studies on yogurt production.

For the ultrafiltration experiments, concentration was continued until the solids level in the retentate reached approximately 16%, measured by the mojonnier method. This corresponds to about 20% solids determined by refractometry. At this point, the flux rate of the permeate decreased to about 8.6 l/m^2 /h. The decline in flux rate (Figure 11a, Appendix 5) is indicative of membrane fouling, presumably due to deposition of protein on the membrane surface. Similar trends in flux rates were also observed for the diafiltration experiments (Figure 12a, Appendix 7).

Figure 11. Changes in flux rate and solids (a) and composition (b) of milk during ultrafiltration through a diaflow hollow fiber membrane (10,000 m.w. cut-off).


o Flux Rate
Δ T.S. (Brix)
□ T.S. (Mojonnier)
a
Total Solids (% Mojonnier)
Flux Rate (l/m²/h)
Total Solids (% Brix)
% Permeate Removed

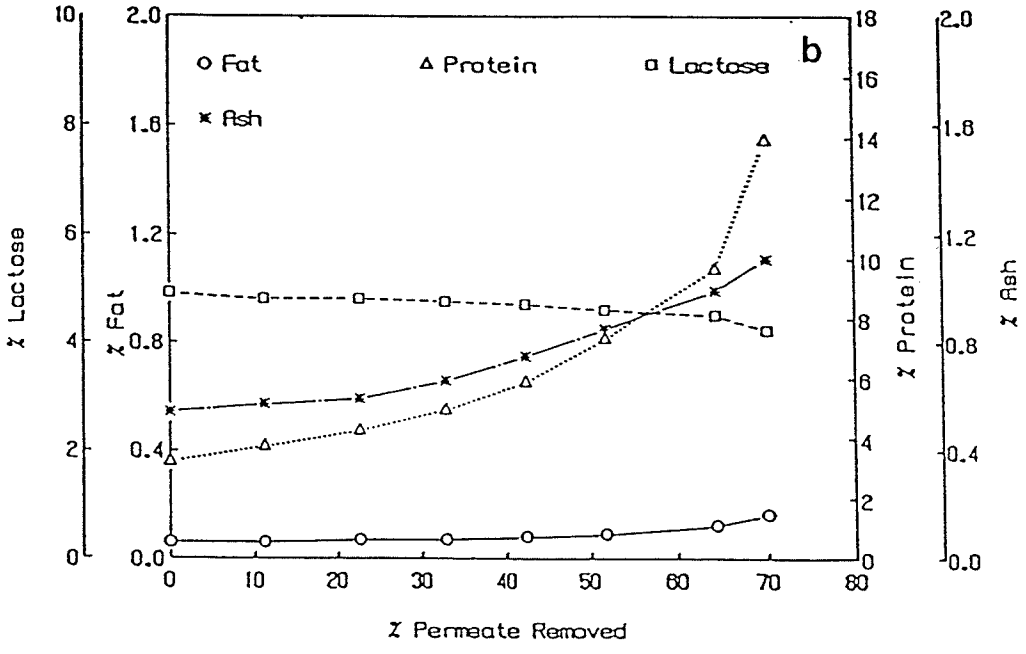
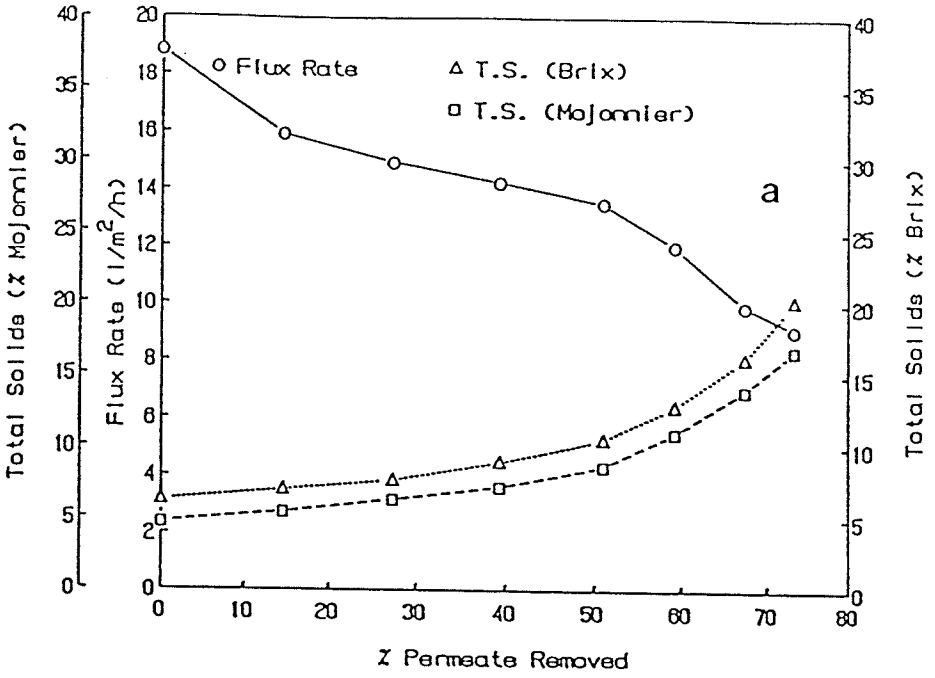
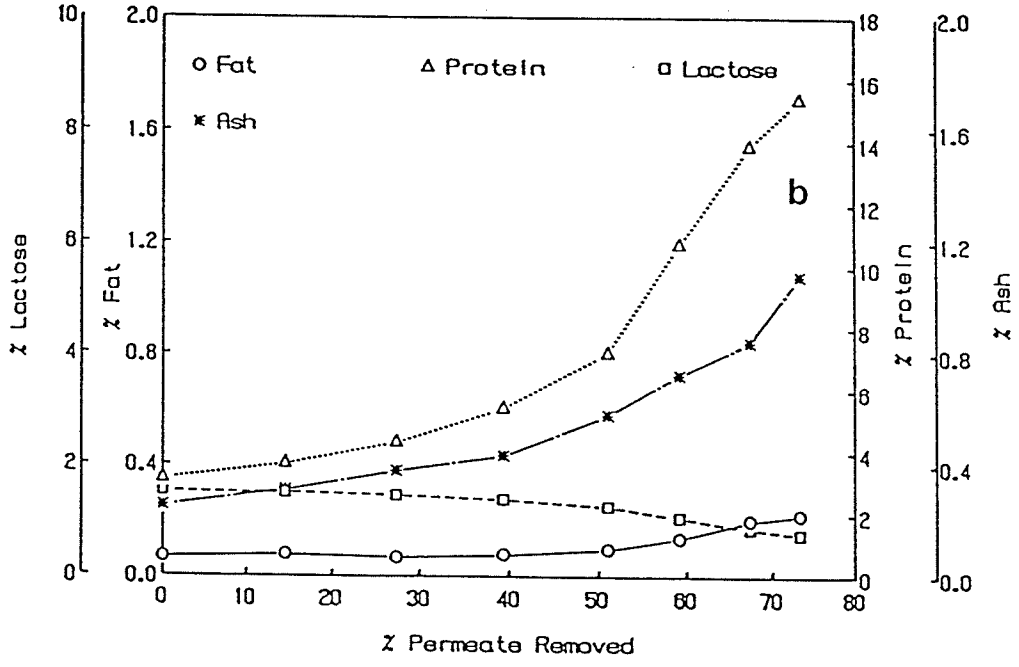

o Fat
Δ Protein
□ Lactose
* Ash
b
% Lactose
% Fat
% Protein
% Ash
% Permeate Removed

Figure 12. Changes in flux rate and solids (a) and composition (b) of skim milk during diafiltration through a diaflow hollow fiber membrane (10,000 m.w. cut off).


o Flux Rate
Δ T.S. (Brix)
□ T.S. (Mojonnier)
a
Total Solids (% Mojonnier)
Flux Rate (l/m²/h)
Total Solids (% Brix)
% Permeate Removed


o Fat
Δ Protein
□ Lactose
* Ash
b
% Lactose
% Fat
% Protein
% Ash
% Permeate Removed

Because of the dilution effects, the initial flux rate of DF (Figure 12a, Appendix 7) was higher than that of direct ultrafiltration (Figure 11a, Appendix 5). At the conclusion of DF (solids level 16.8%, by Mojonnier, or 20.3%, by Brix) the flux rate was still slightly higher than the respective value of the UF experiment (9.1 vs. 8.6 $l/m^2/h$).

4.1.2 Effects on composition

Changes in the composition of retentates upon UF and DF of skim milk are shown in Figure 11b and Appendix 6, and Figure 12b and Appendix 8, respectively. The protein content during UF increased from 3.25 to 13.91%, whereas the corresponding changes upon DF increased from 3.12 to 15.4%. Lactose levels decreased from 4.9 to 4.2% (Figure 11b) during UF and were further reduced during DF to 0.75% (Figure 12b). Ash content in the DF retentates remained at approximately the same level as in the UF retentates, confirming the findings of Covacevich and Kosikowski (1977b) who used membranes with a molecular weight cut-off of 20,000. The slight increases in ash content of the membrane retentates are a consequence of retention of proteins and their associated salts, mainly calcium and phosphorus. Slight increases in the fat content of retentates were also observed during the UF (from 0.06 to 0.16%) and DF (from 0.07 to 0.22%) experiments (Figures 11b and 12b).

The composition of permeates obtained by UF and DF is given in Table 10. The fat level ranged between 0.05 and 0.12% in UF and DF permeates, while the protein content did not change markedly during processing, the

Table 10. Composition of permeate obtained by ultrafiltration & diafiltration using a diaflow hollow fiber membrane (10,000 m.w. cut off; 25° C; Inlet press. 20 psi).

Time (min)	Ultrafiltration Fat (%)	Protein (%)	Lactose (%)	Diafiltration Fat (%)	Protein (%)	Lactose (%)
0	0.05(0.02)[a]	0.12(0.01)	1.93(0.11)	0.04(0.02)	0.10(0.07)	2.04(1.20)
20	0.09(0.03)	0.25(0.02)	5.11(0.11)	0.03(0.02)	0.07(0.00)	1.71(0.45)
40	0.08(0.04)	0.24(0.01)	5.15(0.15)	0.05(0.00)	0.05(0.02)	1.71(0.46)
60	0.08(0.04)	0.23(0.04)	5.18(0.20)	0.07(0.04)	0.11(0.07)	1.68(0.51)
80	0.10(0.02)	0.23(0.05)	5.21(0.24)	0.08(0.04)	0.14(0.09)	1.69(0.53)
100	0.10(0.01)	0.25(0.05)	5.38(0.30)	0.09(0.06)	0.16(0.10)	1.73(0.56)
120	0.10(0.02)	0.28(0.01)	5.50(0.26)	0.10(0.04)	0.18(0.09)	2.28(1.00)
140	0.12(0.01)	0.30(0.01)	5.75(0.10)	0.12(0.04)	0.20(0.07)	2.16(0.54)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

UF permeates had slightly higher amounts of protein than their DF counterparts. The lactose content of UF permeates readily increased during the first 20 min of processing due to the rejection of this solute by the membrane. The lactose levels in DF permeates were much lower due to initial dilution of the UF retentate. The reduction in lactose content of membrane retentates is of great importance since this constituent is the major substrate for microbial fermentation in milk. However, it is noteworthy that only 20-30% of the total lactose in milk is utilized by lactic acid bacteria (Greig and Harris, 1983). On the other hand, high levels of lactose could result in crystallization of this sugar and, therefore, affect adversely the texture of yogurt.

4.2 Composition of Samples Used for Yogurt Making

Four types of milk samples representing three solids (mojonnier) level (12, 14 and 16%) was prepared by either membrane concentration or reconstitution were used for yogurt making. To characterize the control, ultrafiltered, diafiltered and non-fat milk solids their proximate analysis, pH, titratable acidity and total solids were determined and the data are presented in Table 11. As expected, the protein contents of diafiltered samples were higher than those of ultrafiltered milk at equivalent solids level. Some variation for fat and ash content was observed among the samples. Diafiltration resulted in retentates of slightly lower ash content than ultrafiltration, in agreement with the

Table 11. Proximate analysis, pH, titratable acidity (T.A.) and total solids (T.S.) of milk samples used for yogurt production with a mixed starter culture (S.C.).

Sample	T.S. (%)	Ash (%)	Fat (%)	Protein (%)	Lactose (%)	pH	T.A. (%)
Control	12.16[a] (0.08)	0.80 (0.02)	0.14 (0.02)	4.33 (0.15)	6.67 (0.11)	6.45 (0.07)	0.19 (0.01)
UF[b]	12.47 (0.14)	0.87 (0.09)	0.12 (0.02)	6.54 (0.38)	4.50 (0.17)	6.60 (0.01)	0.17 (0.01)
DF[c]	11.94 (0.27)	0.83 (0.01)	0.11 (0.01)	9.95 (0.28)	1.80 (0.03)	6.65 (0.07)	0.18 (0.01)
MSNF[d]	11.98 (0.12)	0.84 (0.05)	0.09 (0.01)	4.65 (0.01)	6.87 (0.01)	6.35 (0.07)	0.21 (0.01)
Control	13.96 (0.09)	0.89 (0.03)	0.10 (0.02)	5.28 (0.03)	7.32 (0.31)	6.45 (0.07)	0.19 (0.01)
UF	13.99 (0.26)	0.94 (0.05)	0.29 (0.09)	9.67 (0.56)	4.34 (0.03)	6.60 (0.00)	0.21 (0.01)
DF	13.57 (0.60)	0.88 (0.02)	0.16 (0.03)	12.16 (0.96)	1.16 (0.09)	6.55 (0.07)	0.19 (0.07)
MSNF	14.01 (0.24)	0.93 (0.09)	0.13 (0.04)	5.52 (0.24)	7.83 (0.42)	6.40 (0.01)	0.21 (0.10)
control	16.01 (0.13)	1.04 (0.10)	0.07 (0.02)	6.02 (0.08)	7.30 (0.33)	6.40 (0.00)	0.22 (0.07)
UF	16.65 (0.91)	1.13 (0.12)	0.24 (0.01)	14.52 (0.03)	4.29 (0.05)	6.65 (0.07)	0.23 (0.07)
DF	16.13 (0.02)	1.03 (0.15)	0.24 (0.03)	16.83 (0.16)	0.58 (0.05)	6.60 (0.01)	0.21 (0.01)
MSNF	16.51 (0.07)	1.14 (0.05)	0.13 (0.01)	6.70 (0.18)	7.77 (0.32)	6.40 (0.00)	0.24 (0.07)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

[b] = ultrafiltered; [c] = diafiltered; [d] = non-fat milk solids.

results of Rash and Kosikowski (1982). The ash contents of UF samples was also higher than the control milk suggesting that UF is an effective means of increasing the total mineral content of yogurt.

Lactose in the UF samples varied from 4.29 to 4.50%, while for both control and reconstituted samples the lactose content ranged between 6.67-7.83%. Considerably lower levels of lactose (1.8-0.58%) were found in diafiltered milk. There were no apparent differences among samples with regard to titratable acidity, whereas the membrane concentrated milks had slightly higher pH than control and reconstituted milk samples.

Elemental composition of the milk preparations used for yogurt production is shown in Table 12. Sodium in ultrafiltered retentates was higher than diafiltered retentates and reconstituted milk samples. Lower amounts of potassium were found in DF retentates compared to any other type of milk. Magnesium levels in membrane processed milks were twice the content of this mineral in control and reconstituted non-fat milk solids. Upon ultrafiltration, there was a marked increase in the calcium and phosphorus content of milk. Diafiltration resulted in even higher retention of calcium compared to UF, while phosphorus was slightly reduced. The high levels of calcium and phosphorus in membrane retentates are a consequence of their association with the casein micelle complex. Similar results have been reported by Covacevich and Kosikowski (1977a), as shown in Table 7. The higher retention of calcium in UF and DF milk based yogurt has direct implications on the nutritional value of this product as well as on its rheological and sensory attributes.

Table 12. Mineral analysis of milk samples used for yogurt production with a mixed starter culture.

	Sodium (%)			Potassium (%)			Calcium (%)			Magnesium (%)			Phosphorus (%)		
T.S.(%)	12	14	16	12	14	16	12	14	16	12	14	16	12	14	16
Control	0.06[a]	0.07	0.09	0.10	0.10	0.10	0.10	0.14	0.15	0.01	0.01	0.01	0.07	0.12	0.14
UF	0.16	0.18	0.21	0.15	0.13	0.19	0.27	0.35	0.44	0.02	0.02	0.03	0.15	0.21	0.26
DF	0.14	0.16	0.20	0.07	0.08	0.09	0.35	0.37	0.48	0.02	0.02	0.03	0.15	0.17	0.21
MSNF	0.07	0.11	0.14	0.12	0.15	0.24	0.14	0.18	0.26	0.01	0.01	0.02	0.08	0.11	0.16

[a] : mean of 2 replicates

4.3 Physicochemical Properties of Milk Samples

4.3.1 Buffering Capacity

The buffering capacity of the various milk samples is shown in Figures 13-15. In general, all UF and DF processed retentates exhibited greater requirements for acid to bring the pH from 6.5 to 4.3-4.5. In every case, there was an exponential relationship between pH and the amount of acid required to reduce the pH. These findings closely agree with the results obtained by Covacevich and Kosikowski (1979) as well as by Mistry and Kosikowski (1985). The increased buffering properties of membrane retentates compared to control milk and reconstituted non-fat milk solids are a direct consequence of the increased amounts of protein and other buffering constituents (e.g phosphates, citrate).

