CHAPTER 1

INTRODUCTION

Ultrafiltration (UF) is increasingly used by dairy industry to concentrate proteins of milk. As a result of this membrane-process, a huge quantity of permeate (by-product) is produced. The solid content of this permeate consists predominantly of lactose (the milk sugar). Lactose is a potential pollutant. Due to high lactose content, the biological oxygen demand (BOD) of UF permeate ranges between 3000 and 4500 mg/L (Renner and Abd-El-Salam, 1991). A liquid with such a high BOD, can not be directly disposed off into sewage as a waste water. Hence from the environmental safety point of view, it needs to be treated. The requirement for the safe disposal of milk UF permeate is commercially disadvantageous to its producer. Another aspect apart from the environmental one, is its low recovery value due to lack of proteins. As per the existing practices, it is blended with some other dairy liquids. But, its rapidly increasing quantity in Australia needs some other commercially viable alternatives than blending. The first of such alternatives is to use milk permeate as a pig-feed. This option is commercially better than the disposal of milk permeate as a waste. However, the profit is not substantial. Second option is to extract lactose from it, but the demand for lactose is showing no significant increase in the world market. Drying of permeate is difficult and costly, making this third option commercially unattractive. Production of lactose derivatives is another option. These derivatives include lactosyl urea, ammonium lactate, biomass, methane, potable and fuel grade alcohols, organic chemicals, and the hydrolysed lactose syrup. The economic analysis of these derivatives indicates the production of potable alcohol as the most viable alternative (Cotton, 1980). However,
the market for this product is limited. Availability of wider market can place the hydrolysed lactose syrup ahead of potable alcohol and on top of the entire options (Cotton, 1980). The fact is that, all communities like sweet products. At the same time, the price of sugar keeps on fluctuating in the world market. The commercial success of hydrolysed lactose syrup can get a further boost, if it finds an ‘in-house’ application, for example in ice cream production.

1.1 Lactose hydrolysis

Hydrolysis of lactose refers to the splitting of lactose (a disaccharide) into its component monosaccharides, glucose and galactose, in presence of water. The site of this hydrolysis is the β-1,4 glycosidic linkage between galactose and glucose. However, the process of hydrolysis is not always confined to the production of glucose and galactose. In fact, the influence of a number of factors during hydrolysis causes oligosaccharide formation through transgalactosidation reactions (Zarate and Lopez-Leiva, 1990).

As a result of hydrolysis, two distinct properties of lactose, change significantly. These properties are the sweetness and the solubility. Lactose itself is very low in sweetness (16% of sucrose), whereas its components, glucose and galactose are significantly sweeter (74% and 32% of sucrose respectively). Similarly, the solubility of lactose is low (13 g/100 g of aqueous solution at 10°C) as compared to its component monosaccharides, glucose (40 g/100 g of aqueous solution) and galactose (28 g/100 g aqueous solution) (Zadow, 1992).

Hydrolysis of lactose can be accomplished by using acid or the enzyme lactase (β-galactosidase). Acidic hydrolysis can be carried out effectively and economically at low pH (1.2) and high temperature (140 °C for 11 min). These conditions make the acidic hydrolysis of lactose, suitable only for protein free streams such as a UF permeate. Moreover, under these conditions, a brown product is produced, which may be required to be neutralised, demineralised and decolourised before use. Despite being economic, the acidic hydrolysis of lactose, appears to have not been adopted commercially (Zadow, 1992).
Enzymic hydrolysis of lactose is practised commercially. The enzymes can be extracted from a number of sources. The source of enzymes affects their characteristics. Fungal enzymes are suitable for lactose hydrolysis at low pH and high temperatures, whereas yeast enzymes have neutral pH optima and are suitable for hydrolysis at lower temperatures. Fungal enzymes do not need co-factors for satisfactory function, whereas bacterial and yeast enzymes need them (Greenberg and Mahoney, 1981). However, fungal enzymes are strongly inhibited by the reaction product, galactose as compared to yeast enzymes. As a result of this inhibition, the rate of conversion slows down and high degree of hydrolysis is not achieved.

Enzymic hydrolysis of lactose can be carried out either using a single-use (soluble) system, enzyme recovery system, or enzyme immobilised system. Enzyme recovery system, however, has rarely been adopted commercially. Single-use system is more suitable for smaller operations, whereas immobilised system will be more cost-effective in larger operations. Although, the cost of enzyme is a concern for single-use system, it does not possess the complexity of and the cost involved in immobilising the enzyme. Moreover, the cost of enzymes is continuing to decrease, which can make the single-use system even more attractive (Zadow, 1992).

Concentration and demineralisation are two important steps in the production of hydrolysed lactose syrup. Concentration of syrup to high total solids (60-70%) is required to reduce the costs of its storage and transportation to distant locations. Concentration also increases its shelf life. Evaporation, freeze concentration, and reverse osmosis (RO) are available methods of concentration. Evaporation is most expensive, followed by freeze concentration and reverse osmosis. They require 140, 87, and 24.5 BTU of steam-equivalent energy respectively to remove 1 pound of water from a 10% dextrose solution at 70°F to reach 50% total solids. However, in practice, RO does not achieve a concentration to 50% solids (Schwartzberg, 1977).

Demineralisation is effective in enhancing the sweetness of syrup. Generally electrodialysis (ED) and ion-exchange (IE) are used for this purpose. But both these methods produce considerable quantities of effluent, which may cause disposal problems (Zadow, 1992). These methods are costly too, making the demineralisation step very critical in deciding the cost-effectiveness of syrup-production. For about 70%
removal of minerals, ED is less expensive than IE, whereas for more than 70% removal of minerals, IE becomes a less expensive process. ED preferentially removes monovalent ions, which have the greatest effect on flavour (Cotton, 1980). This provides ED (with 70% demineralisation) an edge over IE (with 90% demineralisation).

1.2 Nanofiltration

Removal of monovalent ions can also be accomplished by nanofiltration (NF) at the expense of less energy than required by ED or IE. NF or ultraosmosis (UO) is a concentration-cum-demineralisation process. This ‘two-in-one’ approach of this process can enable it to partially or fully substitute (depending on the specific requirement) the traditional concentration and demineralisation techniques. This membrane process involves pumping of liquid across a loose RO membrane at a pressure more than required by UF, but less than required by RO. The operating pressure for NF generally ranges between 1500 KPa and 3000 KPa. NF can remove molecules falling within the nanometer size exclusion range. These molecules include water and monovalent ions. Thin film composite type of membrane is used for nanofiltration. The barrier layer in such type of membrane is a polyamide produced as a result of condensation between trimesoyl chloride (Benzene-1,3,5-tricarboxylic acid chloride) and piperazine. The polymer thus produced, is formed interfacially on a microporous polysulphone support. This results in a barrier layer rich in carboxylate group (Gregory, 1987). Due to the presence of carboxylate group in the active layer of membrane, it is believed that salt rejection during NF is controlled by both the molecular size as well as the charge. In fact, the transport mechanism of NF involves the mechanisms of three processes altogether. These mechanisms are the solution-diffusion of RO, molecular exclusion of UF, and the electro-chemical mechanism of ED (Kelly and Kelly, 1995a; Kelly and Kelly, 1995b).

As NF operates at lower pressure than RO, it will require less steam-equivalent energy. Thus, for the concentration purpose, NF will be the most cost effective process. Coupled with demineralisation, it will outweigh its competitors in favourably influencing the overall economy of producing hydrolysed lactose syrup, without producing much effluent. However, the organic loading of permeate from nanofiltration can become considerable from an environmental point of view, if this process is used after the hydrolysis step. The reason is the loss of
glucose and galactose through the NF membrane (Kelly et al., 1991; Lopez-Leiva and Guzman, 1995). This loss can adversely affect the profit margin as well. Hence the application of NF before hydrolysis will be a better proposition.

Like RO, NF has limitation in concentrating the liquid to a very high level. But, the hydrolysed lactose syrup for in-house application in ice cream formulation may not need concentration to very high total solids. This is due to the fact that water is the major constituent of an ice cream mix and hydrolysed lactose syrup can be a major source of this water, if concentrated to low total solids.

1.3 Ice cream

Ice cream is a frozen mixture of components of milk, sweeteners, emulsifiers and flavourings, with or without egg products, colourings, and starch hydrolysates. This mix, is pasteurised, homogenised, aged and cooled before freezing. Freezing involves rapid removal of heat while agitating vigorously to incorporate air, thus imparting the desirable smoothness and softness of the frozen product (Marshall and Arbuckle, 1996). After freezing, ice cream is immediately kept in a hardening room for storage at -18°C or lower temperature. During storage shelf life and the quality of ice cream are important. Glass transition temperature is an indicator of these parameters. Glass transition is the transformation that occur in amorphous materials from a solid glassy state to a super cooled viscous liquid state. Glass transition in ice cream is important because, molecular mobility and physico-chemical properties change dramatically over its temperature range (Roos et al., 1996).

The composition of ice cream varies widely, depending on the intended market. A typical commercial ice cream with an approximate composition of 38.3% total solids (ranging from 37.5% to 39.0%) contains 12.0% fat, 11.0% non-fat milk solids, 15.0% sweeteners and 0.3% stabilisers and emulsifiers (Marshall and Arbuckle, 1996). According to the Australian Food Standard Code (1993), ice cream should contain not less than 100 g/kg of milk fat and 168 g/L of food solids.

Properties of ice cream are influenced by its constituents. Milk fat imparts rich characteristic flavour to the ice cream and restrict the
growth of undesirable ice crystals. It retards the rate of whipping, but does not lower the freezing point. Non-fat milk solids from skim milk consist of proteins (37%), lactose (55%), and minerals (8%). Lactose adds slightly to the sweet taste, while minerals impart slightly salty taste. Proteins make an ice cream more compact and smooth, and thus tend to prevent a weak body and coarse texture. An excess of non-fat milk solids may result in a salty or overcooked flavour, and increase the risk of lactose crystallisation during storage. Non-fat milk solids increase viscosity and resistance to melting, but also lower the freezing point. The main function of sweeteners is to impart sweetness to the ice cream and enhance the pleasing creamy flavour and the delicate fruit flavour. They also affect the properties such as freezing point, viscosity, and whipping ability. The most important function of stabiliser is to prevent the growth of ice crystals, caused by temperature fluctuations during storage. It also increases the viscosity and tend to limit whipping ability. Freezing point is not affected by stabiliser, but the melting of ice cream is resisted by it. Emulsifiers are used to produce a finished product with a smoother texture and stiffer body and to reduce whipping time. Air is incorporated in ice cream to produce overrun. Production of overrun influences the quality and profits and is involved in meeting legal standards (Marshall and Arbuckle, 1996).

1.4 Aims and objectives

NF, a recently developed membrane process has found applications in the treatment of quite a few dairy liquids including whey permeate. However, there is a dearth of available information on its application in the treatment of milk permeate. A dairy industry troubled with the increasing quantity of milk permeate may need the information on the processing potential of NF.

Lactose hydrolysis has been carried out and the degree of hydrolysis has been studied under various conditions in whey/permeate. However, there is a paucity of information available on lactose hydrolysis in milk permeate, especially its nano-concentrate.

The effects of hydrolysed lactose syrups, prepared from whey/permeate, concentrated by evaporation and demineralised by IE or ED, on the physico-chemical characteristics of ice cream have been studied. However, the effect of a hydrolysed lactose syrup, prepared from milk permeate, and concentrated as well as demineralised by NF,
on the physico-chemical characteristics of ice cream, is yet to be reported.

Thus, in view of these facts, the present study aimed at transforming milk permeate from a liability to an asset to its producer, was undertaken with the following objectives.

1. Concentrating the milk UF permeate to 15% total solids and simultaneously demineralising it using nanofiltration membrane.

2. Converting the nano-concentrate (NC) to sweet sugar syrup by hydrolysing its lactose content by enzyme lactase.

3. Exploring the feasibility of using the hydrolysed lactose nano-concentrate (HLNC) in ice cream formulation as a partial substitute for sucrose.
CHAPTER 2

LITERATURE REVIEW

2.1 Nanofiltration in dairy processing

2.1.1 Flux rate and fouling

Flux rate depends on various processing parameters. These parameters include pH, acidulant, temperature, pressure, pre-treatment, and the type of dairy fluid.

The presence of whey proteins have been predicted to be responsible for the decline in the initial flux rate. This is evident from the fact that the initial flux of salted permeate from cow’s milk is much higher than that of the salted whey obtained by Abd El-Salam and El-Etriby (1992) from domiati cheese.

Flux rates during NF are adversely affected by the presence of NaCl. This is due to the increase in osmotic pressure, which in turn increases the resistance of solution to pass through the membrane (Abd El-Salam and El-Etriby, 1992).

Effect of pH on fouling and flux rate have been studied during NF of skim milk (Eckner and Zottola, 1992a; 1993), acid casein whey (Kelly and Kelly, 1995a), and milk UF permeate (Abd El-Salam and El-Etriby, 1992). According to these studies, with regard to skim milk and milk permeate, the decrease in pH causes decline in flux rate, whereas, opposite trend is observed with regard to acid casein whey. An increase in pH of acid casein whey (from pH 4.6 to 5.9) severely fouls the membrane, leading to flux decline. According to Kelly and Kelly (1995a), the precipitation of calcium phosphate causes fouling during the initial period of NF. They have also reported that NF of acid casein whey at lower pH takes 40 min to reach a volume concentration ratio (VCR) of 4.0 as compared to 60 min taken at higher pH values. However, these results seem to be in contradiction with those obtained by them for skim milk and milk permeate.
Eckner and Zottola (1992a; 1993) have explained the decrease in initial flux rate, as a result of lowering the pH of skim milk with the use of lactic and citric acids. According to them, the decline in flux is probably due to the concentration polarisation and fouling phenomena, under the influence of temperature. According to these authors, the alteration in pH down to 5.2 alters the reactivities of proteins and amino acids by the way of increase in their charged groups. These charged groups form a gel layer and increase the fouling to cause decline in flux rate. According to them, at later stage (after 2 h), pH does not play any role, due to the increased gel layer. The similar trend was observed by Abd El-Salam and El-Etriby (1992) with regard to a pre-treated milk UF permeate (addition of 5% NaCl). They claimed that the alteration in the properties of membrane was responsible for the flux decline as a result of decrease in pH. The type of acidulant, whether lactic or citric acid used to decrease the pH, makes little impact on flux rate (Eckner and Zottola, 1992a; 1993).

The temperature significantly influences the flux rate. For some time (about an hour) from the beginning of the process, an increase in temperature greatly increases the flux, irrespective of the nature of feed (Abd El-Salam and El-Etriby, 1992; Eckner and Zottola, 1993). Abd El-Salam and El-Etriby (1992) used salted milk UF permeate, whereas Eckner and Zottola (1993) used skim milk as the feed for nanofiltration. The temperature affects the hydrodynamic properties of the permeated solution (Abd El-Salam and El-Etriby, 1992). Therefore, a higher initial temperature causes the removal of a greater volume of permeate at the initial stage of processing (Eckner and Zottola, 1993). Gradually, the removal of higher volume of permeate results in an increase in TS concentration, which in turn increases the viscosity of the retentate, even as the temperature attempts to counteract this phenomenon. This increased viscosity reduces the flux at the later stage of processing. High initial temperature also causes membrane fouling, thereby decreasing the flux by the way of enhancing the potential for chemical reaction between the components of the gel layer, and that between the gel-components and the membrane surface. The lower initial temperature, on the other hand, may prevent fouling and increase the flux, because there is less energy in the molecules, decreasing the likelihood of such chemical reactions (Eckner and Zottola, 1993). Fouling occurred as a result of such chemical reactions involving gel layer or the concentration polarisation, is rigid in nature. The rigidity of fouling or its severe nature can be gauged from the fact that while
concentration polarisation can be removed by simply rinsing/flushing the membrane with water, the fouling from membrane can be removed only by the use of cleaning agents. A simple flushing or rinsing of membrane with water may not be enough to get rid of fouling (Eckner and Zottola, 1993).

Abd El-Salam and El-Etriby (1992) studied the effect of transmembrane pressure (TMP) on the flux rate. According to them, an increase in TMP increases the flux rate.

The pre-concentration of acid casein whey adversely affects the flux rate (Kelly and Kelly, 1995b). Consequently, lower TS concentration is achieved (VCR 2 instead of VCR 4). Compared to 37-41 L/m²/h of unconcentrated whey, the initial flux rate of pre-concentrated (concentration level 2:1 achieved through thermal evaporation) whey, was only 21.1 L/m²/h, as observed by Kelly and Kelly (1995b). They further observed that the final flux for the pre-concentrated whey at VCR 2 was 4.4 L/m²/h and that for the unconcentrated whey at VCR 4 was 10-12 L/m²/h.

2.1.2 Effect of nanofiltration on the mineral content of dairy fluids

The NF membrane preferentially permeates monovalent ions of Na, K, and Cl. On the other hand, divalent ions of Ca and Mg are mostly retained by this membrane. Exploiting its property of high permeability to monovalent ions, Gregory (1987) used NF membrane to desalt the sweet type whey salt drippings obtained from the salting tables and the cheese presses of a cheese plant. He employed the Filtration Engineering’s UO membrane for this purpose. The desalted drippings (87% ash removal; 90% NaCl reduction) were found by him quite suitable to be blended with the bulk of whey. However, such a high degree of salt removal was achieved with the inclusion of diafiltration (DF) in the process. Level of concentration reached 4 fold during this process. Similar level of demineralisation (84%) for salt whey was reported by Kelly et al. (1991), with the help of DF. Gregory (1987) observed the increase in flux (as a result of DF inclusion) responsible for such a high degree of salt removal. According to him, salt removal was facilitated due to the reduction of fouling by high flux rate. Contrary to these reports, Kelly et al. (1991) did not find DF substantially instrumental in demineralisation, when applied to some of the other dairy fluids (Table 1). Nevertheless, the flux rate was always
higher during the membrane process, which included DF. It is evident from Table 1 that the type of whey, whether acidic or sweet, has an insignificant effect on salt permeation, particularly when DF is used for 4-fold concentration. The situation is more or less similar, when there is no use of DF, except in the case of rennet casein whey. This whey is significantly different from acid casein and Cheddar cheese wheys.

The presence of whey proteins and the formation of a polarised layer have been found to have no effect on the permeability of NaCl (Abd El-Salam and El-Etriby, 1992). This is evident from their observation that the permeability of NaCl from domiati cheese whey, and that from the salted milk UF permeate is almost the same.

In brief, the membrane fouling adversely affects the permeation of NaCl, as observed by Gregory (1987), whereas the concentration polarisation does not affect it, as reported by Abd El-Salam and El-Etriby (1992).

Minerals other than the monovalent ones, are mostly retained by NF membrane (Kelly et al., 1991; Guu and Zall, 1992; Bird, 1996). However, the report of Guu and Zall (1992) differs from the reports of Kelly et al. (1991) and Bird (1996), with regard to the phosphate retention. The permeation of phosphate from the UF permeates of sweet whey and skim milk, is significantly higher (Guu and Zall, 1992) than that from the sweet whey (Kelly et al., 1991) and salt whey (Bird, 1996), through the NF membrane. The result obtained by Guu and Zall (1992) in this regard, was confirmed by the finding of Eckner and Zottola (1992b).

Effects of various processing parameters on the permeability of certain minerals, have been reported. These parameters include the temperature, transmembrane pressure (TMP), pH, salt content (NaCl and citrate), pre-concentration and the level of concentration.

Report of Kelly and Kelly (1995b) suggests that ash removal during NF is not substantially affected by pre-concentration. They observed 25.8% demineralisation, when the acid casein whey was already thermally concentrated (two fold). Compared to this, the demineralisation observed by them during the NF of uncentrated whey, was 32.8% after four fold concentration.
Table 1

Effects of concentration and diafiltration by NF (HC-50 membrane) on ash and chloride levels of wheys from different sources.

<table>
<thead>
<tr>
<th>Whey source</th>
<th>pH</th>
<th>VCR</th>
<th>Reduction of ash in dry matter after concentration (%)</th>
<th>Reduction of ash in dry matter after diafiltration (%)</th>
<th>Cl' reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid casein</td>
<td>4.6</td>
<td>4</td>
<td>32.8</td>
<td>41.2</td>
<td>41.1</td>
</tr>
<tr>
<td>Acid casein</td>
<td>4.6</td>
<td>2</td>
<td>18.2</td>
<td>28.4</td>
<td>31.5</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>6.29</td>
<td>4</td>
<td>35.2</td>
<td>42.4</td>
<td>71.9</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>6.29</td>
<td>2</td>
<td>18.5</td>
<td>31.0</td>
<td>47.3</td>
</tr>
<tr>
<td>Rennet casein</td>
<td>6.72</td>
<td>4</td>
<td>25.8</td>
<td>40.5</td>
<td>72.9</td>
</tr>
<tr>
<td>Rennet casein</td>
<td>6.72</td>
<td>2</td>
<td>13.8</td>
<td>22.8</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Source: Kelly et al (1991)
Permeability of different minerals responds differently to the levels of concentration. The permeability of potassium, sodium and phosphate increases with the increase in the concentration factor up to 2.0. Then, they gradually level off with further increases in the concentration factor (Guu and Zall, 1992). The chloride follows the same tendency of levelling off, but after three fold concentration (Kelly and Kelly, 1992a). Calcium and magnesium do not show the similar tendency. They remain almost levelled off right from the beginning till the end of the process, irrespective of the level of concentration (Kelly and Kelly, 1995a; Guu and Zall, 1992).

Effects of added salts on the permeation of chloride ion, have been studied. Kelly and Kelly (1995a) used different concentrations of tri sodium citrate (TSC) to reduce the chloride ion content of acid casein whey through Donnon effect. TSC was used by them to bring in the Donnon effect in order to effect the permeation of chloride ion through a HC-50 membrane. On account of the presence of TSC (0.02 M), the reduction of chloride ion (in d.m.) changed from 40.5 to 70.0%. With 0.005M TSC, the reduction changed to 51.7%. According to these authors, a slight increase in pH (from 4.6 to 5.3) was also responsible to some degree for the chloride ion permeation. Addition of NaCl to milk UF permeate has been found to enhance the chloride ion permeation (Abd El-Salam and El-Etriby, 1992), but to a lesser degree than that effected by TSC.

Permeability of NaCl from salted milk UF permeate is negligibly affected by the change in TMP, despite an increase in flux rate with an increase in TMP (Abd El-Salam and El-Etriby, 1992). However, this observation does not hold good for the whey salt drippings. Gregory (1987) found the low flux rate responsible for the slow removal of salt from whey salt drippings by UO.

Presence of proteins has been found to be responsible for the effect of pH on mineral permeation. It is evident from the fact that change in pH of salted milk UF permeate from 6.7 to as low as 5.5 has almost no effect on the permeability of NaCl (Abd El-Salam and El-Etriby, 1992). On the other hand, permeability of minerals changes distinctly with the change in pH values of both the skim milk and the acid casein whey. Lowering the pH of skim milk from 6.8 to 5.6 has been reported to enhance the permeation of minerals with atomic weight below 40 (Eckner and Zottola, 1992b). This is because, at lower pH values, more
and more proteins release these minerals. Consequently, the concentration of these minerals in solution, increases. The increased concentrations of these minerals facilitate their permeability. Larger minerals (typically atomic weight above 40), though released by proteins at lower pH values, do not have sufficient energy to penetrate the concentration polarisation and gel regions on the surface of membrane. This results in their lower permeation (Eckner and Zottola, 1992b). Amphoteric nature of proteins plays a crucial role as far as the effect of pH on mineral permeation is concerned. This is evident from the findings of Kelly and Kelly (1995a). They observed that below pH 4.6 (the isoelectric point of proteins), the permeation of monovalent ions from acid casein whey decreased, but it increased at a near neutral pH. Particularly, chloride was found by them to be very sensitive to these pH changes. These authors reported that below the isoelectric point, proteins remain positively charged. The positive charge of proteins favours the positive retention of anions and the negative retention of cations. This is because of the attraction between opposite charges and the repulsion between the similar charges. Therefore, according to these authors, the negatively charged chloride ion, which is retained by the positively charged proteins, has low permeability below pH 4.6. On the other hand, at pH 6.6, whey proteins carry negative charge. Hence, they positively retain the cations and negatively retain the anions. This is the reason, why the permeation of chloride ion, which is negatively retained, is increased near this pH region.

There exist virtually no differences among the extents of NaCl permeation from salted milk UF permeate at different temperatures (Abd El-Salam and El-Etriby, 1992). These observations for the influence of temperature on NaCl permeation, however, contradict with those made by Eckner and Zottola (1992b), although a different dairy fluid (skim milk) was used by them. It seems that proteins might have played a role to raise this contradiction. Nevertheless, the influence of temperature found to be limited to the minor ash components and the minerals with atomic weight above 40, as reported by Eckner and Zottola (1992b). They observed that the higher temperature resulted in the greater permeation of these minerals, but not without the pH influence. At low pH, when more proteins release the minerals, the larger minerals at low temperature find their permeation difficult. In fact, they need higher energy, which they get at higher temperature to facilitate their permeation by penetrating the gel layer (Eckner and Zottola, 1992b).
2.1.3 Effect of nanofiltration on the fat content of dairy fluids

Fat is a fairly large molecule. Its size lies in micrometer range. Hence it is not able to permeate through a NF membrane, because a NF membrane removes the molecules only within the nanometer size exclusion range. This has been experimentally proved and reported by Kelly and Kelly (1995b) and Eckner and Zottola (1992b). Even the changes in temperature and pH do not force the permeation of fat through the NF membrane (Eckner and Zottola, 1992b).

2.1.4 Effect of nanofiltration on the nitrogenous fractions and protein contents of dairy fluids

According to Renner and Abd El-Salam (1991), milk UF permeate contains 0.25% of crude proteins. These authors reported NPN as the main constituent of crude proteins. According to them, the true protein content of milk UF permeate is one third of its crude protein content. Presence of some peptides in milk permeate was reported by Walstra and Jenness (1984).

Casein fines are completely impermeable to a NF membrane (Kelly and Kelly, 1995b). However, the temperature-dependant permeation of total proteins is possible (Eckner and Zottola, 1992b). Permeation of true proteins is debatable. Bird (1996) reported 100% rejection and 0% permeation of true proteins of salty whey from Cheddar cheese. On the other hand, Kelly and Kelly (1995b) detected 8.12% of true proteins in the NF permeate of acid casein whey. It seems that low pH of acid casein whey is responsible for the permeation of true proteins. Eckner and Zottola (1992b) observed the influence of pH on protein permeation. The α-lactalbumin (α-LA) and the β-lactoglobulin (β-LG) fractions of true proteins permeated in trace amounts, whereas immunoglobulin G (IgG) and bovine serum albumin (BSA) were completely retained (Kelly and Kelly, 1995b).

2.1.5 Effect of nanofiltration on the carbohydrate content of dairy fluids

Literature contradicts on the extent of lactose permeation through a NF membrane. Influence of various processing parameters, the type of dairy fluid used, and the type of membrane employed, could be the reasons for such contradictions.
Kelly et al (1991) reported a less than 0.3% reduction in lactose content of the normal sweet whey, passed through a Filtration Engineering’s thin film composite UO membrane, at the concentration factor of 4, temperature 21°C, and the TMP 25 bars. Bird (1996) reported more than 99% retention of lactose contained in salty whey obtained from hard pressed cheese. But he did not specify the type of membrane, and the processing conditions used. Kelly and Kelly (1995b) reported a little (2.67%) loss of lactose in NF permeate of acid casein whey, nanofiltered through an APV Pasilac HC-50 polymeric membrane, at the concentration factor of 4, pressure more than 30 bars, but at an unspecified temperature. On the other hand, Abd El-Salam and El-Etriby (1992) observed a considerably greater extent of lactose permeation through a Film Tec composite ultrathin membrane, than those reported above. This extent of lactose permeation was in the range of 15-20% with regard to the salted permeate as well as the salted whey. It indicates that the presence of whey proteins does not affect the permeation of lactose through a NF membrane. The finding of Eckner and Zottola (1992b) indicated high permeation of lactose from skim milk, when a DDS HC-50 polyamide membrane was used for 2 h. They found the lactose content (on wet basis) in the range of 4.5-7.87% in NF-permeate.

Various processing factors have been reported to have influenced the permeation of lactose through a NF membrane. Temperature is one of these factors. Abd El-Salam and El-Etriby (1992) observed a significant decrease in lactose permeability at 50°C than at 30°C or 40°C. Eckner and Zottola (1992b) observed an increased lactose permeation through a NF membrane as a result of an increase in temperature. Both of these groups of researchers, however, found no role of pH in lactose permeation. TMP, too, had almost no effect on lactose permeation, but slight increase in lactose permeation was observed due to the influence of NaCl (Abd El-Salam and El-Etriby, 1992).

Glucose, which has lower molecular weight (180) than lactose (342), is reported to have 90% rejection by NF 40 Film Tec membrane (Kelly et al., 1991). Lopez-Leiva and Guzman (1995) fractionated the carbohydrates of hydrolysed lactose whey UF permeate (20% hydrolysis) using NF-PES-5/PP100 membrane. After 3.1 fold concentration, 29.4% of glucose plus galactose was lost in the permeate. Also detected by them in the permeate, were 28.4% of
lactose and 22% of OS. These high degrees of permeation of lactose hydrolysates would not justify the use of NF after the hydrolysis of lactose.

2.1.6 Effect of nanofiltration on the acid content of dairy fluids

Citric acid is completely retained by a NF membrane (Kelly et al., 1991). The permeation of lactic acid seems to be dependant on the nature and the type of feed. Less than 35% reduction of lactic acid has been observed with regard to sweet whey, whereas 42% reduction has been observed with regard to cottage cheese whey (Kelly et al., 1991). The higher initial acidity of acidic cottage cheese whey (0.5% LA) than the sweet whey (0.15% LA) (Renner and Abd El-Salam, 1991) may have forced a greater permeation of lactic acid through the NF membrane.

2.1.7 Effect of nanofiltration on the colour of dairy fluids

There is a paucity of published information on the colour removal by NF from a dairy fluid. A study, however, has been conducted on the ground water. Tan and Sudak (1992) used NF to remove colour from a ground water source. The membrane used, was the softening-NF membrane. This membrane exhibits a sodium chloride rejection of 45-92% and a divalent ion rejection of about 98%. The usefulness of this membrane in removing colour is reflected from the fact, that 79-99% of organic molecules having molecular weights 200 or above, can be rejected. According to this study, more than 97% of colour was rejected by the NF-membranes. The permeate water contained only 3 platinum cobalt units (PCU) of colour against the 65 PCU in raw water.

2.1.8 Use of nano-concentrate

2.1.8.1 Use of the nano-concentrate of whey

Gregory (1987) reported, that 90% demineralised nano-concentrate of whey salt drippings, collected from the salting tables and cheese presses, could be blended with bulk whey for further processing.

The Murray Goulburn Co-operative Co. Limited, Australia transports its partially demineralised nano-concentrate of cheese whey (3.5 to 4.0 fold concentration) to one of its branch. At that branch, the concentrate
is either evaporated and dried directly, or blended with other wheys, demineralised further by ion-exchange, evaporated, and then finally dried (Sanderson, 1991).

Nguyen et al (1994) used a 4-fold concentrated and 50% desalted nano-concentrate of cottage cheese whey in ice cream formulations. They found the substitution of 25 to 50% non-fat milk solids with this nano-concentrate quite acceptable. According to them, some people even preferred the nano-concentrate-incorporated ice cream to the normal one.

Kailasapathy et al (1996) used the nano-concentrate of cottage cheese whey in yoghurt production. It was used in partial replacement of skim milk powder. Considering the overall physical, chemical and sensory characteristics, these authors concluded, that 20% of the MSNF in yoghurt premix could be satisfactorily replaced with this concentrate.

2.1.8.2 Use of the nano-concentrate of UF permeate

Sanderson (1991) reported, that the dried nano-concentrate of the permeate from ultrafiltration of milk or skim milk could be used as a food grade lactose or as a fermentation medium. However, the drying of permeate or its nano-concentrate on the normal spray drying plants, according to this author, is not easy. This is because, the blockages occur frequently and the material sticks to the chamber walls. This results in frequent occurrence of the burnt or scorched particles.

Guu and Zall (1992) used a 3-fold nano-concentrated sweet whey and skim milk permeates for the production of lactose crystals. They reported an 8% increase in the yield of lactose crystals in skim milk permeate and a 10% increase in the yield of lactose crystals in sweet whey permeate as a result of 3-fold nanofiltration.

2.2 Enzymic hydrolysis of lactose in whey/permeate

2.2.1 Effect of substrate concentration on lactose hydrolysis

The concentration of substrate (lactose) significantly affects the degree of hydrolysis (DH). An increase in the concentration of lactose decreases the DH (Giec and Kosikowski, 1983; Brodsky and
Grootwassink, 1986). However, some deviation from this trend was observed by Chiu and Kosikowski (1985).

Hydrolysing the UF permeate of reconstituted spray dried acid whey at 4°C and an adjusted pH of 6.8, by purified yeast lactase (0.7% enzyme concentration), Giec and Kosikowski (1983) found different DH for different concentrations of lactose. For 5% lactose, the DH was 75%, as compared to 70% for the 15% lactose, and 65% for the 25% lactose. The same trend of inverse relationship between the substrate concentration and the DH, was observed by these authors at all other enzyme concentrations. Similar trend was observed at all time intervals during the 168 h hydrolysis.

Brodsky and Grootwassink (1986) evaluated the whole cell yeast lactase instead of the purified one, for use in dairy processing. Studying the effect of lactose concentration on the DH at 40°C and pH 6.1 of the mixed cheese whey (whey from Cheddar cheese and cottage cheese) at a constant lactase/lactose ratio of 25 units/g, they observed an inverse relationship between the lactose concentration and the DH at each interval of time during a 3.5 h hydrolysis. Complete hydrolysis of lactose was observed after 3.5 h in the whey containing 50-250 g of lactose/L. At an elevated lactose concentration of 300-350 g/L, the DH reduced to 90%. With a further increase in lactose concentration to 400-450 g/L, the DH decreased to 80%.

Chiu and Kosikowski (1985) carried out the lactose hydrolysis of the demineralised (90%) permeate from reconstituted sweet whey powder for 5 h at 40°C and pH 6.5 using yeast lactase at a concentration of 0.22% of lactose. Various concentrations of lactose (7.5%, 15%, 22.5%, and 30%) were used for the hydrolysis. The maximum DH was observed in the 15% lactose concentration. The DH in the 7.5% lactose concentration was 70%. However, the trend of inverse relationship between the substrate concentration and the DH, was followed at the lactose concentration more than 15%. The DH in 22.5% and 30% lactose concentrations were found to be 72% and 71% respectively.

2.2.2 Effect of the source of substrate on lactose hydrolysis
Various sources of lactose have been used to study the effect of substrate-sources on the DH. These sources include skim milk, wheys, and whey permeates in either fresh or reconstituted form. These sources have been found to affect the DH. But, they have failed to set a definite
trend to that effect. More hydrolysis was observed in whey (Bernal and Jelen, 1985; Miyamoto et al., 1986), and in whey permeate (Bernal and Jelen, 1985) than in skim milk.

On the other hand, Gregory (1980) reported a higher DH in skim milk than in permeate. Mahoney and Adamchuk (1980) observed a higher activity of lactase in skim milk than in whey. Jakubowski et al. (1975) found similar lactase activity in acid whey and in whey UF permeate. However, Bernal and Jelen (1985) observed higher acidity in whey than in permeate. At 38°C and pH 6.8, they observed 50, 62 and 75% hydrolysis in skim milk, KOH-treated acid whey permeate and the KOH-treated acid whey respectively, using Kluyveromyces lactis (yeast) lactase at a concentration of 1g/L of substrate. The same trend of significant differences in DH was observed at each interval of time during an hour-long hydrolysis. Miyamoto et al. (1986) observed 80-90% of hydrolysis in whey, but only 40-50% in skim milk, after 30 min of incubation in presence of β-galactosidase (from Aspergillus oryzae), at a concentration of 0.1% of the substrate lactose. Gregory (1980) used reconstituted skim milk and the sweet whey permeate as the sources of lactose for the hydrolysis by the yeast lactase at different temperatures. The concentration of enzyme used by him, was higher (lactase/lactose ratio 428.5 EU/%) in permeate than that in skim milk (lactase/lactose ratio 394.7 EU%). Even with the higher enzyme concentration, the DH was found by him less in permeate than that in skim milk. The author reported, that after 6 h of hydrolysis at 20°C, the DH in permeate was 53%, as compared to 56% in skim milk. At 25°C after 6 h, the DH in permeate was 70%, compared to 75% in skim milk.

Differences in DH are not only limited to the substrate-sources differing in their protein content. Significant difference has been observed in different sources of whey itself (Patel and Mathur, 1982). At every interval of time during 6 h hydrolysis by 2% yeast lactase (Saccharomyces lactis) at 30°C and pH 7.0, the highest rate of hydrolysis was observed in paneer whey, followed by casein whey and cheese whey. The DH in these substrate-sources after an hour, were approximately 30, 28, and 26% respectively.

2.2.3 Effect of enzyme activator/inhibitor on lactose hydrolysis
The activity of enzyme lactase is significantly affected by the presence or absence of its activator or inhibitor. These activators/inhibitors include proteins, minerals and galactose.
2.2.3.1 Effect of proteins on lactose hydrolysis

The presence of proteins in milk/whey system has been found by some researchers to affect the enzyme activity and as a consequence, the DH. Mahoney and Adamchuk (1980) observed the highly favourable effect of some milk proteins on the activity of yeast lactase (*K. fragilis*). However, they were not sure about the mechanism by which proteins in general could activate the enzyme to such a high degree. All of the proteins studied, found to be enhancing the enzyme activity. The proteins studied were casein, Ig, BSA, ovalbumin, and the purified α-LA and the β-LG. Both the casein and whey proteins were equally effective towards enhancing the enzyme activity, although whey proteins were effective at a much lower concentration. Even though α-LA and β-LG are the principal proteins present in whey, in purified form, they were less effective as activators than the whey protein fraction. Mahoney and Adamchuk (1980) inferred, that some of the minor whey proteins such as serum albumin and Ig have stronger activating effect on the enzyme activity. This was ultimately confirmed by the higher enzyme activation observed in the presence of BSA. Presence of ovalbumin also showed an enhanced lactase activity. The denaturation of whey proteins by heating, however, found to slightly reduce their enhancing effect on enzyme activity.

The above findings are in disagreement with those of Jakubowski et al. (1975). They observed less activity of the bound fungal lactase (collagen-bound *A. niger lactase*) in acid whey than in the UF permeate of acid whey. This observation led the authors to the conclusion, that whey proteins, particularly those with low molecular weights, inhibited the lactase activity. However, with regard to soluble (free) lactase, these authors did not find whey proteins responsible for the inhibitory action. Their observation with the soluble enzyme is in agreement with that of Sheth et al. (1988). Working with a fungal lactase (*A. oryzae*), Sheth et al. (1988) found the overall rate of lactose hydrolysis independent of isolated proteins in whey permeate or the total whey proteins in the retentate of whey UF. The findings were similar in all of the conditions studied.

2.2.3.2 Effect of minerals on lactose hydrolysis

The presence or absence of certain minerals in the substrate medium has been found to be significantly affecting the lactase activity.
According to Gregory (1980), the yeast lactase requires a monovalent cation for maximum activity. The ions of K and Na can enhance the lactase activity. However, in a situation where pH adjustment of whey is necessary, his suggestion is to prefer the use of KOH to NaOH. This is because, the lactase activity is higher in presence of K ion than Na ion.

Mahoney and Adamchuk (1980) studied the effect of K, Na, Ca and Mg ions on the activity of yeast lactase (K. fragilis) in two substrate media, milk and potassium phosphate buffer of pH 6.6. They found the Na ion inhibitory to enzyme activity in both the buffer as well as the milk at all the concentrations used. This is in contrast to the report of Gregory (1980), who did not deny the activating effect of Na ion on the enzyme activity. However, his report agrees with that of Mahoney and Adamchuk (1980), that the whey neutralised by KOH, will serve as a better substrate medium than the one neutralised by NaOH. Mahoney and Adamchuk (1980) observed some limitations in the activating effect of K ion on lactase activity. At a concentration of 100 mM of K ion in buffer solution, lactase activity found to be inhibited, whereas at the same concentration of K ion in milk, the lactase activity found to be activated. At all the other concentrations of K ion below 100 mM and in both of these substrate media, K ion only activated the lactase activity. Higher concentration of K ion as well as the Na ion (>100 mM) has been found by Mertens and Huyghebaert (1987) to display a limited effect on the stability of K. fragilis lactase in a reconstituted spray dried whey permeate.

Bernal and Jelen (1985) compared the effects of KOH and NaOH on the DH. KOH and NaOH were used at a concentration of 5.0 M to neutralise the acid whey. The hydrolysis was carried out using 0.5g/L concentration of K. lactis lactase at 38°C and pH 6.8 for 2 h. At the end of 2 h, 74% of hydrolysis was achieved using KOH, as compared to 57% hydrolysis using NaOH. KOH proved to be a better neutralising agent every time during the 2 h hydrolysis.

Apart from K and Na ions, Mertens and Huyghebaert (1987) also studied the effect of Mg, Ca and Mn ions on the lactase stability. Influence of Mg and Ca on the lactase stability was almost nil. Mn ion showed relevant effect on enzyme activity. In non-demineralised whey permeate, addition of the optimal concentration of Mn (10^{-3} M) resulted in 100% retention of enzyme activity after 3 h of incubation at 45°C.
Unlike the observation of Mertens and Huyghebaert (1987), Mahoney and Adamchuk (1980) did not find the effect of Ca and Mg ions nil. They, however, had used different substrate media. The latter group of researchers found the Ca ion inhibitory for the lactase activity in buffer at very low concentrations, but had little or no impact, when added to milk. They predicted some reason for this behaviour of Ca ion. According to them, in milk, Ca remains in a non-reactive colloidal form (such as colloidal calcium phosphate) and therefore, is ineffective as a lactase inhibitor. In buffer, however, it remains in a reactive ionic form and hence is effective as a lactase inhibitor.

Disagreeing with the findings of Mahoney and Adamchuk (1980), but agreeing with those of Mertens and Huyghebaert (1987) Jakubowski et al. (1975) observed no effect of Ca and Mg ions on the activity of collagen-bound lactase.

Not only the presence or addition of ions to the substrate medium affects the lactase activity, but the removal of these ions also have been found to affect the activity of lactase. Giec and Kosikowski (1983) observed the effect of demineralisation on the activity of S. lactis lactase in whey permeate containing 15 and 25% lactose. At the resistivity of 30 ohm-cm, the rates of lactose hydrolysis were the lowest. These rates were 50.4% at 24 h and 52.5% at 48 h with regard to 15% lactose concentration, and 42.4% at 24 h and 55.7% at 48 h with regard to 25% lactose concentration. On the other hand, at 160 ohm-cm, they were the highest (69.7% at 24 h and 75.2 % at 48 h with regard to 15% lactose concentration and 56.3% at 24 h and 66.7 % at 48 h with regard to 25% lactose concentartion).

Report by Smart et al (1985) confirms that demineralisation decreases the activity of lactase. These authors observed large reductions in lactase activity in demineralised wheys and whey permeates. The lactase activity reduced to 0.7 units/mg protein from 9.2 units/mg protein, as a result of demineralisation of cheese whey permeate by ion-exchange. Similarly, a reduction from 9.7 units/mg protein to 1.2 units/mg protein was observed, when cheese whey was demineralised by ion exchange. With regard to lactic acid casein whey, the lactase activity declined to 1.7 from 8.2 units/mg protein.

The combined effect of various minerals on the lactase activity has also been studied. Mahoney and Adamchuk (1980) reported that lactase
activities in cold milk and whey are governed by the combined effect of various milk salts. This effect is so strong that the strong activating effects of various milk proteins was not detected in presence of these salts. They found the activity of lactase in milk the same as in the ultrafiltrate of milk. Similarly, according to them, there was no difference between whey and its ultrafiltrate with regard to lactase activity. Gregory (1980) reported that in presence of monovalent cations, certain divalent cations, particularly Mn$^{++}$ and Mg$^{++}$ activated the yeast lactase.

Smart et al (1985) have studied the effect of some individual ions as well as their combinations on the activity of lactase derived from various strains of *Streptococcus thermophilus*. They used a range of substrates for that purpose. These substrates were ONPG and the lactose containing media such as milk, lactic casein whey, rennet whey and cheese whey permeate. Their studies have shown that the hydrolytic activity of lactase is sensitive to the ionic environment. There was a clear distinction between the effect caused by Na ion and that caused by K ion. These two ions found to have opposing effect on lactase activity. With ONPG as the substrate, Na ion showed strong synergistic effect as the activator of lactase activity. On the other hand, with lactase as the substrate, K ion showed strong synergistic effect as the activator of lactase activity. Na ion did not show any synergism with Mg ion, when lactose was the substrate. When all of these cations were present together, Na ion largely abolished the activation caused by K ion. This can be concluded from the results that, when lactose was the substrate, the lactase activity was 22 units/mg proteins in presence of Mg and K ions. With the same substrate, the lactase activity reduced to 6.3 units/mg protein in presence of Mg, K and Na ions, and to 3.1 units/mg protein in presence of Mg and Na ions. Ca ion affected the lactase activity in the same way as Na ion did. However, the extent of inhibition by Ca ion was significantly less than that by Na ion in presence of Mg and K ions. Phosphate found to be an activator with lactose as the substrate.

Smart et al (1985) also observed, that addition of the combination of Mg and K ions as well as Mg and Na ions to the demineralised cheese whey permeate enhanced the lactase activity. However, the enhancement by K ion was much greater than that by Na ion. Addition of Na ion along with Mg ion to the undemineralised cheese whey permeate decreased the lactase activity. These authors suggested that a
satisfactory rate of hydrolysis could be achieved in cheese wheys after neutralising them with KOH. But, with regard to lactic casein whey, the neutralisation with KOH was not sufficient to achieve a satisfactory rate of hydrolysis. This was due to a relatively low pH and high Ca ion-concentration of lactic casein whey. This whey, however, could be rapidly hydrolysed following its demineralisation, neutralisation with KOH and addition of activating amounts of Mg ion to it.

Another synergism was observed by Giec and Kosikowski (1983). They observed an increased rate of lactose hydrolysis, when concentrated NH₄OH was added to the demineralised acid whey permeate (25% lactose) in the presence of 0.1% potassium citrate used to adjust the pH. When used alone, NH₄OH reduced the rate of hydrolysis.

2.2.3.3 Effect of galactose on lactose hydrolysis

Effect of galactose on the rate of enzymic hydrolysis is dependant on the type of substrate and the source of lactase. With ONPG as the substrate, galactose (100 mM concentration) caused 30% inhibition of bacterial (S. thermophilus) lactase. However, the same concentration of galactose did not have any effect either on the initial rate or on the degree of hydrolysis, when lactose (0.14 M) was used as a substrate (Smart et al., 1985). Fungal lactase, on the other hand, was strongly inhibited by galactose, when lactose was the substrate (Zadow, 1992; Bernal and Jelen, 1989). Bernal and Jelen (1989) observed a considerable reduction in the activity of fungal lactase (A. oryzae). They found that the inhibitory effect of galactose on lactase activity was strongly concentration-dependent. Compared to about 70% lactose hydrolysis (without galactose addition) after 90 min at 55°C and pH 4.65, only about 40% hydrolysis was achieved in cottage cheese whey on addition of 13.1 mM of galactose to it. Inhibition of lactase by galactose prevents this enzyme from hydrolysing all of the lactose. Therefore, aiming at 100% conversion in the final product is not practical. Yeast lactases, however, are generally less affected by the reaction product, galactose (Zadow, 1992).

The α-anomer of galactose acts as a competitive as well as a non-competitive inhibitor, whereas its β-anomer acts only as a competitive inhibitor. Competitive inhibition by α-galactose is approximately 12 times higher than that by β-galactose (Flaschel et al., 1982). Furthermore, the enzyme lactase can distinguish between the anomers
of galactose. Due to this reason, the rate of lactose hydrolysis is not linearly correlated to the concentration of lactase (Flaschel et al., 1982).

2.2.4 Effect of the source of enzyme lactase on lactose hydrolysis

Lactases are obtained from various sources, which include plants, animal organs and the micro-organisms such as yeasts, bacteria and fungi (Gekas and Lopez-Leiva, 1985). However, the lactases of microbial origin are in common use. The source of enzyme affects its characteristics. Enzymes of different origins have different optimum processing conditions (Table 2). At their specific optima, they are expected to exhibit maximum degree of hydrolysis. However, the difference in their specific characteristics causes the difference in their degrees of hydrolysis even at their specific optimum conditions.

Miller and Brand (1980) compared the lactase of yeast origin (*K. lactis*) with that of fungal origin (*A. niger*) for their effects on the DH in milk ultrafiltrate. The hydrolysis was carried out at specific optimum conditions required for them. An enzymic dosage of 12 ONPG units/mL was used for the yeast lactase at 30°C and pH 6.7. The similar enzymic dosage was used for the fungal lactase at 55°C and pH 4.3. After 2 h, 100% hydrolysis was obtained by using yeast lactase, whereas less than 70% was obtained by using fungal lactase. On the other hand, when the lactases of yeast and fungal origins were compared for the DH at the similar conditions of pH and temperature, fungal lactases were found hydrolysing the lactose to significantly higher degrees than the yeast lactases (Patocka and Jelen, 1988). At pH 3.7 and temperature 30°C, a 0.5 mg /g dosage of yeast enzyme produced only 12-14% hydrolysis in a whey-butter milk mixture after 4 h. Under similar conditions, fungal enzyme produced 24-34.3% hydrolysis.

Not only the enzymes obtained from different microbial sources differ in hydrolysing lactose to different degrees, but those obtained from the same microbial origin, have also been found to differ from each other.
## Table 2

### Properties of microbial lactases

<table>
<thead>
<tr>
<th>Source</th>
<th>Optimum pH</th>
<th>pH stability</th>
<th>Optimum temperature (°C)</th>
<th>Co-factors needed</th>
<th>$K_m$ for lactose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>3.0-4.0</td>
<td>2.5-8.0</td>
<td>55.0-60.0</td>
<td>none</td>
<td>85.0</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>5.0</td>
<td>3.5-8.0</td>
<td>50.0-55.0</td>
<td>none</td>
<td>50.0</td>
</tr>
<tr>
<td>Kluyveromyces fragilis</td>
<td>6.6</td>
<td>6.5-7.5</td>
<td>37.0</td>
<td>Mn, K</td>
<td>14.0</td>
</tr>
<tr>
<td>Kluyveromyces lactis</td>
<td>6.9-7.3</td>
<td>7-7.5</td>
<td>35.0</td>
<td>Mn, Na</td>
<td>12.0-17.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.2</td>
<td>6.0-8.0</td>
<td>40.0</td>
<td>Na, K</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactobacillus thermophilus</td>
<td>6.2</td>
<td>nd</td>
<td>55.0</td>
<td>nd</td>
<td>6.0</td>
</tr>
<tr>
<td>Leuconostoc citrovorum</td>
<td>6.5</td>
<td>nd</td>
<td>60.0</td>
<td>none</td>
<td>7.8</td>
</tr>
</tbody>
</table>

nd - not determined.

Source: Greenberg and Mahoney (1981).
Observations made by Bernal and Jelen (1989) confirmed it. They observed that every time and with each enzymic dosage, the degrees of hydrolysis in cottage cheese whey (pH adjusted to 6.8) obtained by using *K. lactis* (an yeast) lactase were different from those obtained by using *K. fragilis* (an yeast) lactase. For example, after 60 min, at 38°C, a dosage of 0.5 g/L of *K. fragilis* lactase hydrolysed 60% of lactose. Under similar conditions, *K. lactis* lactase hydrolysed 56% of lactose.

### 2.2.5 Effect of enzymic dosage on lactose hydrolysis

A number of studies have shown that an increase in the dosage of enzyme increases the rate and the degree of hydrolysis. However, this relationship is not linear, particularly at high dosage of enzyme. For enzymic dosages less than or equal to 0.3% of demineralised whey permeate, Giec and Kosikowski (1983) observed near linearity between the enzymic dosages and the degrees of hydrolysis at all of the substrate concentrations used (5, 15 and 25% lactose). These hydrolysates, carried out using the yeast lactase (*S. lactis*) at 4°C for 24 h at pH 6.8 showed departure from linear relationship between the dosage of lactase and the degree of hydrolysis at the enzymic dosages more than 0.3% of substrate-concentrations used. Studying the effect of different concentrations of bacterial (*S. thermophilus*) lactase on the DH (in terms of glucose produced) in cheese whey permeate, Smart et al. (1985) observed almost the similar trend as stated above. In fact, the linearity was perfect at a concentration of 1 ONPG units/mL of whey. However, there was gradual departure from the linearity, when the dosages of enzyme were increased to 2.5, 5.0, and 10.0 ONPG units/mL of whey.

A number of other studies confirms that an increase in DH is not in the same proportion to an increase in the dosage of enzyme. On a 4-fold increase in the dosage of *K. fragilis* lactase (from 0.5g/L to 2.0 g/L of cottage cheese whey), Bernal and Jelen (1989) observed only 1.33-fold increase in the DH (from 60 to 80%) after 60 min at pH 6.8 and temperature 38°C. On a 2-fold increase in the dosage of *S. lactis* lactase (from 1% to 2%), Patel and Mathur (1982) observed only 1.62-fold increase (from 40% to 65%) in the DH of 5% buffer solution of lactose after 60 min at 30°C. The DH reported by Chiu and Kosikowski (1985) after 1 h at 50°C of concentrated (15% lactose) sweet cheese whey permeate were 33, 46, 67, and 74% at *A. oryzae* lactase dosages of 0.5, 1, 2, and 2.5% respectively. Patocka and Jelen (1988) using *A. oryzae*
lactase and Mertens and Huyghebaert (1987) using *K. fragilis* lactase observed the same trend. After 2 h lactose hydrolysis of whey-butter milk mixture (pH 3.7) at 30°C, Patocka and Jelen (1988) observed 25.5, 34.6, and 46.4 % of lactose hydrolysis at enzymic dosages of 0.5, 1, and 2 mg/g respectively. Similarly, Mertens and Huyghebaert (1987) observed 50, 70, and 86% hydrolysis at enzymic dosages of 100, 200 and 400 mg/L of whey permeate (pH 6.6) after 21 h at 8.5°C.

### 2.2.6 Effect of pH of substrate on lactose hydrolysis

Greenberg and Mahoney (1981) compared the optimum pH values for the activities of lactases obtained from yeasts, fungi and bacteria (Table 2). The pH optima for the lactases of fungal origin (*A. niger* and *A. oryzae*) are in the acidic region. Therefore, the enzymes of fungal origin are suitable for lactose hydrolysis in acidic wheys and permeates. The enzymes of yeast (*K. fragilis* and *K. lactis*) and bacterial (*E. coli, L. thermophillus* and *L. citrovorum*) origin have neutral or near neutral pH optima. These enzymes are therefore suitable for use in milk, sweet wheys and sweet permeates. They can also be used in acidic wheys and permeates, but after the adjustment of the pH of substrate to neutrality.

Greenberg and Mahoney (1981) also reported the effect of optimum pH on the activity of yeast lactase. Using lactose as a substrate, they found the optimum pH almost exactly to that found in milk and sweet whey, with the optimal stability region being pH 6-8. From pH 5.8 to pH 5, the enzymic activity dropped rapidly and below pH 5, it was lost irreversibly. Due to this reason, these authors concluded that the use of yeast lactase in acid whey was not possible without prior adjustment of the pH. However, the practice of pH adjustment is costly and generally results in an increase in ash content of the end product (Zadow, 1992).

Purification of enzyme is a factor that determines the optimum pH for its activity. This was reported by Brodsky and Grootwassink (1986). Using impure whole cell yeast (*K. fragilis*) lactase, they observed maximum DH (about 50%) in cheese whey at 40°C, after 1 h between pH 5.6 and 6.0. Compared to that for a purified lactase (Greenberg and Mahoney, 1981; Gregory, 1980), the range of optimum pH was much broader and lower. Consequently, the optimum pH for the activity of whole cell lactase can ensure greater acid stability of this enzyme than the one generally reported for the purified lactase.
Effect of pH on lactose hydrolysis of lactic casein whey by bacterial lactase (*S. thermophilus*) was studied by Smart *et al* (1985). The fact that bacterial lactases are suitable at neutral pH values for their optimal activities was confirmed by the report of these authors. Compared to 2.5% hydrolysis at pH 4.6, they achieved 86% at pH 7.0 after 3 h. This hydrolysis was carried out at 50°C, with an enzymic dosage of 5 ONPG units/mL of whey.

### 2.2.7 Effect of temperature on lactose hydrolysis

Greenberg and Mahoney (1981) reported the optimum temperatures for the lactases of yeast, fungal and bacterial origin (Table 2). Compared to the lactases of fungal and bacterial origins, the yeast lactases have low optimum temperatures. The optimum temperatures for fungal, yeast, and bacterial lactases reported by them were 50-60, 35-37, and 55-60°C. The optimum temperature for lactase from *E. coli* was lower than the other bacterial lactases. Guarda *et al* (1988) reported 37°C as the optimum temperature for *S. lactis* lactase. A temperature range of 35-40°C for the optimal activity of yeast lactases was reported by Gregory (1980). He further reported that the temperature stability of yeast lactase was such that normal pasteurisation completely inactivated the enzyme. According to him, the yeast enzyme was effective at cold storage temperatures (5-10°C). This property is extremely important when the microbiological quality of the product has to be maintained during hydrolysis.

The report by Miller and Brand (1980), however, presents a different picture of the temperature profile of yeast (*K. lactis*) lactase. According to this report, the optimum temperature of hydrolysis by this enzyme was out of the range of 35-37°C. This was evident from the fact that in order to achieve 98% hydrolysis in milk ultrafiltrate at a fixed enzyme dosage of 60 ONPG units/mL, only about 45 min were needed at 24°C. In contrast, it took around 2 h and 45 min at 35°C. At 10°C, 98% hydrolysis was achieved after 1 h, whereas at 5°C, it was achieved after 6 h.