4.3.2 Flow Properties of Fluid Milk

The flow behaviour of the four types of processed fluid milk and membrane retentates under steady shear is shown in Figures 16-18, while the rheological data for these samples are given in detail in Appendices 9-11. The flow curves of most samples indicated linear responses between viscosity and shear rate, thus suggesting Newtonian behaviour of these fluids over the shear rate range of 116-924 s^{-1} . Slight shear thining properties were exhibited by UF and DF milks at 14 and 16% solids. Other rheological studies on fluid milks (Whitnah et al., 1956; Yamamoto et al., 1986; Wayne and Shoemaker, 1988) also suggested that fluid milk shows a slight pseudoplastic behaviour. The results of Wayne and Shoemaker (1988) on skim milk flow profiles over a concentration range of

Figure 13. Buffering capacity of processed skim milk (control), skim milk retentates (UF,DF) and non-fat milk solids (MSNF) at 12% total solids level (sample weight 10 g).

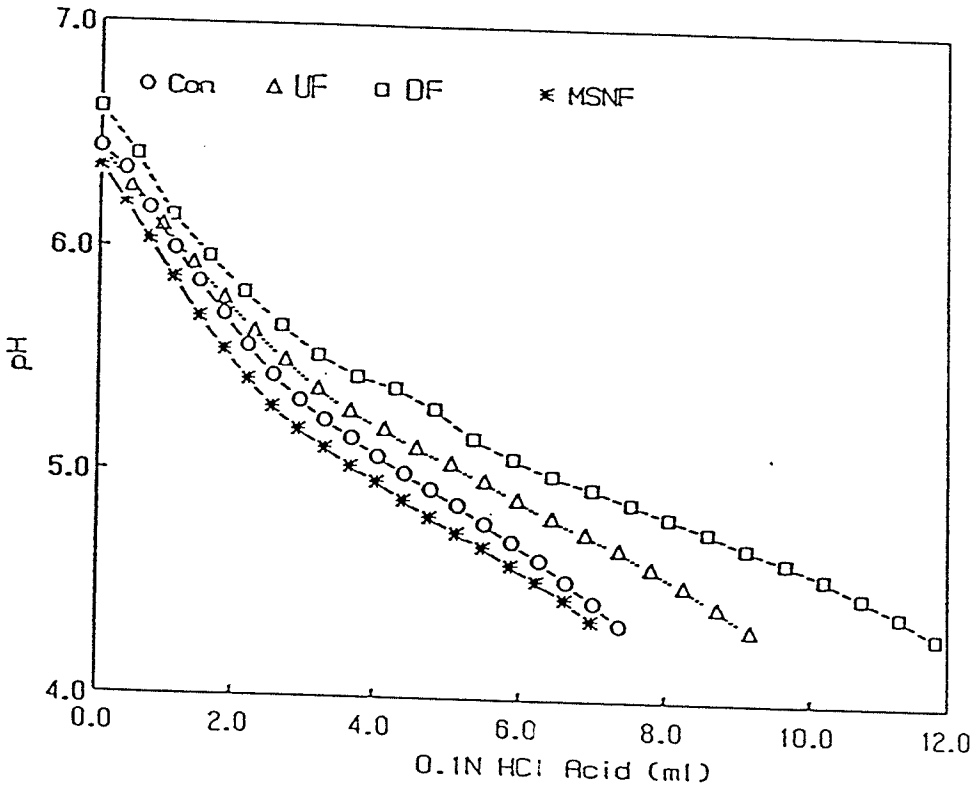
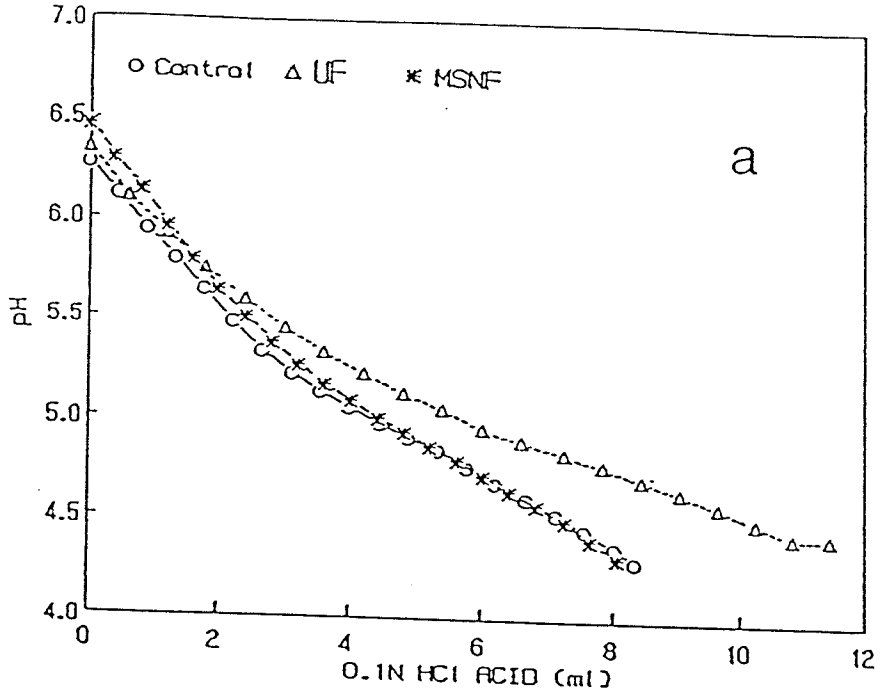

7.0
6.0
5.0
4.0
pH
o Con
Δ UF
□ DF
* MSNF
0.0
2.0
4.0
6.0
8.0
10.0
12.0
0.1N HCl Acid (ml)

Figure 14. Buffering capacity of processed skim milk (control), skim milk retentate (UF) and non-fat milk solids (MSNF) at 14% (a) and 16% (b) total solids level (sample weight 10 g).


o Control Δ UF * MSNF
a
pH
0.1N HCl ACID (ml)

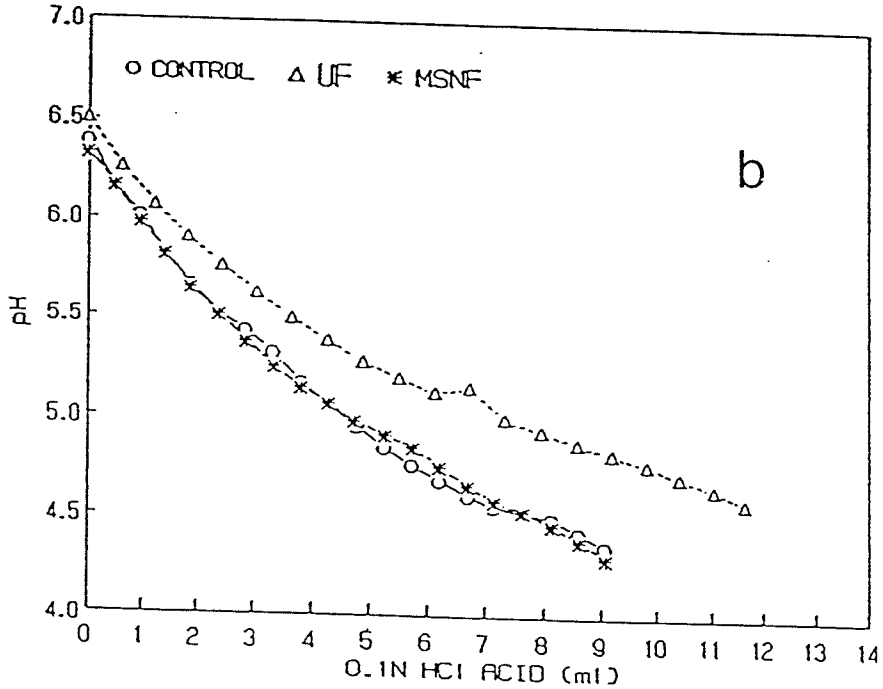
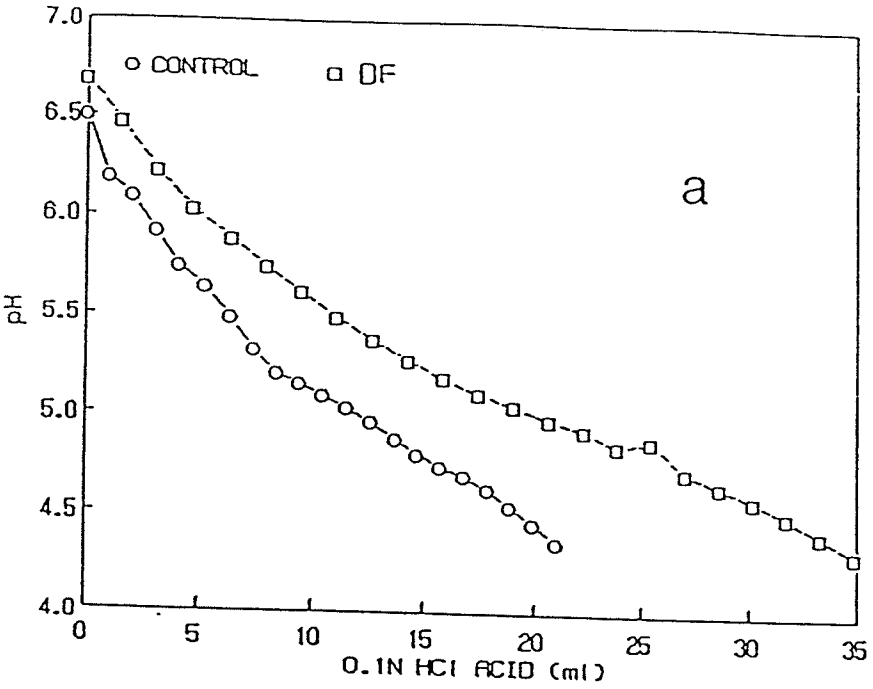

o CONTROL Δ UF * MSNF
b
pH
0.1N HCl ACID (ml)

Figure 15. Buffering capacity of processed skim milk (control) and diafiltered skim milk retentate (DF) at 12% (a) and 14% (b) total solids level (10 g sample diluted with 20 ml distilled water).


7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
o CONTROL
□ DF
a
0
5
10
15
20
25
30
35
0.1N HCl ACID (ml)

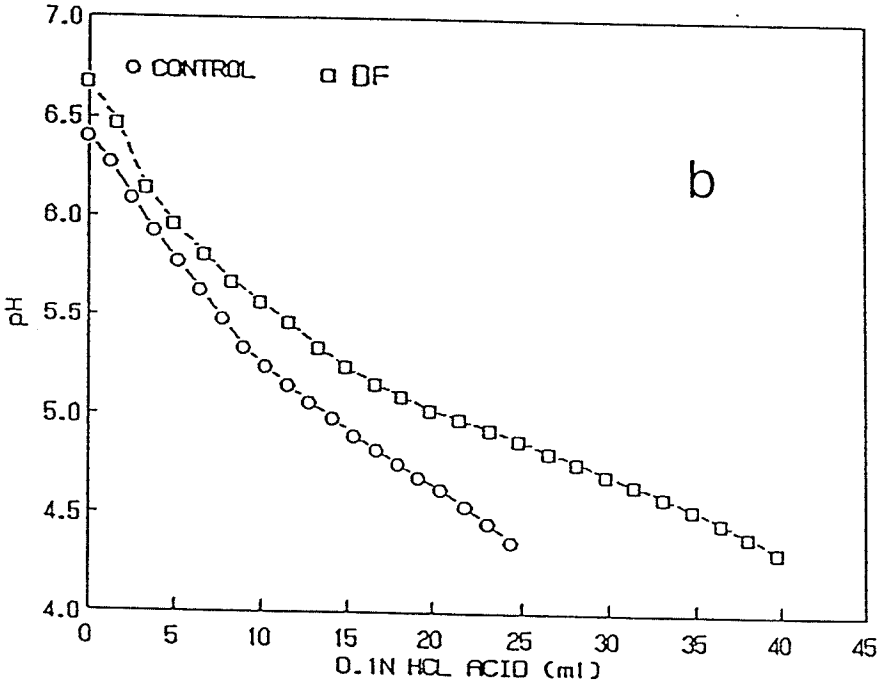

7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
o CONTROL
□ DF
b
0
5
10
15
20
25
30
35
40
45
0.1N HCL ACID (ml)

Figure 16. Viscosity (m.Pas) of processed fluid milk samples (12% total solids) at 20° C; UF and DF correspond to ultrafiltered and diafiltered retentates, respectively.

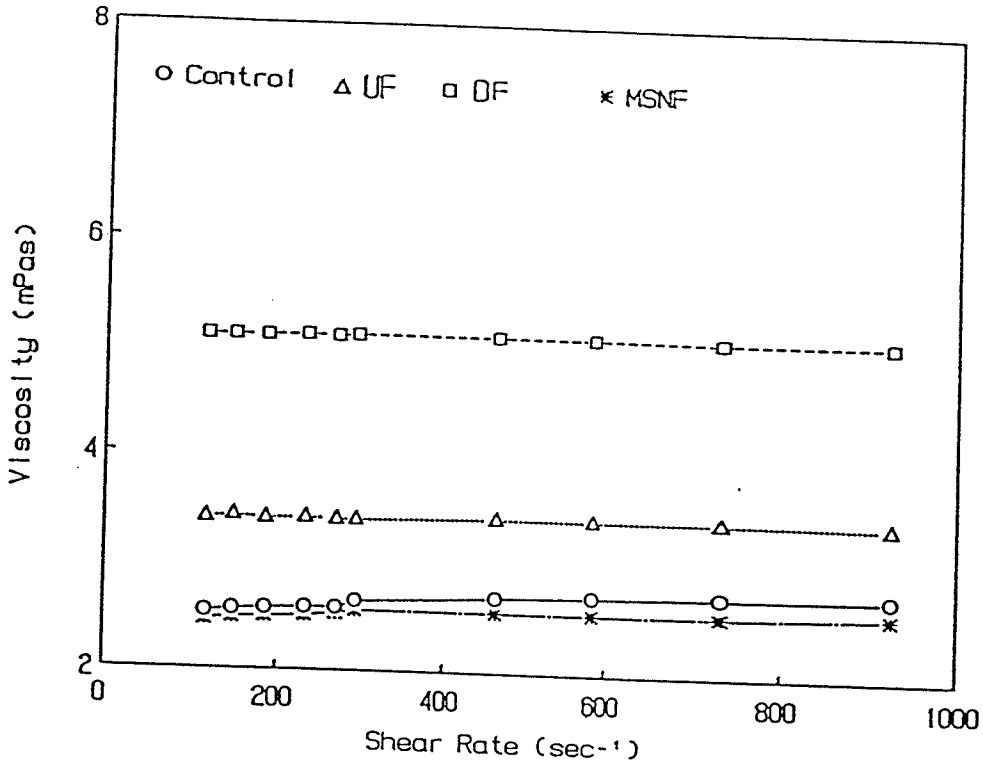

8
6
4
2
0
200
400
600
800
1000
Viscosity (mPas)
Shear Rate (sec-1)
o Control
Δ UF
□ DF
* MSNF

Figure 17. Viscosity (m.Pas) of processed fluid milk samples (14% total solids) at 20° C; UF and DF correspond to ultrafiltered and diafiltered retentates, respectively.

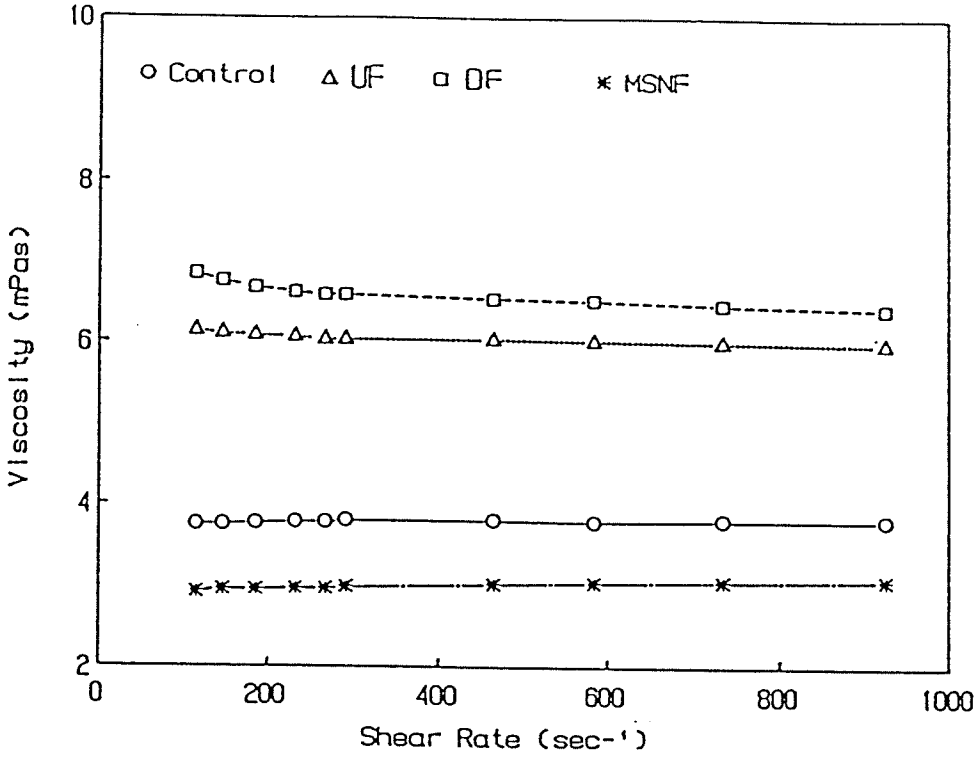
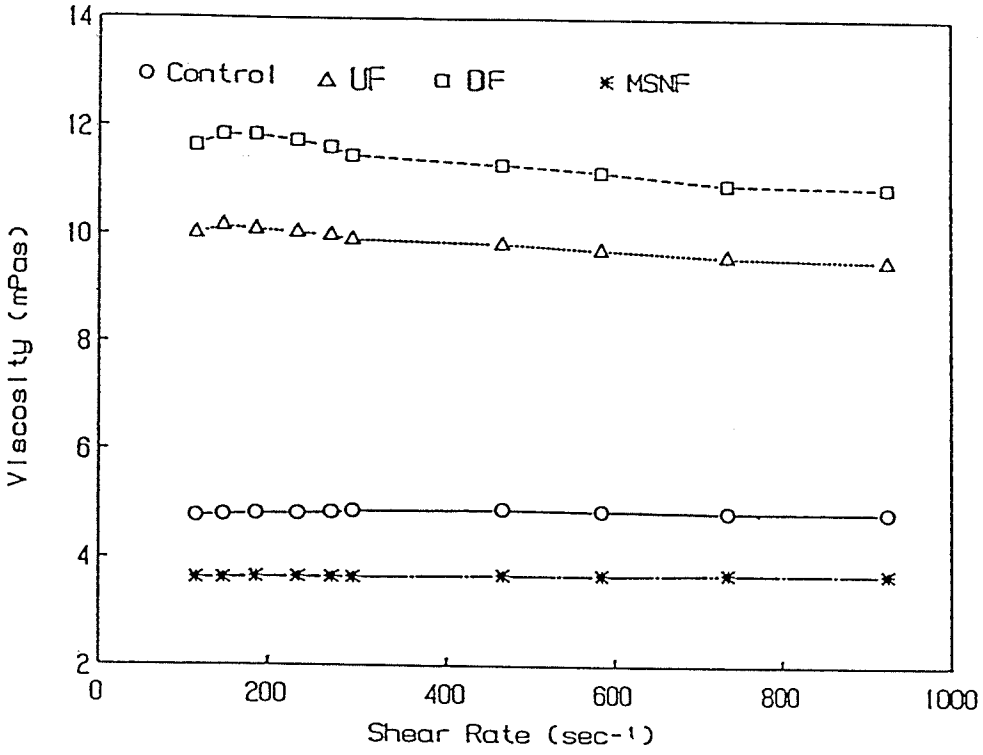

10
8
6
4
2
0
200
400
600
800
1000
Viscosity (mPas)
Shear Rate (sec-1)
o Control
Δ UF
□ DF
* MSNF

Figure 18. Viscosity (m.Pas) of processed fluid milk samples (16% total solids) at 20° C; UF and DF correspond to ultrafiltered and diafiltered retentates, respectively.


o Control
Δ UF
□ DF
* MSNF
Viscosity (mPas)
Shear Rate (sec-1)
14
12
10
8
6
4
2
0
200
400
600
800
1000

9.7 to 20.2% total solids revealed a Newtonian flow between 121-485 s^{-1} and are consistent with our findings (Figures 16-18). However, despite the linear relationships between shear stress and shear rate, the linear lines did not pass from zero; the occurence of small but not zero intercept was interpreted as reflecting shear thining dependence of apparent viscosity. The ability to measure the rheological behaviour differences and among samples over a broad range of shear rates, as adopted in the present study, is important when considering processing of these fluids in the milk industry.

4.4 Changes in Chemical and Physical Properties of Milk Upon Fermentation

4.4.1 Titratable acidity and pH

The pH and titratable acidity of milk during fermentation are related to the starter culture activity as well as the buffering properties of milk. The results of such measurements, as obtained during yogurt production of various milk samples, are given in Tables 13-15. The objective of this study was to monitor the fermentation progress in terms of pH and acidity development (% titratable acidity) by a mixed starter culture of S. thermophilus and L. bulgaricus at 42° C.

A period of 5h was required to attain a pH range 4.45-4.3 for control and non-fat milk solids. The corresponding pH values for UF and DF milk were 4.55 and 4.50 over the same time period. At the end of fermentation (5 h, 12% solids, Table 13) very little variation in titratable acidity was apparent among samples (1.04-1.14%). The amount of titratable acidity required to attain a pH range of 4.45-4.55 (i.e. 1.05-1.10)

Table 13. Changes in pH and titratable acidity (T.A.) during fermentation of milk samples (12% T.S.) with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Control		UF		DF		MSNF	
	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)
0	6.45[a] (0.1)	0.19 (0.0)	6.60 (0.0)	0.17 (0.0)	6.65 (0.1)	0.18 (0.0)	6.35 (0.1)	0.21 (0.0)
1	6.15 (0.1)	0.22 (0.0)	6.45 (0.1)	0.21 (0.0)	6.30 (0.0)	0.25 (0.1)	6.00 (0.0)	0.24 (0.1)
2	5.95 (0.1)	0.24 (0.0)	6.10 (0.1)	0.24 (0.1)	5.95 (0.1)	0.31 (0.1)	5.45 (0.1)	0.38 (0.1)
3	5.45 (0.1)	0.41 (0.0)	5.85 (0.1)	0.31 (0.1)	5.00 (0.1)	0.48 (0.0)	4.80 (0.0)	0.76 (0.0)
4	4.85 (0.1)	0.55 (0.1)	5.15 (0.1)	0.47 (0.0)	4.75 (0.1)	0.80 (0.0)	4.55 (0.1)	1.02 (0.1)
5	4.40 (0.0)	1.14 (0.1)	4.55 (0.1)	1.04 (0.1)	4.50 (0.1)	1.05 (0.0)	4.30 (0.0)	1.10 (0.1)

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 14. Changes in pH and titratable acidity (T.A.) during fermentation of milk samples (14% T.S.) with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Control		UF		DF		MSNF	
	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)
0	6.45[a] (0.1)	0.19 (0.1)	6.60 (0.0)	0.21 (0.0)	6.55 (0.1)	0.19 (0.1)	6.40 (0.0)	0.21 (0.0)
1	6.25 (0.1)	0.24 (0.1)	6.40 (0.0)	0.22 (0.1)	6.45 (0.1)	0.22 (0.1)	6.05 (0.1)	0.24 (0.1)
2	6.15 (0.1)	0.27 (0.0)	6.20 (0.0)	0.25 (0.1)	6.10 (0.0)	0.29 (0.0)	5.75 (0.1)	0.36 (0.0)
3	5.40 (0.0)	0.49 (0.0)	5.95 (0.1)	0.37 (0.0)	5.80 (0.0)	0.44 (0.1)	4.95 (0.1)	0.76 (0.0)
4	4.75 (0.1)	0.84 (0.1)	5.25 (0.1)	0.56 (0.0)	5.05 (0.1)	0.70 (0.1)	4.70 (0.0)	0.97 (0.0)
5	4.45 (0.0)	1.04 (0.1)	4.95 (0.1)	0.75 (0.0)	4.80 (0.0)	0.94 (0.0)	4.40 (0.0)	1.12 (0.1)
6	-----	-----	4.45 (0.1)	1.05 (0.1)	4.55 (0.1)	1.10 (0.1)	-----	-----

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 15. Changes in pH and titratable acidity (T.A.) during fermentation of milk samples (16% T.S.) with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Control		UF		DF		MSNF	
	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)
0	6.40[a] (0.0)	0.22 (0.1)	6.65 (0.1)	0.23 (0.1)	6.60 (0.0)	0.21 (0.1)	6.40 (0.0)	0.22 (0.1)
1	6.20 (0.0)	0.27 (0.0)	6.40 (0.0)	0.25 (0.1)	6.45 (0.1)	0.25 (0.1)	6.20 (0.0)	0.27 (0.1)
2	5.95 (0.1)	0.35 (0.0)	6.15 (0.1)	0.29 (0.1)	6.25 (0.1)	0.28 (0.0)	5.85 (0.1)	0.34 (0.0)
3	5.15 (0.1)	0.63 (0.1)	5.95 (0.1)	0.39 (0.0)	5.96 (0.1)	0.36 (0.1)	4.90 (0.0)	0.71 (0.0)
4	4.70 (0.0)	0.95 (0.1)	5.55 (0.1)	0.56 (0.0)	5.30 (0.0)	0.65 (0.0)	4.55 (0.1)	1.02 (0.1)
5	4.45 (0.1)	1.12 (0.1)	4.90 (0.0)	0.76 (0.0)	5.05 (0.1)	0.81 (0.0)	4.35 (0.1)	1.19 (0.0)
6	----	----	4.55 (0.1)	1.15 (0.0)	4.80 (0.0)	0.84 (0.0)	----	----

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

for the UF and DF samples was reached over a 6 h fermentation period at 14% solids level. With higher solids level milk retentates (16%) it was not possible to reach similar pH values under identical conditions of fermentation (temperature-time-starter culture level). The results in Table 15 clearly indicate that due to increased protein content of the 16% solids membrane retentates a longer time is required to lower the pH to 4.5. This behaviour is dependent on protein concentration and is in agreement with the findings of Covacevich and Kosikowski (1985). These researchers have reported that the higher buffering capacity of membrane-processed milks places higher demand on the starter culture to produce more lactic acid for pH reduction than normally found in fermented milk. In our study, the higher ash and protein contents of UF and DF samples did not appear to hinder the metabolic activity of the starter culture since lactic acid production was similar to those of control and MSNF milk (section 4.4.2).

4.4.2 Organic Acids

The organic acid composition of the various yogurt samples made from control, UF and DF milk (14% solids, starter culture 2%, 42° C) throughout the course of fermentation is presented in Figures 19-21; the corresponding data are given in Appendices 12-14. Organic acid analysis was performed by HPLC using acetonitrile as a denaturant and solvent; the solvent peak occured at about 6.5 min (Figure 22). A few unidentified peaks were also evident in the chromatograms of the yogurt samples; e.g a shoulder peak eluting after the lactic acid peak at about 13.2 min. Coelution of orotic and citric acid was observed at 65° C and a flow

Figure 19. Changes in organic acid composition of control milk (14% total solids) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

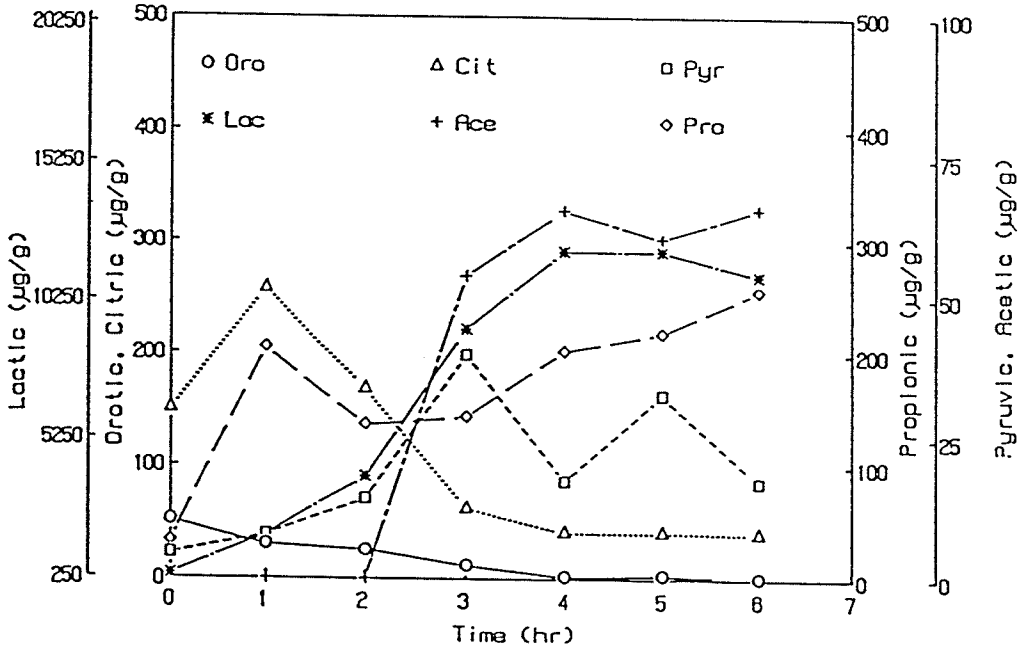

Oro
Cit
Pyr
Lac
Ace
Pro
Lactic (µg/g)
Orotic, Citric (µg/g)
Propionic (µg/g)
Pyruvic, Acetic (µg/g)
Time (hr)
20250
15250
10250
5250
250
500
400
300
200
100
0
500
400
300
200
100
0
100
75
50
25
0
0
1
2
3
4
5
6
7

Figure 20. Changes in organic acid composition of ultrafiltered milk (14% total solids) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

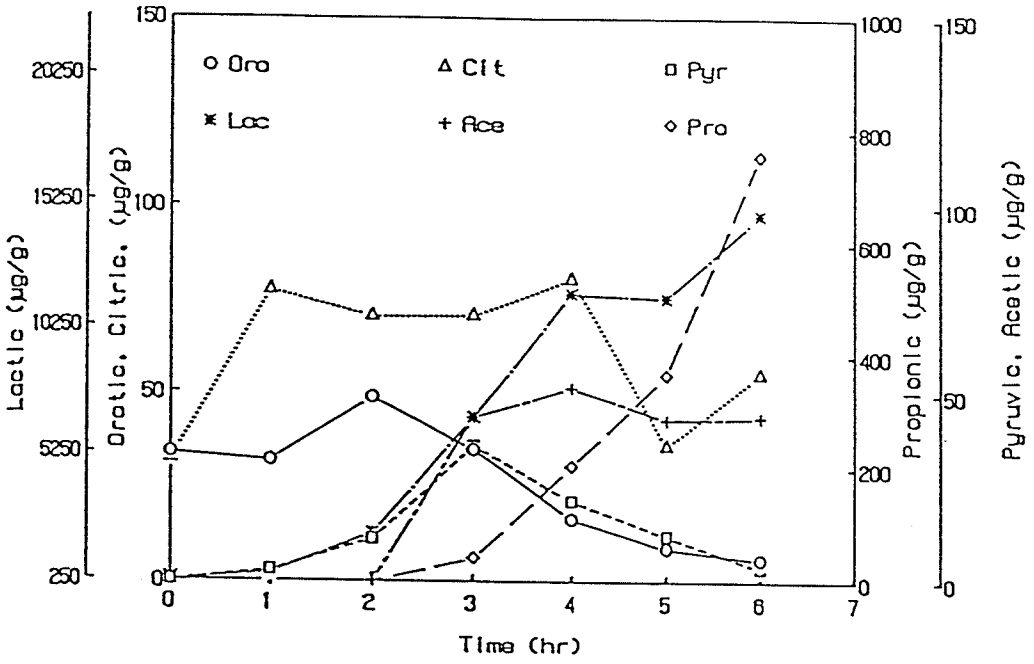

o Ora
Δ Cit
□ Pyr
* Lac
+ Ace
◇ Pro
Lactic (μg/g)
Orotic, Citric, (μg/g)
Propionic (μg/g)
Pyruvic, Acetic (μg/g)
Time (hr)
20250
15250
10250
5250
250
150
100
50
0
1000
800
600
400
200
0
0
1
2
3
4
5
6
7

Figure 21. Changes in organic acid composition of diafiltered milk (14% total solids) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

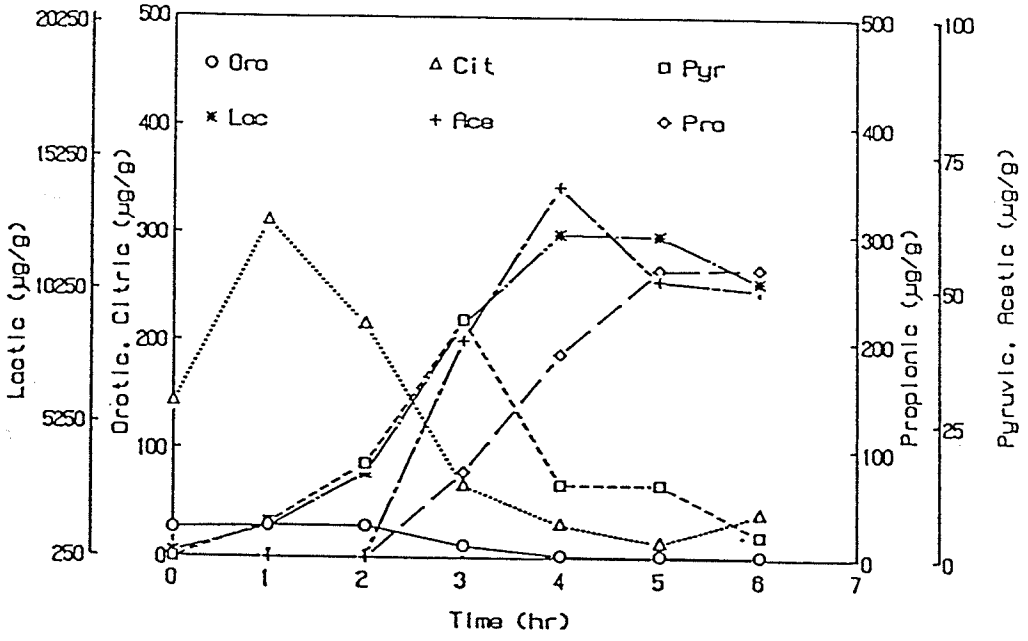
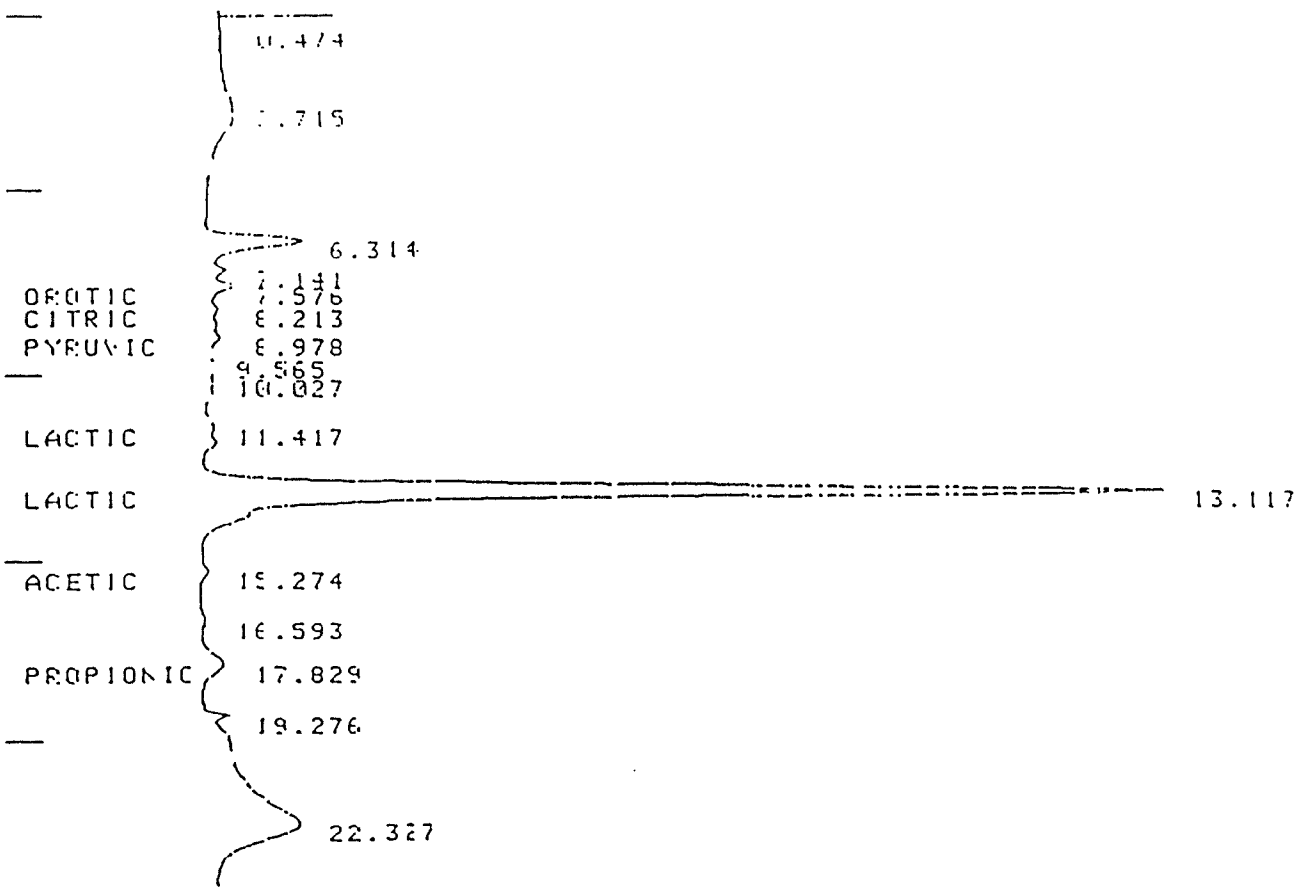