### 2.2.8 Effect of time on lactose hydrolysis

A number of studies have shown that the DH is strongly influenced by the time-period. Initially, the hydrolysis takes place very rapidly. It keeps on increasing throughout the period of hydrolysis, however, with
much slower rates. Lack of substantial increases in the DH with the progress of time, can make the economic viability of the process of lactose hydrolysis questionable. Under such condition, an aim of 100% hydrolysis is not prudent. Observations by a number of workers confirm it.

During a 2 h hydrolysis of casein whey at 30°C using 2% yeast lactase, Patel and Mathur (1982) observed 40% hydrolysis of lactose. During the next 4 h, they observed only about 6% increase. Similar trend was observed in other whey streams such as the cheese and paneer wheys and at a different dosage of enzyme (1%). During initial 30 min using *K. fragilis* lactase (2g/L), at 38°C, Bernal and Jelen (1989) observed 77% hydrolysis in cottage cheese whey. During next 2 h, the increase was only about 6%. Situation was not different when Giec and Kosikowski (1983) carried out the hydrolysis in concentrated (25% lactose) or unconcentrated (5% lactose), demineralised whey permeate at 4°C using yeast (*S. lactis*) lactase. During initial 24 h, the DH observed by them were as high as 62% and 78% in concentrated and unconcentrated permeates respectively. During next 6 days (144 h), the increase was only 13% in each type of permeate. Hydrolysing concentrated sweet whey permeate (15% lactose) at 50°C using fungal lactase (0.5%), Chiu and Kosikowski (1985) reduced about 34% lactose in the first hour. The reduction after the next 4 h was 54% (20% increase). A similar trend was observed at all other enzymic dosages.

Using the lactose of milk permeate (pH 6.7), as a substrate for hydrolysis by yeast (*K. fragilis*) lactase (12 ONPG units/mL), at 30°C, Miller and Brand (1980) reported 75% hydrolysis during the first hour. Although, the hydrolysis was complete by the end of 2 h, only 25% increase was observed during the next hour. Using the fungal enzyme at 55°C and pH 4.3 of a lactose phosphate buffer, the same authors reported 78% hydrolysis in total of 3 h, compared to 58% in the first hour. Thus, compared to a 25% increase in the later 1 h by yeast lactase, the increase was only 20% in later 2 h, when fungal lactase was used. This points out the reason behind the slower progress of hydrolytic activity at later stages. It is already mentioned elsewhere that the reaction product galactose acts as an inhibitor of lactase and prevents it from hydrolysing all of the lactose (Bernal and Jelen, 1989). At the initial stages, the production of galactose is low, hence less inhibition of lactase results. At the later stages, the galactose production is high, hence there is higher inhibition of lactase. The higher inhibition
causes the slower progress in hydrolytic process. As already mentioned, it is more so with regard to fungal lactase than with yeast lactase.

2.2.9 Formation of oligosaccharides during lactose hydrolysis

Several reports are available on the formation of OS during lactose hydrolysis. The extents of their formation were as high as 44.6 % to as low as 1 % (Zarate and Lopez-Leiva, 1990). Formation of OS during lactose hydrolysis was attributed to a number of factors, such as the degree of lactose conversion, nature and concentration of substrate, type of process (free or immobilised enzyme), salts, pH, temperature, source of enzyme, and the enzymic dosage.

Formation of OS is strongly dependent on the degree of lactose conversion. Mozaffar et al. (1985) observed rehydrolysis of almost all of the OS after 4 h incubation. At shorter reaction times, however, OS concentrations were quite high (15% at 80% conversion; 10-20% at 90-98 % conversion). Burvall et al. (1979) on the other hand, found the OS unhydrolysed even after a long incubation of 14 days.

Reviewing the reports on the influence of the nature of substrate on the OS formation during lactose hydrolysis, Zarate and Lopez-Leiva (1990) reported higher hydrolytic activity in milk and milk products than in buffer solutions. With regard to milk and milk products, the OS concentrations ranged between 1 and 25 %, as against 25 and 44.6 % with regard to buffer solutions.

The initial lactose concentration strongly influences the formation of OS. If the initial lactose concentration is high, the formation of OS is high. The maximum amounts of OS obtained by Prenosil et al. (1987) were approximately 3, 5, 9, 11 and 15 % of total sugars, when the initial lactose concentrations were 5, 10, 15, 20 and 30 % respectively. The maximum concentration of OS observed by Jeon and Mantha (1985) was 11.3 % of total lactose, for 5 % lactose solution and 16 % for 20 % lactose solution. Near the completion of hydrolysis, the OS concentration decreased to 5.5 % for 5 % lactose solution and 10.8 % for 20 % lactose solution. Burvall et al. (1979) observed a similar trend. For the 5 % lactose solution in buffer, they obtained a maximum OS concentration of 5 % of the total sugars; this increased to 8.5 and 12.5 %, when 10 and 20 % lactose were used respectively. According to Burvall et al. (1979), higher amount of OS at higher initial lactose
concentration could be expected, since the higher lactose concentration increased the possibility of lactose becoming an acceptor of galactose, transferred by the enzyme.

Prenosil et al. (1987) reported that the immobilised enzyme produced less amount of OS than the free or soluble enzyme. They observed a decrease by 14 % in the maximum concentration of trisaccharides (TIS) and by 28 % with regard to tetrasaccharides. However, this trend was limited to higher initial lactose concentration (15 %). The change in OS production was very little, when lower initial concentration of lactose was used. They also observed, that with immobilised enzyme, at higher initial lactose concentration, the maximum OS production shifted towards lower degree of conversion.

Zarate and Lopez-Leiva (1990) have reported that higher amounts of OS are produced at higher temperature, pH and salt concentration. Prenosil et al. (1987) on the other hand, did not observe any significant influence of a temperature-increase of 10°C and a 10 fold increase in salt concentration on the production of OS.

Source of enzyme is another factor, which influences the production of OS. Prenosil et al. (1987) compared two fungal lactases with two yeast lactases. The maximum amount of OS was produced by the fungal lactase of A. oryzae origin. Another fungal lactase from A. niger produced considerably less amount of OS. There was no difference in this regard between the lactases from K. fragilis and K. lactis. However, the yeast enzymes produced substantially less OS than their fungal counterparts. Furthermore, the maximum production of OS by the yeast lactases shifted towards a high degree of conversion compared to the fungal lactases. On the other hand, higher number of OS was observed by Toba and Adachi (1978) with yeast lactase than the fungal lactase compared to 10 OS produced by its fungal counterpart. However, with respect to the bacterial lactase from C. pseudotropicalis, the yeast lactase from K. fragilis did not differ as far as OS production was concerned (Jeon and Mantha, 1985). Both of those lactases produced equal number of 5 OS each.

Jeon and Mantha (1985) studied the effect of different enzymic dosages on the production of OS. They found the effect of the levels of enzymic dosages (2 μmol ONP and 1 μmol ONP) the similar on the trend of DIS production, first increasing, then decreasing. However, the
maximum DIS production was observed at different times. The trend with regard to TIS was similar, when higher dosage was used. With a lower dosage, TIS first increased, but remained stable later on. Moreover, the formation of TIS with lower dosage was less than that with higher dosage.

2.2.10 Calculations for the degree of lactose hydrolysis

Ideally, the hydrolysis of lactose should involve the liberation of 1 M of glucose (molecular weight 180) and 1 M of galactose (molecular weight 180) from 1M of lactose (molecular weight 342) in presence of 1 M of water (molecular weight 18), according to the following equation:

Lactose + H₂O → Glucose + Galactose

Thus, only glucose and galactose should be considered as the products of hydrolysis. However, in practice, this ideal situation normally does not exist. The cause of that deviation is the production of OS, which is not through the hydrolysis, rather through the transgalactosidation reactions. Hence a clear distinction has to be made between the products of hydrolysis and those of transgalactosidation or transfer reactions, so that the products of transfer reactions can not influence the calculation of the DH. Jeon and Saunders (1986) reported the influence of OS formation on DH. These authors compared two methods to determine the DH. These methods were HPLC and Cryoscopy. HPLC was able to detect all of the carbohydrates produced, whereas cryoscopic method determined the freezing point of the hydrolyzed samples. The cryoscopic method determined the DH on the basis that the depression in freezing point is directly proportional to the amounts of monosaccharides, glucose and galactose formed (Nijpels et al., 1980). Formation of OS, as reported by Jeon and Saunders (1986), does not contribute to depression in freezing point. That results in a lower freezing point depression than a simple breakdown of lactose to glucose and galactose with no transgalactosidation products formed. The lower depression in freezing point results in lower DH, than that determined by HPLC. The calculation of DH by HPLC was accomplished by these authors by deducting the lactose remaining in the sample after hydrolysis, from the initial lactose content. These authors reported, that the differences between the DH measured by the two methods were correspondingly larger with the formation of increasingly higher concentrations of OS.
Various researchers calculated the DH differently. Smart et al. (1985), using the spectrophotometric method, calculated the DH as follows.

**Extent of hydrolysis = Amount of glucose released.**

Bernal and Jelen (1985), Sheth et al. (1988), Patocka and Jelen (1988) and Bernal and Jelen (1989) calculated the DH as follows, using the glucose analyser method.

\[
\text{Hydrolysis (\%)} = \frac{\text{mM glucose produced}}{\text{mM initial lactose}} \times 100
\]

Nijpels et al. (1980) enzymatically analysed the DH and the method of calculation used by them was as follows.

\[
\text{DH} = \text{Amount of galactose formed.}
\]

In the methods of calculation mentioned above, the formation of only one of the hydrolytic product of lactose, either glucose or galactose was considered. The reported literature (Greenberg and Mahoney, 1983) shows that due to the involvement of OS, the concentrations of glucose and galactose do not remain constant. At high lactose concentration, the value for glucose tends to overestimate the amount of lactose remaining, but gives a fairly good estimate of the total number of glycosides remaining to be hydrolysed. At lower lactose concentration, the glucose value underestimates the remaining glycosides. Determination of galactose and the use of the same assumption that a mole of either glucose or galactose represents a mole of lactose hydrolysed, leads to even worse overestimate of amount of lactose remaining and also overestimation of the remaining glycosides. Hence the DH can not be calculated by the determination of a single monosaccharide, except where the DH approaches 100% (Greenberg and Mahoney, 1983).

Patel and Mathur (1982) and Buera et al. (1990) used colorimetric method to determine the DH. The method of calculation used by them was as follows.

\[
\text{DH} = \text{Amount of glucose and galactose released.}
\]
This method of calculation considers both of the hydrolytic products, glucose and galactose for the calculation of the DH. In colorimetric method, the amount of initial lactose and that of glucose and galactose are estimated and the DH is expressed in terms of glucose and galactose formed, with respect to total initial concentration of lactose. However, the drawback of this method is that, the release of glucose and galactose is considered only from lactose. But, in fact, after attaining their maximum concentrations, the OS too begin to release glucose and galactose. Thus, not only lactose, but also the OS are involved in the production of these monosaccharides.

Boer and Robbertsen (1981) and Jeon and Saunders (1986) used HPLC for the determination of DH. The method of DH calculation used by them was as follows.

\[
\text{Hydrolysis (\%)} = \frac{\text{Initial amount of lactose} - \text{the amount of lactose remained after hydrolysis}}{}
\]

The method of calculation mentioned above, considers OS too as the products of hydrolysis, instead of the products of transfer reactions. The amount of lactose remained after hydrolysis, if deducted from the initial amount of lactose, will include in the resultant the OS formed along with glucose and galactose. The DH in this case, will be overestimated. However, Jeon and Saunders (1986) calculated the ‘percent of lactose hydrolysis’ by this method, not the ‘DH’.

Mertens and Huyghebaert (1987) used GC method for the analysis of lactose hydrolysis and calculated the DH as follows.

\[
\text{DH (\%)} = \frac{\%\text{glucose} + \%\text{galactose}}{\%\text{glucose} + \%\text{galactose} + \%\text{lactose}} \times 100
\]

The method mentioned above to estimate the DH, does not involve OS. But, as already mentioned, glucose and galactose also produce the OS at later stages of hydrolysis.

Using HPLC for the analysis of lactose hydrolysis, Prenosil et al. (1987) calculated the DH as follows.
Initial lactose concentration - Disaccharide concentration

\[ DH = \frac{\text{Initial lactose concentration} - \text{Disaccharide concentration}}{\text{Initial lactose concentration}} \times 100 \]

The method of calculation mentioned above considers the OS other than those having two monomeric residues, as the products of hydrolysis. In fact, only glucose and galactose are the hydrolytic products. The irony is that the disaccharides other than lactose, remaining after the hydrolysis, are not considered as the result of hydrolysis.

In view of the fact that OS affects the calculation of the DH, the method suggested by Novo Nordisk Bio-industrial Pty Ltd (1995), for which HPLC was used for the analysis of lactose hydrolysis, seems to be the most appropriate one. This method of DH calculation is as follows.

\[ DH \ (\%) = \frac{\text{Peak height of glucose} + \text{Peak height of galactose}}{\text{Peak heights of total carbohydrates}} \times 100 \]

This method considers only glucose and galactose as the products of hydrolysis and also considers OS involvement in the production of glucose and galactose. The OS are considered in this method, as part of total carbohydrates.

### 2.3 The hydrolysed lactose ice cream

Application of lactose hydrolysis in ice cream production, has been studied for quite some time. The application has been in different forms. It has been either through the hydrolysis of lactose in mix or through the use of hydrolysed lactose ingredients. The ingredients used were fresh or reconstituted milk/skim milk, wheys, and the permeates obtained from the ultrafiltration of milk, skim milk and wheys. Except permeates, the hydrolysed lactose products have been used both in substituting sucrose as well as the traditional sources of MSNF. Due to lack of proteins, permeates have been used only as sugar substitutes. The feasibility of such substitutions has been investigated through studies of their impacts on various physico-chemical characteristics of ice cream.
2.3.1 Sensory characteristics of hydrolysed lactose ice cream

2.3.1.1 Colour and appearance of hydrolysed lactose ice cream

Application of lactose hydrolysis did not significantly affect the colour or appearance of ice cream as reported by Arndt and Wehling (1989). In fact, the ice cream, in which 50% of sucrose was replaced with 90% lactose hydrolysed whey permeate, was slightly more appealing than the control ice cream. However, the ice cream, in which 25% sucrose was replaced, found to be less appealing than the control one. All of the ice creams prepared by El-Neshawy et al. (1988) obtained equal score for colour, irrespective of the degree of lactose hydrolysis in reconstituted skim milk, used for ice cream production. This 75% lactose hydrolysed reconstituted skim milk had been partially substituted for sucrose.

Effect of lactose hydrolysis on the colour of ice cream was also studied by Huse et al (1984). They measured the colour using a colorimeter. The colour parameters measured, were lightness, blueness/yellowness, redness/greenness and yellowness index. Increases in the levels of substitution of hydrolysed skim milk and whey solids, increased the lightness. However, blueness/yellowness and redness/greenness were insignificantly affected. At 0.05 level of significance, yellowness index was barely affected.

2.3.1.2 Body and texture of hydrolysed lactose ice cream

Effect of lactose hydrolysis on the body and texture of ice cream and other frozen desserts have been studied by a number of workers. There was no significant effect on the body and texture of ice creams, in which sucrose was replaced to levels up to 25 and 50% by the incorporation of 90% lactose hydrolysed whey permeate (Arndt and Wehling, 1989). Similar results were obtained by Rexroat and Bradley (1986), when sucrose was partially or fully substituted with 90% lactose hydrolysed and concentrated whey permeate in soft serve ice milk, hardened ice milk and milk shakes. The observation made by Patel and Mathur (1982) was not different to those made by the former two groups of researchers. In a mix formulation, 3% of non fat dry milk (NFDM) and 2.5% of sucrose were replaced by Patel and Mathur (1982) by the incorporation of 5.5% (with regard to the total solids of mix) hydrolysed lactose whey. In another formulation, they replaced
6% NFDM and 5% sucrose by the incorporation of 11% hydrolysed lactose whey.

In some other studies, the body and texture of ice creams, which involved lactose hydrolysis were found to be improved. El-Neshawy et al. (1988) observed these improvements in direct proportion to the DH in reconstituted skim milk used in ice cream formulations. Irrespective of the periods of storage at -15°C (0, 15 and 30 days), control ice creams always received less scores than the hydrolysed lactose ice creams (50 and 75% lactose hydrolysis). These authors found that the effect of sucrose substitution on the body and texture of lactose hydrolysed (75% hydrolysed reconstituted milk) ice cream, even more favourable than the effect of DH at the same sucrose level. However, there was limitation with regard to sucrose substitution. Ice cream with 14% sucrose was definitely better than the control one (having 16% sucrose), whereas, that with 12% sucrose, was similar to the control one.

Gregory (1980) prepared ice cream batches, to which enzyme lactase was either added directly to the mix (for a maximum of 50% hydrolysis) or the already hydrolysed milk (80% hydrolysis) was used in the formulations. Sensory evaluation found the texture of all of the hydrolysed lactose ice creams softer than the control one. After a week’s storage, the control ice cream in fact developed a detectable rough texture.

Some studies have been attributed to the effect of lactose hydrolysis on some textural properties of ice cream. Huse et al (1984) reported the reduction in mean score of sandiness as a result of lactose hydrolysis. Even the heat shocking (storage of ice cream at fluctuating temperatures) did not induce sandiness to any significant degree. Reduction of sandiness due to lactose hydrolysis prompted Gregory (1980) to suggest the replacement of non fat milk solids with lactose hydrolysed whey solids in ice cream formulations. No sandiness was detected by Guy (1980) in any ice cream prepared with lactose hydrolysed sweet whey solids (67 or 79% lactose hydrolysis) substituted for both the MSNF and the cane sugar.

With regard to the ice cream not subjected to heat shock, the increase in lactose hydrolysis decreases the iciness. However, the trend is not the same for the heat shocked ice cream, as reported by Huse et al. (1984).
According to them, the heat shocked ice cream containing 100% hydrolysed milk and 72% hydrolysed whey solids, was more icy than the unhydrolysed ice cream. But, the one containing these ingredients, hydrolysed to only 50% level, was less icy than the unhydrolysed ice cream. The lack of significant consistency in iciness/courseness was observed by Guy (1980) in unshocked sample of ice cream made with lactose hydrolysed whey solids.

Both the unshocked as well as the heat shocked ice cream found to be smoother than the control one by Huse et al (1984). Gumminess, on the other hand, showed inconsistent pattern (Huse et al., 1984). At 50\% lactose hydrolysis of skim milk and whey solids, gumminess obtained more score than the control in an unshocked ice cream, whereas less score than the control ice cream was received in the heat shocked sample. At maximum level of hydrolysis (100\% skim milk; 72\% whey solids), it received less score in unshocked and more in heat shocked sample than the control ice cream. At a constant level of sucrose, application of neutralised and hydrolysed cottage cheese whey resulted in an ice cream with predominantly coarse and icy texture. It was particularly in the samples neutralised with Ca(OH)\_2 (Young et al., 1980). After 22 weeks of storage at -18±1°C, Young et al. (1980) found all the Ca(OH)\_2-neutralised products developed sandiness defect.

Lindamood et al(1989) determined the firmness of lactase treated ice cream instrumentally. The measurement was accomplished by the work required to penetrate ice cream with a probe of specified dimensions to a specified depth in the ice cream at -15°C. They observed consistent reduction in firmness with decrease in lactose content as a result of increased enzymic hydrolysis. Compared to the control ice cream (0.44 J), the firmness of 25\% treated (0.29 J), 50\% treated (0.18j) and 100\% treated (0.16 J) ice cream was significantly low. On the other hand, Huse et al. (1984) found the hardness (firmness) increased as a result of lactose hydrolysis. This was observed through the reduction in the depth of penetration by the penetrometer.

2.3.1.3 Taste and flavour of hydrolysed lactose ice cream

2.3.1.3.1 Sweetness of hydrolysed lactose ice cream
The main purpose of lactose hydrolysis or the use of lactose hydrolysed ingredient in ice cream formulation is to reduce its cost of production
without significantly affecting the sweetness. Generally, 14-16% of solid content of ice cream is sugar. Sucrose and/or glucose syrup are generally used to maintain the required sugar level. Different sugars, however, have different sweetening capabilities. The components of hydrolysed lactose syrup have less sweetening capacities than sucrose. With respect to 100 units of sweetness of sucrose, glucose has 74 and galactose only 32 (Zadow, 1992). Due to less sweetening power than sucrose, incorporation of hydrolysed lactose in ice cream as a sucrose-substitute is limited. Complete substitution of sucrose by the way of either 100% lactose hydrolysis of mix, or incorporation of hydrolysed ingredients may not be possible. However, a number of studies were conducted to find to what extent such a replacement could be practically feasible.

Arndt and Wehling (1989) did not find the sweetness of either the control or reference ice cream significantly different from that, in which 25 or 50% of sucrose was replaced with hydrolysed lactose permeate syrup. The control ice cream had 15% sucrose content. In the reference ice cream, 25% of sucrose had been replaced with dextrose. The DH of hydrolysed lactose syrup was 97.2%. Its ash content had been reduced to mere 0.03% by ion exchange. Gregory (1980) used lactose hydrolysed milk (80%) for partial substitution of sucrose (by 28.5%). He found the sweetness of the ice cream similar to that of a control. Partial substitution of both NFDM as well as sucrose with lactose hydrolysed sweet whey solids did not show any significantly consistent pattern with respect to whey solids level, type used in the formulation, or the period of storage (Guy, 1980). However, when lactose hydrolysed ingredients (skim milk and whey) were used to facilitate the substitution of only non fat milk solids and not the sucrose, a metallic sweetness developed (Huse et al., 1984). This development was perceived, when high amounts of hydrolysed solids were incorporated in the mix.

There was a definite increase in sweetness, when enzyme lactase was added directly to the mix to hydrolyse 25, 50, or 100% of its lactose content. The calculated relative sweetness of the most hydrolysed sample was 134% of the control (Lindamood et al., 1989). However, neither the untrained nor the expert judges found the sample excessively sweet.
2.3.1.3.2 Saltiness of hydrolysed lactose ice cream

Guy (1980) used hydrolysed lactose sweet whey solids to partially replace non-fat milk and cane sugar in an ice cream formulation. This formulation comprised of 12% fat, 11% MSNF, 15% sucrose, 0.28% stabiliser and 0.5% vanilla flavour. Contents of MSNF and sucrose were reduced to varying degrees through the incorporation of 67% or 79% lactose hydrolysed sweet whey solids. The levels of whey solids added, were 2.75%, 5.5%, 8.25% and 11%. The ash contents of whey solids at these levels of addition were 1, 1.1, 1.2 and 1.3%; that of control ice cream was 0.9%. The author reported that the perception of saltiness by the trained judges correlated well with the increased ash content of ice creams. At 2.75% level, the perception was not significantly different from the control. However, the sample containing 5.5% of hydrolysed whey was found to be salty and the samples with higher contents of hydrolysed whey were significantly salty. According to the author, though the ice creams containing 11% lactose hydrolysed whey were rated much salty, their scores averaged only close to ‘slightly salty’ on a 4-point scale.

2.3.1.3.3 Flavours and off-flavours of hydrolysed lactose ice cream

According to El-Neshawy et al (1988), the use of lactose hydrolysed reconstituted skim milk (50% or 75% hydrolysed) as the source of MSNF, improved the flavour of fresh as well as stored (-15°C for 15 and 30 days) ice creams, when the level of sucrose was the same in each formulation. At the same degree of lactose hydrolysis (75%), reductions in sucrose level (by 12.5 or 25%), however, did not significantly affect ice cream’s flavour (El-Neshawy et al., 1988). Huse et al (1984) reported the maximum flavour perception, when 100% lactose hydrolysed skim milk and 72% lactose hydrolysed whey solids were used in combination with each other as the sources of MSNF in an ice cream formulation. Addition of hydrolysed lactose whey to partially substitute both the MSNF as well as sucrose content in ice cream formulations, did not influence the flavour of the final product (Patel and Mathur, 1982).

According to Arndt and Wehling (1989), substitution of 25 or 50% of sucrose with lactose hydrolysed whey permeate resulted in some off-flavour perception. It was also detected in control ice cream. In fact, during the sensory evaluation of off-flavour by untrained panellists, the
control ice cream scored higher than the experimental ice creams. The difference in sensory scores, however, was not significant. Similar trend was reported by Rexroat and Bradley (1986) with regard to soft serve ice milk, where sucrose was partially substituted with the concentrated hydrolysed whey permeate. With regard to hardened ice milk too, these authors reported the presence of off-flavour, though with statistically the same intensity in both the control as well as the experimental sample. Decolourisation of hydrolysed permeate, however, significantly reduced the off flavour intensity. With regard to milk shake, the lactose hydrolysed sample received less score than the control one for off-flavour intensity.

Application of lactose hydrolysed dairy ingredients in ice cream formulation gives rise to the development of cooked flavour. This was reported by Huse at al (1984). Sensory score for cooked flavour increased with the increases in the extent of lactose hydrolysis. As these authors presumed, the reaction between milk protein and the reducing sugars formed during hydrolysis, was the basis for the cooked flavour development. At a constant level of sucrose content, addition of neutralised hydrolysed lactose cottage whey resulted in cooked or caramelised flavour defect (Young et al., 1980). Young et al (1980) presumed that it was a result of the heat treatment involved in vat pasteurisation.

2.3.1.4 Overall acceptability of hydrolysed lactose ice cream

Lactase treated (to accomplish 60 to 70% hydrolysis) mix containing whey, did not significantly differ from the control mix in its overall acceptability (Martinez and Speckman, 1988). To some extent, a similar observation was made by Arndt and Wehling (1989). The latter group of researchers observed insignificant difference in the overall acceptability between a control ice cream and the one, in which 25% of sucrose had been replaced with demineralised lactose hydrolysed concentrated whey permeate. Similar observation was also made by these latter group of researchers, with regard to the reference ice cream, in which 25% sucrose had been replaced with dextrose. The overall acceptability of ice cream with 50% sucrose substitution, was the highest. Overall acceptability of ice cream, in their observation, was based on sensory parameters such as appearance, body, texture, sweetness and off-flavours. Harju (1987) reported that the replacement of 36.4% MSNF and 50% sucrose with the hydrolysed whey syrup
produced favourable overall quality of ice cream. The parameters taken into consideration in his study were texture, taste and melting properties. Mitchell (1991) reported that an acceptable ice cream could be manufactured by using lactose hydrolysed (70% hydrolysis) whey to replace upto 50% of the non fat milk solids and sucrose.

Overall preference of some frozen desserts other than ice cream was reported by Rexroat and Bradley (1986). The study had taken into consideration, sensory parameters such as sweetness intensity, off-flavour intensity, texture, appearance, thickness and vanilla perception. The soft serve ice milk, in which sucrose was partially replaced with undecolourised lactose hydrolysed whey permeate was highly preferable to a control sample. According to these authors, decolourisation is a critical step during the production of hydrolysed lactose syrup. Removal of colour made possible the reduction in off-flavour intensity. The effect of colour removal on the overall acceptability of ice cream was such that even complete substitution of sucrose found to be possible. Unlike soft serve ice milk, an ice cream made with partial substitution of sucrose by undecolourised hydrolysed permeate, was found to be poor in the overall preference. It was because, sucrose did not have the masking effect in the latter case, as it did with the soft serve ice milk. Furthermore, unlike the soft serve ice milk, complete substitution of sucrose even with the decolourised hydrolysed permeate in hardened ice milk was not feasible. With regard to milk shake, partially lactose substituted, but undecolourised sample was significantly preferred to the control sample. The reason cited by these authors, was the low total solids in the shake mix, where the concentrated hydrolysed whey permeate (CHWP) was more diluted. This dilution effect was responsible for the lower off-flavour intensity of undecolourised CHWP. The presence of some sucrose also contributed to raise its overall preference. However, a complete substitution of sucrose with CHWP in milk shake, like the hardened ice milk was not feasible. The reason was the inadequate sweetness provided by the CHWP containing low total solids.

2.3.2 Viscosity of hydrolysed lactose ice cream mix

Viscosity of ice cream mix containing partially hydrolysed lactose milk, is higher than that of the mix with no lactose hydrolysis. Furthermore, an increase in the DH, results in an increase in mix viscosity. This was observed by El-Neshawy et al (1988). They found out the viscosity of
unhydrolysed mix as 618 Centipoise (Cp), which increased to 658 and 692 Cp at 50 and 75% lactose hydrolysis respectively. The same group of researchers investigated the effect of alteration in sucrose level (at the same level of lactose hydrolysis of mix) on the mix viscosity. Their findings indicated a slight reduction in viscosity (from 672 to 651 Cp) as a result of reduction in sucrose level (from 14 to 12%).

In the mixes, where the application of lactose hydrolysed whey solids had been to partially substitute for both the MSNF and sucrose contents, the viscosity found to be affected inversely. In that case, an increase in the level of lactose hydrolysed whey solids, reduced mix viscosity. Guy (1980) observed a definite trend in this regard. Significant differences were observed, when the levels of incorporation were higher than 5.5%. The DH of incorporated whey solids did not have a well defined effect on the mix viscosity. At lower levels of incorporation upto 5.5%, a lower DH (67%) resulted in a higher viscosity. On the other hand, at higher levels of incorporation, between 5.5 and 11%, a higher DH (79%) resulted in a higher viscosity. However, these differences in viscosities as a result of differences in the DH, were not significant.