Oro
Cit
Pyr
Lac
Ace
Pro
Lactic (µg/g)
Orotic, Citric (µg/g)
Propionic (µg/g)
Pyruvic, Acetic (µg/g)
Time (hr)
20250
15250
10250
5250
250
500
400
300
200
100
0
75
50
25
0
1
2
3
4
5
6
7

Figure 22. High performance liquid chromatograhic profile of a representative yogurt sample (Control milk, 14% total solids, 2% starter culture, 42° C).


11.474
7.719
6.314
7.141
ORGTIC
CITRIC
PYRUVIC
7.576
8.213
8.978
9.565
10.027
LACTIC
11.417
LACTIC
13.117
ACETIC
15.274
16.593
PROPIONIC
17.829
19.276
22.327

rate of 0.7 ml/min. This difficulty was overcome by increasing the temperature to 69° C along with a reduction of flow rate to 0.6 ml/min. The results for pyruvic, lactic, acetic and propionic acids (Figure 19, Appendix 12) were comparable to those reported by Marsili et al. (1981) for a variety of cultured dairy products. However, citric and orotic acids were present in substantially lower amounts than the levels given by Marsili et al. (1981). During the course of fermentation, orotic and citric acid contents decreased as a result of bacterial activity, while the amounts of acetic and propionic acids remained relatively constant. As expected, high levels of lactic acid were found in the products at the end of the fermentation period; there have been similar concentrations of lactic acid between control and membrane-processed milk samples. The course of lactic acid production clearly followed the lag, growth and declining phases of lactic acid producing bacteria. When the growth phase of lactic acid fermentation occured (at about 2 h), there was a concurrent increase in the production of acetic acid. The latter remained relatively constant until the end of the fermentation experiments. Pyruvic acid levels reached a maximum in the middle of fermentation for all three samples (Figures 19-21). Although propionic acid fermentation commenced at the early stages of fermentation of control milk, there have been no detectable amounts of this acid for the UF and DF milks.

4.4.3 Structure Development of Milk Upon Fermentation

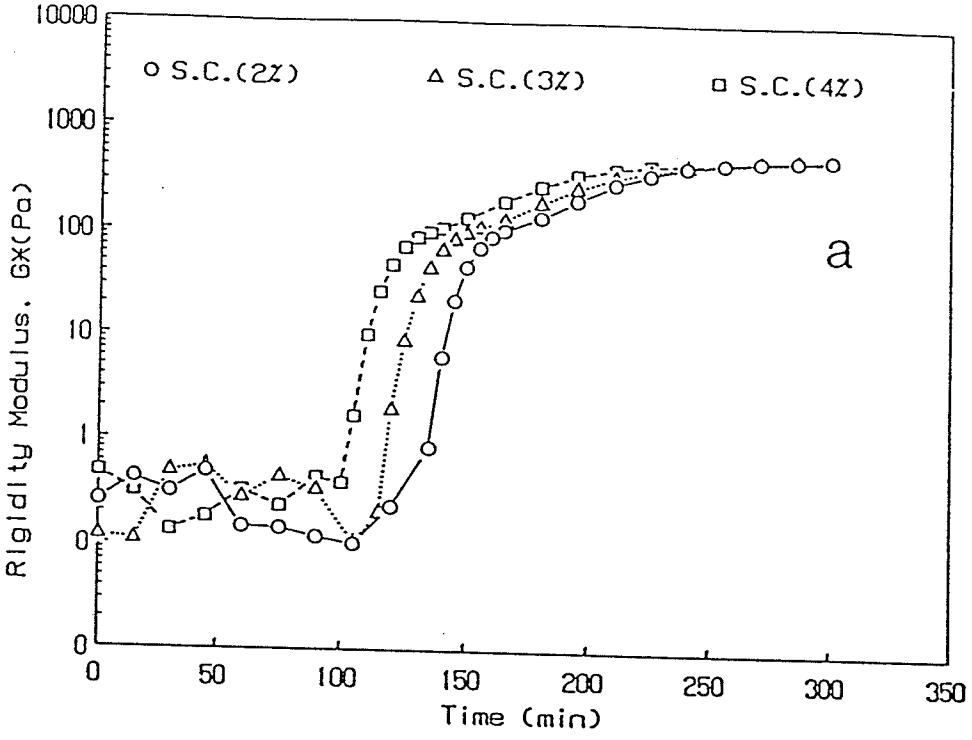
Small amplitude shear stress oscillatory testing is the rheological method of choice in studying the time-dependency of structure of food

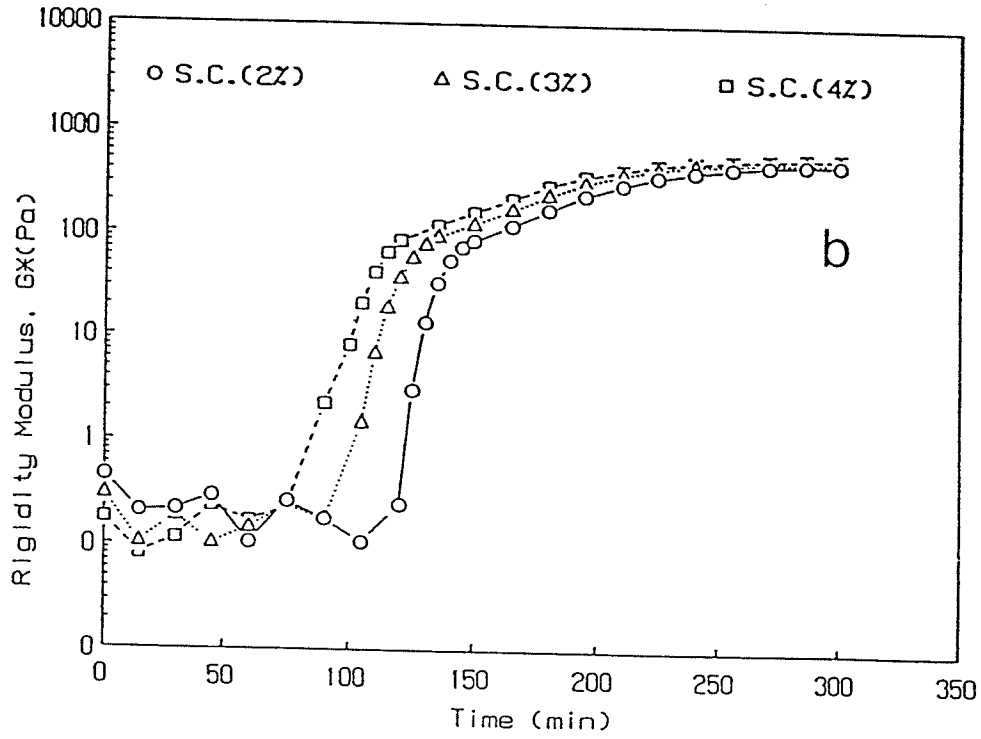
systems undergoing changes as a result of physicochemical and biological reactions, e.g. milk coagulation. While operating at a fixed frequency (1 Hz) this technique allows continuous monitoring of the viscoelastic properties of the system as a function of time. Dynamic rheological testing thus provides information on the storage (G') and loss (G'') moduli, dynamic viscosity ($\eta' = G''/\omega$), tan δ (= G''/G') as well as on the complex or rigidity modulus (G*). The relative contributions of the viscous and elastic components in the viscoelastic properties of a food material are expressed by the phase shift angle (δ) between the sinusoidal shear stress and shear strain waves. For example, the shear stress for a viscous fluid will be 90^0 out of phase with respect to strain wave, while for an elastic solid the phase angle is 0^0 . Viscoelastic materials on the other hand, have an intermediate phase shift angle.

4.4.3.1 Effect on Starter Culture Concentration

In the studies of the rheological behaviour of milk samples during fermentation, as probed by the complex modulus (G*), all samples (at 14% solids level) exhibited complex reaction kinetics during the initial stages or lag phase of clot formation. The time of onset of gelation at which gelation started decreased with increasing starter culture concentration whereas the slope of the rigidity modulus, G*, versus time varied. The onset of gelation differed with the concentration of culture as well as types of milk samples used for fermentation. Figure 23a shows the effect of starter culture on the rigidity modulus of control milk during the coagulation process. In this figure, the rigidity modulus starts to increase at about 135 min after inoculation of 2% starter

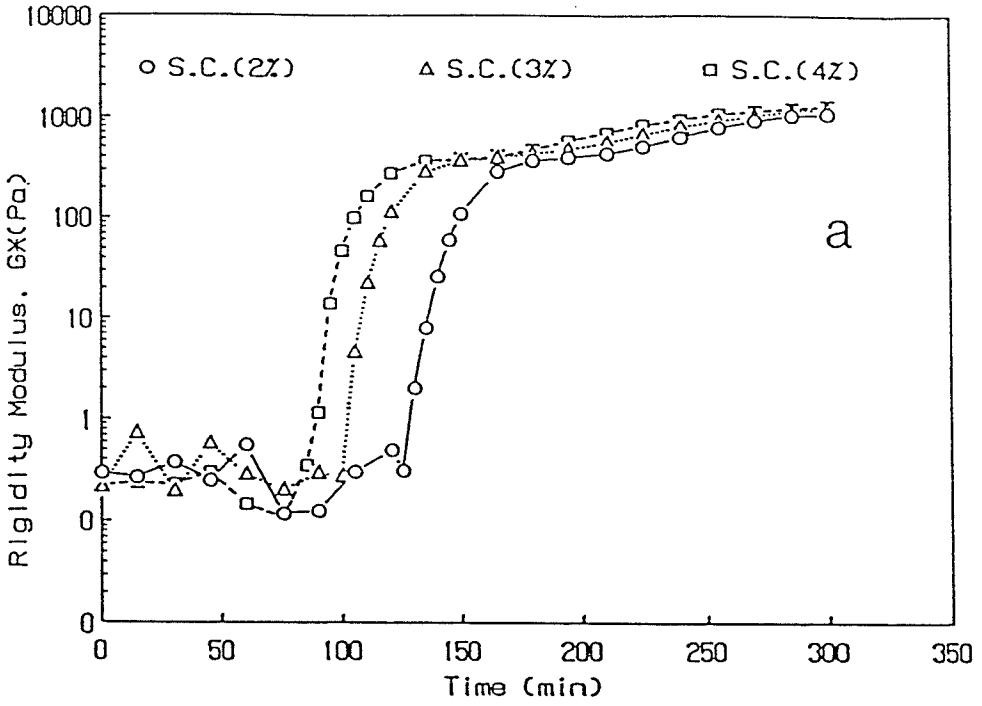
Figure 23. Rigidity modulus, G*, of milk samples (14% TS) fermented with a mixed starter culture (1:1, 42° C): a) Control; b) MSNF.

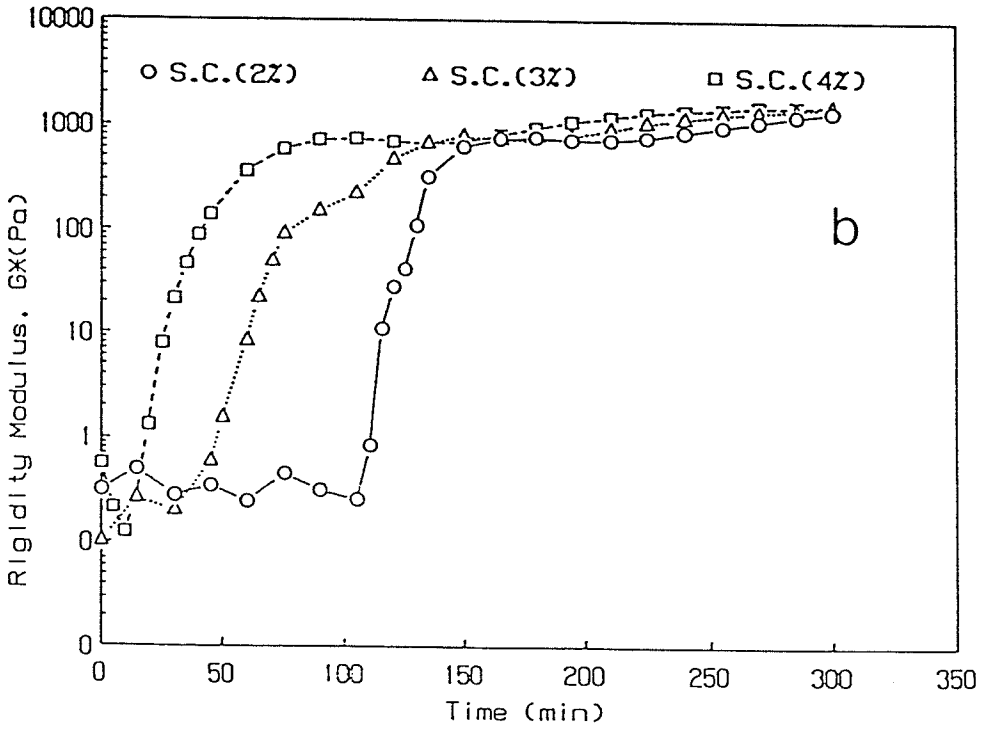

10000
1000
100
10
1
0
0
50
100
150
200
250
300
350
Rigidity Modulus, G*(Pa)
Time (min)
○ S.C.(2%)
△ S.C.(3%)
□ S.C.(4%)
a


10000
1000
100
10
1
0
0
50
100
150
200
250
300
350
Rigidity Modulus, G*(Pa)
Time (min)
○ S.C.(2%)
△ S.C.(3%)
□ S.C.(4%)
b

culture (S.C.), indicative of the onset of gelation (time to attain a G* value of 1 Pa). This phenomenon took place more rapidly when the starter culture level was increased; a culture level of 3% caused initiation of gelation at 120 min while that of 4% caused gelation at 105 min. A sigmoidal curve of G* versus time was obtained for all three starter culture levels tested. However, all curves reached similar plateau values (533-551 Pa), suggesting that final network rigidity is mainly governed by the milk solids level and is not affected by the kinetics of the coagulation process. Similar trends were observed in the case of bacterial culture-induced coagulation of MSNF milk (Figure 23b) and UF (Figure 24a). However, marked differences in the range of plateau values were observed among samples at 14% solids; 533-551 Pa control, 508-573 Pa MSNF, 1200-1300 Pa UF and 1360-1600 Pa DF. For the DF milk, a much shorter onset time of gelation was found (Figure 24b), compared to other samples, presumably due to high concentration of polymeric constituents (proteins). In this case, the gelation started at 110 min with 2% culture, 50 min for 3% and approximately at 20 min with a 4% starter culture level. Therefore, it is reasonable to assume that gelation is the result of major conformational changes induced by acid coagulation of proteins in milk. According to Heertje et al. (1985), network formation in acid-coagulated milk gels is influenced by many factors such as heat treatment, milk composition, salt system, pH, starter culture etc. Structure formation, therefore, appears to be a much more complex process than just an association of the milk casein micelles. It involves subtle dissociation and association phenomena of the caseins of milk upon acidification. The sequence of events can be described as partial micellar disintegration (induced by solubilization of calcium phosphate)

Figure 24. Rigidity modulus, G*, of milk samples (14% TS) fermented with a mixed starter culture (1:1, 42° C): a) UF; b) DF.


10000
1000
100
10
1
0
Rigidity Modulus, GX(Pa)
o S.C.(2%)
Δ S.C.(3%)
□ S.C.(4%)
a
0
50
100
150
200
250
300
350
Time (min)


10000
1000
100
10
1
0
Rigidity Modulus, GX(Pa)
o S.C.(2%)
Δ S.C.(3%)
□ S.C.(4%)
b
0
50
100
150
200
250
300
350
Time (min)

with the release of β-casein (pH 5.5-5.2) and formation of a loosely aggregated αs_1 -casein network. This is followed by a contraction stage (pH 5.2-4.8), where discrete aggregated structures (greater in size than the original micelles) are apparent. In the final stage of bacterial fermentation (pH 4.5) rearrangement and aggregation of casein particles take place with the establishment of the ultimate milk protein network.

Compared to control and MSNF samples, the modulus-time profiles of UF and DF milks exhibited gradual changes in the "plateau" region (Figure 24). The progressive increase in G' in this region is indicative of further strengthening of the gel structure. The high buffering properties of UF and DF retentates could be responsible for the delay in reaching a constant G* value within the time-frame of the rheological experiments.

With increasing concentration of starter culture there was a parallel displacement of the G* curves for all types of milk. Similar observations on milk clotting times were made by Olson and Bottazzi (1977) for rennet-coagulated milk. Using a thrombelastograph, it was shown that the clotting times decreased with increasing level of rennet. In contrast to our findings on lactic acid-coagulated milk (Figures 23, 24), these researchers have shown a slight increase in the attained maximum rigidity for milk gels with rennet concentration.

4.4.3.2 Effect of Temperature and Milk Solids Level

The effects of three levels of solids (12, 14 and 16%) on the onset of gelation of fermented milk at three different temperatures (39, 42 and 45° C) are shown in Tables 16-18; the data also include the rheolo-

Table 16. Onset of gelation (time to attain a G* value of 1 Pa) and plateau values of rigidity modulus (G*, Pa) of fermented milk samples with a mixed starter culture (1:1, 39° C, 5 h).

Total Solids (%)		12			14			16		
S. Culture (%)		2	3	4	2	3	4	2	3	4
Control	Time(min)	235	190[a] (10)	170	170	145 (5)	115	160	140 (10)	135
	G*	231	223 (12)	276	666	653 (15)	704	820	759 (22)	820
UF	Time(min)	185	175 (15)	165	145	130 (15)	110	135	135 (10)	105
	G*	654	813 (18)	842	1550	1420 (20)	1480	2160	2090 (30)	2200

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 17. Onset of gelation (time to attain a G* value of 1 Pa) and plateau values of rigidity modulus (G*, Pa) of fermented milk samples with a mixed starter culture (1:1, 42° C, 5 h).

T.S. (%)		12			14			16		
S.C. (%)		2	3	4	2	3	4	2	3	4
Control	Time (min)	140	115[a] (8)	105	135	120 (2)	105	125	115 (10)	105
	G*	344	363 (15)	366	551	526 (12)	533	780	791 (15)	804
UF	Time (min)	135	85 (5)	80	130	105 (8)	90	115	90 (5)	85
	G*	770	663 (7)	674	1200	1220 (20)	1300	1850	2230 (25)	2260
DF	Time (min)	95	65 (9)	45	110	30 (6)	20	95	50 (5)	20
	G*	1200	1200 (28)	1200	1360	1580 (30)	1600	2340	2210 (25)	2000
MSNF	Time (min)	120	100 (5)	90	125	105 (5)	90	120	110 (10)	100
	G*	395	307 (10)	342	508	536 (12)	573	659	684 (19)	692

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 18. Onset of gelation (time to attain a G* value of 1 Pa) and plateau values of rigidity modulus (G*, Pa) of fermented milk samples with a mixed starter culture (1:1, 45° C, 5 h).

T.S. (%)		12			14			16		
S.C. (%)		2	3	4	2	3	4	2	3	4
Control	Time (min)	195	165[a] (10)	130	130	115 (5)	105	140	125 (15)	115
	G*	172	143 (12)	198	466	480 (10)	481	716	752 (18)	719
UF	Time (min)	140	100 (5)	85	135	105 (5)	95	125	110 (10)	95
	G*	174	222 (20)	231	1130	900 (15)	1140	1520	1550 (25)	1750

[a] : mean values (N=2); number in parenthesis refers to standard deviation.

gical responses of each system by varying the starter culture level (2-4%).

In general, the onset time of gelation decreased with increasing level of total solids, particularly between 12 to 14%. A faster gelation was also observed at 42° C compared to the other two temperatures under identical solids and starter culture levels. The G* plateau values did not differ greatly with the starter culture level for each type of milk tested at a particular solids concentration and temperature of incubation. However, there have been pronounced differences in G* between membrane filtered and control or reconstituted MSNF samples; UF and DF yogurts exhibited G* values of at least twice the magnitude of those for the control milk. These data are a direct consequence of the higher protein content of membrane-processed milks. Since gels from acidified milk originate from the coagulation of particles consisting of several hundred casein molecules, there should be very strong casein concentration dependence of the dynamic moduli (G*, G'). Double logarithmic plots of log G* (plateau, Pa) against % protein concentration gave linear relationships between the two variables for the samples produced at 42° C: $G^* < P^{2.38}$ for control; $G^* < P^{1.37}$ for UF; $G^* < P^{1.11}$ for DF; and $G^* < P^{1.83}$ for MSNF.

Upon coagulation, formation of semi-permanent bonds between casein molecules at the boundary of adjacent particles will be very likely; this results in establishment of a continous gel network. The mechanical properties of such inhomogeneous gel macrostructure, as reflected by its dynamic moduli and tan δ values, would depend not only on the number and nature of bonds between particles, but also on their spatial distri-

bution. Consequently, in addition to casein concentration, other variables such as rate of acid production and temperature would be expected to affect the final network structure via changes in the rate of casein micelle solubilization and/or intermolecular interactions. These factors have been reported as the principal determinants of the spatial structure in acidified milk gels. For example, using HCL or glucono-δ-lactone (acid precursor) Roefs (1986) reported differences in the permeability and stiffness of acidified skim milk and caseinate gels as a function of ageing temperature. A higher temperature during gel formation resulted in greater inhomogeneity of gel structure which was accompanied by a larger permeability. Furthermore, gels aged at high temperatures were much stronger thus suggesting the effective number of interparticle associations increases with ageing temperature.

For the UF and DF samples, particularly at high concentrations, G* did not reach at a steady plateau value within the experimental timeframe. It would appear that rearrangements in the gel network of these samples occur at a slower rate, compared to control or reconstituted MSNF presumably due to their higher buffering capacity and casein content.

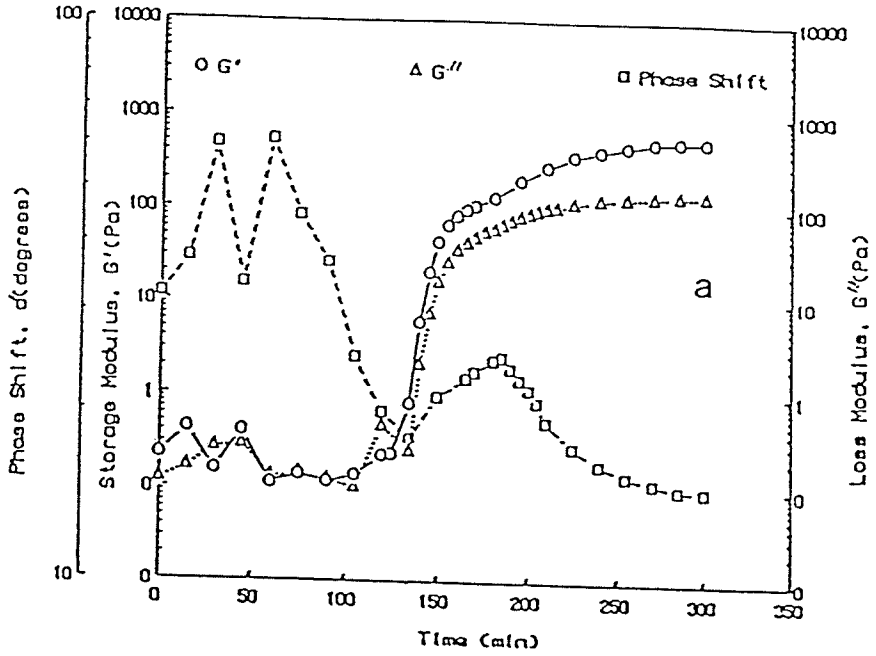
At the completion of the fermentation experiments, the recorded G* for the yogurt samples varied considerably with the temperature when considering identical solids levels for each type of milk. In general, a decrease in the dynamic moduli with increasing temperature was found. Since the temperature range within which fermentation experiments were conducted was fairly narrow (39-45^{0} C), the character of the interaction forces operating during gelation of casein aggregates (hydrogen bonding,

steric repulsion and electrostatic forces, hydrophobic interactions) is expected to vary little with temperature. Consequently, the observed variation in G* with temperature most likely arises from the well known inverse relationship between network regidity and temperature.

Besides the complex modulus, the rheometer also provided information on the viscous (G") and storage or elastic (G') moduli as well as the phase shift angle, δ. The latter or its more commonly used tan value ($\tan \delta = G''/G'$) is a parameter very sensitive to changes in G' and G'' with respect to each other; a high value ($\tan \delta > 0.1$) points to a more liquid like character in a system, while a low value of $\tan \delta$ (<0.1) points to a more elastic character.

The phase shift angle, storage and loss moduli for the different milk samples (control, ultrafiltered, diafiltered and reconstituted MSNF) at 14% solids (2% starter culture, 42° C) during fermentation are depicted in Figures 25 and 26. In addition to the rapid rise in moduli values, the transition from sol to gel was evident by the sudden decrease in the phase angle. Following the onset of gelation, a local maximum in δ values was observed at the early stages of the gelation process for all samples; e.g. about 170 min for control milk (Figure 25a) and 190 min for UF milk (Figure 26a). A similar but less pronounced maximum was been reported also in the case of rennet-coagulated milk by Bohlin et al. (1984). Such rheological response is indicative of a partial loosening in the casein gel network. These effects do not necessarily reflect identical molecular processes in view of the different mechanisms of casein coagulation between rennet-treated and fermented milks. For the latter, the most likely explanation is partial micellar disintegration,

Figure 25. Kinetics of milk coagulation (14% total solids, 2% starter culture, 42° C): a) Control b) MSNF.


O G'
Δ G''
□ Phase Shift
a
Phase Shift, d(degrees)
Storage Modulus, G'(Pa)
Loss Modulus, G''(Pa)
Time (min)

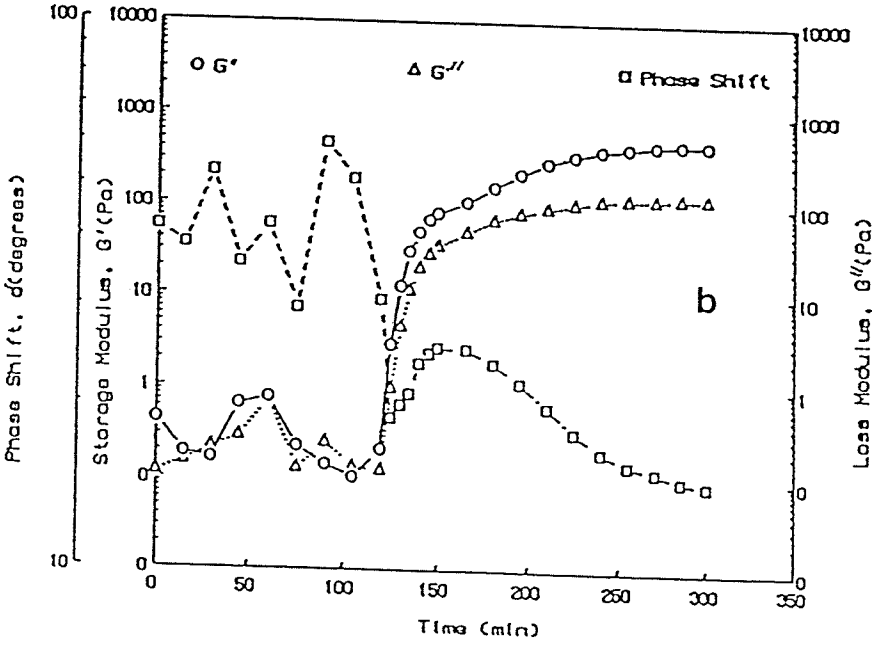
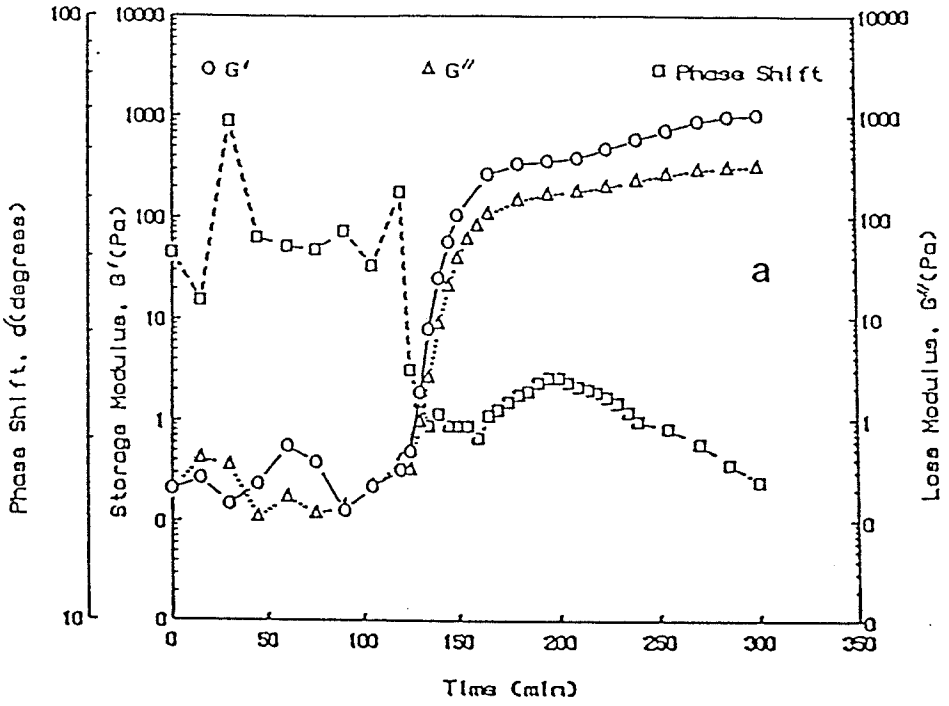
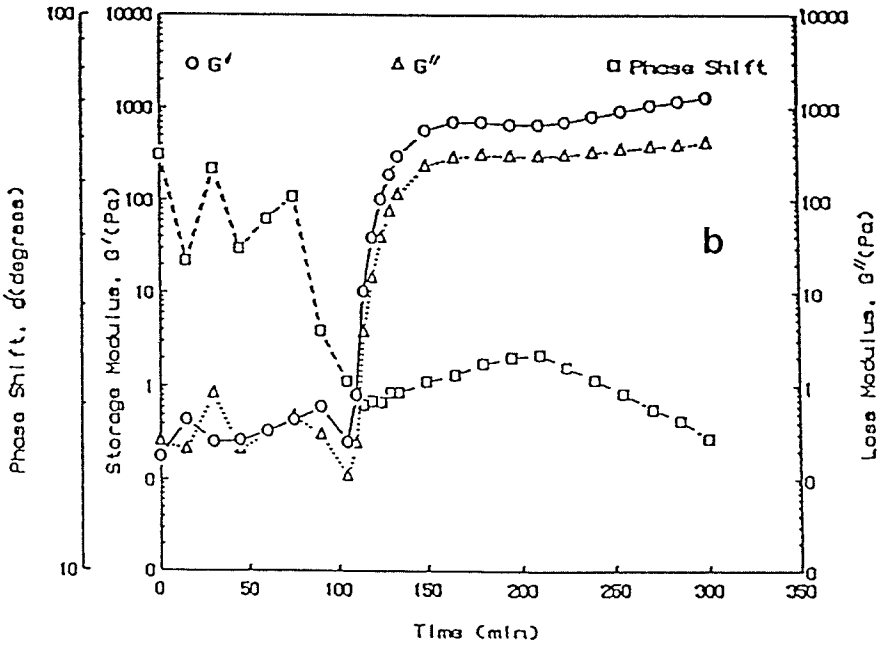

O G'
Δ G''
□ Phase Shift
b
Phase Shift, d(degrees)
Storage Modulus, G'(Pa)
Loss Modulus, G''(Pa)
Time (min)

Figure 26. Kinetics of milk coagulation (14% total solids, 2% starter culture, 42° C): a) UF b) DF.


O G'
Δ G"
□ Phase Shift
a
Phase Shift, d(degrees)
Storage Modulus, G'(Pa)
Loss Modulus, G"(Pa)
Time (min)


O G'
Δ G"
□ Phase Shift
b
Phase Shift, d(degrees)
Storage Modulus, G'(Pa)
Loss Modulus, G"(Pa)
Time (min)

due to solubilization of colloidal calcium phosphate, before the final aggregation stage of caseins commences. This suggestion is supported by the observation that the increase in phase shift occured within the pH range of 5.2-5.7 for all samples in the present study. It is well known that at this pH range calcium removal from native micelles causes dissociation of weakly bound β- and κ-caseins from their structure, thus leaving a loosely connected framework of αs-caseins. Changes in micellar composition (increase in αs-casein content) also enhance the calcium sensitivity and thereby the rapid development of the αs-casein network (Heertje et al., 1985). Upon further acidification (later stages of fermentation), the released β-casein molecules start to precipitate (at their isoelectric point, pH 5.1-5.2) and readsorb on the aggregated as-caseins. This causes the formation of new particles and initiates the final stage of gel structure development, as evidenced by attainment of much lower δ values (Figures 25, 26).