Unlike Guy (1980), Patel and Mathur (1982) did not observe a well defined trend of inverse relationship between the viscosity of mix and the incorporation of lactose hydrolysed whey solids. Compared to a control mix, the latter group of researchers observed lower viscosity at 5.5% level of incorporation of hydrolysed whey solids. At 11% level of incorporation, however, the viscosity was not significantly different from that of the control mix.

2.3.3 Freezing property of hydrolysed lactose ice cream mix

Freezing point is an important physical property of frozen dairy products, because storage stability and therefore quality is affected by it. Low freezing point makes the product prone to heat shock and undesirable textural changes. The product may also become too soft, when stored in a conventional home freezer. High freezing point, however, can make the product too hard (Martinez and Speckman, 1988).

Freezing point of a product depends on the molal concentration of substances in its water phase. They include carbohydrates and salts
(Rexroat and Bradley, 1986). Several researchers have reported that hydrolysis of lactose (a disaccharide) in a mix or the incorporation of lactose hydrolysed ingredients in a mix is accompanied by a decrease in the freezing point. This decrease is due to an increase in the number of monosaccharide units, glucose and galactose. An increase in the number of monosaccharide units increases the effective molecular weight of the mix. Effective molecular weight is the average weight of all the molecules in the mix and it is known to affect the freezing point (Smith et al., 1984). The depression in freezing point as a result of lactose hydrolysis, is governed by the following relationship (Nijpels et al., 1980):

\[
\frac{XK}{AT} = \frac{1}{M}
\]

where, \( AT \) = Depression in freezing point,
\( X \) = Concentration of the non-ionic substance in solution (molality),
\( K \) = Cryoscopic constant for water = 1.86, and
\( M \) = Molecular weight of the substance.

Martinez and Speckman (1988) observed very little effect of lactose hydrolysis on the depression of freezing point of ice cream mix, which had been treated with lactase. The freezing point of hydrolysed mix was -1.77°C, compared to -1.68°C of the unhydrolysed mix. Similar observations were recorded for some other desserts such as ice milk, milk shake and low calorie frozen dessert. In all of these products, where 60 to 70% of lactose had been hydrolysed, 25% of MSNF was substituted with whey solids. Similarly, a reduction in sucrose content by 28.5 % and the hydrolysis of 80% of lactose in milk, did not significantly depress the freezing point of an ice cream mix (Gregory, 1980). Lindamood et al. (1989) observed substantial effect of lactase treatment to ice cream mixes, on the depression of their freezing points. MSNF of these mixes, however, were not substituted with whey solids. According to their observations, the f.p. of 25, 50, and 100% lactose hydrolysed mixes were -1.62, -1.67, and -1.92°C respectively as compared to -1.45°C of unhydrolysed mix.

Partial substitution of both MSNF as well as sucrose with the already hydrolysed sweet whey solids (67% lactose hydrolysis) did not result in any significant reduction in the freezing point of mix. Guy (1980)
observed this phenomenon even when 54.5% of MSNF and 33.3% of sucrose in ice cream were replaced with hydrolysed whey solids. The incorporation of hydrolysed whey solids was as high as 11% of total solids of mix to effect these replacements. However, the freezing point was reduced to a greater extent, when whey solids with higher DH (79%) was incorporated in the mix. The author also observed a small, but definite reduction in freezing point of mix, when higher amount of 79% lactose hydrolysed whey solids was incorporated into it. This reduction in freezing point, according to him, was in association with the longer freezing time. According to this author, the freezing point of mix decreased from -4.5 to -6.0°C as a result of replacing 54.5% of MSNF and 33.3% of sucrose with hydrolysed whey solids. The associated increase in freezing time was from 8.0 to 9.5 min. Arndt and Wehling (1989) also reported longer lengths of time taken to freeze the mixes containing lactose hydrolysed ingredients. When 25 and 50% of sucrose contents were substituted with demineralised lactose hydrolysed whey permeate (97.2% hydrolysis), the lengths of time taken to freeze those mixes were 7.8 and 8.8 min respectively, as compared to 7.6 min taken by the control mix.

2.3.4 pH and acidity of hydrolysed lactose ice cream mix

Use of lactose hydrolysed whey in ice cream formulation to partially replace MSNF as well as sucrose, resulted in acidity, in presence of heat, also accelerates protein denaturation and imparts cooked flavour to ice cream (Marshal and Arbuckle, 1996).

Application of lactose hydrolysed whey in an ice cream formulation to partially substitute for MSNF as well as sucrose content, resulted in a decrease in the mix pH (Guy, 1980). Incorporation of 11% of 67% hydrolysed whey solids, decreased the pH to 6.31 from 6.59, as observed by this author. According to him, when 79% hydrolysed whey was used, the pH of the mix reduced from 6.6 to 6.26. Patel and Mathur (1982) observed a small decrease in mix pH (from 6.68 to 6.56), when 11% of mix total solids, constituted of 6% NFDM and 5% sucrose, was substituted with lactose hydrolysed whey. According to them, the pH of mix remained unchanged, when 5.5% of the total solids of mix, constituted of 3% NFDM and 2.5% sucrose, was substituted with lactose hydrolysed whey.
2.3.5 Overrun of hydrolysed lactose ice cream

Overrun is the volume of the product obtained in excess of the volume of the mix. The increase in volume is due to the incorporation of air during the freezing process (Martinez and Speckman, 1988). According to Martinez and Speckman (1988), there is a range of percent overrun for the optimum quality of each type of product of a particular composition, above or below which, the product is heavy or thin-bodied. According to these authors, overrun of a normal ice cream falls within the range of 70 and 80%, that for ice milk falls between 50 and 80%, whereas that for milk shake is between 10 and 15%.

Overrun can be kept constant during the freezing process through mechanically controlling the incorporation of air into the product. Through this practice, the influence of any change in ice cream formulation on overrun, can be negated. Patel and Mathur (1982) adopted this approach. They kept the overrun constant at 80% in all of the batches of ice cream made by them. They did so in order to overcome the effect of lactose hydrolysed whey, used to partially substitute for both the NFDM and sucrose in an ice cream formulation.

Huse et al (1984) observed considerable variation in overrun, when a particular make of freezer (Dairy Queen) was used. With this freezer, they experienced difficulty in controlling overrun. Random variation in overrun was observed by Arndt and Wehling (1989). They did not observe any influence of lactose hydrolysed whey permeate on the overrun of ice cream. The hydrolysed permeate had been used by them to reduce sucrose content by 25 or 50%. Some of the effects of lactose hydrolysis on overrun were reported by El-Neshawy et al (1988). They observed slightly higher overrun as a result of using lactose hydrolysed reconstituted skim milk instead of unhydrolysed one, in an ice cream formulation. According to them, the higher mix viscosity resulting from an increase in lactose hydrolysis, was responsible for the higher overrun.

2.3.6 Glass transition and melting temperatures of hydrolysed lactose ice cream

Freezing of foods or their storage at sub zero temperatures, is frequently practiced to ensure long shelf life. However, frozen foods too, possess a finite shelf life. This is despite a tremendous reduction in unfrozen water content. Frozen foods stored at or near the conventional -18°C
storage temperature, are neither completely frozen nor are they inert. Several detrimental reactions take place in the unfrozen water phase at this storage temperature. These detrimental reactions include those associated with the ice phase, related to the size and growth of ice crystals and sublimation (dehydration or ‘freezer burn’), and those related to the non-ice phase, including enzymatic and chemical reactions (Goff, 1992).

In the non-ice phase, enzymatic reactions can lead to changes in texture, colour, flavour and even the nutritional quality of a frozen food. Reid (1990) reported four enzyme-groups responsible for these changes. Lipoxygenase, lipase, and protease can cause off-flavour development. Pectic enzymes and cellulases can cause textural changes. Polyphenol oxidase, chlorophyllase and peroxidase can cause colour changes and ascorbic acid oxidase can cause nutritional changes.

Several physico-chemical properties of food components can be affected by the process of freeze concentration. These properties of the unfrozen phase include pH, titratable acidity, ionic strength, viscosity, freezing point, surface tension, and the oxidation-reduction potential. These altered properties can be detrimental to many food components. For example, protein denaturation can lead to curdling and drip in thawed foods and freezing of emulsions may cause coalescence of fat. Furthermore, rapid freezing can lead to the survival of many microorganisms, due to the formation of smaller ice crystals and possibly less ice phase (Goff, 1992).

During the frozen storage, ice crystals undergo changes in number, size and shape, collectively known as recrystallisation. According to Goff (1992), this is probably the most important reaction leading to quality losses in frozen foods. Recrystallisation is the result of temperature fluctuations during frozen storage. When temperature increases, the proportion of unfrozen water increases and smaller crystals melt, because of their lower melting temperature. On the other hand, when temperature decreases, water gets deposited on the surface of larger crystals. It does not nucleate. As a net result, total number of crystals decreases, but the mean crystal size increases. This phenomenon leads to poor food quality. Recrystallisation can be minimised by maintaining a low and constant temperature. Constant temperature, however, gives rise to the ‘Ostwald ripening’. In Ostwald ripening, larger crystals grow and smaller ones disappear (Goff, 1992). Thus, maintaining a constant
normal temperature during frozen storage, is not the complete protection to foods against the deteriorative changes.

Deteriorative changes during frozen storage of foods can be minimised or almost stopped, if they can be maintained in their glassy or non-crystalline metastable state. A glass can be characterised as a liquid with an extremely high viscosity \((10^{12} - 10^{14} \text{ Pa.s})\) that will flow, but has a molecular diffusion rate of many years (Goff, 1992). The critical temperature at which polymeric materials change from a viscoelastic or supercooled liquid (flexible rubber) to a solid glassy (amorphous) state, with an associated increase in viscosity, is called the glass transition temperature \((T_g)\). For frozen food systems, this temperature is defined as the glass transition temperature of a maximally freeze-concentrated solution and is denoted by \(T'_g\) (Goff, 1992). Thus, \(T_g\) is a general denomination and \(T'_g\) is a specific one for the glass transition temperature. \(T_g\) is not always a single, well defined temperature. In fact, the transition takes place over a range of temperatures (Goff, 1992).

The most common method, used to determine \(T_g\), is the differential scanning calorimetry (DSC). This method detects the change in heat capacity, occurring over the transition temperature range (Roos et al., 1996). \(T_g\) is determined by taking the midpoint of the near discontinuous rise in heat capacity at the transition (Ollett and Parker, 1990).

According to Roos et al (1996), there is the occurrence of one more transition, different from glass transition. This transition takes place at the melting temperature \((T_m)\) of a material. For a maximally freeze-concentrated material, this transition is related to ice melting. This temperature for the onset of ice melting, also called ice dissolution temperature or the melting temperature, is denoted by \(T'_m\). Thus \(T_m\) is a general denomination and \(T'_m\) is a specific one for the melting temperature.

The melting temperature is higher than the glass transition temperature. The most dramatic and obvious changes in mobility occur at the temperatures above \(T'_m\). These changes are practically very important. Between \(T_g\) and \(T_m\), the material is a supercooled liquid (Herrington and Branfield, 1984). This practical state of material is significantly
prone to changes in mechanical properties. The equilibrium melting temperature of several glass formers including most of the sugars, is located at about $T_g + 100^\circ C$ (Roos et al., 1996).

The rate of changes in shelf life and quality of frozen foods above $T_g$ and below $T_m$ are determined by the temperature-difference $T-T_g$. This temperature-difference is the function of the ratio ($a_T$) of the relaxation times of configurational rearrangements at respective temperatures $T$ and $T_0$. $T$ can be any temperature between $T_g$ and $T_m$, such as the storage temperature of a frozen food. This relationship follows the Williams-Landel-Ferry (WLF) equation (Roos et al., 1996), given below.

$$\log a_T = \frac{-C_1 (T-T_0)}{C_2} + (T-T_0)$$

Where, $C_1$ and $C_2$ are constants. $T_0$ is a reference temperature. $T_g$ or $T_m$ can be this reference temperature. This equation is applicable over the temperature range from $T_g$ to $T_g + 100^\circ C$.

Various factors influence the glass transition temperature. These factors are the chemical structure, plasticisers (e.g. low molecular weight additives), branching, cross-linking, entanglement of polymer chains, and the molecular weight. In solution, $T_g$ is strongly influenced by concentration. For the mixtures of solutes, the $T_g$ can be determined as the weighted mean of the $T_g$ for each solute. The polymer $T_g$ can be lowered by adding small molecular weight components (Goff, 1992). On the other hand, cryostabilisation of frozen food can be accomplished by elevation of $T_g$ through ingredient formulation. It can also be accomplished by the storage of foods at temperatures below $T_g$. However, as Goff (1992) suggested, storage of foods below $T_g$ may neither be practical nor economical to maintain them in glassy state, due to the involvement of an extremely low temperature. Similarly, formulation of foods to raise $T_g$ may not be practical and economical, because the formulation is limited by the sensory properties of many of the large molecular weight stabilisers.

One of the frozen foods on which $T_g$ or $T_m$ has tremendous influence, is ice cream. Ice cream is a frozen food of four-phase system. This four-phase system comprises fat globules, air bubbles, ice crystals, and a concentrated serum phase containing many of the soluble components including sugars and polysaccharide stabilisers. Air bubbles are
embedded in a continuous phase. In this continuous phase of sucrose-lactose syrup or glass are distributed fat globules, ice crystals, proteins etc. (White and Cakebread, 1966; Goff et al., 1993). This continuous phase will change to sucrose-lactose-glucose-galactose- (and possibly oligosaccharides), if the ice cream involves lactose hydrolysis in its formulation.

After aeration and freezing, ice cream is usually transferred to a hardening room at about -20°F (-28.8°C). This temperature is well below the one reported by White and Cakebread (1966). On the basis of some evidences cited in literature, White and Cakebread (1966) reported -10°F (-23.3°C) as the $T_g$. They further stated that this temperature was likely to vary somewhat with composition. Goff (1992) reported a range of $T_g$ of ice cream. This range is -23 to -43°C, which depends on the formulation. Since, it is the composition of serum phase, which affects the $T_g$ of ice cream, this temperature can be the average of $T_g$ of individual components of this phase. As already mentioned, this phase includes sugars and stabilisers. Stabilisers do not significantly affect the $T_g$ (Goff et al., 1993). Hence it should be assumed that the $T_g$ of ice cream is the $T_g$ of individual sugars. However, the $T_g$ of individual sugars have been controversially reported in the literature. Goff (1992) reviewed those literature. According to this review, some groups of researchers reported $T_g$ as -43°C for glucose, -42°C for fructose, -41.5°C for galactose, -32°C for sucrose, and -29.5°C for maltose. Some of the other groups of researchers termed $T_g$ of individual sugars as the temperatures at the onset of melting (Goff, 1992). The latter groups of researchers reported the $T_g$ of individual sugars differently. According to them, the values for $T_g$ were -52, -53, -56, -40, and -36°C for glucose, fructose, galactose, sucrose, and maltose respectively.

There is a paucity of published information on the effect of lactose hydrolysis on the $T_g$ of ice cream. However, Jouppila and Roos (1994) have reported that lactose hydrolysis reduces the $T_g$ of dried milk.
CHAPTER 3
MATERIALS AND METHODS

3.1 Nanofiltration of milk UF permeate

A Desal-3, cross-linked polyamide ultraosmosis membrane of thin film composite type, manufactured by Desalination Systems, Inc., CA, was used for the present study. The photograph of the membrane unit is shown in Fig 1 and its schematic diagram is presented in Fig 2. The membrane equipment including the two spiral wound membrane modules, was supplied by the Filtration Engineering Australasia Ltd., Auckland. During present study, however, only one module was used. The total membrane area of each module, with 95% NaCl permeability, was 6m² (4' x 39''). The pH tolerance of the membrane ranged from 2.3 to 11.0. The maximum operating temperature for this membrane was 57°C. The maximum operating pressure for this membrane was 41 bar (4100 KPa). The membrane unit was also fitted with a flowmeter (only in the permeate line), a temperature indicator, a pressure gauge (only at the inlet of the module), a feed tank (capacity 150 L), and a positive displacement pump (maximum capacity 20 gpm and maximum pressure 1000 psi).

Milk UF permeate (or milk permeate), the feed for nanofiltration, was received from the Australian Co-operative Foods Ltd, NSW. It was transported to the pilot food processing plant of the University of Western Sydney Hawkesbury, NSW, and was received there at 5°C. The pH, TA and TS of milk permeate were 6.74, 0.16% LA and 5.25% respectively. The colour of milk permeate was greenish yellow.

At the beginning of the process (before taking feed), the membrane was washed by circulating water (30°C) for 1 hr at 500 KPa of transmembrane pressure (TMP). TMP here is defined as the reading shown by the pressure gauge installed at the inlet of the module. This washing was followed by the circulation of fresh water, beginning at 500 KPa of TMP. During this circulation, temperatures and flux rates of water were measured at 500, 800, 1000, 1200, 1500, and 2000 KPa of TMP.
Fig 1

Photograph of spiral wound thin film composite Ultraosmosis membrane unit (Filtration Engineering Australasia Ltd, New Zealand), with a membrane module separately kept on top of the unit.
Fig 2

Schematic diagram of spiral wound thin film composite Ultraosmosis membrane unit (Filtration Engineering Australasia Ltd, New Zealand).
The flux rate was determined by collecting the permeated water in a 5 L capacity plastic bucket for 1 min with the help of a stop watch; measuring the volume of this water by a 1 or 2 L capacity measuring cylinder; and expressing the measurement in terms of litre per square meter of membrane area per hour. The flux rate obtained at each TMP was corrected to 25°C, using the correction factors, supplied by the membrane manufacturer. The measurements of flux and temperature of water were carried out 9 min after setting up of each TMP. The water was drained off after 1 h circulation.

The feed (milk UF permeate) was taken after the washing of membrane-unit. The nanofiltration was set to yield just enough TS (15%) in the concentrate to be suitable (after lactose hydrolysis) for replacing a maximum of 50% of sucrose in an ice cream formulation, containing 38.5% TS and 15.0% sugar (Refer to Appendix 1 for calculation). For this, 80 Kg of feed was processed in duplicate batches through a batch recirculation mode. The applied TMP was increased gradually from 800 to 1500 KPa during the 55 min run to maintain satisfactory flux rates, which began to fall as the concentration increased. Flux rate and temperature were recorded during the process more frequently at later stages than initial stages. The flux rate was determined by directly collecting the nano-permeate in a 2 L capacity plastic measuring cylinder for 1 min with the help of a stop watch, and expressing the measured volume of the nano-permeate in terms of litre per square meter of membrane area per hour. The weight of the permeate collected at regular intervals during nanofiltration, was determined with a platform balance (Avery Pty Ltd, NSW). The mass concentration ratio (MCR) was calculated with the help of weight of permeate collected at a particular interval of time (Refer to section 3.4.17). Concentrate samples were collected at regular intervals of time for the analyses of %TS (section 3.4.2), pH (section 3.4.1), TA (section 3.4.4), and colour (section 3.4.5).

The final concentrate as well as the feed (milk UF permeate) was also analysed for protein (section 3.4.7), lactose (section 3.4.7), ash (section 3.4.3), K⁺ (section 3.4.6) and Na⁺ (section 3.4.6) contents in addition to pH (section 3.4.1), %TS (section 3.4.2), TA (section 3.4.4) and colour (section 3.4.5). Furthermore, the TA (section 3.4.4), %TS (section 3.4.2), crude protein (section 3.4.7), and lactose (section 3.4.7) contents of the nano-permeate collected during NF were also determined.
The unit was flushed at the end with water (30°C). The flushing was
drained off. Water was then circulated for 1 h, beginning at an applied
TMP of 500 KPa. During this circulation, temperatures and flux rates
of water at 500, 800, 1000, 1200, 1500 and 2000 KPa of TMP were
measured. The flux obtained at each TMP was corrected to 25°C. The
unit was cleaned with 1% aqueous solution (30°C) of Ultrasil 56
(Ecolab Pty Ltd, Castle Hill, NSW), which was circulated for 1 h.
Cleaning solution was then drained off, followed by the rinsing with
water. It was followed by a 1 hr circulation of water, beginning at a
TMP of 500 KPa. During this circulation, the temperatures and the flux
rates of water were measured at 500, 800, 1000, 1200, 1500 and 2000
KPa of TMP. The flux obtained at each TMP was corrected to 25°C.
The water was drained off. The unit was then sanitised with a 30 min
circulation of 0.2% aqueous solution (30°C) of Bio-klenz MBS
(Ecolab Pty Ltd, Castle Hill, NSW) and shut down. The membranes
were kept soaked in this sanitizer solution till further use. However, this
soaking was allowed for a maximum of one week. When not in use, the
unit was kept sanitised every week with the circulation of a fresh
sanitiser solution.

The final nano-concentrate was heated in a 50 L capacity closed lid
steam-jacketed kettle with continuous and vigorous stirring to 72°C.
To simulate pasteurisation, the heating was allowed to continue for 15
sec at this temperature, during which, however, the temperature slightly
increased to 73°C. The heat treated nano-concentrate, removed from
the kettle and collected in 20 L capacity plastic buckets, was
immediately taken to the chilled room for cooling to 40°C. The cooling
process was hastened by the use of ice slabs, jacketed between the
bucket and a stainless steel drum, in which the bucket had been kept.
The nano-concentrate was continuously stirred with a stainless steel
stirrer during the cooling process. At 40°C, enzymic hydrolysis of
lactose was carried out.

3.2 Lactose hydrolysis of the nano-concentrate of milk UF
permeate

Food grade lactase (Lactozym 3000; type HP-G; source yeast K.
fragilis; activity 3000 LAU/mL) was obtained from the Novo Nordisk
Bio-industrial Pty Ltd, NSW. The enzyme was in liquid form.
Lactose hydrolysis of nano-concentrate (pH 6.6; lactose 13%) was carried out for 0, 5, 10, 15, 30, 60 min and 18 h (overnight) in a waterbath maintained at 40°C. In each of 7 test tubes, used separately for 7 time intervals mentioned above, 25 mL of nano-concentrate (40°C) was taken. Into each of the test tubes (except the one used for 0 min interval) 0.3 mL of enzyme was pipetted. The enzymic dosage used, was 12.0 mL/L (Equivalent to 1.0 mL of enzyme per 10.83 g of lactose) of nano-concentrate. The sample without any enzyme addition (0 min interval) was treated as control. After the completion of each of specified periods of hydrolysis, the enzyme was inactivated by placing the test tube in an 80°C waterbath. The enzyme inactivation was carried out for 5 min at this temperature. The enzyme inactivated samples in test tubes were immediately transferred to the chilled room, and kept there overnight at 3°C. Two trials of experiments were conducted. Samples were analysed by HPLC for the degree of hydrolysis (DH) and the amount of oligosaccharides formed during hydrolysis.

The hydrolysis for 60 min (with the other conditions remaining the same) was selected for the production of HLNC to be used in ice cream formulations. The selection was mainly made on the basis of optimum DH and the amount of oligosaccharides formed.

3.3 Ice cream production

3.3.1 Ingredients used for ice cream formulations

Ice creams were prepared with spray dried skim milk powder (Allowrie Foods Ltd, NSW), pasteurised cream (United Dairies Ltd, NSW), and the pasteurised, homogenised milk (University of Western Sydney Hawkesbury Dairy, NSW). Other ingredients were the pure granulated cane sugar (C.S.R. Ltd, NSW), stabiliser/emulsifier mix (Gemcol brand, Germantown Co., NSW), vanilla flavour (Bush Boake Allen Australia Ltd, NSW), and the hydrolysed lactose nano-concentrate (HLNC).

3.3.2 Composition of dairy ingredients used for ice cream formulations

Composition of dairy ingredients used for ice cream formulations, as shown in Table 3.
3.3.3 Formulations of ice cream

Formulations containing (w/w) 11% fat, 12% MSNF (excluding HLNC), 15% sugar (including HLNC), 0.5% stabiliser/emulsifier, 0.04% vanilla flavour, and 38.5% TS were used as shown in Table 4 (Refer to Appendix 1 for the calculation).

The 15.0% of total solids achieved in nano-concentrate was capable of providing the required amount of water to maintain a 38.5% TS in ice cream. By this way, the need to add extra water or any other sources of water such as milk or skim milk to maintain a 38.5% TS was eliminated.

3.3.4 Experimental procedure used for ice cream production

Each of the ice creams— the control, treatment 1 (25% sucrose substitution), and treatment 2 (50% sucrose substitution) was produced in two trials according to the flow process chart (Fig 3). Each batch of size 12Kg was produced at the Research and Development Laboratory of the Australian Co-operative Foods Ltd, Lidcombe, NSW. All of the liquid ingredients (milk and cream with or without HLNC, depending on the treatment), except vanilla, were mixed and transferred to a 15 L capacity hot well (made locally by the R & D Lab, Australian Co-operative Foods Limited, Lidcombe, NSW). At 49°C, skim milk powder was added to liquid ingredients. Solid ingredients (sucrose and stabiliser/emulsifier) were mixed, and transferred to hot well at 60°C. Heating was continued. At 80°C, vanilla was added. Mix was held at this temperature for pasteurisation for 5 min. In order to maintain the uniformity of temperature during the entire process of heating, the mix was continuously stirred with a power-operated stirrer. The pasteurised mix was homogenised (2200 psi first stage and 800 psi second stage) (Model 15 M lab homogeniser and sub-micron disperser; Gaulin Corporation, Everett, Massachusetts). It was then taken in a plastic bucket, transferred to adjacent chilled room, and kept there overnight at 3°C for aging and cooling. The next day, mix was frozen in a batch freezer (Gelmark 80; α-laval Company, Hoyer, Italy). Each of the frozen mixes was collected in 125 mL and 1 L capacity plastic containers for various analyses. All of the ice cream samples were hardened at -30°C for a week in a hardening chamber at the R&D Lab,
Table 3

Composition of dairy ingredients used for ice cream formulations.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Total solids (%)</th>
<th>Fat (% wet basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMP</td>
<td>93.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Cream</td>
<td>42.8</td>
<td>40.0</td>
</tr>
<tr>
<td>Milk</td>
<td>12.7</td>
<td>4.0</td>
</tr>
<tr>
<td>HLNC</td>
<td>15.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

All the data points are the means of duplicate analyses. Total solids was determined by AOAC (1990) method (section 3.4.2). Fat was determined by Babcock method (section 3.4.9). HLNC = Hydrolysed lactose nano-concentrate.
### Table 4

**Ice cream formulations**

<table>
<thead>
<tr>
<th>Ingredients (% w/w)</th>
<th>Control (0% sucrose substitution with HLNC)</th>
<th>Treatment 1 (25% sucrose substitution with HLNC)</th>
<th>Treatment 2 (50% sucrose substitution with HLNC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk powder</td>
<td>7.06</td>
<td>9.11</td>
<td>11.8</td>
</tr>
<tr>
<td>Cream</td>
<td>21.9</td>
<td>24.5</td>
<td>27.1</td>
</tr>
<tr>
<td>Milk</td>
<td>55.5</td>
<td>29.6</td>
<td>3.06</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15.0</td>
<td>11.25</td>
<td>7.50</td>
</tr>
<tr>
<td>HLNC</td>
<td>0.0</td>
<td>25.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Stabiliser/emulsifier</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vanilla</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Refer to Appendix 1 for the calculation of above formulations.

HLNC = Hydrolysed lactose nano-concentrate.

Ice cream for each treatment including the control, was produced in two trials.
Fig 3

Flow process chart, used for the production of ice cream.
Flow process chart for the production of ice cream

Mixing of liquid ingredients in hot well with power-operated stirrer

Checking the temperature of mix

Addition of skim milk powder at 49°C

Checking the temperature of mix

Addition of sucrose and stabiliser/emulsifier at 60°C

Checking the temperature of mix

Addition of vanilla flavour at 80°C

Holding the mix for 5 min at 80°C

Homogenisation (1st stage 2200 psi; 2nd stage 800 psi)

Ageing and cooling of mix overnight at 3°C

Freezing of mix, along with the measurement of freezing time and overrun

Hardening of ice cream at -30°C for a week

Transportation of ice cream (kept in dry ice) from R&D lab of ACF Ltd, Lidecombe, NSW to UWS, Hawkesbury, Richmond, NSW

Storage of ice cream in a -20°C freezing chamber
Australian Co-operative Foods Limited, Lidcombe, NSW. Twenty four hours prior to various examinations, ice cream samples were transferred from the Australian Co-operative Foods Limited, Lidcombe, NSW to the Food Science Department of the University of Western Sydney Hawkesbury, Richmond, NSW, under dry ice (temperature \(-79^\circ C\)) within an hour. Samples were immediately transferred to a \(-20^\circ C\) freezing chamber, located in the pilot food processing plant of the university.