The dynamic viscosity (η'), storage modulus (G') and phase shift angle values (measured at 1 Hz) of all yogurt samples after fermentation (starter culture at 2, 3 and 4%) for 5 h at 42° C are given in Tables 19-21. Notably, there were no significant differences in these rheological parameters between control and reconstituted non-fat dry milk solids. As expected, the membrane processed milk samples had higher η' and G' values (parameters highly dependent on cross-link density of the gel network) than control and MSNF. The UF and DF yogurts also exhibited greater phase angle values which suggests a more viscous character for the gel network of these samples.

Table 19. Dynamic viscosity, η'(Pas) at 1 Hz of yogurts obtained after fermentation of various milk samples for 5 hr with a mixed starter culture (1:1, 42° C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	30.8	28.5[a] (5.4)	36.1	44.1	41.3 (1.5)	40.4	58.5	57.7 (1.8)	59.4
UF	44.5	52.8 (2.3)	53.3	101.0	98.8 (2.9)	103.0	151.0	152.0 (11.0)	167.0
DF	96.1	92.5 (4.7)	90.4	133.0	135.0 (6.4)	132.0	257.0	248.0 (9.0)	226.0
MSNF	30.9	25.3 (2.0)	27.2	38.4	40.4 (3.5)	43.0	49.4	50.1 (5.1)	51.6

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 20. Phase shift angle, δ(degrees) at 1 Hz of yogurts obtained after fermentation of various milk samples for 5 hr with a mixed starter culture (1:1, 42° C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	15.0	14.4[a] (0.1)	14.2	14.1	13.9 (0.1)	13.8	13.6	13.5 (0.0)	13.4
UF	16.3	14.7 (0.1)	14.5	16.6	14.8 (0.1)	14.4	14.9	14.1 (0.2)	13.8
DF	14.8	14.0 (0.2)	13.8	17.9	15.7 (0.1)	15.3	20.2	20.6 (0.1)	20.8
MSNF	14.1	14.9 (0.1)	14.5	14.0	13.8 (0.2)	13.7	13.6	13.1 (0.0)	13.0

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

Table 21. Storage modulus, G'(Pa) at 1 Hz of yogurts obtained after fermentation of various milk samples for 5 hr with a mixed starter culture (1:1, 42° C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	357	348[a] (35)	350	534	511 (18)	518	764	769 (17)	782
UF	738	638 (10)	648	1040	1200 (28)	1260	1780	2010 (42)	2030
DF	1140	1170 (27)	1160	1380	1500 (25)	1520	2200	2070 (35)	1870
MSNF	380	298 (8)	330	474	516 (10)	553	641	649 (5)	672

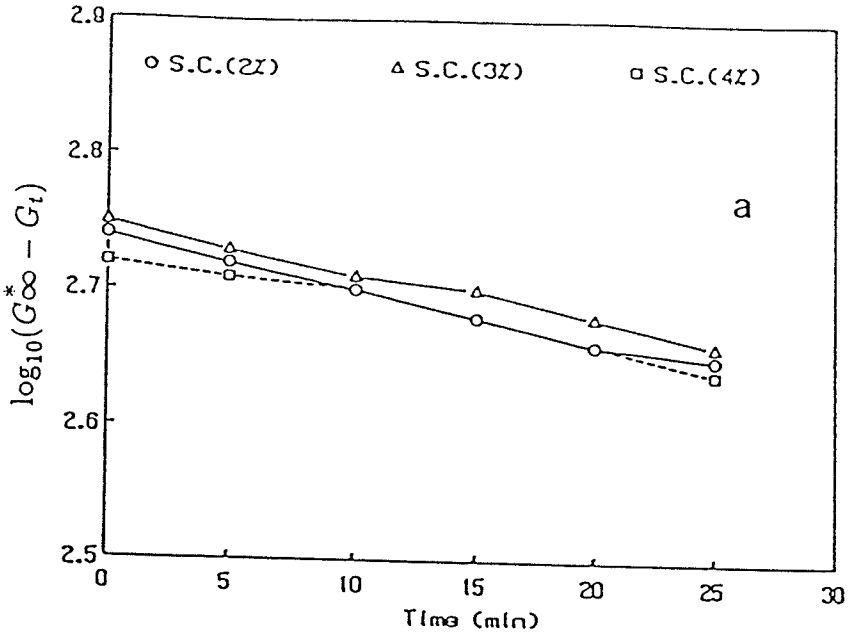
[a] : mean values (n=2); number in parenthesis refers to standard deviation.

4.4.3.3 Kinetic Analysis of Rheological Data on Milk Gelation

The kinetics of the conversion from liquid milk to a solid gel are expected to influence both the different stages of yogurt manufacture and quality attributes of the resultant gels. Clotting time is widely used to characterize changes in the physical state of milk during fermentation. However, clotting time determinations offer no direct information about the kinetics of the process, particularly during the phase of rigidity development.

Rheological techniques have been used in previous kinetic studies of rennet-coagulated (Culioli and Sherman, 1978; Garnot and Olson, 1982) and acidified (Roefs, 1986) milk gels. In the present study, using a first-order reaction model, the rheological data (G*) of gel structure development were used to calculate the apparent rate constants (K) of the fermentation experiments. The relationships between complex modulus (G*) and time are shown in Figure 27 for the control and UF milks at 14% (w/v) solids at all three starter culture levels (2, 3 and 4%). The corresponding estimates of the rate constants are summarized in Tables 22-24. The highly significant correlation coefficients (0.99-0.84, $p<0.01$) for the kinetic plots, also given in Tables 22-24, indicate that the experimental data can be described by first-order reaction kinetics. The reproducibility of the rheological data and the estimates of K derived therefrom was assessed by conducting duplicate experiments using a 3% starter culture for control, reconstituted MSNF, UF and DF milks under identical fermentation conditions. The data presented in Tables 22-24 suggest that there are no clear trends in the K values with respect to temperature of incubation, solids content and starter culture

Figure 27. Kinetic plots of milk gelation upon fermentation with a mixed starter culture (1:1, 14% total solids, 42° C): a) Control; b) UF.


o S.C.(2%)
Δ S.C.(3%)
□ S.C.(4%)
a
$\log_{10}(G^*_\infty - G_t)$
Time (min)
2.9
2.8
2.7
2.6
2.5
0
5
10
15
20
25
30

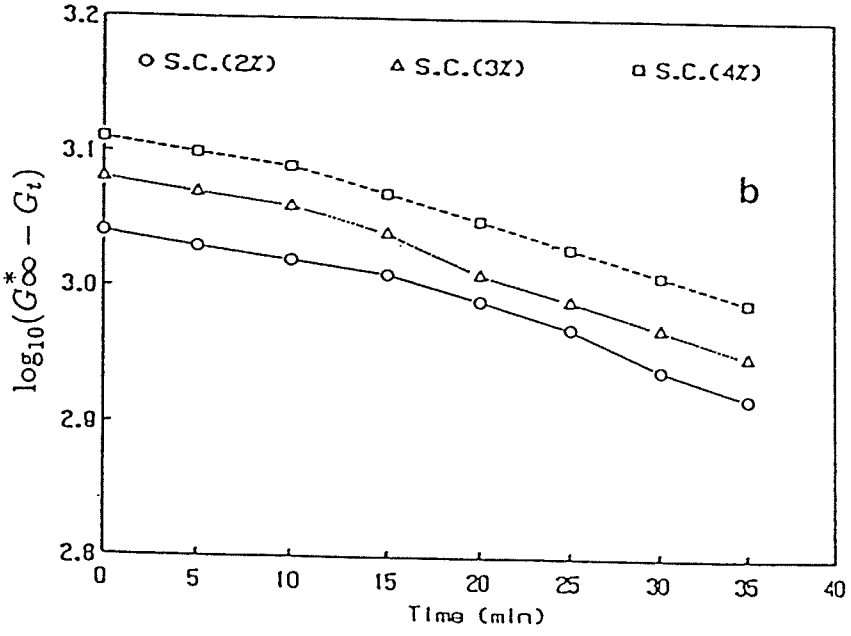

o S.C.(2%)
Δ S.C.(3%)
□ S.C.(4%)
b
$\log_{10}(G^*_\infty - G_t)$
Time (min)
3.2
3.1
3.0
2.9
2.8
0
5
10
15
20
25
30
35
40

Table 22. Apparent reaction rate constants ($Kx10^3$ min^{-1}) for gelation of milk samples during fermentation with a mixed starter culture (1:1, 39° C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	9.4	10.0[a] (1.1)[b]	8.4	7.9	7.7 (1.1)	7.7	6.8	7.5 (0.6)	7.2
	r=.94	r=.96	r=.97	r=.97	r=.95	r=.98	r=.95	r=.99	r=.97
UF	9.2	8.1 (0.7)	7.7	6.1	5.3 (0.5)	6.0	5.4	5.3 (0.8)	4.7
	r=.92	r=.95	r=.94	r=.90	r=.92	r=.90	r=.92	r=.92	r=.91

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

[b] : r values significant at $p< 0.01$

Table 23. Apparent reaction rate constants ($Kx10^3$ min^{-1}) for gelation of milk samples during fermentation with a mixed starter culture (1:1, 42° C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	7.1	7.4[a]	7.0	8.4	7.0	7.5	6.9	6.4	6.7
		(1.5)			(1.0)			(0.7)	
	r=.98[b]	r=.99	r=.99	r=.99	r=.97	r=.98	r=.97	r=.96	r=.97
UF	11.0	6.4	7.0	9.0	9.6	8.7	8.5	8.0	8.3
		(0.9)			(2.9)			(1.2)	
	r=.96	r=.91	r=.92	r=.93	r=.98	r=.97	r=.95	r=.92	r=.95
DF	14.4	8.2	4.5	14.6	6.0	5.5	15.6	10.0	6.6
		(1.1)			(1.4)			(3.2)	
	r=.98	r=.94	r=.90	r=.92	r=.90	r=.90	r=.85	r=.85	r=.84
MSNF	8.5	5.9	5.6	7.6	6.7	5.6	8.6	7.3	6.8
		(0.8)			(0.5)			(0.6)	
	r=.98	r=.99	r=.99	r=.99	r=.99	r=.90	r=.99	r=.97	r=.95

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

[b] : r values significant at $p< 0.01$

Table 24. Apparent reaction rate constants ($Kx10^3$ min^{-1}) for gelation of milk samples during fermentation with a mixed starter culture (1:1, 45^0 C).

T.S. (%)	12			14			16		
S.C. (%)	2	3	4	2	3	4	2	3	4
Control	14.5	17.7[a] (2.0)	8.9	8.9	8.1 (1.1)	7.2	7.7	7.0 (0.6)	7.2
	r=.95[b]	r=.97	r=.98	r=.98	r=.99	r=.98	r=.98	r=.99	r=.98
UF	7.6	5.8 (1.2)	5.2	12.3	12.2 (2.2)	8.7	11.4	9.1 (1.1)	9.5
	r=.93	r=.94	r=.90	r=.96	r=.98	r=.96	r=.96	r=.92	r=.95

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

[b] : r values significant at $p< 0.01$

level among the various samples or treatments employed. Thus in contrast to onset time of gelation (Tables 16-18), which is sensitive to total milk solids, starter culture level and protein content, the rate of G* development during the gelation stage is not related to these variables.

4.5 Chemical and Sensory Attributes of Yogurts During Storage

4.5.1 Effect of Storage on pH and Acetaldehyde Content

Acetaldehyde has been identified as the compound responsible for the characteristic flavor and aroma of yogurt (Schulz and Hingst, 1954). In fact, sour milks possessing a well-developed yogurt flavor contain a relatively high concentration of acetaldehyde. In Table 25 the results of acetaldehyde content and pH for control and ultrafiltered milk yogurts are shown during storage at 4^0 C for 1, 7 and 14 days. As expected, there was a progressive decrease in pH for all yogurt samples during storage. Similar trends were also observed for acetaldehyde. It is worth noting that the acetaldehyde content of UF milk yogurts remained higher than the control throughout the entire storage period for samples of equivalent solids level. However, acetaldehyde content decreased markedly in all samples during storage which supports the findings of Bills et al. (1972) who have reported that acetaldehyde in yogurt decreased upon storage for 14 days at 5^0 C, presumably due to conversion of acetaldehyde to ethanol (Marsili, 1981).

Table 25. Changes in pH and acetaldehyde content of yogurt during storage at 4° C.

Time (days)		1		7		14	
Sample	T.S. (%)	pH	acetaldehyde (μg/g)	pH	acetaldehyde (μg/g)	pH	acetaldehyde (μg/g)
Control	12	4.43[a] (0.09)	8.54 (0.29)	4.29 (0.01)	7.30 (0.14)	4.12 (0.03)	5.82 (0.55)
UF	12	4.46 (0.04)	9.40 (0.10)	4.31 (0.01)	9.15 (0.12)	4.17 (0.03)	8.42 (0.41)
Control	14	4.48 (0.05)	10.14 (0.03)	4.33 (0.02)	8.62 (0.85)	4.17 (0.03)	7.18 (0.29)
UF	14	4.49 (0.06)	10.28 (0.53)	4.36 (0.02)	9.66 (0.31)	4.22 (0.03)	8.54 (0.48)
Control	16	4.51 (0.02)	11.75 (0.35)	4.38 (0.02)	10.79 (0.20)	4.25 (0.07)	8.97 (0.98)
UF	16	4.52 (0.03)	13.10 (0.22)	4.40 (0.00)	11.98 (0.29)	4.29 (0.05)	11.18 (0.33)

[a] : mean values (n=2); number in parenthesis refers to standard deviation.

4.5.2 Changes in Organic Acid Profiles of Yogurt

Figures 28 and 29 present the profiles of various organic acids in yogurt samples at 14% total solids during storage for 14 days for control and UF milk, respectively. Both orotic acid and pyruvic acids remain unchanged during storage at 4^0 C. A slight decrease was observed with citric and propionic acids, while the acetic acid content continuously increased with storage time. Finally, the lactic acid content did not show any trends and remained relatively constant after 7 days storage.

The profiles of all six organic acids in the control and UF milk yogurt samples at all three different levels of solids (12, 14 and 16%) during storage are summarized in Tables 26-28. These data illustrate that in most cases the organic acid composition of yogurts did not changed considerably under refrigeration temperatures for 14 days. Among the changes in the organic acid contents observed for the control and UF milk yogurt, the most pronounced effect was that of citric acid at 16% solids (Tables 26-28) where after storage for two weeks the profiles showed no evidence for the presence of citric acid in both control and UF yogurts.

4.6 Sensory Analysis

The mean scores for the sensory parameters of control and ultrafiltered yogurts at a particular storage time are given in Tables 29-31. These tables also depict the differences from comparisons in the sensory characteristics between control and ultrafiltered yogurt stored for two weeks at 4^0 C.

Figure 28. Changes in organic acid composition of control milk yogurt (14% total solids) over storage time (14 days) at 4° C.

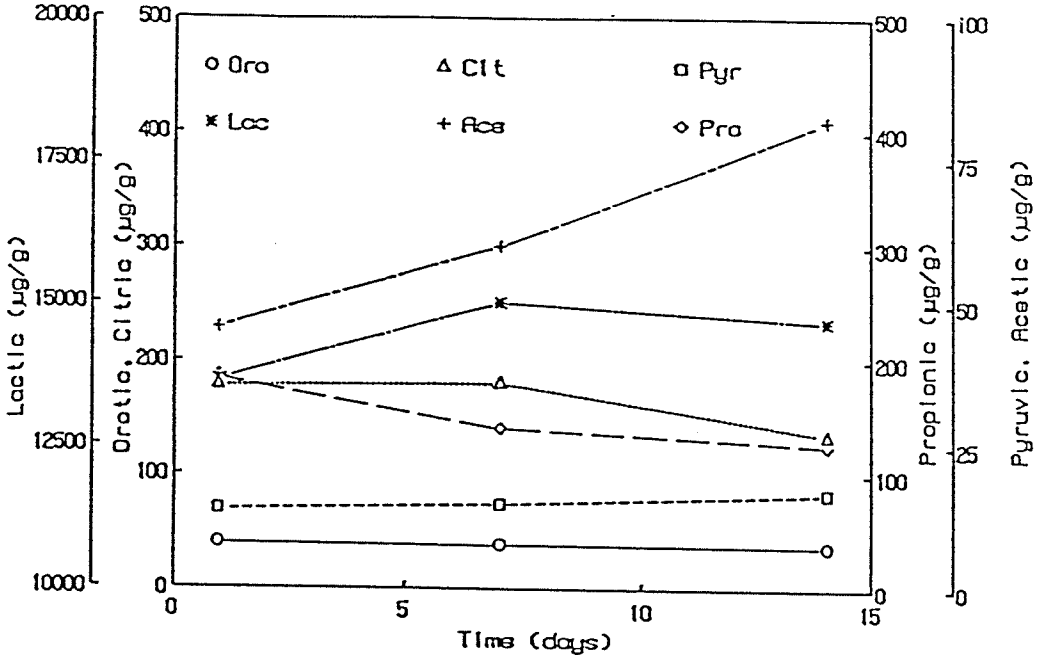

Lactic (µg/g)
Orotic, Citric (µg/g)
Propionic (µg/g)
Pyruvic, Acetic (µg/g)
Time (days)
Oro
Cit
Pyr
Lac
Ace
Pro
20000
17500
15000
12500
10000
500
400
300
200
100
0
75
50
25
0
5
10
15

Figure 29. Changes in organic acid composition of ultrafiltered milk yogurt (14% total solids) over storage time (14 days) at 4° C.