At the mix stage, ice creams were tested for TS (section 3.4.2), ash (section 3.4.3), pH (section 3.4.1), TS (section 3.4.2), freezing point (section 3.4.8), fat (section 3.4.9), viscosity (section 3.4.13) and colour (section 3.4.5). The finally prepared ice creams (after freezing and hardening) were examined for texture (section 3.4.11), sensory perception (section 3.4.18), glass transition and melting temperatures (section 3.4.12). Ovverun (section 3.4.14) and freezing time (section 3.4.15) were measured during the freezing process.

3.4 Methods of Analysis

3.4.1 Determination of pH

pH was determined using a pH meter (Model HI 8519; Hanna Instruments Ltd) at 25°C. Calibration of pH meter was done using acidic buffer pH 4.0, and neutral buffer pH 7.0. Every time the pH meter was equilibrated for 30 sec before recording the reading.

3.4.2 Determination of total solids

Total solids of the samples, other than those of milk UF permeate, its nano-concentrates, and the nano-permeate were determined gravimetrically. AOAC (1990) method was used with some modifications. Five grams of each sample were weighed (using an electronic balance, model AA-200, Denver Instrument Limited) into a flat bottom alluminium dish (dia 5 cm). Weighed samples were heated at 100°C for overnight in a hot air oven. They were cooled in a desiccator and analytically weighed. The percent residue was reported as total solids. Each determination was duplicated. Fat containing samples such as milk, cream and ice cream mixes were heated on a steam bath for 30 min before weighing them into dishes.
The TS of the nano-concentrates at various stages during nanofiltration were determined refractometrically.

3.4.3 Determination of ash

Ash content was determined gravimetrically, using AOAC (1990) method with some modifications. Three to five grams of the samples were placed in dried crucibles. Samples were charred (to prevent them igniting in the muffle furnace) before placing them into a muffle furnace overnight at 525°C. Crucibles were removed from the furnace, cooled for 10 min on an asbestos mat and then transferred to a desiccator for further cooling. Crucibles were analytically weighed. The percent of grey-white residue was reported as ash content.

3.4.4 Determination of titratable acidity

Titratable acidity (TA) was determined using the method of Kirk and Sawyer (1991), with slight modification. Diluted samples were used for the analysis. To 10 mL of a sample, 1 mL of phenolphthalein indicator was added. The sample was then titrated against 0.1 M NaOH with continuous stirring. The colour of the titrated sample was matched with that of the control. Control was made up of solution of phenolphthalein (1 mL) in distilled water (10 mL). TA was calculated as %lactic acid (LA) using the following equation.

\[ 1 \text{ mL } 0.1 \text{ M NaOH } = 0.009 \text{ g LA.} \]

3.4.5 Colour measurement

Colour was measured with a Nippon Denshoku colour difference meter (model ND-101 DP; Nippon Denshoku Kogyo Co. Ltd). Among the accessories, DC power supply model-9463 (Nippon Denshoku Kogyo Co. Ltd) and stepdown transformer, model KD-300 (Toyozumi Dengenki Co. Ltd) were used. Commission Internationale de l’Eclairage (CIE) method was used for colour measurements.

Standardisation was done by removing the black tile from the light base, thus exposing a vertical light beam. The light beam was then covered with the standard white plate. White dot on the plate was adjusted to enable it to face the operator. ‘YXZ’ button was pressed to
set the machine on to the ‘YXZ’ mode. ‘YL’, ‘Xa’, and ‘Zb’ buttons were pressed and the respective knobs were used for calibration.

For translucent liquid samples, such as milk UF permeate, its nano-concentrates, and the nano-permeate, rectangular cell was used for colour reading. The sample was poured into the cell carefully to avoid air bubbles. With the help of a holder, sample cell was placed in the light path. The removed hatch colour was replaced to the light box. Standard white tile was left on the light beam. Four readings were recorded for each sample.

For the non translucent liquid sample, such as ice cream mix, cylindrical cell was used for colour reading. The samples were placed firmly in the cell to avoid development of air pockets between the sample and the bottom surface of the cell. The black tile was removed from the light box and the cell was placed over the vertical light beam, completely covering the hole. Four readings were recorded for each treatment.

X, Y and Z values were expressed in terms of ‘x’ and ‘y’, according to the following relationships.

\[
Y = \frac{Y}{Y+X+Z}
\]

\[
X = \frac{X}{Y+X+Z}
\]

The %colour intensity or %radiant energy was determined by measuring the distance between the point at the illuminant ‘C’ and the point of intersection of ‘x’ vs ‘y’ on the CIE chart (Fig 7). Wavelength and the type of colour were determined by extrapolating this distance towards the periphery of the horse shoe shaped curve of the CIE chart (Fig 7).

3.4.6 Atomic Absorption Spectroscopy

Concentrations of K⁺ and Na⁺ in unconcentrated (original) milk UF permeate and its final nano-concentrate (3 fold concentration) were determined using the flame atomic absorption spectroscopy (AAS), model A-600 (Varian Australia Pty Ltd, Victoria). The operation was controlled by the OS/2 interface software.
KCl (A.R. grade) was used to prepare standard for $K^+$. In distilled deionised water 1.907 g of dried KCl was dissolved and the solution was diluted to 1 L to give 1 mg/mL concentration of KCl. The standard curve (concentration vs absorbance) for $K^+$ was obtained using concentrations of 2, 4, 6 and 10 mg/L.

NaCl was used to prepare standard for $Na^+$. In distilled deionised water 2.542 g of dried NaCl was dissolved and the solution was diluted to give 1 mg/mL concentration of NaCl. The standard curve (concentration vs absorbance) for $Na^+$ was obtained using concentrations of 2, 4, 6 and 10 mg/L.

The atomic absorption for both of the monovalent cations was carried out with the fixed working conditions, which included 5 mA lamp current, acetylene as fuel, air as support, and the oxidizing flame stoichiometry. Flame emission for $K^+$ was carried out at wavelength 766.5 nm and for $Na^+$ at wavelength 589.0 nm. Slit width, fuel and support for of these ions were 0.1 nm, acetylene and air respectively.

Test samples were adequately diluted with distilled deionised water. Concentrations of $K^+$ and $Na^+$ in test samples were determined by plotting their absorbances on their respective standard curves.

3.4.7 Milkoscan

Crude protein and lactose contents of the unconcentrated (original) milk UF permeate, its final nano-concentrate, and the nano-permeate were determined by milkoscan 104 type 19900 (Foss Electric Australia Pty Ltd). De-aeration of the flow system, zero setting, calibration check, and the purging of the flow system were carried out as described in the milkoscan manual. Temperature for all of the samples was maintained at 40°C at the time of their analyses. Each sample was analysed in duplicate.

3.4.8 Cryoscopy

Freezing points of ice cream mixes were determined using a Fiske Cryoscope, model J-66. Samples were diluted 10 times with the 40°C distilled, deionised water. Two mL aliquots of each sample were used for the analysis. Each analysis was replicated 4 times. Cryoscope was calibrated against deionised water (0.000°C), 6.859% (w/v) NaCl (-
0.408°C), 8.645 % (w/v) NaCl (-0.512), and 10.155% (w/v) NaCl (-6.00). Calibration was done as described in the manual for cryoscopy.

3.4.9 Babcock method for fat analysis

Babcock method (AOAC, 1990) was used to determine the fat content of milk, cream, SMP, HLNC and ice cream mixes. For the determination of fat in milk, 17.6 mL of a representative sample (heated to 40°C), cooled to 20°C, was pipetted into a Babcock milk flask. After the addition of some sulphuric acid to it, it was mixed thoroughly, but slowly (to prevent charring), with a circular, swirling motion. Another volume of sulphuric acid was added and mixed. The contents of the flask were centrifuged for 5 min. Hot water (80°C) was added to the flask to adjust the volume. Flask was again centrifuged for 1 min, and the fat level was recorded from the reading shown on the flask.

SMP was diluted with 40°C distilled water, cooled to 20°C and pipetted into a Babcock milk flask. The rest of the procedure was the same as described above for milk.

Fat content of HLNC was determined by the same procedure as used for milk. For cream also, the same procedure was followed, but in this case, 9 g of representative sample had been pipetted into a Babcock cream flask.

For the ice cream mix, 9 g of sample was weighed into a 10% Babcock milk flask. Fifteen mL of Minnesota reagent was added to it. The flask was placed into a water bath near boiling point for 10 min with occasional agitation. When the fat appeared in a clear layer, the flask was centrifuged for 30 sec. The hot water (60°C) was added to within 5 mm of the top graduation and then the flask was centrifuged for a further 30 sec. The flask was placed into water bath at 60°C for 5 min and then the fat percentage was read. The reading was doubled and recorded. As a Babcock flask, which had been graduated to handle 18 g (17.6 mL) of milk, was used and only 9 g was used in this test, the percentage of fat had to be doubled to achieve the correct result.

3.4.10 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was used to determine the degree of lactose hydrolysis in the final nano-concentrate
of milk UF permeate. A set of various concentrations of standard solutions of analytical grade lactose, glucose, and galactose (Ajax Chemicals, NSW) was prepared with distilled, deionised water. These concentrations were 0.4, 0.8, 1.0, 1.6, 2.0 and 2.5% (w/v). Each of the standard solutions was filtered through a 0.45 μm Millex-HV filter (Millipore Corporation, USA), before its injection into the HPLC system. Both the unhydrolysed and the hydrolysed samples were diluted before their injection into the system. Each of the diluted samples was filtered through the same type of filter as used for the standards. All of the samples were maintained at 3°C during their HPLC analysis.

The HPLC system consisted of a Waters 510 HPLC pump, Waters 410 differential refractometer detector, a personal computer set with Baseline 810 Chromatography software, and a printer (Dynamic solutions, Millipore Corporation, USA). Carbohydrates were separated on a Benson polymeric carbohydrate column (7.8 mm i.d. x 30 cm length; 10 μm particle size (Alltech Australia Pty Ltd, NSW). The column was heated to 90°C by the detector’s heating system. A Benson polymeric guard column (4.6 mm i.d. x 7.5 mm length) was used to filter out harmful particles and to remove lactic and other organic acids (Jeon et al., 1984). Distilled-deionised- degassed water was used as the solvent (mobile phase). The solvent flow rate of 0.6 mL/min was maintained throughout the experiment. The temperature of solvent kept in a glass container, was maintained at 60°C in a water bath. The HPLC flow system was purged with the solvent for 2 h at the same flow rate before the injection of the first sample. The sample size of each injection was 20 μL. Retention time was used to identify lactose, glucose and galactose in the sample and the peak height was used as the detector response. The peaks other than those of these 3 sugars were considered as the oligosaccharides. The DH was calculated using the following equation:

\[
\text{Peak heights of glucose and galactose} \\
\text{DH (\%)} = \frac{\text{Peak heights of total carbohydrates}}{\text{Peak heights of total carbohydrates}} \times 100
\]

Two sets of analyses corresponding to the duplicate batches of experiments were conducted separately.
3.4.11 Textural profile analysis

Textural profile of each ice cream sample was analysed using a texture analyser (Stable Micro System, model TA.XT 2), equipped with XT.RA Dimension V3.7 G software for the data acquisition. The temperature of the samples at the time of analysis was -20°C (samples directly taken from the freezing chamber). Textural profile was plotted as force vs time with an acquisition rate of 200 pps. Force units were measured in g and the distance format in mm. The contact area was 1.0 mm² and the contact force was 5.0 g. Pre-test speed, test-speed and post-test speed were set at 2.0, 1.0 and 5.0 mm/sec respectively. Distance was set up for 15.0 mm, with an interval of 5 sec and an auto trigger force of 5 g. The analysis of each treatment of ice cream was replicated four times.

3.4.12 Differential scanning calorimetry

A Perkin-Elmer 7 series (Perkin-Elmer Corporation, Norwalk, CT) thermal analyser equipped with a Perkin-Elmer differential scanning calorimeter (DSC) cell was utilised. The DSC was calibrated with high purity melting point standards and the heat of fusion of indium (m.p. 156.6°C, ΔHₘ 28.45 J/g, Perkin-Elmer standard). Nitrogen was used as the purge gas at the rate of 20 mL/min. Approximately 6 mg of samples were hermetically sealed in aluminium pans using the Perkin-Elmer sample encapsulation press. Samples were rapidly cooled from the ambient temperature to -80°C using liquid nitrogen in the quench cooling assembly. They were scanned at a heating rate of 10°C/min from -80 to 20°C. Each treatment was replicated 4 times. The two response variables represented by the two separate shifts on the DSC curves were identified as the glass transition temperature (T_g) and the melting temperature (T_m).

3.4.13 Determination of viscosity

Viscosity of aged mix of ice cream was measured with a Brookfield viscometer, model DVII+ (Selby Scientific Ltd, NSW). All of the samples were diluted with distilled deionised water, before analysis. Temperature of each sample at the time of analysis was 20°C. UL adaptor at 20 r.p.m. was used for the measurement of viscosity of the diluted samples. Analysis for each treatment was replicated 4 times.
3.4.14 Measurement of overrun

Overrun of ice cream was measured during the freezing process. Weight of a fixed volume of the aged mix as well as the ice cream was recorded, and the overrun was calculated using the following equation:

\[
\text{Overrun (\%)} = \frac{(\text{Weight of the mix} - \text{Weight of ice cream})}{\text{Weight of ice cream}} \times 100
\]

The measurement for each treatment was replicated 4 times.

3.4.15 Determination of freezing time

The freezing time of the mix was determined by overrun measurement and visual observation during freezing. It was the period of time from the start of the compressor to let the mix into the freezer to when the product (ice cream) was drawn from the outlet.

3.4.16 Refractometry

An Abbe refractometer No. 302 (Atago Optical Works Co. Ltd) was used for the quick check of the solid content of the concentrate during nanofiltration of milk UF permeate. It was also used for the determination of TS of the unconcentrated (original) milk UF permeate and the nano-permeate. The method for measurement was followed as described for transparent liquid in the instructional manual of Abbe refractometer. Refracting prism was opened by turning the required knob with the left hand. The prism was fully opened with the right hand. A few drops of sample were applied on the main prism and the refracting prism was then closed. The illuminator was switched on and the secondary voltage was raised at 7 V. The field of view was observed through the eyepiece. When the refractive index was matched, the boundary line between the dark and bright field appeared. Colour, along the boundary line was eliminated by turning the dispersion scale knob. Then a sharp boundary line appeared. Boundary line was adjusted to coincide with the crossed point of the diagonal lines. The %TS (°Brix) was read on the lower scale and recorded. Each of the analyses was duplicated.
3.4.17 Methods of calculation used during nanofiltration of milk UF permeate

\[
\text{Kg of feed} \\
\text{MCR} = \frac{\text{Kg of NC} \times \text{Concentration of material in NC}}{\text{(Kg of feed - Kg of nano-permeate)}}
\]

\[
\text{Recovery} \, (\%) = \frac{\text{Kg of feed} \times \text{Concentration of material in feed}}{\text{Kg of NC} \times \text{Concentration of material in NC}} \times 100
\]

\[
\text{Loss in NP} \, (\%) = \frac{\text{Kg of NP} \times \text{Concentration of material in NP}}{\text{Kg of feed} \times \text{Concentration of material in feed}} \times 100
\]

Loss in membrane unit \( (\%) \) = 100 - (\% recovery in NC + \% loss in NP)

Total loss \( (\%) \) = 100 - \% recovery in nano-concentrate

3.4.18 Sensory evaluation of ice cream

Five sensory attributes of each of the ice cream samples were evaluated by 24 untrained panellists aged 21-52 years (15 males and 9 females). These panellists included staff members and students of the Department of Food Science and Technology of the University of Western Sydney Hawkesbury, Richmond, NSW. The sensory attributes evaluated, were sweetness, saltiness, cooked flavour, iciness, and the overall acceptability. The sensory evaluation was conducted under normal lightening conditions between mid morning and noon. This period comprised four sessions, each of 30 min duration. Each session accommodated 6 panellists, seated in 6 partitioned booths. Each of the panellists was provided with week-long stored (at -20°C) 6 samples of ice cream. Throughout the sensory evaluation period, the samples were maintained at -20°C in a freezing chamber, adjacent to the sensory evaluation laboratory and taken directly from there for sensory evaluation. The 6 samples of ice cream were provided to each of the panellists in 6 different plastic cups. These 6 ice cream samples comprised the duplicates of the control and the two treatments (substitution of 25 and 50% sucrose with HLNC). One sample was provided at a time, followed by 5 others in succession at the regular intervals. Each of the 36 samples (6 panellists x 6 sample-cups) used in
a session, was randomly coded with a three-digit number, selected from the random access table. Each of the samples had different random number. The same set of 36 three-digit numbers was used in other sessions also, but in different orders. Drinking water was provided to the panellists for rinsing their mouths between samples. A scale-sheet was provided to each of them for indicating his/her choices. The sensory scale used, was an unstructured line or graphic scale (Land and Shepherd, 1984). The two verbal anchors used at the two ends of the graphic scale were ‘none’ (representing 0% distance) and ‘extremely’ (representing 100% distance). For the overall acceptability, however, the two ends were marked as ‘disliked extremely’ and ‘liked extremely’.

3.4.19 Statistical analysis

Student’s t test

For Table 7 (T.A. and pH), Table 8 (Colour intensity), Table 9 (Production of glucose and galactose), Table 10 (Effect of OS on the calculation of DH), Table 11 (pH and colour intensity), Table 13 (Colour intensity), Table 17 (Ash content of ice cream mix), Figure 4 & 5 (Flux rate during nanofiltration), and Figure 6 (Fouling study), Student’s t tests were used to compare two variables (Refer to Appendix 3) (IGNOU Handbook, 1997).

One way ANOVA & DMRT

Completely randomised design was used to study the effect of two levels (25 and 50% of sucrose substitution) of HLNC-treatment on ice cream properties (Table 12, 14, 15 and 16). Results were statistically analysed by SAS system (SAS Institute Inc., 1990) using one way ANOVA. Any variable with significant difference between two treatment means was evaluated using Duncan’s multiple range test (DMRT). The level of significance was set at 0.05 (Refer to Appendix 5) (SAS Institute Inc., 1990). For the sensory data, the sensory attributes were used as variables and the panellists as the replicates (a total of 48 replications comprising 24 panellists evaluating each sample twice).
PLEASE NOTE

The greatest amount of care has been taken while scanning the following pages. The best possible results have been obtained.
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Nanofiltration of milk UF permeate

4.1.1 Flux rate during nanofiltration of milk UF permeate

Figure 4 shows the flux rate of nano-permeate as a function of mass concentration ratio (MCR) and Figure 5 shows the flux rate of nano-permeate as a function of time during nanofiltration of milk UF permeate with the temperature existent and transmembrane pressure applied at each point. The initial flux (after 5 min at MCR 1.2) was 16.35 L/m²/h at 13°C and 800 KPa. This flux finally dropped to 8.90 L/m²/h at 3 fold mass concentration (after 55 min). The initially applied TMP of 800 KPa was kept constant for 30 min (MCR 1.73). At 30 min, the flux significantly decreased to 8.70 L/m²/h, eventhough the temperature increased (to 18.5°C), as an increase in temperature has been reported to greatly increase the rate of flux, because it affects the hydrodynamic properties of the permeated solution (Abd El-Salam and El-Etrioby, 1992). Application of 1000 KPa of TMP at that stage (at 30 min) did not increase the flux significantly, measured at 40 min, although the temperature, by that time increased to 21.5°C. The increase in flux at this stage was from 8.70 to 9.50 L/m²/h. Hence the applied TMP was increased to 1200 KPa (at 40 min, MCR 2.09) in order to improve the rate of flux. Even this TMP did not significantly increase the flux, which found to be 10.8 L/m²/h, measured at 45 min (MCR 2.40). The temperature by that time had further increased to 24°C. Allowing the persistence of this 1200 KPa of TMP for further 5 min resulted in significant reduction in flux rate to 6.40 L/m²/h, measured at 50 min (MCR 2.64), though by that time, the temperature had further increased to 26°C. At 50 min, the TMP was increased to 1500 KPa. This TMP did not increase the flux significantly, which found to be 8.9 L/m²/h, measured at 55 min (MCR 3.01) of processing, eventhough the temperature had increased to 30°C. By that time, however, the desired 3 fold mass concentration was achieved and therefore any further TMP was not required to be applied.

The significant reduction in flux rate at fixed transmembrane pressures, and the insignificant effect of increased transmembrane pressures on
Flux and T.S. vs MCR during nanofiltration of milk UF permeate.

$\text{LMH} = \text{L/m}^2\text{h} = \text{Litre per meter square per hour.}$

$\text{TMP} = \text{Transmembrane pressure.}$

$\text{KPa} = \text{Kilo-pascal.}$

Two trials of nanofiltration were conducted.

Flux was determined by the method mentioned in section 3.1.

Percent T.S. was determined by refractometer (section 3.4.16).

The MCR was calculated by the method described in section 3.4.17.

Refer to Appendix 3 for comparison between fluxes at various MCR.
Fig 5

Flux and MCR vs Time during nanofiltration of milk UF permeate.

LMH = L/m²/h = Litre per square meter per hour.
TMP = Transmembrane pressure.
KPa = Kilo-pascal.

Two trials of nanofiltration were conducted.

Flux was determined by the method mentioned in section 3.1.

The MCR was calculated by the method described in section 3.4.17.

Refer to Appendix 3 for comparison between fluxes at various time intervals.
the flux rate, even in presence of increased temperature during present study, indicate to some factors capable of counteracting and overcoming the influence of temperature, and subsequently decreasing the rate of flux. These factors, as reported by Jelen (1978) include the osmotic pressure, the lactose-insolubility, the viscosity, the diffusivity and the membrane-fouling.

The osmotic pressure of a dairy fluid is contributed by lactose and minerals. With an increase in concentration of lactose, osmotic pressure increases. Jelen (1978) reported approximately a 2.5 fold increase in osmotic pressure of an aqueous solution of lactose, when the concentration of lactose was increased 3 fold. In addition to lactose, salt has tremendous effect on the osmotic pressure. This was evident from the observation of Jelen (1978) that, with a similar level of 3 fold increase in the concentration of whey, the osmotic pressure increased by approximately 4 times. Similarly, an increase in osmotic pressure of milk UF permeate (virtually a solution of lactose) could be expected as a result of concentration of almost all of lactose by NF membrane, which allowed the permeation of only 2.70 % of it (Table 6). Most of the salt/ash content was also concentrated by the NF membrane (Table 5 and 6). To maintain a satisfactory flux rate, a TMP was required to be applied to overcome the effect of osmotic pressure. Initially, the applied TMP was kept low (800 KPa). This was because of the low osmotic pressure perceived to be exerted by the milk UF permeate, which contained lower solids concentration, particularly those of lactose and salts than its concentrated form. However, the TMP was increased at latter stages, as the osmotic pressure increased, owing to increase in solids concentration. Kelly and Kelly (1995) also reported the increase in applied TMP, as the retentate solids increased during the nanofiltration of acid casein whey.

Another factor capable of significantly reducing the flux, is the poor solubility of lactose in water. Solubility of lactose is temperature dependant. However, within a temperature range suitable to membrane processing, lactose still remains poorly soluble. At 0, 25, 40 and 60°C, the solubility of lactose has been estimated to be only 10.6, 17.4, 24.2 and 37.2% respectively (Jelen, 1978). Poor solubility of lactose leads to the formation of its crystals, which become deposited
Table 5

Concentration of components in feed, nano-concentrate and nano-permeate.

<table>
<thead>
<tr>
<th>Component</th>
<th>Feed</th>
<th>Nano-concentrate</th>
<th>Nano-permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (%)</td>
<td>5.25</td>
<td>15.2 (±0.2)</td>
<td>0.45</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>0.3 (±0.01)</td>
<td>0.83 (±.02)</td>
<td>0.05</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.46</td>
<td>13.0 (±0.01)</td>
<td>0.18 (±0.02)</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.37 (±0.05)</td>
<td>0.73 (±0.03)</td>
<td>nd</td>
</tr>
<tr>
<td>Potassium (mg/100 mL)</td>
<td>144.0 (±3.6)</td>
<td>249.6 (±0.3)</td>
<td>nd</td>
</tr>
<tr>
<td>Sodium (mg/100 mL)</td>
<td>43.6</td>
<td>94.8</td>
<td>nd</td>
</tr>
<tr>
<td>TA (%LA)</td>
<td>0.16</td>
<td>0.38 (±0.01)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Each of the above figures is the average of the results of two trials of nanofiltration of milk UF permeate.
Table 6

Percent recoveries and losses of the components of milk UF permeate during 3 fold nanofiltration.

<table>
<thead>
<tr>
<th>Component</th>
<th>Recovered in NC (%)</th>
<th>Lost in NP (%)</th>
<th>Lost in membrane unit (%)</th>
<th>Total loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>29.2</td>
<td>66.8</td>
<td>4.0</td>
<td>70.8</td>
</tr>
<tr>
<td>Total Solids</td>
<td>84.5</td>
<td>5.71</td>
<td>9.79</td>
<td>15.5</td>
</tr>
<tr>
<td>Crude protein</td>
<td>79.1</td>
<td>10.8</td>
<td>10.1</td>
<td>20.9</td>
</tr>
<tr>
<td>Lactose</td>
<td>85.4</td>
<td>2.70</td>
<td>11.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Ash</td>
<td>57.4</td>
<td>nd</td>
<td>nd</td>
<td>42.6</td>
</tr>
<tr>
<td>Potassium</td>
<td>50.4</td>
<td>nd</td>
<td>nd</td>
<td>49.6</td>
</tr>
<tr>
<td>Sodium</td>
<td>62.8</td>
<td>nd</td>
<td>nd</td>
<td>37.2</td>
</tr>
<tr>
<td>T.A.</td>
<td>69.4</td>
<td>12.5</td>
<td>18.1</td>
<td>30.6</td>
</tr>
</tbody>
</table>

nd = not determined.

The figures were derived from those mentioned in Table 5.

All of the above calculations were made according to the methods described in section 3.4.17.
onto the surface of membrane. These lactose crystals could be responsible for the reduction in flux. Formation of crystals, however, is resisted by salts. Salts form complexes with lactose (Guu and Zall, 1992). These complexes are water soluble. Hence in a membrane process, which increases the lactose concentration without demineralisation (e.g. R.O.), the effect of lactose solubility on flux rate may not be relevant. The same, however, may not be true for NF, because as a result of NF, the ash to lactose ratio in the nano-concentrate of milk UF permeate decreases, as observed during present study (Table 5). Such a decrease in ash to lactose ratio facilitates the insolubility of lactose, which results in its crystallisation (Guu and Zall, 1992). Hence an increased amount of lactose depositing onto the membrane surface with an increase in concentration level, seems possible.

Reduction in flux rate is also due to an increase in viscosity, which increases with an increase in concentration. Viscosity affects the mass transfer through its effect on diffusivity and on the fluid flow properties of the system (Jelen, 1978). It is contributed generally by milk proteins. It is not appreciably contributed by the solution of lactose and milk salts (Jelen, 1978). Although, the concentration of protein in milk UF permeate is very low (Table 5), its increased concentration due to nanofiltration (Table 5) could be expected to contribute to the viscosity of milk UF permeate to some extent. Consequently, some reduction in flux may be attributed to the viscosity of nano-concentrate of milk UF permeate.

A very important factor, which affects the flux rate, is the solute diffusivity. In a process, such as RO or NF (a loose RO), insufficient back diffusion of the retained components of a dairy fluid results in the formation of a concentrated boundary layer. The osmotic pressure of this boundary layer is high. The components of concern for milk UF permeate are protein and lactose, because salts have very high diffusivity compared to these two components. Protein is substantially lower in concentration than lactose in milk UF permeate or its nano-concentrate (Table 5) and has much less diffusivity than lactose. Moreover, diffusivity of lactose is less than that of salts (Jelen, 1978). Furthermore, diffusivity depends on the concentration of components mentioned above. For instance, Jelen (1978) reported that rise in concentration of lactose by four fold decreased the diffusivity by 44.7%. Thus, the low diffusivity of protein (despite its low
concentration) and high concentration of lactose (resulting in its decreased diffusivity) could be one of the reasons for flux decline during the nanofiltration of milk UF permeate. Furthermore, towards higher ratio of mass concentration (MCR) during nanofiltration, the diffusivity is lower and hence boundary layer formation becomes more intense than that at the lower MCR. As a result, higher TMP is required at later stages than at initial stages during the nanofiltration in order to maintain a satisfactory flux rate (Jelen, 1978).

The incidence of loose adherence of boundary layer to the membrane surface is called the concentration polarisation, which can be washed away even by flushing the membrane with water, without the use of cleaning agents (Eckner and Zottola, 1993). On the other hand, when the adherence of boundary layer to the membrane surface becomes severe in nature, a new phenomenon called fouling comes into existence (Eckner and Zottola, 1993). Fouling is caused by the deposition of colloidal material onto the membrane surface, giving rise to an additional hydrolytic resistance. Fouling always starts with adsorption and it is the protein which adsorbs (Walstra and Jenness, 1984). The phenomenon of adsorption causes a change of surface. Onto this surface, deposition of other materials takes place. This adsorbed layer and the deposited materials causing fouling, may not be possibly washed away by simply flushing the membrane with water. However, a cleaning agent containing the protein splitting enzymes and complexing agents in its formulation, can remove the fouling. In a nutshell, if the flushing of membrane with water restores the original water-flux, the reduction in process flux, measured at the end of membrane processing of dairy fluid, can be attributed to concentration polarisation or else, it can be attributed to fouling.

It is evident from Fig 6 that, thorough flushing/rinsing of membrane by circulating water for 2 h, before the onset of cleaning process, failed to restore the original flux of water at any TMP applied and the difference between the original water-fluxes and those obtained after flushing was significant (Appendix 3). It shows that concentration polarisation was not the only phenomenon responsible for reduction in process flux during nanofiltration of milk UF permeate, fouling was definitely responsible for that. This was further evident from the fact that, the circulation of 1% aqueous solution (30° C) of the Ultrasil 56 (Ecolab Pty Ltd, NSW) enzyme cleaner for an hour restored the original water-fluxes and there was no significant difference between the original
Fig 6

Fluxes at various applied transmembrane pressures, before the start of the nanofiltration of milk UF permeate; after rinsing/flushing the membrane with water at the end of the nanofiltration of milk UF permeate; and after cleaning the membrane with the solution of Ultrasil 56 after the rinsing step at the end of the nanofiltration of milk UF permeate.

LMH = L/m²/h = Litre per square meter per hour.

KPa = Kilo-pascal.

Two trials were conducted.

Flux was determined by the method mentioned in section 3.1.

Refer to Appendix 3 for the statistical analysis of the effect of membrane cleaning on flux rates.
water-fluxes and those obtained after the cleaning of membrane (Appendix 3). This enzyme cleaner in powder form contains a combination of organic and inorganic complexing agents as well as protein splitting enzymes. Probably, the enzyme present in cleaning solution may have removed the fouling caused by protein; the organic complexing agent may have removed the fouling caused by lactose & other organic components of milk UF permeate, and the inorganic complexing agent may have removed the fouling caused by salts.

4.1.2 Effect of nanofiltration on pH and titratable acidity of milk UF permeate

After 3 fold nanofiltration, the pH of milk UF permeate significantly decreased from 6.74 to 6.6 (Table 7; Appendix 3). The significant reduction in pH seems to be the result of significant increase in the titratable acidity (TA) from 0.16 to 0.38% LA after 3 fold nanofiltration (Table 7; Appendix 3). However, only 69.4% of TA was recovered in the nano-concentrate. The rest 30.6% was lost, 12.5% in nano-permeate and 18.1% in membrane unit (Table 6).

Reduction in lactic acid content by nanofiltration has been reported by Kelly et al. (1991). According to them, the acidity of sweet whey reduced by 35%, whereas that of cottage cheese whey by 42% after 4 fold nanofiltration.

4.1.3 Effect of nanofiltration on the colour of milk UF permeate

Table 8 shows the changes in the type and the intensity of colour of milk UF permeate as a result of nanofiltration (NF). Its greenish yellow colour changed to yellow. Yellow colour existed at all the levels of mass concentration. The wavelengths between MCR of 1.2 and 3.01 ranged between 576 and 580 m\(\mu\) (Fig 7). This is the range of yellow colour. The greenish tint disappeared from the milk UF permeate and permeated through NF membrane at the very beginning of the process. This can be evident from the colour of NF permeate, which was greenish yellow, though with only 6% intensity (Table 8). The intensity of the changed colour (yellow) increased significantly from 20% (at MCR 1.2) to 49.0% (at MCR 3.01) (Table 8; Appendix 3).
Table 7

The changes in titratable acidity, pH, and temperature of milk UF permeate during nanofiltration.

<table>
<thead>
<tr>
<th>MCR</th>
<th>Titratable Acidity (% LA)</th>
<th>pH (At 25°C)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.16 (S.D. 0.0)</td>
<td>6.74 (S.D. 0.0)</td>
<td>10.0</td>
</tr>
<tr>
<td>1.20</td>
<td>0.18 (S.D. 0.01)</td>
<td>6.74 (S.D. 0.0)</td>
<td>13.0 (±1.0)</td>
</tr>
<tr>
<td>1.37</td>
<td>0.21 (S.D. 0.01)</td>
<td>6.74 (S.D. 0.0)</td>
<td>15.0 (±1.0)</td>
</tr>
<tr>
<td>1.73</td>
<td>0.26 (S.D. 0.01)</td>
<td>6.72 (S.D. 0.01)</td>
<td>18.5 (±0.5)</td>
</tr>
<tr>
<td>2.09</td>
<td>0.29 (S.D. 0.0)</td>
<td>6.68 (S.D. 0.0)</td>
<td>21.5 (±0.5)</td>
</tr>
<tr>
<td>2.40</td>
<td>0.33 (S.D. 0.007)</td>
<td>6.66 (S.D. 0.0)</td>
<td>24.0</td>
</tr>
<tr>
<td>2.64</td>
<td>0.34 (S.D. 0.007)</td>
<td>6.63 (S.D. 0.01)</td>
<td>26.0</td>
</tr>
<tr>
<td>3.01</td>
<td>0.38 (S.D. 0.02)</td>
<td>6.60 (S.D. 0.0)</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Each data point is the mean of values from two trials.

Difference between T.A. at MCR 1.0 and 3.01 was significant at 5% level of significance; Difference between the pH at MCR 1.0 and 3.01 was significant at 5% level of significance (Appendix 3).

Titratable acidity was determined by the method described in section 3.4.4.

pH was determined by the method described in section 3.4.1.

Temperature was recorded from the temperature recorder attached to the membrane unit.
Table 8

Effect of nanofiltration on the colour of milk UF permeate.

<table>
<thead>
<tr>
<th>Mass concentration ratio</th>
<th>Type of colour</th>
<th>Intensity of colour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Greenish yellow</td>
<td>20.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>1.2</td>
<td>Yellow</td>
<td>20.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>1.37</td>
<td>Yellow</td>
<td>22.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>1.73</td>
<td>Yellow</td>
<td>31.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>2.09</td>
<td>Yellow</td>
<td>31.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>2.4</td>
<td>Yellow</td>
<td>36.0 (S.D. 0.0)</td>
</tr>
<tr>
<td>2.64</td>
<td>Yellow</td>
<td>43.8 (S.D. 0.28)</td>
</tr>
<tr>
<td>3.01</td>
<td>Yellow</td>
<td>49.0 (S.D. 4.24)</td>
</tr>
<tr>
<td>Nano-permeate</td>
<td>Greenish yellow</td>
<td>6.0 (S.D. 0.0)</td>
</tr>
</tbody>
</table>

Each data point is the mean of four values, two from each of the two trials.

Difference between the intensity of yellow colour at MCR 1.2 and 3.01 was significant at 5% level of significance (Appendix 3).

Mass concentration ratio was determined by the method described in section 3.4.17.

The type and the intensity of colour were determined by the method described in section 3.4.5.
Fig 7
The horse shoe shaped CIE (Commission International de l'Eclairage) colour chart.

(Source: The manual, Nippon Denshoku colour difference meter, model ND-101 DP; Nippon Denshoku Kogyo Co. Ltd.)

X-axis: $x$ denotes the ratio of the value of $X$ and the sum of the values of $X$, $Y$ and $Z$, obtained from the colour difference meter.

Y-axis: $y$ denotes the ratio of the value of $Y$ and the sum of the values of $X$, $Y$ and $Z$, obtained from the colour difference meter.

The numerical figures located at the peripheri of the horse shoe shaped curve, denote the wavelengths.
However, some yellowish tint too, was detected in NF permeate along with the green colour. The lower intensity of the greenish yellow colour (6%) of NF permeate (wavelength 574 m\(\mu\)) than that of milk UF permeate (20%) could be due to huge difference in dilutions of these two streams (TS of milk UF permeate being 5.25% and that of NF permeate being 0.45%) (Table 5).

The greenish yellow appearance of nano-permeate and the non-existence of greenish tint in the yellow coloured nano-concentrate imply that the green colour agent has a smaller molecular size than its yellow counterpart. The greenish tint of milk is due to the presence of riboflavin (Johnson, 1978), whereas carotenoids are responsible for the yellow colour (Hartman and Dryden, 1978). The molecular weight of riboflavin is 376, whereas that of \(\beta\)-carotene, the most prominent carotenoid is 536. Although, from the view point of the pore size of the NF membrane, a molecular weight of 376 is high, some of its permeation through this membrane, however, can be expected. On the other hand, comparatively less carotenoids than riboflavin may have permeated due to higher molecular weight of the former.

The water soluble riboflavin and carotenoids exist in plant. They appear in milk due to the ingestion of their sources by cow. Carotenoids are converted to fat-soluble vitamin A in cow, but not all of them (Hartman and Dryden, 1978). The conversion depends on the ability of a particular breed of cow (Walstra and Jenness, 1984). Only 11 to 50% of carotenoids are involved in vitamin A activity (Hartman and Dryden, 1978). It means, the remaining 50-89% of carotenoids will still remain water soluble and can appear in milk UF permeate, which is fat-free.

Due to their low molecular weight with regard to the pore size of an UF membrane, the water soluble carotenoids would have permeated through the UF membrane and appeared in milk UF permeate. However, due to its large molecular weight with regard to the pore size of a NF membrane, these carotenoids would have concentrated, and not permeated through the NF membrane. This may have resulted in the increased intensities of nano-concentrates at different stages of mass concentration during nanofiltration of milk UF permeate.
4.1.4 Effect of nano-filtration on the mineral content of milk UF permeate

After 3 fold concentration by NF, the milk UF permeate was demineralised by 42.6%, which included the mineral lost in nano-permeate as well as that lost in membrane unit. The rest 57.4% remained with the nano-concentrate (Table 6). The extent of demineralisation achieved during present investigation is higher than that reported by Kelly and Kelly (1995b). They obtained 32.8, 35.2, and 25.8% ash reductions at the volume concentration ratio (VCR) of 4 with regard to acid casein, Cheddar cheese, and rennet casein wheys respectively.

The concentrations of monovalent cations, Na⁺ and K⁺ in milk UF permeate were determined as 43.6 mg/100 mL (0.83% dry matter) and 144 mg/100 mL (2.74% dry matter) respectively (Table 5). These values are comparable to those reported in literature. Renner and Abd El-Salam (1991) reported the concentrations of Na⁺ and K⁺ in milk UF permeate as 40 and 140 mg/100 mL.

At the end of 3 fold nanofiltration of milk UF permeate in the present study, Na⁺ decreased (lost) by 37.2%, whereas K⁺ decreased (lost) by 49.6% (Table 6). In the results obtained during present study, the extent of reduction of monovalent ions are not entirely comparable to those reported in the literature. The plant manual of the membrane indicates 95% permeation of NaCl (Filtration Engineering plant manual, 1995). Using a plate and frame HC-50 membrane for the nanofiltration of acid casein whey, Kelly and Kelly (1995b) achieved 55% reduction (on dry matter basis) in the levels of both of these monovalent cations after 4 fold concentration. With hollow fibre HC-50 membrane, Guu and Zall (1992) achieved 45.4 and 42.4% reductions in Na⁺ and K⁺ respectively after 3 fold concentration of sweet whey UF permeate.

4.1.5 Effect of nanofiltration on the total solid content of milk UF permeate

The total solids (TS) of milk UF permeate increased with an increase in mass concentration ratio (MCR) (Fig 4). However, the trend of increase in TS was slightly different from that of MCR. Unlike MCR, the increase in TS was steady throughout the process. The difference between these two trends were probably due to higher amount of solids
permeation during the last 15 min than the initial 40 min of processing. During the initial 40 min, an average of only 3.08% of solids permeated, as compared to the 4.86% (average) during the last 15 min. Higher amount of solids permeation at a higher level of concentration was also reported by Kelly et al (1991). At the VCR of 2, 3, and 4, they observed 29.2, 30.8, and 37.8% permeation of solids during the NF of casein whey.

The total amount of solids in 80 Kg of feed was 4.2 Kg (80 x 5.25%). The concentration of solids recovered in 23.4 Kg of nano-concentrate after 3 fold nano-filtration was 15.2% (Table 5). Thus, 84.5% of solids contained in feed was recovered in nano-concentrate (Table 6). The concentration of solids in 53.5 Kg of nano-permeate was 0.45% (Table 5). Thus, 5.71% of solids contained in feed was lost in nano-permeate (Table 6). The rest 9.79% of solids was lost in membrane unit (Table 6).

4.1.6 Effect of nanofiltration on the fat content of milk UF permeate

No fat was detected either in milk UF permeate or its nano-concentrate or nano-permeate. The composition of milk UF permeate reported by Renner and Abd El-Salam (1991) did not include fat. No fat was detected by Kelly and Kelly (1995b) in the nano-permeate of acid casein whey. It is understandable, because the pore size of NF or even UF-membrane is small enough to allow the permeation of fat molecules.

4.1.7 Effect of nanofiltration on the crude protein content of milk UF permeate

The concentration of crude protein in feed was 0.3% (Table 5). The total amount of crude protein in feed was 0.24 Kg (80 x 0.3%). The concentration of crude protein in 23.4 Kg of nano-concentrate was 0.83% (Table 5). Thus, 79.1% of crude protein contained in feed was recovered in nano-concentrate. The concentration of crude protein in 53.5 Kg of nano-permeate was 0.05% (Table 5). Thus, 10.8% of the amount of crude protein contained in feed was lost in nano-permeate. The remaining 10.1% of crude protein was lost in membrane unit (Table 6).
Out of 5.8% TS of milk permeate, reported by Renner and Abd Elsalam (1991), the crude protein content was 0.25%. According to them, NPN was the main constituent of crude protein. The true protein content was one third of the crude protein. According to Walstra and Jenness (1984), milk UF permeate also contains some peptides.

The recovery of crude protein in the present study was less than those reported by Kelly and Kelly (1995b). However, they found that the recovery was pH dependant. After a 4 fold concentration of acid casein whey, they observed the recovery of 92.1, 91.2 and 90.5% of proteins (on a dry matter basis) at pH 3.6, 4.6, and 6.6 respectively.

Eckner and Zottola (1992b) observed a loss of 2.2 to 7.24% of protein (on a dry matter basis) in the permeate during the NF of skim milk. According to Kelly and Kelly (1995b), more than half (51%) of the protein content of acid casein whey was detected in NF-permeate. With regard to the raw whey level, 8.12% true protein and 39.3% NPN were found in permeate. The NPN content of permeate was 77% of the total protein permeated. Bird (1996) observed 50% recovery of NPN and 100% recovery of true protein. No true protein was detected by him in the NF permeate of salty whey from Cheddar cheese. Of the true protein, only α-LA and β-LG were found in the nano-permeate by Kelly and Kelly (1995b).

4.1.8 Effect of nanofiltration on the lactose content of milk UF permeate

The concentration of lactose in feed was 4.46% (Table 5). The total amount of lactose in 80 Kg of feed was 3.57 Kg (80 x 4.46%). The concentration of lactose in 23.4 Kg of nano-concentrate was 13.0% (Table 5). Thus, 85.4% of the amount of lactose contained in feed was recovered in nano-concentrate (Table 6). The concentration of lactose in 53.5 Kg of nano-permeate was 0.18% (Table 5). Thus, 2.70% of the amount of lactose contained in feed was lost in nano-permeate (Table 6). The remaining 11.9% of lactose was lost in membrane unit (Table 6). Thus, the total loss of lactose was 14.6%.

The total loss of lactose observed during present study was higher than that reported by Kelly and Kelly (1995b). After a 4 fold NF of acid casein whey, they observed a lactose-loss of 2.67%. This reported loss,
however, is comparable to the loss of lactose in nano-permeate found during present study.

The losses of organic solids in the form of proteins or nitrogenous fractions and lactose may necessitate the treatment of nano-permeate as an effluent. Thus, from an environmental point of view, NF could be less attractive than RO. The advantage of NF over RO, however, is in the extent of demineralisation, which was one of the main objectives of the present study. Kelly and Kelly (1995b) quantified the organic losses in terms of chemical oxygen demand (COD) at each VCR. The COD values resulted in an increase from 1000 mg/L at VCR 1 to 2592 mg/L at VCR 4, with the mean value of 1592 mg/L. On the basis of this result, they suggested biological treatment of nano-permeate.

4.2 Hydrolysis of nano-concentrate

4.2.1 HPLC Chromatogram

The calibration curves obtained from HPLC for lactose (Fig 8), glucose (Fig 9) and galactose (Fig 10) show good correlations among the concentrations used for individual sugars. The correlation coefficients for lactose, glucose, and galactose were 0.999, 0.999, and 0.996 respectively.

The HPLC chromatogram for unhydrolysed nano-concentrate (Fig 11) shows only one peak. This peak appeared at a retention time of 11.87 min and was identified as lactose. At approximately the same retention time, this peak appeared in all of the lactose hydrolysed samples (Fig 12, 13, 14, 15, and 16), except in one, kept overnight for hydrolysis. In fact, no lactose peak was detected in the sample hydrolysed overnight (Fig 17).

Glucose, in all of the samples appeared at retention times ranging between 12.98 and 13.04 min (Fig 12, 13, 14, 15, 16 and 17). The retention times of galactose ranged between 15.01 and 15.08 min (Fig 12, 13, 14, 15, 16 and 17). Separation of all of these sugars was achieved within 19 min. Apart from these 3 sugars, some additional peaks were also detected in the hydrolysed samples. These peaks might be of oligosaccharides formed through transgalactosidation reactins. One of the OS peaks with retention times ranging between 10.38 and 10.43 min was detected in all of the hydrolysed samples, except the
Fig 8

HPLC calibration curve for lactose.

Correlation coefficient 0.999.
Fig 9

HPLC calibration curve for glucose.

Correlation coefficient 0.999.
Fig 10

HPLC calibration curve for galactose.

Correlation coefficient 0.996.
one, kept overnight (Fig 12, 13, 14, 15 and 16). Another OS peak did not appear for the initial 15 min. It showed some sign of its appearance after 30 min, but was not fully resolved (Fig 15). The sign of appearance of this OS peak was evident from a shift in the symmetrical shape of lactose peak (Fig 15). The resolution of this OS peak, appearing at a retention time of 12.3 min, however, was complete after 60 min of hydrolysis. At the same retention time, this OS peak appeared in the overnight sample also. Thus, a total of two OS peaks were detected on the resin based column used during the present investigation.

Using sweet whey UF permeate as a substrate, Jeon et al. (1984) observed only one peak other than the peaks of lactose, glucose and galactose in the hydrolysed sample, separated on a resin based μ-spherogel column. The hydrolysis of lactose had been carried out by them at 37°C for 30 min, with the use of Maxilact β-galactosidase, at a rate of 2000 μ mol ONP/L. Using the same type of column, Jeon and Mantha (1985) observed three OS peaks (two of them in substantial proportion) after 60 min of hydrolysis of 20 % lactose solution at 37°C with yeast lactase at the rate of 1 to 2 μ mol ONP/mL lactose solution. On a different column, however, these authors detected higher number of OS peaks. Under the similar conditions of hydrolysis as stated above, they observed 5 OS peaks, detected on a polar bonded column. By this comparison, they concluded that a resin based column had poor separation of OS than a polar bonded column. Scott (1992) too reported that resolution was the major problem with a resin based column. However, a resin based column possesses several commendable characteristics. With this column, generally water (deionised) is used as the solvent. Therefore, the problem of toxicity or concern for safety does not exist, except in case organic modifiers are used with water. One remarkable benefit of using water as solvent over a mixed solvent is that, water does not lower the refractive index of saccharides (Jeon and Mantha, 1985). The use of a resin based column provides a good baseline stability, which is helpful in minimising baseline noise. Stability of column itself is very good below the pressure of 1500 psi. Columns of this type contain 10 μm sphere of sulphonated, polystyrene divinyl benzene (PDVB) resin with 4-8 % cross-linking. The strongly cationic PDVB resin is loaded with cationic counter ions such as Pb²⁺ (used during present investigation). The interaction of sugars with these
HPLC chromatogram for the unhydrolysed nano-concentrate of milk UF permeate.

pH of the nano-concentrate --- 6.6
Lactose content of the nano-concentrate --- 13.0% (wet basis)
Analysis was conducted in duplicate.
Fig 12

HPLC chromatogram for the nano-concentrate of milk UF permeate after 5 min of lactose hydrolysis.

- pH of the nano-concentrate: 6.6
- Lactose content of the nano-concentrate: 13.0% (wet basis)
- Temperature of hydrolysis: 40°C
- Source of the enzyme lactase used: Yeast *K. fragilis*
- The enzymic dosage used: 12.0 mL/L of the nano-concentrate.
  (Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
Fig 13

HPLC chromatogram for the nano-concentrate of milk UF permeate after 10 min of lactose hydrolysis.

pH of the nano-concentrate --- 6.6
Lactose content of the nano-concentrate --- 13.0% (wet basis)
Temperature of hydrolysis --- 40°C
Source of the enzyme lactase used--- Yeast *K. fragilis*
The enzymic dosage used --- 12.0 mL/L of the nano-concentrate.
Analysis was conducted in duplicate.
**Fig 14**

HPLC chromatogram for the nano-concentrate of milk UF permeate after 15 min of lactose hydrolysis.

pH of the nano-concentrate --- 6.6
Lactose content of the nano-concentrate --- 13.0% (wet basis)
Temperature of hydrolysis --- 40°C
Source of enzyme lactase used --- Yeast *K. fragilis*
The enzymic dosage used --- 12.0 mL/L of the nano-concentrate.

(Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
**Fig 15**

**HPLC chromatogram for the nano-concentrate of milk UF permeate after 30 min of lactose hydrolysis.**

- pH of the nano-concentrate: 6.6
- Lactose content of the nano-concentrate: 13.0% (wet basis)
- Temperature of hydrolysis: 40°C
- Source of enzyme lactase used: Yeast *K. fragilis*
- Enzymic dosage used: 12 mL/L of the nano-concentrate. (Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
HPLC chromatogram for the nano-concentrate of milk UF permeate after 60 min of lactose hydrolysis.

pH of the nano-concentrate ---- 6.6
Lactose content of the nano-concentrate --- 13.0% (wet basis)
Temperature of hydrolysis --- 40°C
Source of enzyme lactase used --- Yeast *K. fragilis*
Enzymic dosage used --- 12 mL/L of nano-concentrate.

(Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
HPLC chromatogram for the nano-concentrate of milk UF permeate after 18 h (overnight) of lactose hydrolysis.

pH of the nano-concentrate --- 6.6  
Lactose content of the nano-concentrate ---- 13.0% (wet basis)  
Temperature of hydrolysis --- 40°C  
Source of enzyme lactase used --- Yeast *K. fragilis*  
Enzymic dosage used --- 12 mL/L of nano-concentrate.  
  (Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
counterions results in the formation of strong complexes. The one loaded with Pb$^{++}$ counterions is particularly useful in the analysis of dairy products. Apart from these benefits, a resin based column can prevent the interferences from salts, organic acids, and methanol (Scott, 1992). It means, de-salting of sample is not a critical step in sample preparation with this column, and the need of a guard column can also be ignored. During present investigation, however, partially demineralised samples (nanofiltered) were used as well as a guard column was connected in line prior to the main column.

A resin based column elutes the components of a mixture in the order of decreasing molecular size (Jeon and Mantha, 1985). This is evident from the present study. Among three sugars, the disaccharide lactose eluted first, followed by the monosaccharides, glucose and galactose.

On the basis of the fact, that a resin based column elutes the components of a mixture in the order of decreasing molecular size, the OS detected at the retention time of 10.38 - 10.43 min, can be considered as a trisaccharide (TIS). Another peak, appearing at a retention time of 12.3 min was separated after lactose, but before glucose. It could be considered as a disaccharide (DIS), provided it was an OS. The reason behind the doubt whether this peak was of an OS is that, an OS consists of at least 2 monomeric residues joined together through glycosidic bonds (Lopez-Leiva and Guzman, 1995). It is known that glycosidases catalyse both the hydrolytic as well as the transfer reaction. A transfer reaction is one, in which the sugar residue forming the glycone part of the substrate, is transferred to either water or to some other hydroxylic acceptor, such as another sugar or an alcohol. The OS will form only when the hydroxylic acceptor is a sugar (Zarate and Lopez-Leiva, 1990). Then the peak, with a retention time of 12.3 min may not be of an OS. This is because, in the event of water being the acceptor, this peak would be of a monosaccharide. However, the possibility of its being a disaccharide can not be omitted merely on the basis of the idea, that only the molecule ‘smaller’ in size than lactose could have appeared after the lactose peak. It is because, the peaks with the same molecular size can appear one after another. This argument can be justified by the fact that despite the same molecular size, glucose and galactose appeared one after another. Thus the peak appearing between the peaks of lactose and glucose could be considered as that of an OS (a DIS).
4.2.2 Concentrations of oligosaccharides and the hydrolysates of lactose

Appearance of only one peak in the unhydrolysed sample (Fig 11) indicates that no hydrolysis took place in that sample and only lactose was present there as the carbohydrate. However, as a result of hydrolysis, the concentration of lactose began to decrease and after 18 h, no lactose peak was detected in the HPLC chromatogram (Fig 17). The concentrations of lactose, remaining unhydrolysed at various intervals of time were 50.8, 37.3, 29.9, 15.2, 6.6 and 0.0% after 5, 10, 15, 30, 60 and 1080 min of hydrolysis respectively (Table 9). Reduction in lactose resulted in the formation of its hydrolysates, glucose and galactose. After 5, 10 15, 30, 60 and 1080 min, the concentration of glucose was 27.3, 34.2, 37.5, 46.6, 46.0 and 50.7%, whereas after the same intervals of time, the galactose concentration was 14.6, 18.8, 22.1, 29.7, 34.1 and 45.4% respectively (Table 9). According to the statistical analysis, the concentration of galactose during hydrolysis, was significantly lower than that of glucose (Appendix 3). For the initial 30 min, the rate of increase in glucose concentration was higher than that of galactose. After 30 min, till the end of hydrolysis, the rate of increase in galactose concentration became higher than that of glucose.

From 5 min till 10 min of hydrolysis, the increase in glucose concentration was 6.9% (34.2% - 27.3%), as compared to 4.2% increase in galactose concentration (from 14.6 to 18.8%). Between 10 and 15 min of hydrolysis, the increase in glucose concentration was 3.3% (37.5% - 34.2%). The same percentage of increase was observed in galactose concentration (from 18.8 to 22.1%) during this period. Between 15 and 30 min of hydrolysis, the increase in glucose concentration was 9.1% (46.6% - 37.5%) compared to 7.6% increase (29.7% - 22.1%) in galactose concentration. After 30 min of hydrolysis, the rate of increase in galactose concentration became more than that of glucose. This is evident from the result (Table 9) that between 30 and 60 min, glucose concentration decreased by 0.6% (46% - 46.6%), whereas, the galactose concentration increased by 4.4% (34.1% - 29.7%). The increase in the rate of galactose concentration between 60 min and 1080 was the highest, by 11.3% (45.4% - 34.1%). Glucose concentration during this period increased by only 4.7% (50.7% - 46%).
Table 9

Concentration of different carbohydrates in hydrolysed lactose nano-concentrate (as %total carbohydrates) at various time intervals during the hydrolysis of lactose.

<table>
<thead>
<tr>
<th>Time of hydrolysis (Min)</th>
<th>Lactose (%)</th>
<th>Glucose (%)</th>
<th>Galactose (%)</th>
<th>Total OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5.0</td>
<td>50.8 (±0.4)</td>
<td>27.3 (±0.9)</td>
<td>14.6 (±1.0)</td>
<td>7.1 (±0.4)</td>
</tr>
<tr>
<td>10.0</td>
<td>37.3 (±0.2)</td>
<td>34.2 (±0.6)</td>
<td>18.8 (±1.7)</td>
<td>9.5 (±0.1)</td>
</tr>
<tr>
<td>15.0</td>
<td>29.9 (±0.5)</td>
<td>37.5 (±3.2)</td>
<td>22.1 (±2.1)</td>
<td>9.7</td>
</tr>
<tr>
<td>30.0</td>
<td>15.2</td>
<td>46.6 (±2.6)</td>
<td>29.7 (±2.7)</td>
<td>19.3 (±0.3)</td>
</tr>
<tr>
<td>60.0</td>
<td>6.6 (±0.6)</td>
<td>46.0 (±3.0)</td>
<td>34.1 (±3.3)</td>
<td>12.0 (±0.2)</td>
</tr>
<tr>
<td>1080</td>
<td>0.0</td>
<td>50.7 (±3.2)</td>
<td>45.4 (±4.0)</td>
<td>3.8 (±0.68)</td>
</tr>
</tbody>
</table>

OS = Oligosaccharides

Each data point is the mean of values from two trials.

Concentration of glucose was significantly higher than that of galactose at 5% level of significance (Appendix 3).
The concentration of total OS after 5 min was 7.1% of total carbohydrates, which increased to 9.5 and 9.7% respectively after 10 and 15 min of hydrolysis (Table 9). Only TIS was present during this period of hydrolysis, as the OS (Fig 12, 13, and 14). After 15 min of hydrolysis, the TIS concentration began to decrease, which almost disappeared after 1080 min (Fig 17). Its concentration after 30 min, 60 min and 1080 min of hydrolysis was 7.3, 5.0 and 0.6% respectively. The concentration of DIS (12%) at 30 min was higher than that of TIS (7.3%). It decreased from 12% to 7.0% and 3.2% after 60 min and 1080 min of hydrolysis respectively. The concentration of total OS was 7.1, 9.5, 9.7, 19.3, 12.0 and 3.8% after 5, 10, 15, 30, 60 min and 1080 of hydrolysis respectively. Thus, the highest concentration of OS was after 30 min of lactose hydrolysis (at 84.8% conversion of lactose). The lowest concentration of OS was after 1080 min (at 100% conversion of lactose).