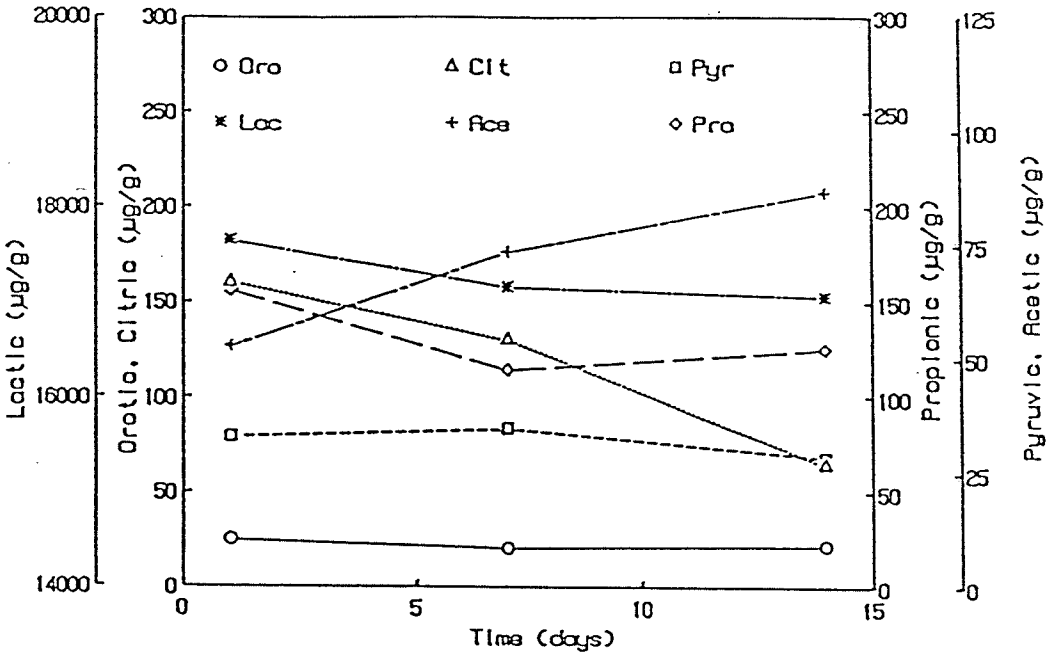

Oro
Cit
Pyr
Lac
Ace
Pro
Lactic (µg/g)
Orotic, Citric (µg/g)
Propionic (µg/g)
Pyruvic, Acetic (µg/g)
Time (days)
20000
18000
16000
14000
300
250
200
150
100
50
0
125
100
75
50
25
0
5
10
15

Table 26. Organic acid composition of yogurt samples (1 day) after fermentation with a mixed starter culture (1:1, 2%, 42° C).

Samples	T.S. (%)	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
		concentration (μg/g)					
Control	12	47.3[a] (1.2)	271.4 (61.1)	21.7 (5.8)	13800.5 (51.6)	42.6 (4.6)	143.5 (55.7)
UF	12	38.5 (1.2)	144.3 (4.6)	24.4 (2.1)	14990.5 (197.9)	46.7 (3.8)	73.5 (2.5)
Control	14	40.4 (4.1)	177.5 (11.6)	13.8 (1.2)	13653.8 (139.7)	45.7 (4.1)	184.9 (28.6)
UF	14	24.6 (4.1)	160.5 (6.8)	32.8 (4.9)	17640.9 (201.0)	52.7 (3.7)	155.8 (10.1)
Control	16	45.3 (1.2)	167.0 (85.0)	20.1 (0.4)	13580.3 (16.5)	47.3 (4.1)	202.3 (2.8)
UF	16	21.6 (2.5)	23.4 (2.5)	37.1 (6.9)	15970.5 (108.6)	54.6 (6.2)	62.5 (9.1)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Table 27. Organic acid composition of yogurt samples (7 day) after fermentation with a mixed starter culture (1:1, 2%, 42° C).

Samples	T.S.	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
	(%)	concentration (μg/g)					
Control	12	42.9[a] (4.1)	268.4 (34.5)	21.7 (4.1)	14331.0 (114.6)	65.4 (4.1)	66.1 (3.7)
UF	12	34.9 (1.2)	264.8 (7.9)	33.5 (0.0)	16434.0 (53.8)	64.9 (10.4)	106.1 (23.7)
Control	14	39.5 (4.5)	180.8 (45.5)	14.9 (0.4)	15028.0 (86.3)	60.1 (12.0)	139.8 (11.5)
UF	14	20.8 (0.4)	130.2 (24.4)	34.8 (16.9)	17167.2 (620.0)	73.5 (10.3)	114.5 (0.4)
Control	16	45.5 (2.0)	28.0 (1.1)	19.8 (0.8)	15182.0 (207.0)	69.2 (2.9)	125.5 (4.3)
UF	16	15.47 (0.6)	15.8 (2.2)	16.3 (1.6)	15364.0 (118.0)	61.3 (3.3)	108.6 (21.5)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Table 28. Organic acid composition of yogurt samples (14 day) after fermentation with a mixed starter culture (1:1, 2%, 42° C).

Samples	T.S.	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
	(%)	concentration (μg/g)					
Control	12	39.1[a] (1.2)	229.0 (7.0)	20.3 (0.4)	13883.8 (387.0)	66.1 (4.6)	61.1 (4.9)
UF	12	29.1 (3.7)	145.2 (16.6)	28.5 (5.4)	14824.0 (139.8)	62.3 (9.1)	107.2 (5.0)
Control	14	37.2 (0.4)	133.9 (4.5)	16.9 (1.7)	14685.0 (125.0)	82.0 (0.0)	125.1 (11.6)
UF	14	21.7 (0.8)	64.5 (41.4)	28.4 (9.5)	17056.0 (21.5)	86.7 (0.8)	125.1 (21.1)
Control	16	39.1 (6.6)	----	12.5 (0.4)	14274.0 (186.6)	67.1 (0.8)	103.6 (14.4)
UF	16	19.2 (1.6)	----	27.7 (6.1)	17038.0 (74.7)	94.6 (4.1)	132.2 (45.8)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

4.6.1 Thickness

The yogurt samples varied significantly in thickness/viscosity (Table 29). The mean score values after one day storage varied from 14.37 to 57.32. Similar ranges in thickness were observed for the seven day (11.78 to 57.27) as well as the 14 day yogurt samples (11.55 to 57.75), based on the 60 point rating scale; the lowest score was given to a very thin sample, while the sample with the highest score was considered very thick by the panelists. The thickness score for ultrafiltered yogurt (12% solids) and control sample (16% solids) appeared to be in the middle of the rating grid at all three storage periods, indicating that these samples had moderately viscous properties. The consistency of the samples ranged from very thin/watery (control, 12% solids) to thick (UF, 14% solids), to gelled (UF, 16% solids). Control yogurts at 12 and 14% solids level were moderately thin products compared to the membrane processed milk yogurts.

The thickness mean score of a particular sample over two weeks storage obtained by multiple comparison test, as listed, showed no significant differences between control and ultrafiltered samples at 14 and 16 per cent solids levels (Table 29). The lower scores (11.78 and 11.55), however, for the seven and 14 days control samples, respectively, were significantly different from the one day sample (14.37) at 12% solid level. For the 12% ultrafiltered sample the thickness score decreased to 25.75 after seven days and finally increased to 30.84 following 14 days of storage. These changes were accompanied by visual signs of syneresis initially (7 days) and subsequent recovery of gel strength thereafter.

Table 29. Comparison of perceived thickness mean scores between Control and ultrafiltered (UF) yogurts at 3 different storage times at 4° C.[x,y]

Treatment	T.S. (%)	Day 1	Day 7	Day 14
Control	12	14.37[f,1]	11.78[e,2]	11.55[f,2]
UF	12	32.66[c,1]	25.73[c,2]	30.84[c,1]
Control	14	17.70[e,1]	18.50[d,1]	19.04[e,1]
UF	14	47.46[b,1]	46.82[b,1]	47.54[b,1]
Control	16	28.03[d,1]	26.00[c,1]	28.20[d,1]
UF	16	57.32[a,1]	57.27[a,1]	57.75[a,1]

x : Means with the same letter (column) are not significantly different at $p < 0.05$ level.

y : Means with the same number (row) are not significantly different at $p < 0.05$ level.

The F values from the analysis of variance indicated that replications and panelists were not significantly different at 5% level and thus the interaction between them is not significantly different.

4.6.2 Chalkiness or Texture

The taste panel results for chalkiness scores between control and ultrafiltered yogurt samples at equivalent solids content were significantly different at 0.05 level after 1, 7 and 14 days storage. However, control samples at 12 and 14 percent solids were not statistically significant after 1 and 14 day analyses. The highest score for chalkiness was for the ultrafiltered yogurt at 16% solids level, while the control yogurt at 12% total solids exhibited the least but was characterized as being "very thin/watery". The respective mean scores obtained after each storage day for both control and ultrafiltered yogurt samples increased significantly with increasing total solids level. The scores for the 12% total solids UF milk yogurts corresponded to products with moderate texture, as assessed by the 60 point rating grid. The sample having the highest score (UF, 16% total solids) was perceived as a product with extreme chalkiness as far as texture is concerned.

To determine if there was a change in this sensory attribute of yogurt samples after two weeks of storage (at 4^0 C), a separate test was performed. The data summarized in Table 30 indicated that there were no significant changes in chalkiness of the yogurts throughout the entire storage period.

Table 30. Comparison of perceived chalkiness mean scores between Control and ultrafiltered (UF) yogurts at 3 different storage times at 4° C.[x,y]

Treatment	T.S. (%)	Day 1	Day 7	Day 14
Control	12	8.45[e,1]	7.43[f,1]	6.98[e,1]
UF	12	30.46[c,1]	29.43[c,1]	31.45[c,1]
Control	14	11.93[e,1]	12.66[e,1]	9.64[e,1]
UF	14	47.60[b,1]	46.61[b,1]	46.78[b,1]
Control	16	19.70[d,1]	18.25[d,1]	22.36[d,1]
UF	16	56.68[a,1]	54.75[a,1,2]	54.25[a,2]

[x] : Means with the same letter (column) are not significantly different at $p < 0.05$ level.

[y] : Means with the same number (row) are not siginificantly different at $p < 0.05$ level.

4.6.3 Sourness

Mean scores for sourness are presented in Table 31. The sourness scores for all samples following storage for one day suggested that the control (12% solids), and UF (14% and 16% solids) yogurts did not vary significantly in perceived sourness. Slight differences in sourness were observed between control (14 and 16% solids) and UF (12% solids). Similar patterns of variation in sourness for the same samples were found after seven and 14 days of storage (Table 31). Nevertheless, the narrow range of sourness mean scores found among samples (Table 31) as well as their similar pH values (Table 25) suggest that the panelists could not readily distinguish small differences in levels of acid in the yogurts. Scores for sourness generally increase with increased acid content in yogurt, as the level of acid has an influence on the taste and flavour. A moderate level of sourness appears to be important in the perception of flavour pleasantness. The sourness mean score for UF (12% solids) was rated in the middle range of the rating scale. Thus indicating a product with pleasant flavour.

A comparison of sourness mean scores among yogurt samples throughout the 14 days storage (4^0 C) is also shown in Table 31. In general, there have been no significant differences in sourness ($p< 0.05$) for each yogurt sample during storage, although the pH values (Table 25) showed a slight decrease. For the UF yogurt at 16% total solids only the sourness seemed to decrease after 14 days of storage. The constant scores for all other samples at a particular solid level indicated that the perceived acidity for these products was relatively stable.

Table 31. Comparison of perceived sourness mean scores between Control and ultrafiltered (UF) yogurts at 3 different storage times at 4° C.[x,y]

Treatment	T.S. (%)	Day 1	Day 7	Day 14
Control	12	39.45 ab,1	39.98 ab,1	39.44 abc,1
UF	12	32.84 c,1	36.64 b,1	33.00 d,1
Control	14	37.27 b,1	40.18 ab,1	37.43 bc,1
UF	14	41.07 ab,1	39.37 ab,1	40.00 ab,1
Control	16	41.73 a,1	42.00 a,1	43.12 a,1
UF	16	40.82 ab,1	42.11 a,1	35.86 cd,2

x : Means with the same letter (column) are not significantly different at $p < 0.05$ level.

y : Means with the same number (row) are not significantly different at $p < 0.05$ level.

Chapter V

CONCLUSIONS AND RECOMMENDATIONS

The present investigation was undertaken to study the effect of concentration of skim milk by various methods on the physicochemical properties and sensory attributes of yogurt. Skim milk samples concentrated by four different techniques were chemically characterized to assess their suitability for the manufacture of quality yogurts. The ultrafiltration and diafiltration experiments were effective in increasing the concentration of milk solids and protein; diafiltration alone reduced the amount of lactose significantly in the final retentates. Although the flux rate decreased gradually as expected during membrane processing of milk, it did not result in any major experimental setback with regard to severity of membrane fouling.

The chemical analyses for pH and titratable acidity demonstrated little variation among the milk samples. The retentates, however, differed from reconstituted (skim milk and non-fat skim milk solids) samples during fermentation in terms of length of time required to reach a particular pH level. In fact, the buffering capacity of membrane retentates was much greater than those of control and reconstituted MSNF samples. The flow properties of membrane retentates at 25^{0} C suggested that they are essentially non-Newtonian fluids over the shear rate range of 116-947 sec^{-1} examined; a slight shear thining dependence of apparent viscosity was observed. Both control and MSNF samples exhibited Newtonian behavior over the same shear rate range.

High performance liquid chromatography (HPLC) was found an effective means of analysing for organic acids in various milk samples during fermentation and storage. This study showed the lactic acid being the major organic acid present, followed by acetic, propionic and citric acid. Trace amounts of orotic and pyruvic acids in the final product were also detected. Citric acid was not found in the diafiltered milk yogurt.

The rheological responses of four types of milk samples during fermentation were monitored by small amplitude oscillatory measurements. Using this nondestructive dynamic testing method both the rate and extent of structure formation in various processed milk samples fermented by a mixed starter culture of S. thermophilus and L. bulgaricus (1:1) were determined. Based on the results of gel formation kinetics at various culture and milk solids level, it became apparent that the onset of gelation is dependent on both these two variables. The rheological studies were also extended to examine the effect of incubation temperature; the onset of gelation appeared to be faster at 42^0 C compared to 39 and 45^0 C. The transition from sol to gel during fermentation was evident by the sudden decrease in phase angle. Among other rheological parameters measured (G^*, G', G'', η'), the phase shift angle (δ) was very sensitive to changes in the liquid- and solid-like character of milk gels during fermentation. Following the onset of gelation, a local maximum in δ values was evident for all samples which suggested partial loosening of the casein network. Micellar disintegration, due to solubilization of colloidal calcium phosphate, was invoked as the origin of such rheological responses. Ionic strength might also be an important parameter of protein coagulation in acidified milks and consequently a

more extensive examination of the onset and kinetics of gelation and denaturation requires further study.

Kinetic analysis of the rheological data (G* vs. time) provided estimates of a first-order reaction rate constant, K, between 6.4-8.4 X 10^3 min^{-1} for control; 6.4-11 X 10^3 min^{-1} for UF; 4.5-15.6 X 10^3 min^{-1} for DF; as well as 5.6-8.5 X 10^3 min^{-1} for MSNF sample at all three culture and solids levels at 42° C. There have been no apparent differences or clear trends in K values with respect to temperature of fermentation, solids content and starter culture level among the various milk samples or different treatments employed.

Moreover, a higher amount of acetaldehyde found in UF yogurts, compared to control, is indicative of proper development of this major flavor component in fermented retentates at any solids level. The investigations on organic acid composition of yogurts upon storage revealed very little changes in most of the acids except for acetic acid content which increased. Furthermore, citric acid was not detected in both control and UF samples at 16% total solids after 14 days storage.

The perceived sensory characteristics were considered as an important aspect for the determination of acceptability of yogurts made from UF retentates. The sensory analysis showed that yogurt samples varied significantly (control vs. UF) in perceived thickness and chalkiness but not in sourness at all solids levels. Yogurts made from retentates at 12% total solids were comparable to that of control skim milk at 16% solids. Thickness and chalkiness scores increased with an increase in the solids level particularly for the membrane treated retentates. In

contrast, the perceived sourness scores did not show any significant differences between control and membrane processed milk yogurts. Yogurts samples made from UF retentates at 12% total solids scored in the middle of a 60 point rating grid with regard to perceived thickness, chalkiness and sourness, suggesting a highly desirable product.

There is a need to expand the fermented milk market which requires new product formulation and development. In order to ensure proper fermentation processes by lactic acid producing bacteria, the correct ratio of buffering capacity to lactose as well as casein to non-casein protein ratio should be established in the final UF retentates. This would facilitate appropriate fermentation of lactose and attainment of the desired pH level in the finished product at a minimal time. For this reason, more information is required concerning the metabolic processes of starter organisms. This would permit the development of skim milk retentate-based dairy products of improved texture and extended shelf life. For yogurt products, there should be no gel shrinkage which results in whey separation. The increased viscosity by the higher solids level in fermented products would prompt the manufacturers to design retentates of certain chemical composition that would yield products of desirable texture and other sensory attributes. Finally, the greater viscosity and hardness of yogurts made from ultrafiltered and diafiltered milk samples at higher solids level (e.g 16%) are problems that require further investigations. Variables such as casein to non-casein protein ratio and calcium, phosphorus contents in the retentates have to be optimized to minimize the detrimental effects of increased protein and mineral contents on texture.

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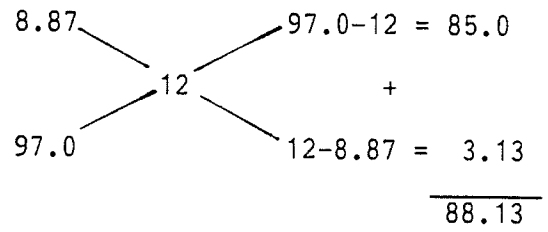
APPENDICES

Appendix 1.

Pearson Square Model utilized for adjusting of total solids in milk.

e.g. Control Milk (12% TS):



Calculation for 100 ml:

$$\frac{85}{88.13} \times 100 = 96.45 \text{ ml of skim milk}$$

$$\frac{3.13}{88.13} \times 100 = 3.55 \text{ g of MSNF milk}$$

Blending was based on total solids content (8.87%) of skim milk. Hence, in the above example, 3.55 g of non-fat milk solid (MSNF) was mixed with 96.45 ml of skim milk in order to obtain control milk of 12% solids level.