Higher concentration of glucose than galactose throughout the period of hydrolysis was reported in the literature (Burvall et al., 1979; Greenberg and Mahoney, 1983; Jeon and Mantha, 1985; Zadow, 1986). According to these reports, galactose is more involved in the synthesis of OS than glucose. Jeon and Mantha (1985) reported the transfer of more galactosyl residue of galactose than that of glucose in the formation of OS. This was evident from the fact that at least 3 of the 5 OS detected during their study, contained only galactose units. However, as the findings of the present study also indicate in most of the cases (Table 9), the difference between glucose and galactose was greater than the concentration of total OS. Burvall et al. (1979) predicted the reason for this. According to them, this discrepancy could be attributed to the formation of DIS containing two galactose molecules (galactobiose). These DIS were not detected by paper chromatography used by them. The same explanation could be applied to the findings of the present study. DIS was detected by HPLC system, during the present investigation, but not from the beginning. The existence of discrepancy, however, was there from the beginning. It suggests that some undetected DIS might have been present at the beginning.

The maximum glucose/galactose ratio reported by Greenberg and Mahoney (1983) was 2.19, when 35.3% of lactose was still unhydrolysed. Glucose was 1.89 times higher than galactose in their study, when there was only 6.16% of lactose to be hydrolysed. The
### Table 10

**Effect of the formation of oligosaccharides on the calculation of the degree of hydrolysis.**

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>5 min (±0.4)</th>
<th>10 min (±0.2)</th>
<th>15 min (±0.5)</th>
<th>30 min</th>
<th>60 min (±0.6)</th>
<th>1080 min</th>
<th>100.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (%)</td>
<td>0</td>
<td>49.2</td>
<td>62.7</td>
<td>70.1</td>
<td>84.8</td>
<td>93.4</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>DH (%)</td>
<td>0</td>
<td>42.0</td>
<td>53.0 (±0.1)</td>
<td>60.2 (±0.5)</td>
<td>67.1</td>
<td>80.1 (±0.2)</td>
<td>96.2 (±0.7)</td>
<td></td>
</tr>
</tbody>
</table>

Each data point is the mean of values from two trials.

LC (%) = Amount of lactose converted to its products

DH (%) = Degree of hydrolysis

According to the statistical analysis, the difference between LC and DH was significant at 5% level of significance (Appendix 3).
present results differed from their findings. The maximum glucose/galactose ratio in present study was 1.87. This maximum ratio was at the very beginning of the process, when 50.8% of lactose was still to be hydrolysed. The minimum glucose/galactose ratio obtained by Greenberg and Mahoney (1983) was less than 1 (0.98). It means, when 98.8% of lactose was hydrolysed, the concentration of galactose surpassed that of glucose. During the present study, even when 100% of lactose was hydrolysed, more than 1 (1.11) ratio of glucose/galactose was observed. However, the observation in trial 2 sample found to be in agreement with that reported by Greenberg and Mahoney (1983).

The difference between the results obtained during present investigation and those reported, could be attributed to different sources of lactose and lactase used. Greenberg and Mahoney (1983) used reconstituted skim milk as the substrate, and the source of enzyme was the bacterium *S. thermophilus*.

The results of the present study, with regard to the rates of increase in the concentrations of glucose and galactose are in agreement with those of Jeon and Mantha (1985). They also observed two different trends with regard to the formation of glucose and galactose. During initial stages of hydrolysis, the rate of production of glucose was higher than that of galactose. On the other hand, at later stages, the rate of production of galactose dominated that of glucose. The ‘turning point’, with regard to the rates of production of glucose and galactose during the present study was the hydrolysis at 30 min. This was due to the participation of OS in the production of galactose (Jeon and Mantha, 1985). The remaining lactose concentration at that time was 15.2 %. Jeon and Mantha (1985) stated that at or after the ‘turning’ or ‘crossover point’, the concentration of remaining lactose was too low to increase the concentrations of galactose over those of glucose. According to them, something else other than the remaining lactose is responsible for the increase in galactose concentration. They pointed out to the changes in concentrations of two major OS observed by them. As observed in the present study, Jeon and Mantha (1985) observed the decrease in the concentrations of both DIS and TIS towards the later stage of hydrolysis, after attaining their maxima. The maximum concentration of TIS obtained during present study was at 15 min and that of DIS was at 30 min. Both of these OS kept on decreasing thereafter, suggesting that part of them formed during the
early stage of hydrolysis, was hydrolysed by the same enzyme (Jeon and Mantha, 1985). The explanation by these authors suggest that the two OS detected by them (also in the present study) were predominantly containing galactose residues. In fact, the TIS was β-D-galactopyranosyl-(1,6)-β-D-galactopyranosyl-(1, 6)-D-galactose and the DIS was 6-O-β-D-galactopyranosyl-D-glucose. Thus, all of the monosaccharide residues of TIS were galactose, whereas DIS contained one galactose and one glucose residues. This indicates that at longer reaction times, when OS tend to disappear, being hydrolysed to monosaccharides (Lopez-Leiva and Guzman, 1995), significantly higher galactose will form than glucose. Greenberg and Mahoney (1983) quantified the monosaccharide components of OS formed. Sixty percent of the total OS detected during their study was found to be allolactose (containing both the glucose and the galactose) and about 30% was found to be as digalactose. Thus, about three-quarter of the sugars in the OS, they presumed to be galactose.

The amount of OS obtained during present study (3.8 to 19.3%) fits well within the large range (1% and 44.6%) reported by Zarate and Lopez-Leiva (1990). According to these authors, the production of OS during lactose hydrolysis, is directly proportional to temperature, pH and salt concentration of the substrate. These factors could be, in part the reason for the production of OS as high as 19.3% of total carbohydrates during the present investigation. This is because, in the present study, the hydrolysis of the nano-concentrate, with pH 6.6 (Table 11) and ash content 0.73% (Table 5), was carried out at 40°C.

The production of OS is important from both the nutritional as well as the technological point of view. However, whether the OS are nutritionally beneficial or harmful, is a matter of controversy (Refer to section 4.2.4). For the present study, the consideration of the nutritional aspect of OS was important in view of the HLNC treated ice creams being subjected to sensory evaluation by human panellists. From the technological point of view, the concentration of OS present in the HLNC, used in ice cream during the present study, could have adversely affected the sweetness of ice cream, because OS are not the hydrolytic products of lactose. The higher the production of OS, the lower becomes the production of glucose and/or galactose. It means less sweetness of HLNC. Smith et al. (1984) have reported, that the apparent viscosity of ice cream mix is influenced by OS. Another technological importance of OS, as far as its presence in ice cream is
concerned, lies in the freezing point of ice cream. Jeon and Saunders (1986) have reported, that since OS do not contribute to the depression of freezing points, their presence would cause less freezing point depression than a simple breakdown of lactose to glucose and galactose.

4.2.3 The calculation for the degree of hydrolysis

The method suggested by Novo Nordisk Bio-industrial Pty. Ltd. (1995) (Section 2.2.10 and Section 3.4.10) was used during the present study for the calculation of the degree of lactose hydrolysis. Table 10 shows the influence of OS on the calculation of DH. Due to the non-inclusion of oligosaccharides as the hydrolytic products of lactose, the degree of hydrolysis (DH) calculated, was significantly less than the amount (%) of lactose converted to its products, which included the oligosaccharides (Appendix 3). The DH calculated was plotted as a function of time of hydrolysis (Fig 18). As expected, the DH was very rapid at the initial stage, so much so that 42% was achieved within 5 min. Thereafter, there was definite increases in the DH, but at continuously slower rates. The DH after 1 h was 80.1 % and between 1 h and 18 h, it increased to 96.2 %.

The other methods mentioned in section 2.2.10 were not considered for the calculation of the degree of hydrolysis, because of their being faulty owing to the reasons mentioned in section 2.2.10.

4.2.4 Bulk production of hydrolysed lactose nano-concentrate for use in ice cream

On the basis of the results obtained for lactose hydrolysis, one of the time intervals was selected for the bulk production of hydrolysed lactose nano-concentrate (HLNC) to be substituted for sucrose in ice cream formulations. Mainly two criteria were considered to select the best available option. These criteria were the DH and the amount of OS formed. Some other factors were also taken into consideration. These factors were the nutritional aspect of OS, time involved in lactose hydrolysis, microbial contamination of the product, and the equisweetness effect.
Table 11

Effect of lactose hydrolysis on the pH and colour of the nano-concentrate of milk UF permeate.

<table>
<thead>
<tr>
<th></th>
<th>pH (at 25°C)</th>
<th>Colour</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type</td>
<td>Intensity (%)</td>
</tr>
<tr>
<td>Nano-concentrate,</td>
<td>6.6 a (S.D. 0.0)</td>
<td>Yellow</td>
<td>49.0 a (S.D. 0.0)</td>
</tr>
<tr>
<td>without hydrolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrolysed lactose</td>
<td>6.5 b (S.D. 0.014)</td>
<td>Yellow</td>
<td>41.0 b (S.D. 0.0)</td>
</tr>
<tr>
<td>nano-concentrate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each data point for pH is the mean of values from two trials.

Each data point for colour intensity is the mean of four values, two from each of the two trials.

Means in a column with different letters are significantly different at 5% level of significance (Appendix 3).
Fig 18

Degree of lactose hydrolysis of the nano-concentrate of milk UF permeate as a function of the time of hydrolysis.

pH of the nano-concentrate --- 6.6
Lactose content of the nano-concentrate --- 13.0% (wet basis)
Temperature of hydrolysis --- 40°C
Source of enzyme lactase used --- Yeast *K. fragilis*
Enzymic dosage used --- 12 mL/L of nano-concentrate.

(Equivalent to 1 mL of enzyme per 10.83 g of lactose)

Analysis was conducted in duplicate.
Nutritional aspect of OS was taken into consideration, because the ice cream samples containing HLNC were subjected to sensory evaluation by the panellists.

Literature reports contradict on the nutritional aspect of OS. According to the review published by Zarate and Lopez-Leiva (1990), OS can be either nutritionally harmful or beneficial. One group of researchers with in vitro experiments evaluated the digestibility of OS by the human intestinal enzymes. They found that the activity of human small intestine β-galactosidase on OS was less than 10% of the activity on lactose. This led them to the conclusion that OS would pass mostly undigested through small intestine into the large bowel, where bacterial degradation took place. This explains the gastrointestinal discomfort after OS ingestion.

On the other hand, another group of researchers observed the effect of galactosyl-OS on the growth of bifidobacteria. They found that all types of OS used, were utilised by the bifidus bacteria. One other group of researchers complemented this report. Thus, these latter two groups of researchers believed that the OS were an excellent source of carbohydrates for the bifidobacteria. These bacteria are known to improve a number of body functions (Lopez-Leiva and Guzman, 1995). Wijsman et al. (1989) reported several physiological effects of bifidobacteria.

The reported ambiguity of the nutritional effect of OS led to lay greater emphasis on the DH instead of OS in the present study. With regard to OS, however, a compromise was made and the hydrolytic conditions either producing a very high or very low concentration of OS were neglected for selection to produce HLNC.

The DH obtained during the initial 15 min were considered to be too low to provide requisite sweetness. Hence either 42.0% DH (obtained after 5 min of hydrolysis), 53.0% DH (obtained after 10 min of hydrolysis) or 60.2% DH (obtained after 15 min of hydrolysis) was not considered for selection. Thus only the latter three contenders, the hydrolysis for 30 min, 60 min and 18 h were left for the selection to produce HLNC. Among these three, the minimum DH (67.1%) was obtained after 30 min of hydrolysis. This DH was still too low to provide adequate sweetness. At this time, the OS production was the maximum (19.3%). Due to these reasons, this option was neglected for
the selection. Remaining two options were the strongest contenders. The overnight hydrolysis (18 h) had the maximum DH (96.2%) and minimum OS (3.8% of total carbohydrates).

With respect to 80.1% hydrolysis after 1 h, the overnight sample took considerable time (18 h) for 96.2% hydrolysis. However, it could be acceptable, had time not been an important issue. If time was manageable, another factor might become a stumbling block. This was the association of microbial contamination with the long time of hydrolysis at a temperature such as 40°C. This is evident from the observation of Chiu and Kosikowski (1985). After only 5 h of hydrolysis at 40°C with yeast (Saccharomyces lactis) enzyme, the standard plate count of the hydrolysed permeate of sweet whey increased from a range of 5.0 x 10^-2 - 6.1 x 10^2/mL to a range of 1.8 x 10^3 - 8.8 x 10^3/mL. If the microbial contamination was also manageable with the use of bacteriostatic agent, such as methyl-p-oxybenzoic acid (Burvall et al, 1979), one other factor would still be worth considering. This factor was the equi-sweetness effect. Shah and Nickerson (1978) reported that sugar mixtures equivalent to hydrolysed lactose syrups had good sweetening properties due to the synergism among the sugars present. On this basis, they suggested that 100% hydrolysis was unnecessary, because even a 70% hydrolysis could produce the same sweetening effect.

In view of above discussion, hydrolysis for 60 min seemed to be the best option and was selected for the bulk hydrolysis for the production of hydrolysed lactose nano-concentrate (HLNC). Concentration of OS formed during this 60 min of hydrolysis was 12% of the total carbohydrates. This 12% concentration of OS was neither too high (the maximum being 19.3% of total carbohydrates) nor too low (the minimum being 3.8% of total carbohydrates).

After 60 min of hydrolysis, the pH of nano-concentrate significantly reduced from 6.6 to 6.5 and the intensity of yellow colour significantly reduced from 49% to 41% (Table 11; Appendix 3).
Table 12

Effect of hydrolysed lactose nano-concentrate (HLNC) on some physico-chemical properties of ice cream mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH** (at 25°C)</th>
<th>Viscos.** (Cp)</th>
<th>Overrun** (%)</th>
<th>Freezing time* (sec)</th>
<th>Freezing point** (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.70a</td>
<td>25.0a</td>
<td>62.7a</td>
<td>180b</td>
<td>-1.62a</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>6.70a</td>
<td>22.8b</td>
<td>53.0b</td>
<td>215a</td>
<td>-1.76b</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>6.64a</td>
<td>21.9b</td>
<td>63.7a</td>
<td>225a</td>
<td>-1.98c</td>
</tr>
</tbody>
</table>

* Mean of values from two trials.
** Mean of four values, two from each of the two trials.

pH was determined by the method described in section 3.4.1.
Viscosity was determined by the method described in section 3.4.13.
Overrun was determined by the method described in section 3.4.14.
Freezing time was determined by the method described in section 3.4.15.
Freezing point was determined by the method described in section 3.4.8.
Means in a column with the same letter, are not significantly different at 5% level of significance (Appendix 5).
4.3 Use of hydrolysed lactose nano-concentrate in ice cream

4.3.1 Effect of hydrolysed lactose nano-concentrate on the properties of ice cream mix

4.3.1.1 Effect of hydrolysed lactose nano-concentrate on the pH of ice cream mix.

Table 12 shows the effect of HLNC on the pH of ice cream mix. Replacing 25% of sucrose with HLNC did not have any effect on the pH. Both the control as well as the 25% HLNC substituted ice cream mix had pH 6.70. The pH at 50% level of sucrose substitution in ice cream decreased slightly to 6.64. However, it was not significantly different from either the control mix or the one in which 25% of sucrose was substituted with HLNC.

Patel and Mathur (1982) observed an insignificant effect on the pH of ice cream, when 5.5% (with respect to the TS of mix) of hydrolysed whey solids were incorporated to replace 16.6% of sucrose and 28.3% of NFDM contents. No significant effect was observed, eventhough 11% (with respect to the TS of mix) whey solids were incorporated to replace 33.3% of sucrose and 56.6% of NFDM contents. The pH of control mix (containing 15% sucrose and 10.6% NFDM), observed by them, was 6.68 and the pH values for 5.5% and 11% whey incorporations were 6.65 and 6.56 respectively. On the other hand, Guy (1980) observed a substantial decrease in pH, when hydrolysed whey was used to partially substitute for both the sucrose and MSNF contents. The pH of control ice cream (containing 15% sucrose and 11% MSNF) was 6.59. This pH decreased to 6.31 and 6.26, when 11% whey solids with 67% and 79% lactose hydolysis, were incorporated in the mix.

The difference between the results obtained during present study and those obtained by Guy (1980) could be attributed to the pH/acidity of hydrolysed products, used during these two studies. The pH of nano-concentrate used during present study was 6.60 which slightly changed to 6.5 during the 60 min hydrolysis. On the other hand, Guy (1980) used whey obtained from the Cheddar cheese made of lactose hydrolysed milk. During cheesemaking, starter culture is added to milk to decrease its pH through the production of lactic acid, before the
addition of rennet. Bacterial activity is faster, when hydrolysates of lactose (glucose and galactose) are provided in the growth medium, instead of lactose itself. The acidity of cheesemilk used by Guy (1980) may have thus increased. As a result, the sweet whey used by him in ice cream production may have contained high acidity. The high acidity of whey could have been the reason for the decrease in pH of ice cream made by him.

4.3.1.2 Effect of hydrolysed lactose nano-concentrate on the viscosity of ice cream mix

Substitution of sucrose with HLNC, either at 25% or at 50% level significantly decreased the viscosity of mix (Table 12). Furthermore, there was a definite downward trend in the viscosity of mix as a result of increase in the level of sugar substitution. However, the difference between 25 and 50% levels of sugar substitution was not significant. The viscosities observed, were 25.0, 22.8 and 21.9 Cp for the control, 25% sucrose substituted, and 50% sucrose substituted mixes respectively.

Reduction of sucrose content from 14 to 12% by hydrolysing the mix (75% lactose hydrolysis) resulted in a slight reduction in the viscosity (from 672 Cp to 651 Cp), as observed by El-Neshawy et al. (1988). Guy (1980) also observed a similar trend. An increase in the level of lactose hydrolysed whey solids (79% hydrolysis) to partially substitute for both the MSNF and sucrose, resulted in the reduction of mix viscosity (Guy, 1980). When the sucrose content was reduced from 15 to 10% in his study, the viscosity decreased from 183 Cp to 141 Cp at 4.4°C. Patel and Mathur (1982) reported a decrease in viscosity from 75.4 to 66.5 sec/100 revolutions, when 5.5% hydrolysed lactose whey was incorporated in ice cream mix to partially replace sucrose and NFDM. No significant change, however, was observed by them, when the substitution level was increased to 11%.

Smith et al. (1984) on the other hand reported, that the partial replacement of sucrose in an ice cream mix with 36 DE corn syrup resulted in a higher viscosity. They explained that the presence of long chained OS and the greater texturing capability of glucose than sucrose were the factors responsible for this phenomenon. This explanation seems to be in stark contrast with the findings of the present study and those reported by others. According to Smith et al. (1984), larger
molecules resist alignment in the shearing gradient, thus providing higher viscosity at any given shear rate relative to the mix containing only sucrose. HLNC produced during the present study, however, did not contain any long chained OS. The largest OS it contained, was probably a trisaccharide (TIS), which was only 5% of the total carbohydrates. Hence any special effect of significance, produced by OS did not seem to be possible during the present investigation. Therefore any significant effect on the higher viscosity of ice cream mix, as a result of lactose hydrolysis could be expected through the greater texturising capability of glucose than sucrose. However, the present study indicated that the mix viscosity was favourably influenced more by the presence of larger size of sucrose than the texturing capability of glucose. Due to its larger size, sucrose can resist alignment in the shearing gradient at any given rate relative to the mix containing greater amount of glucose and galactose than sucrose.

According to Marshall and Arbuckle (1996), high viscosity was believed essential at one time. But for the modern equipment, a lower viscosity was desirable, because low viscosity could increase the rate of whipping. According to these authors, low viscosity also results in low melting resistance and the decrease in smoothness of texture.

4.3.1.3 Effect of hydrolysed lactose nano-concentrate on the overrun of ice cream

At 25% level of substitution, the overrun of mix (53%) was significantly less than the control ice cream (62.7%). There was no significant difference, however, between the control mix and the one, in which 50% of sucrose was replaced with HLNC (63.7%) (Table 12). Thus the overrun varied randomly and was independent of the HLNC substitution. Similar observation was reported by Arndt and Wehling (1989). Overrun, however, can be mechanically controlled to maintain its constant level in order to nullify the effect of experimental treatment on it (Patel and Mathur, 1982).

Overrun is very important in ice cream both from commercial as well as from technological point of view. Martinez and Speckman (1988) have reported that overrun affects the quality of ice cream. According to these authors, the overrun above or below the one required for optimum quality, can result in a heavy or thin body. According to them, overrun normally falls within the ranges of 70 and 80%. El-Neshawy
et al. (1988) observed the effect of lactose hydrolysis on overrun. According to their observation, overrun slightly increased as a result of using lactose hydrolysed reconstituted skim milk. These authors further reported that an increased mix viscosity resulting from an increase in lactose hydrolysis, was responsible for the higher overrun.

4.3.1.4 Effect of hydrolysed lactose nano-concentrate on the freezing property of ice cream mix

Substitution of 25 or 50% sucrose with HLNC significantly increased the mix freezing time. The increase was, however, not significant, when the substitution level was raised from 25 to 50%. Table 12 shows the time taken by each mix for freezing. It was 180 sec for the control, 215 sec for the 25% sucrose substitution and 225 sec for the 50% sucrose substitution.

Corresponding to the trend of increase in freezing time, was the trend of depression in freezing point (f.p.). The f.p. for each treatment was significantly different from each other. The f.p. of the control mix was -1.62°C, which decreased to -1.76 and -1.98°C at 25 and 50% levels of substitution respectively (Table 12).

Arndt and Wehling (1989) reported, that compared to 7.6 min taken by control mix, the mix in which 25% sucrose was replaced with hydrolysed lactose syrup (HLS) took 7.8 min for freezing. The time taken for freezing by the mix, in which 50% sucrose had been substituted with HLS, was 8.8 min.

When hydrolysed whey was used to reduce MSNF content from 11% to 5% and sucrose content from 15 to 10%, Guy (1980) observed the time taken to freeze the mix increased from 8 to 9.5 min. They observed an associated decrease in f.p. from -4.5 to -6.0°C.

Martinez and Speckman (1988) observed the depression in mix f.p. from -1.687 to -1.777°C, as a result of lactase treatment of mix.

Depression in f.p. or increase in freezing time as a result of lactose hydrolysis, or the incorporation of hydrolysed lactose product in mix formulation is an expected phenomenon. This is because, the hydrolysis of lactose produces the sugars of smaller molecular weight. Production
of these sugars results in the decrease of average molecular weight, which causes the depression in f.p. (Arndt and Wehling, 1989). Naturally, a mix aged at 3-4°C will take longer to reach the depressed f.p. The OS produced during lactose hydrolysis, however, do not influence the freezing point of an ice cream mix (Jeon and Saunders, 1986).

Apart from lactose hydrolysis, the lower freezing points of HLNC treated mixes than the control one, can also be attributed to the higher overall mineral contents of HLNC treated mixes than the control one (Table 17).

4.3.1.5 Effect of hydrolysed lactose nano-concentrate on the colour of ice cream mix

The effect of HLNC treatment on the colour of ice cream is given in Table 13. At 25% level of sucrose substitution with HLNC, there was no change in colour of ice cream. Both the control as well as the 25% HLNC ice cream had the same greenish yellow colour, with very low intensity of 14.0%. The colour intensity of control ice cream was not significantly different from that of 25% HLNC ice cream at 5% level of significance (Table 13; Appendix 3). At 50% level of sucrose substitution, however, the colour of ice cream changed to yellow. The intensity of the colour of 50% HLNC ice cream was also very low (17%), but significantly higher than that of both the control and the 25% HLNC ice cream. The colour difference meter did not show any brown colour in any ice cream mix. The result is in agreement with those reported in the literature (Arndt and Wehling, 1989; El-Neshawy et al., 1988).

The greenish tint of a milk product is due to the presence of riboflavin (Johnson, 1978), whereas the yellow colour is imparted by carotenoids (Hartman and Dryden, 1978).

4.3.2 Effect of hydrolysed lactose nano-concentrate on the glass transition and melting temperatures of ice cream

Fig 19, 20 and 21 show two peaks (baseline shifts) in the DSC thermograms. The two peaks relate to the occurrence of two transitions during the heating of maximally freeze concentrated material. One of these two transitions is the change from subcooled liquid to a solid
Table 13

Effect of hydrolysed lactose nano-concentrate on the colour of ice cream mix

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colour Type</th>
<th>Intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Greenish yellow</td>
<td>14.0 a (S.D. 0.0)</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>Greenish yellow</td>
<td>14.0 a (S.D. 0.31)</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>Yellow</td>
<td>17.0 b (S.D. 0.0)</td>
</tr>
</tbody>
</table>

Each data point is the mean of four values, two from each of the two trials.

Means in a column with the same letters are not significantly different at 5% level of significance (Appendix 3).

Colour parameters were determined by the method described in section 3.4.5
Fig 19

The Differential Scanning Calorimetric thermogram of the Control ice cream (containing 0% hydrolysed lactose nano-concentrate).
Fig 20

The Differential Scanning Calorimetric thermogram of ice cream, in which 25% of sucrose was substituted with hydrolysed lactose nano-concentrate.
Fig 21

The Differential Scanning Calorimetric thermogram of icecream, in which 50% of sucrose was substituted with hydrolysed lactose nano-concentrate.
glassy state of ice cream. Another is the onset of ice melting. The former is the glass transition temperature (T_g) and the latter is the melting temperature (T_m). Roos et al. (1996) reported that the T_m is higher than the T_g. On this basis, the T_m and T_g peaks can be identified in the thermograms.

The mean T_g and T_m values for the control ice cream, observed during present investigation were - 64.9°C and - 0.25°C (Table 14). Substitution of 25% sucrose with the HLNC did not significantly reduce the T_g. It was - 68.1°C for 25% sucrose substitution. However, the T_m was significantly reduced (- 0.71°C) at this level of substitution. T_g at 50% level of sucrose substitution (- 70.7°C) was significantly less than that of the control, but not significantly less than the 25% level of sucrose substitution. The T_m at 50% level of sucrose substitution (-2.43°C) was, however, significantly less than those of both the control and 25% sucrose substituted ice creams (Table 14). Jouppila and Roos (1994) reported reduction in T_g due to lactose hydrolysis.

The results mentioned above can be interpreted in practical terms. The indication is that the HLNC treated ice creams need to be stored at slightly lower temperature of glass transition than the untreated one in order to almost completely stop the deteriorative changes. However, as Goff (1992) suggests, storage of foods below their T_g is neither practical nor economical. Then the alternative is to store the ice creams at a temperature between the temperatures of their glass transition and melting. Although, this is a ‘danger zone’, storage of a food product at a temperature lower than T_m can slow down the deteriorative changes. The greater the difference between the temperature of melting and the one lower than this, the slower will be the changes such as ice crystallisation. This difference in temperature for the control ice cream was 19.7°C, which was nearly similar to that for 25% sucrose substitution (19.3°C), when the samples were stored at -20°C. Hence not much difference in the changes between the control and 25% sucrose substituted ice creams can be expected during their frozen storage. Comparatively greater changes, however, can be expected with regard to 50% sucrose substitution. The difference between the melting and storage temperatures of 50% sucrose substituted ice creams was 17.5°C. Thus from this point of view, the ice cream, in which 50% sucrose was substituted with HLNC, can be expected to be of little inferior in quality than either the control or the 25% sucrose substituted ice cream.
Table 14

Effect of hydrolysed lactose nano-concentrate on the glass transition temperature (T$_g$) and the melting temperature (T$_m$) of ice cream.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T$_g$ (°C)</th>
<th>T$_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-64.9a</td>
<td>-0.25a</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>-68.1ab</td>
<td>-0.71b</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>-70.7b</td>
<td>-2.43c</td>
</tr>
</tbody>
</table>

All the data points are the means of four values, two from each of the two trials.

Means in a column with the same letter are not significantly different at 5% level of significance (Appendix 5).

The method for the Differential Scanning Calorimetry is given in section 3.4.12.
From another point of view, however, the lower melting temperature can be a desirable characteristic of ice cream, because such an ice cream will take less time to melt and spread on the tongue, resulting into better sensory perception. The reason is that, the difference between the melting temperature and the storage temperature (-20.0°C) will be less with regard to the ice cream with lower melting temperature than that with higher melting temperature. Thus the ice cream with a narrower difference between its melting and storage temperatures, will meet its melting temperature sooner than the one with a wider such temperature-difference. For example, the difference between the melting and storage temperatures of 50% sucrose substituted ice cream during the present study was 17.57°C {(-2.43°C)-(-20.0°C)}, whereas such a temperature-difference with regard to the control ice cream was 19.75°C {(-0.25°C)-(-20.0°C)}. Thus, less time would be required by the 50% sucrose substituted ice cream to overcome such a temperature-difference for melting, than by the control ice cream.