Appendix 2.

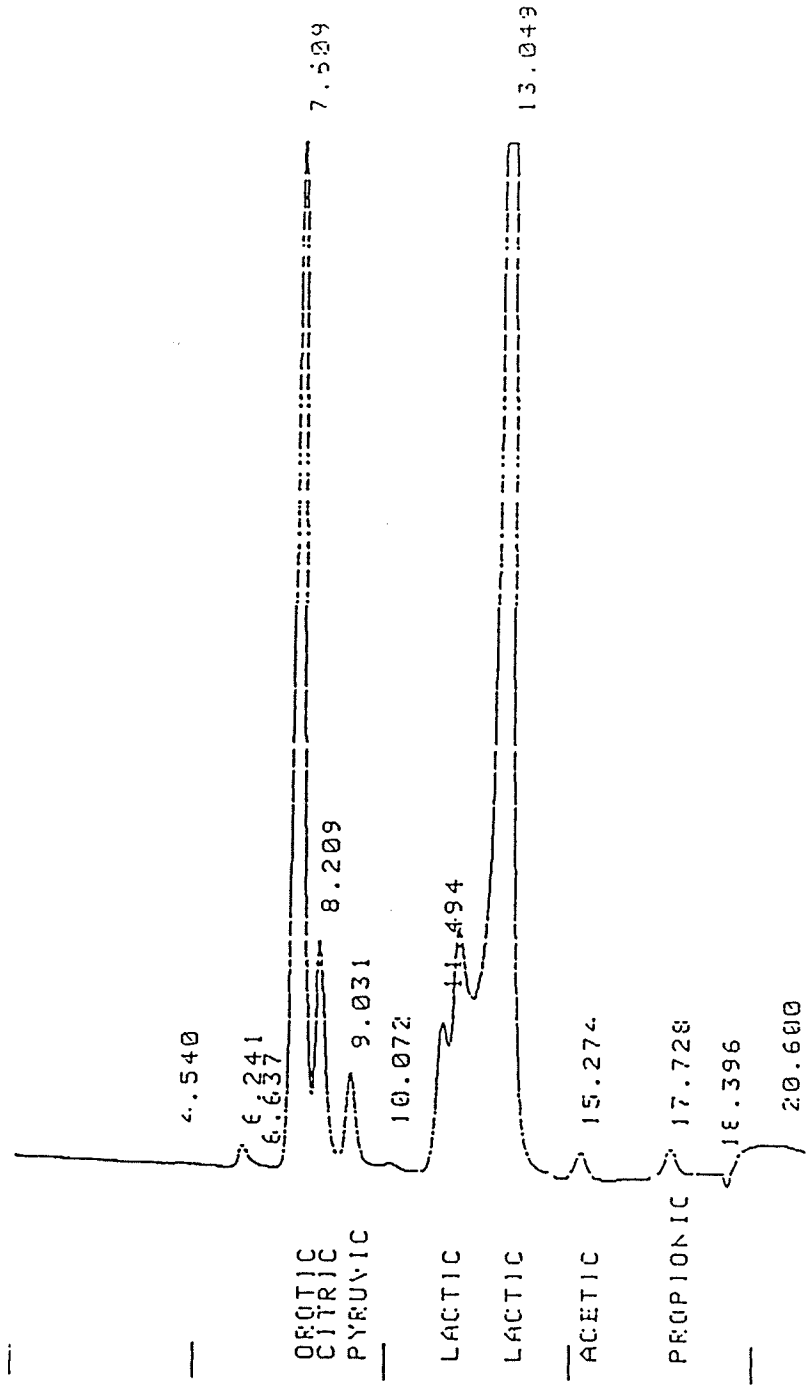
Concentration and elution time of the standard organic acid mixture used for HPLC calibration and analysis.

Organic acid	Concentration[a] (mg/ml)	Elution Time (min)
Orotic	.0125	7.62
Citric	.1294	8.21
Pyruvic	.0091	9.04
Lactic	2.956	13.07
Acetic	.040	15.28
Propionic	.0529	17.73

[a] : in acetonitrile solution.

Appendix 3.

Typical chromatogram of a standard mixture of organic acids. Chromatographic conditions: Column, Aminex HPX-87H; Temp., 69° C; Eluent, 0.006 N H_2SO_4 , Isocratic; Flow rate, 0.6 ml min^{-1} ; Detector, UV at 214 nm.



Appendix 4.

Ballot used for the evaluation of yogurt samples.

Name _____
Date _____

Yogurt Evaluation

Place a vertical mark on the horizontal line, with the code number, corresponding to the intensity of the parameter in the sample. Taste the samples in the following order:

_____ _____ _____ _____ _____ _____

Thickness/Firmness

Determine the thickness/firmness by evaluating how the sample is perceived between tongue and palate.

_____

thin/watery thick/solid

Chalkiness

After rubbing the sample between tongue and palate determine the amount of chalkiness.

_____

none moderate extreme

Sour

Take enough sample to spread over the tongue. Hold the sample in the mouth until the maximum amount of sourness is perceived. "S" is provided as the standard for "moderate".

_____

weak moderate strong

Comments:

Appendix 5.

Changes in flux rate and solids of skim milk during ultrafiltration (UF) through a diaflow hollow fiber membrane of 10,000 m.w. cut off (Temp. 25° C; Inlet press. 20 psi).

Time (min)	Flux rate (l/m^2 /h)	Permeate removed (%)	Total Solids	
			Brix (%)	Mojonnier (%)
0	17.02 (1.96)[a]	-----	9.93 (0.11)	8.66 (0.18)
20	14.09 (0.53)	11.18 (1.85)	10.80 (0.28)	9.05 (0.25)
40	12.84 (0.53)	22.58 (2.84)	11.46 (0.41)	9.60 (0.37)
60	12.09 (0.31)	32.72 (3.40)	12.40 (0.36)	10.16 (0.30)
80	11.29 (0.55)	42.38 (3.92)	13.40 (0.52)	11.04 (0.55)
100	10.84 (0.41)	51.77 (4.03)	14.83 (0.76)	12.10 (0.67)
120	10.13 (0.22)	64.32 (1.45)	16.70 (1.22)	13.73 (0.95)
140	8.57 (0.46)	70.02 (1.55)	19.60 (0.96)	15.97 (0.29)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Appendix 6.

Composition of skim milk during ultrafiltration through a diaflow hollow fiber membrane (10,000 m.w. cut off).

Time (min)	Permeate removed (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
0	-----	0.06 (0.01)	3.25 (0.04)	4.90 (0.03)	0.54 (0.07)
20	11.18 (1.85)[a]	0.06 (0.01)	3.73 (0.09)	4.82 (0.03)	0.57 (0.08)
40	22.58 (2.84)	0.07 (0.01)	4.27 (0.17)	4.80 (0.04)	0.59 (0.10)
60	32.72 (3.40)	0.07 (0.01)	4.97 (0.17)	4.75 (0.05)	0.66 (0.11)
80	42.38 (3.92)	0.08 (0.01)	5.89 (0.39)	4.70 (0.04)	0.75 (0.07)
100	51.77 (4.03)	0.09 (0.02)	7.32 (0.66)	4.60 (0.06)	0.85 (0.08)
120	64.32 (1.45)	0.12 (0.03)	9.66 (1.22)	4.51 (0.04)	0.99 (0.06)
140	70.02 (1.55)	0.16 (0.04)	13.91 (1.17)	4.24 (0.07)	1.11 (0.03)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Appendix 7.

Changes in flux rate and solids of skim milk during ultrafiltration (UF) & diafiltration (DF) through a hollow fiber membrane (10,000 m.w.cut off; 25° C; Inlet press. 20 psi).

Trial	Time (min)	Flux rate (l/m^2 /h)	Permeate removed (%)	Total Solids	
				Brix (%)	Mojonnier (%)
UF	0	16.46 (0.09)[a]	-----	9.90 (0.14)	8.56 (0.01)
	140	8.75 (0.50)	69.60 (1.94)	19.25 (1.06)	15.80 (0.32)
DF	0	18.86 (3.10)	-----	6.30 (0.14)	4.74 (0.16)
	20	15.93 (1.78)	14.59 (4.30)	7.05 (0.77)	5.54 (0.19)
	40	14.93 (0.75)	27.28 (3.30)	7.70 (0.90)	6.34 (0.33)
	60	14.26 (0.19)	39.42 (7.60)	9.00 (1.40)	7.25 (0.77)
	80	13.53 (0.47)	51.05 (8.40)	10.50 (2.10)	8.69 (1.13)
	100	12.07 (1.98)	59.19 (4.50)	12.80 (1.69)	10.96 (1.90)
	120	10.02 (1.05)	67.34 (2.90)	16.15 (1.48)	14.02 (0.38)
	140	9.10 (1.30)	73.25 (2.20)	20.30 (1.80)	16.80 (0.42)

[a] : mean values (n=3); number in parenthesis refers to standard

Appendix 8.

Composition of skim milk during ultrafiltration (UF) and diafiltration (DF) through a diaflow hollow fiber membrane (10,000 m.w. cut off; 25^{0} C; Inlet press. 20 psi).

Trial	Time (min)	Permeate removed (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
UF	0	-----	0.06(0.02)	3.28(0.01)	4.92(0.01)	0.62(0.01)
	140	69.60(1.94)[a]	0.15(0.05)	13.57(1.34)	4.28(0.07)	1.10(0.05)
DF	0	-----	0.07(0.02)	3.12(0.11)	1.52(0.48)	0.25(0.04)
	20	14.59(4.30)	0.08(0.02)	3.64(0.12)	1.49(0.48)	0.31(0.04)
	40	27.28(5.38)	0.07(0.03)	4.32(0.36)	1.44(0.52)	0.38(0.04)
	60	39.42(7.60)	0.08(0.04)	5.44(0.82)	1.37(0.54)	0.43(0.05)
	80	51.05(8.40)	0.10(0.03)	7.20(1.47)	1.25(0.53)	0.58(0.09)
	100	59.19(4.50)	0.14(0.00)	10.74(3.20)	1.06(0.51)	0.72(0.09)
	120	67.34(2.90)	0.20(0.08)	13.90(0.48)	0.87(0.24)	0.84(0.07)
	140	73.25(2.20)	0.22(0.04)	15.40(0.37)	0.75(0.16)	1.08(0.06)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Appendix 9.

Viscosity (mPas) of four types of fluid milk (12% T.S.) at 20° C.

Shear rate (sec^{-1})	Control	UF	DF	MSNF
116.3	2.53 (0.10)[a]	3.40 (0.01)	5.10 (0.04)	2.46 (0.09)
146.7	2.56 (0.08)	3.42 (0.01)	5.10 (0.05)	2.48 (0.09)
184.6	2.57 (0.10)	3.40 (0.02)	5.10 (0.03)	2.49 (0.07)
232.2	2.58 (0.10)	3.41 (0.02)	5.11 (0.03)	2.51 (0.07)
268.2	2.59 (0.03)	3.40 (0.05)	5.10 (0.04)	2.54 (0.06)
292.3	2.65 (0.03)	3.40 (0.05)	5.12 (0.03)	2.55 (0.07)
463.4	2.70 (0.05)	3.42 (0.05)	5.11 (0.01)	2.56 (0.04)
583.3	2.73 (0.06)	3.43 (0.05)	5.11 (0.02)	2.56 (0.04)
734.3	2.74 (0.01)	3.42 (0.06)	5.10 (0.02)	2.56 (0.04)
924.6	2.75 (0.01)	3.41 (0.04)	5.10 (0.01)	2.58 (0.01)

[a] : mean values (n=4); number in parenthesis refers to standard deviation.

Appendix 10.

Viscosity (mPas) of four types of fluid milk (14% T.S.) at 20° C.

Shear rate (sec^{-1})	Control	UF	DF	MSNF
116.3	3.74 (0.40)[a]	6.13 (0.45)	6.85 (0.42)	2.91 (0.09)
146.7	3.75 (0.30)	6.10 (0.37)	6.77 (0.33)	2.95 (0.06)
184.6	3.76 (0.25)	6.08 (0.28)	6.68 (0.26)	2.95 (0.05)
232.2	3.78 (0.21)	6.07 (0.20)	6.62 (0.21)	2.96 (0.03)
268.2	3.78 (0.14)	6.04 (0.24)	6.59 (0.15)	2.96 (0.02)
292.3	3.79 (0.13)	6.04 (0.14)	6.59 (0.10)	2.97 (0.03)
463.4	3.79 (0.15)	6.03 (0.08)	6.54 (0.09)	2.98 (0.01)
583.3	3.78 (0.14)	6.01 (0.01)	6.52 (0.10)	3.02 (0.02)
734.3	3.80 (0.17)	6.00 (0.05)	6.48 (0.09)	3.04 (0.01)
924.6	3.80 (0.20)	5.98 (0.07)	6.43 (0.08)	3.05 (0.01)

[a] : mean values (n=4); number in parenthesis refers to standard deviation.

Appendix 11.

Viscosity (mPas) of four types of fluid milk (16% T.S.) at 20° C.

Shear rate (sec^{-1})	Control	UF	DF	MSNF
116.3	4.77 (0.02)[a]	10.01 (0.00)	11.64 (0.31)	3.62 (0.05)
146.7	4.79 (0.01)	10.16 (0.02)	11.85 (0.29)	3.63 (0.04)
184.6	4.81 (0.00)	10.09 (0.01)	11.84 (0.24)	3.64 (0.03)
232.2	4.83 (0.01)	10.03 (0.02)	11.73 (0.21)	3.65 (0.02)
268.2	4.85 (0.02)	9.98 (0.02)	11.60 (0.19)	3.66 (0.01)
292.3	4.88 (0.02)	9.91 (0.04)	11.46 (0.19)	3.66 (0.03)
463.4	4.89 (0.01)	9.82 (0.03)	11.31 (0.16)	3.67 (0.03)
583.3	4.87 (0.01)	9.72 (0.02)	11.17 (0.13)	3.67 (0.04)
734.3	4.86 (0.02)	9.61 (0.01)	10.95 (0.14)	3.70 (0.01)
924.6	4.85 (0.02)	9.52 (0.02)	10.90 (0.14)	3.70 (0.00)

[a] : mean values (n=4); number in parenthesis refers to standard deviation.

Appendix 12.

Changes of organic acid composition of control skim milk (T.S. 14%) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
	concentration (μg/g)					
0	50.7[a] (1.2)	150.1 (10.5)	4.4 (2.9)	380.3 (97.0)	-----	32.8 (4.1)
1	30.8 (0.4)	257.5 (15.3)	7.9 (1.2)	1826.2 (85.7)	-----	204.8 (21.9)
2	25.5 (0.4)	168.4 (32.7)	14.1 (2.5)	3890.7 (60.9)	-----	136.5 (61.1)
3	12.0 (0.4)	61.8 (13.6)	39.8 (3.3)	9227.4 (97.3)	53.9 (12.4)	143.9 (14.0)
4	2.0 (0.4)	41.0 (16.5)	17.3 (1.2)	12073.0 (20.5)	65.6 (5.8)	202.4 (1.2)
5	3.2 (2.0)	41.0 (2.9)	32.8 (15.6)	12055.0 (129.0)	60.6 (0.4)	218.8 (17.8)
6	1.5 (1.2)	40.3 (15.3)	17.0 (3.3)	11166.0 (364.0)	65.9 (1.2)	256.4 (7.0)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Appendix 13.

Changes of organic acid composition of ultrafiltrated milk (T.S. 14%) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
	concentration (μg/g)					
0	33.7[a] (19.5)	32.2 (4.3)	-----	326.4 (60.5)	-----	-----
1	32.0 (7.9)	77.35 (6.8)	2.9 (0.8)	586.4 (117.0)	-----	-----
2	48.6 (0.8)	70.2 (12.4)	11.4 (3.7)	2164.9 (186.0)	-----	-----
3	34.8 (11.0)	70.5 (5.6)	36.0 (12.8)	6692.3 (236.0)	43.4 (8.3)	41.0 (0.8)
4	16.4 (1.6)	80.6 (31.9)	21.1 (0.0)	11608.0 (85.7)	51.3 (3.7)	202.5 (36.9)
5	8.8 (0.8)	36.0 (36.8)	12.0 (2.9)	11434.0 (88.8)	42.7 (4.9)	366.8 (6.6)
6	5.8 (0.8)	55.3 (24.5)	3.5 (0.8)	14728.0 (326.0)	43.4 (11.6)	756.2 (92.4)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.

Appendix 14.

Changes of organic acid composition of diafiltrated milk (T.S. 14%) during fermentation with a mixed starter culture (1:1, 2%) at 42° C.

Time (hr)	Orotic	Citric	Pyruvic	Lactic	Acetic	Propionic
	concentration (μg/g)					
0	26.1[a] (5.4)	141.5 (13.8)	-----	448.0 (139.0)	-----	-----
1	29.6 (0.4)	311.0 (52.6)	6.4 (0.4)	1398.0 (29.0)	-----	-----
2	29.0 (4.0)	214.7 (3.7)	17.3 (0.4)	3363.0 (57.0)	-----	-----
3	12.0 (1.2)	66.5 (13.7)	43.9 (9.9)	8998.0 (251.0)	40.1 (5.4)	78.8 (33.6)
4	2.9 (0.8)	31.0 (2.8)	13.5 (1.7)	12378.0 (152.0)	68.8 (1.2)	187.5 (4.1)
5	2.6 (0.4)	14.0 (1.2)	13.5 (0.0)	12336.0 (32.0)	51.3 (9.5)	267.0 (4.1)
6	2.0 (0.4)	----	4.1 (1.6)	10606.0 (79.0)	49.5 (12.8)	267.5 (46.8)

[a] : mean values (n=3); number in parenthesis refers to standard deviation.