Martinez and Speckman (1988) found the melting characteristic of lactose hydrolysed frozen dairy product comparable to one without hydrolysis. Furthermore, they have reported that a desirable melting quality in a frozen product is that the melted product should have the appearance and consistency of the original mix. According to this report, a high quality ice cream should show little resistance towards melting on its exposure to the room temperature.

Accelerated melting, being a desirable characteristic with the associated softening effect, has been reported also by Lindamood et al.(1989). They found the melting rates of lactose hydrolysed ice creams significantly higher, when more than 50% of the mix was treated with enzyme lactase.

In the opinion of Patel and Mathur (1982), superior melting resistance is a desirable characteristic of an ice cream. According to them, an ice cream with superior melting resistance may be preferred by the customers. Such an ice cream will be preferable from the view point of convenience in storage and serving. In this regard, the ice cream containing lactose hydrolysed product was found to be inferior in quality by them, because that ice cream had lower melting resistance.
4.3.3 Effect of hydrolysed lactose nano-concentrate on the textural profile of ice cream

Six textural attributes for each treatment were analysed by the texture analyser. These attributes were springiness, cohesiveness, chewiness, gumminess, adhesiveness and hardness. Hardness is defined as the peak force during the first compression cycle. Cohesiveness is defined as the ratio of the positive area during the second compression cycle to the positive force area during the first compression cycle. Adhesiveness is defined as the negative force area for the first bite, representing the work required to pull the plunger away from the food sample. Springiness (elasticity) is defined as the height to which the food recovers during the time that elapses between the end of the first bite and the start of the second bite. Gumminess is defined as the product of hardness and cohesiveness. Chewiness is defined as the product of gumminess and springiness (Rao and Skinner, 1986).

Table 15 shows the effect of HLNC treatments on the textural profile of ice cream. According to the statistical analysis (one way ANOVA), the effect of either of the HLNC treatments on any textural attribute, was not significant, except that on gumminess. According to Duncan's multiple range test (DMRT), the gumminess of 50% sucrose substituted ice cream was significantly lower than that of the control ice cream. The gumminess of 25% sucrose substituted ice cream, however, was not significantly different from that of the control ice cream. Furthermore, there was no significant difference between the gumminess of 25% and that of the 50% sucrose substituted ice creams.

Gregory (1980) found lactose hydrolysed ice creams softer than the unhydrolysed ice creams during sensory evaluation. Lindamood et al. (1989) reported that the lactose hydrolysed ice creams gave lower firmness (hardness) values than the corresponding unhydrolysed ice cream. Contrary to these reports, Huse et al. (1984) observed an increase in firmness of ice cream, as a result of addition of lactose hydrolysed material to the ice cream mix. The pattern of gumminess, however, was inconsistent during sensory evaluation in their study.

In spite of substantial differences in the mean values of the most of the textural attributes, statistical analysis did not find those differences significant. The effect of error being more than the effect of treatment in this regard, could be the reason. The occurrence of experimental
Table 15

Effect of hydrolysed lactose nano-concentrate on the textural profile of ice cream.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Chewiness</th>
<th>Gumminess</th>
<th>Adhesiveness</th>
<th>Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.912a</td>
<td>0.184a</td>
<td>226.8a</td>
<td>270.0a</td>
<td>-825.8a</td>
<td>1565.9a</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>1.14a</td>
<td>0.216a</td>
<td>213.4a</td>
<td>180.2ab</td>
<td>-564.0a</td>
<td>877.4a</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>0.916a</td>
<td>0.210a</td>
<td>103.6a</td>
<td>118.6b</td>
<td>-352.7a</td>
<td>605.0a</td>
</tr>
</tbody>
</table>

All the data points are the means of four values, two from each of the two trials.

Means in a column with the same letter are not significantly different at 5% level of significance (Appendix 5).

Texture analyser was used to determine the texture of ice cream (section 3.4.11).
Fig 22

The textural profile of the Control ice cream (containing 0% hydrolysed lactose nano-concentrate).

The four profiles represent the four observations of the same ice cream at different locations in the sample.

Texture analyser was used to determine the textural profile of the ice cream (section 3.4.11).
Sample Rate: 200.00 pps
Threshold: 20.0 g
Area: 1.00 mm²

Test Time: 53.60 s
Distance Threshold: 0.50 mm
Contact Force: 5.0 g

Speed: 1.0 mm/s
Pre Test Speed: 2.0 mm/s
Post Test Speed: 5.0 mm/s
Logger Type: Auto @ 5 g
Distance: 15.0 mm
Ex: 5.00 s

Stable Micro Systems
Texture Analyser
Thu Dec 19 13:31:49 1996
The textural profile of ice cream, in which 25% of sucrose was substituted with hydrolysed lactose nano-concentrate.

The four profiles represent the four observations of the same ice cream at different locations in the sample.

Texture analyser was used to determine the textural profile of the ice cream (section 3.4.11).
Sample Rate: 200.00 pps  Test Time: 53.60 s
Tare Threshold: 20.0 g  Dist. Threshold: 0.50 mm
Sample Area: 1.00 mm²  Contact Force: 5.0 g

BED: 1.0 mm/s  PRE TEST SPEED: 2.0 mm/s  POST TEST SPEED: 5.0 mm/s
HUGER TYPE: Auto @ 5 g  DISTANCE: 15.0 mm
ME: 5.00 s

Table Micro Systems  Texture Analyser  Thu Dec 19 13:47:34 1996
The textural profile of ice cream, in which 50% of sucrose was substituted with hydrolysed lactose nano-concentrate.

The four profiles represent the four observations of the same ice cream at different locations in the sample.

Texture analyser was used to determine the textural profile of the ice cream (section 3.4.11).
Sample Rate : 200.00 pps  Test Time : 53.92 s

Gripping Threshold : 20.0 g  Dist. Threshold : 0.50 mm

Sample Area : 1.00 mm²  Contact Force : 5.0 g

ED: 1.0 mm/s  PRE TEST SPEED: 2.0 mm/s  POST TEST SPEED: 5.0 mm/s
TRIGGER TYPE: Auto @ 5 g  DISTANCE: 15.0 mm
PRE: 5.00 s

Stable Micro Systems  Texture Analyser  Thu Dec 19 14:02:13 1996
error is evident from the average textural profile depicted in Fig 22, 23 and 24. Fig 22 is the average textural profile of the control ice cream; Fig 23 is that for the 25% HLNC treated ice cream; and Fig 24 is that for the 50% HLNC treated ice cream. The difference was substantial when the measurements were taken at different locations within a particular sample of ice cream. The situation was more or less the same with all the treatments. The experimental error points out to the heterogeneous structure of ice cream. Ice cream is a complex mixture of fat globules, ice crystals and a concentrated serum phase containing sugars and stabilisers. The serum phase acts as the continuous phase, and the other components are distributed in this phase (White and Cakebread, 1966). The distribution of these components are not always uniform and homogeneous. Due to this, the texture lacks smoothness. Texture refers to the finer structure of the product and depends on the size, shape and arrangement of the small particles (Sherman, 1976). These small particles of particular importance include fat globules, ice and lactose. All of them are capable of crystallisation at lower temperatures. Owing to temperature fluctuation during the frozen storage, recrystallisation of ice creams also takes place. This is because, the normal storage temperature of ice cream (about -20°C) is well above its T_g. All of these phenomena add to the heterogeneity of ice cream texture. As a result, the probe of a texturometer experiences unequal amount of force at different locations within the same sample and gives rise to an experimental error.

4.3.4 Effect of hydrolysed lactose nano-concentrate on the sensory properties of ice cream

4.3.4.1 Effect of hydrolysed lactose nano-concentrate on the sweetness of ice cream

Table 16 shows significant decrease in sweetness of ice cream on account of partial replacement of sucrose with HLNC, either by 25%, or by 50%. However, the sweetness of 50% HLNC treated ice cream was insignificantly less than that of the 25% HLNC treated ice cream. The sweetness of ice cream without any HLNC was preferred by 62.5 % of the panellists. The ice cream with 25% HLNC was preferred for its sweetness by 20.8% of the panellists, whereas 16.7% of them found the ice cream with 50% HLNC the sweetest.
4.3.4.2 Effect of hydrolysed lactose nano-concentrate on the saltiness of ice cream

DMRT showed the HLNC treated ice creams significantly saltier than the control ice cream (Table 16). Ice cream with 25% HLNC was 1.8 times and that with 50% HLNC was 2.7 times saltier than the one without HLNC treatment. Ice cream with 50% HLNC treatment, was the saltiest for 75% panellists; for 12.5% of the panellists, 25% HLNC treated ice cream was the saltiest; for the remaining 12.5% panellists, the control ice cream was the saltiest one.

The perception of saltiness by the panellists seems to be in agreement with the ash content of ice cream samples (Table 17). The ash content of 50% HLNC ice cream was significantly higher than that of both the control as well as the 25% HLNC ice cream. The ash content of 25% HLNC ice cream was significantly higher than the control ice cream (Appendix 3). The increased ash content of HLNC treated ice creams may have been due to incomplete or insufficient demineralisation of the nano-concentrate. Only 34.5% demineralisation was achieved during 3 fold nanofiltration of milk permeate and 65.5% of minerals was recovered in the nano-concentrate (Table 6). However, it was probably the more than expected recovery of monovalent ions in the nano-concentrate, which might have enhanced the sensory perception of saltiness of the HLNC treated ice creams (Cotton, 1980). The recovery of $K^+$ was 57.5% and that of $Na^+$ was 72.2% (Table 6).

From these results, it seems that NF alone is unable to reduce the saltiness of HLNC treated ice cream to a satisfactory level. Additional demineralisation by IE or ED seems to be unavoidable. Alternatively, some improvement can be expected through the inclusion of DF and/or higher than the 3 fold NF of milk UF permeate. Demineralisation by IE or ED alone is a costly process. Application of NF prior to IE or ED can make the demineralisation process a remarkably cost effective step in the production of hydrolysed lactose concentrated milk UF permeate.

4.3.4.3 Effect of hydrolysed lactose nano-concentrate on the cooked flavour of ice cream

According to DMRT analysis, the cooked flavour perception of the 25% HLNC treated ice cream was not significantly higher than that of the control ice cream. (Table 16). However, the ice cream containing 50% HLNC gave significantly higher (about 2 times) cooked flavour
Table 16

Effect of hydrolysed lactose nano-concentrate on some sensory properties of ice cream.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sweetness</th>
<th>Saltiness</th>
<th>Cooked flavour</th>
<th>Iciness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.6a</td>
<td>14.1c</td>
<td>20.9b</td>
<td>25.3a</td>
<td>67.2a</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>47.7b</td>
<td>25.5b</td>
<td>27.4b</td>
<td>25.2a</td>
<td>57.1a</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>42.4b</td>
<td>38.1a</td>
<td>40.7a</td>
<td>23.9a</td>
<td>44.6b</td>
</tr>
</tbody>
</table>

All the data points are the means of four values from each of the two trials.

Means in a column with the same letter are not significantly different at 5% level of significance (Appendix 5).

Sensory evaluation of the ice cream was made according to the method described in section 3.4.18.
Table 17

Ash content of the ice cream mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ash (% wet basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.04 a (S.D. 0.02)</td>
</tr>
<tr>
<td>25% HLNC</td>
<td>1.23 b (S.D. 0.01)</td>
</tr>
<tr>
<td>50% HLNC</td>
<td>1.39 c (S.D. 0.04)</td>
</tr>
</tbody>
</table>

Ash was determined by the AOAC (1990) method (section 3.4.3).

Each data point is the mean of values from two trials.

Means in a column with different letters are significantly different at 5% level of significance (Appendix 3).
scores than the control ice cream. The cooked flavour score of 50% HLNC treated ice cream was also significantly higher than that of the 25% HLNC treated ice cream. For 79.1% panellists, 50% HLNC treated ice cream was the most cooked in taste perception. For 12.5% of them, 25% HLNC treated ice cream was the most cooked in taste. For the remaining 4.3% of the panellists, the ice cream without any HLNC (control ice cream) was the most cooked in taste perception. The remaining 4.1% of the panellists found all of the ice creams including the control, similar in cooked flavour perception. Huse et al. (1984) reported an increase in sensory score for the cooked flavour as a result of lactose hydrolysis. They presumed the reaction between milk protein and the reducing sugars, formed during hydrolysis, responsible for it. In fact, the free amino groups of proteins, in association with the reducing sugars in presence of heat are responsible for the cooked flavour development through the Maillard Browning reactions. About 90% of these free amino groups are from lysine (Parry, 1972).

4.3.4.4 Effect of hydrolysed lactose nano-concentrate on the iciness of ice cream

Presence of ice was detected in each of the ice creams including the control one. For 33.3% panellists, the control ice cream was the most icy. For 37.5% of them, 25% HLNC treated ice cream was the most icy. For the remaining 29.1% of the panellists, 50% HLNC treated ice cream was the most icy. However, with overall consideration, HLNC treatment had no significant effect on the iciness of ice cream (Table 16).

To some extent, the perception of iciness can be expected in ice cream, if it is stored at a temperature substantially higher than its \( T_g \), despite containing stabilisers. The ice creams used for the sensory evaluation in the present study, had been stored for a week at -20°C. This temperature is very high compared to the \( T_g \) of any of the ice creams, including the control one. The storage temperature of -20°C was in between the \( T_g \) and the \( T_m \) of any of the ice creams. The temperature range between \( T_g \) and \( T_m \) is conducive to the formation of ice crystals. In the event of fluctuations in the storage temperature, recrystallisation can occur, enabling either the deposition of water on the surface of larger ice crystals, or the melting down of the smaller ice crystals (Goff, 1992). In the present study, some recrystallisation as a result of
temperature fluctuation, may have taken place, because the same freezing chamber was being used by others, too.

Huse et al. (1984) found a high degree of lactose hydrolysis (100% in skim milk and 72% in whey) resulting in reduced iciness with regard to both the heat shocked as well as the unshocked ice creams. Though, not significant, the same trend regarding the effect of lactose hydrolysis on iciness of ice cream, can be found also in the present study (Table 16). This was well supported by the fact, that on HLNC treatment, f.p. depressed and longer time was taken to freeze the mix (Table 12).

4.3.4.5 Effect of hydrolysed lactose nano-concentrate on the overall acceptability of ice cream

The ice cream containing no HLNC was preferred to the ice cream, in which either 25% or 50% of sucrose had been replaced with HLNC. The overall acceptability of 50% HLNC treated ice cream was significantly lower than that of the control ice cream. The overall acceptability of the 50% HLNC treated ice cream was significantly less than that of the 25% HLNC treated ice cream, too. However, the overall acceptability of the 25% HLNC treated ice cream was not significantly less than that of the control ice cream.

In view of no significant difference between the overall acceptability of the control ice cream and that with 25% HLNC, ice cream manufacturers can cut down the use of cane sugar by one fourth and this reduction in sucrose requirement can be replaced by HLNC. However, the replacement of 50% of sucrose is not possible, unless a major portion of salt, especially the monovalent ions is removed from the HLNC.
CHAPTER 5

OVERALL SUMMARY

The milk UF permeate was nanofiltered using an Ultraosmosis membrane unit. The desired 15% TS in the nano-concentrate was obtained after 3 fold concentration of milk UF permeate by nanofiltration. The flux (corrected to 25°C) decreased from 22.1 to 7.9 L/m²/h during a 55 min run. The applied transmembrane pressure ranged between 800 and 1500 KPa during this period. The recovery of TS in the nano-concentrate was 94.9%. The protein content, recovered in the nano-concentrate was 91.9%, corresponding to an increase from 0.3% to 0.83%. The recovery of lactose content in the nano-concentrate was 96.8%, corresponding to an increase from 4.46% to 13.0%. The ash content of milk UF permeate reduced by 34.5%, whereas concentrations of monovalent cations K⁺ and Na⁺ decreased by 42.4 and 27.8% respectively. The pH slightly decreased from 6.74 to 6.6. The titratable acidity was recovered by 78.9% (an increase from 0.16 to 0.38% LA), whereas 21.1% was lost. The colour of milk UF permeate changed from greenish yellow (20% intensity) to yellow (49% intensity). Thorough rinsing of membrane with water at the end of nanofiltration of milk UF permeate, failed to completely return the original flux of water. However, cleaning of membrane with Ultrasil 56 returned the original flux of water.

The lactose content of the nano-concentrate was hydrolysed by the enzyme lactase of yeast origin at 40°C. The degree of lactose hydrolysis obtained in the nano-concentrate after 5 min, 10 min, 15 min, 30 min, 60 min and 18 h of lactose hydrolysis were 42.0, 53.0, 60.2, 67.1, 80.1 and 96.2% respectively. The lactose conversion at these time intervals were 49.2, 62.7, 70.1, 84.8, 93.4 and 100%. The difference between the degree of hydrolysis and the %lactose conversion was equivalent to the amount of oligosaccharides (OS) formed. For the initial 30 min, the concentration of OS increased, the maximum concentration being 19.3% of total carbohydrates. After reaching its maximum, the OS concentration began to decrease. After 60 min, it was 12.0% and after 18 h, it was only 3.8% of total carbohydrates. Concentration of glucose was higher than galactose throughout the period of hydrolysis. However, the rate of increase in galactose concentration was higher than that in glucose concentration.
after 30 min of hydrolysis. Hydrolysis for 60 min was selected for the bulk production of hydrolysed lactose nano-concentrate (HLNC) to be substituted for 25 and 50% of sucrose contents in ice cream formulations.

The substitution of 25% of sucrose with HLNC did not affect the pH of ice cream mix, whereas substitution of 50% of sucrose with HLNC insignificantly decreased the pH of ice cream mix. Viscosity of ice cream mix was significantly reduced by HLNC treatments both at 25 and 50% levels of sucrose substitution. Overrun showed inconsistent trend on HLNC treatments. It was significantly decreased at 25% level of sucrose substitution, but insignificantly increased at 50% level of sucrose substitution. Freezing point of the ice cream mix was significantly reduced by HLNC treatments at both the levels of sucrose substitution. The reduction in freezing point was in association with significantly longer time taken by HLNC treated mixes to freeze. At 25% level of sucrose substitution with HLNC, the greenish yellow colour of ice cream remained unchanged, with the similar low intensity (14%). However, at 50% level of sucrose substitution, the greenish yellow colour (14% intensity) of ice cream changed to yellow (17% intensity). Substitution of 25% sucrose with HLNC did not significantly reduce the glass transition temperature of ice cream. Melting temperature of ice cream, however, was significantly reduced at this level of sucrose substitution. The glass transition temperature as well as the melting temperature was significantly reduced at 50% level of sucrose-substitution. Springiness and cohesiveness of ice cream were insignificantly increased by both the 25 and 50% levels of HLNC treatments. Chewiness, adhesiveness and hardness were insignificantly decreased by both the 25 and 50% levels of HLNC treatments. The gumminess of ice cream significantly decreased as a result of substituting 50% of sucrose with HLNC, but insignificantly decreased as a result of substituting 25% of sucrose with HLNC. The sweetness of ice cream was significantly reduced by both the levels of HLNC treatments. The 25% as well as the 50% sucrose substituted ice cream was significantly higher in saltiness than the control ice cream. Likewise, the cooked flavour perception of ice cream was significantly enhanced by HLNC treatments. HLNC treatments insignificantly reduced the iciness of ice cream. The overall acceptability of ice cream was significantly reduced on replacing 50% of sucrose with HLNC. However, there was insignificant reduction in overall acceptability of ice cream, when 25% of sucrose was replaced with HLNC.
CHAPTER 6

OVERALL CONCLUSIONS

During nanofiltration, the flux rate kept on decreasing, with an increase in TS concentration. In order to achieve the desired level of TS concentration within a reasonable period of time, a satisfactory flux rate has to be maintained. The gradual increase in transmembrane pressure is required to maintain such a satisfactory rate of flux.

Milk UF permeate is capable of fouling the NF membrane, despite having very little protein content. Concentration polarisation is not the only phenomenon of reducing the flux rate during the nanofiltration of milk UF permeate, fouling too, has some role in it.

Not all of the solids are retained in the nano-concentrate of milk UF permeate. Some of them are lost by either being retained by the membrane or are lost in the nano-permeate. These lost solids include some organic matters such as lactose and the nitrogenous fractions. The loss of these organic matters may necessitate the treatment of the nano-permeate as a waste.

The extent of removal of sodium ion from milk UF permeate by nanofiltration was low during present study, compared to that reported in the literature. However, the overall demineralisation of milk UF permeate by nanofiltration during the present study was comparable to those reported in the literature.

Nanofiltration is unable to decolorise the milk UF permeate. It can only change the greenish yellow colour of milk permeate to yellow. Hence to decolorise the milk UF permeate or its nano-concentrate, some other methods need to be applied. However, for up to 25% replacement of sucrose with HLNC in ice cream, decolorisation of nano-concentrate or HLNC is not necessary.

During nanofiltration, the pH of milk permeate is slightly reduced, and the titratable acidity is increased on concentration. However, the titratable acidity is not completely retained in the nano-concentrate; some deacidification also takes place.
Degree of lactose hydrolysis should not be considered as the percent of lactose converted, as the oligosaccharides formed during lactose hydrolysis is responsible for the difference between these two. Hence the oligosaccharides formed, should not be overlooked at the time of calculating the degree of lactose hydrolysis.

The incorporation of hydrolysed lactose nano-concentrate of milk UF permeate to replace as high as 50% of sucrose in an ice cream formulation, does not significantly affect the pH of ice cream.

Substitution of sucrose with hydrolysed lactose nano-concentrate significantly reduces the viscosity of ice cream mix. The reduced viscosity of ice cream mix may suit the functioning of a modern freezer.

It is uncertain, whether the incorporation of hydrolysed lactose nano-concentrate will affect the overrun of ice cream.

The use of hydrolysed lactose nano-concentrate of milk UF permeate prolongs the time taken for freezing the ice cream mix, as a result of depression in freezing point. The prolonged freezing time will result into slightly higher energy consumption in freezing the mix, than is normally required.

Hydrolysed lactose nano-concentrate slightly changes the colour of ice cream, if used to replace 50% of sucrose. However, this change in colour is unable to have a negative impact on the consumers’ appeal for the ice cream.

Both the glass transition and the melting temperatures of ice cream are significantly reduced as a result of replacing sucrose with the hydrolysed lactose nano-concentrate. The reduced glass transition temperature is an unfavourable outcome, as a further low temperature will be required for the storage of ice cream to save it completely from deteriorative changes. However, the fact is that, even a normal glass transition temperature is rarely used for the storage of ice cream, because it is neither practical nor economical. Therefore, an ice cream should be stored at a temperature as close as possible to its glass transition temperature, but as far away as possible from its melting temperature to minimise the deteriorative changes.
The lower melting temperature of an ice cream, containing hydrolysed lactose nano-concentrate, can be a desirable characteristic, because this ice cream will take less time to melt and spread on the tongue.

As a whole, the texture of ice cream is not significantly affected by the incorporation of hydrolysed lactose nano-concentrate into it. Sensorily, the ice cream, having one fourth of its sucrose content replaced with hydrolysed lactose nano-concentrate, is less sweet and more salty, but has almost the same iciness and cooked flavour as a normal ice cream. On the other hand, an ice cream, having half of its sucrose replaced with hydrolysed lactose nano-concentrate, is more cooked, apart from being almost the same icy, less sweet and more salty than the normal ice cream. Overall, replacing 25% of sucrose with hydrolysed lactose nano-concentrate can be sensorily acceptable, but not the replacement of 50% of sucrose. The replacement of 50% of sucrose can be possible if the hydrolysed lactose nano-concentrate is substantially demineralised, particularly, the substantial removal of monovalent ions. It is possible by electrodialysis. Electrodialysis by itself, may not be a cost effective technique, if high level of demineralisation is required. But if the already partially demineralised nano-concentrate is used as a feed for electrodialysis, the cost effectiveness of the demineralisation process will remarkably increase.
CHAPTER 7

RECOMMENDATIONS FOR FURTHER RESEARCH

On the basis of the methods applied for investigation and the results obtained during the present study, the following recommendations are made for further research.

1. Application of diafiltration during nanofiltration of milk UF permeate for higher extent of demineralisation and to find out how much sucrose could be successfully replaced with this hydrolysed lactose nano-concentrate.

2. Application of electrodialysis or ion-exchange to the nano-concentrated milk UF permeate and to find out how much sucrose could be successfully replaced with substantially demineralised hydrolysed lactose nano-concentrate.

3. To find out whether 100% degree of hydrolysis of lactose could be successful in replacing 50% of sucrose in an ice cream formulation.

4. To predict the shelf-life and the storage stability of hydrolysed lactose nano-concentrate, by determining the glass transition temperatures of the samples stored at various sub-zero temperatures for different periods of time, using a differential scanning calorimeter.

5. To predict the shelf-life and storage stability of ice cream made with hydrolysed lactose nano-concentrate, by determining the glass transition temperatures of the samples stored at various sub-zero temperatures for different periods of time, using a differential scanning calorimeter.

6. Production of spray dried powder of hydrolysed lactose nano-concentrate of milk UF permeate and to study its physico-chemical properties.

7. Decolorisation of hydrolysed lactose nano-concentrate (HLNC) using charcoal, incorporating this decolorised HLNC in ice cream formulations, and to study the effect of decolorised HLNC on the properties of ice cream.
8. Concentration of HLNC to higher total solids (60-70%) using vacuum evaporation and to study the shelf-life and storage stability of concentrated HLNC at the room, refrigeration and sub-zero temperatures.

9. Production of HLNC using immobilised lactase and to study the production efficiency of the process as well as the quality of the final product.

10. Carrying out the hydrolysis of lactose during nanofiltration of milk UF permeate and to study the flux pattern and the extent of permeation of glucose, galactose and oligosaccharides. This process can save time, separately required for lactose hydrolysis.

11. To study the physico-chemical and sensory characteristics of ice cream, in which sucrose could be partially or completely substituted with a number of combinations of HLNC and high fructose corn syrup (HFCS). Sucrose is sweeter than HLNC, but less sweet than HFCS. Hence the study should be conducted to find out to what extent of substitution, the sweetness provided by sucrose, would match the net sweetness imparted by the combination of HLNC and HFCS.
APPENDIX 1

Calculations for the formulations of ice cream mixes

Desired composition of mixes
Refer to section 3.3.4

Composition of available ingredients
Refer to section 3.3.2

The amounts of three unknown ingredients of mix (SMP, Cream and milk) were calculated using the simultaneous equations.

Let X be the %weight of SMP,
Let Y be the %weight of Cream, and
Let Z be the %weight of milk

Fat equation

The desired content of fat for all the mixes was 11%. Fat was available from three sources, SMP (0.2%), Cream (40%) and milk (4%). Accordingly the algebraic equation for fat was as follows.

\[
\begin{align*}
0.2 \times & \quad 40.0 \times & \quad 4.0 \times \\
---------- & \quad ---------- & \quad ---------- \\
100 & \quad 100 & \quad 100
\end{align*}
\]

\[
0.2X + 40.0Y + 4.0Z = 11.0
\]

or, 0.2 X + 40.0 Y + 4.0 Z = 1100 ........................................ (1)

Milk solid not fat (MSNF) equation

The desired content of MSNF in all of the mixes was 12.0% of the weight of the mix. MSNF was available from three sources, SMP (93.0%), Cream (2.8%) and milk (8.7%). The solid content of HLNC was not included as the source of MSNF. It was considered only as a sugar source. Accordingly, the equation for MSNF was as follows.
93.0 X + 2.8 Y + 8.7 Z = 1200  ........................................... (2)

1. Calculation for the Control mix (containing no HLNC)

*Weight equation for the Control mix*

In addition to SMP, cream and milk the total weight of the control mix included sucrose (15.0%) and vanilla (0.04%). Accordingly, the equation was as follows

\[ X + Y + Z + 15.0 + 0.5 + 0.04 = 100.0 \]

Or, \[ X + Y + Z = 84.46 \]  .................................................. (3)

The simultaneous equation of (1) and (2) was obtained by multiplying both the left hand side (LHS) and right hand side (RHS) of equation (1) by the first number of equation (2) to derive equation (4); multiplying both the LHS and RHS of equation (2) by the first number of equation (1) to derive equation (5); and substracting equation (5) from (4) to derive equation (6). Accordingly, the equation (6) was derived as follows.

\[ 18.6 X + 3720.0 Y + 372.0 Z = 102300.0 \]  ........................................... (4)

\[ 18.6 X + 0.56 Y + 1.74 Z = 240.0 \]  ................................................ (5)

\[ - \quad - \quad - \quad - \]

\[ 3719.44 Y + 370.26 Z = 102060.0 \]  .................................... (6)

The simultaneous equation from equations (1) and (3) was derived as follows to get equation (9)

\[ 0.2 X + 40.0 Y + 4.0 Z = 1100.0 \]  ................................................ (7)

\[ 0.2 X + 0.2 Y + 0.2 Z = 16.892 \]  ................................................ (8)

\[ - \quad - \quad - \quad - \]

\[ 39.8 Y + 3.8 Z = 1083.1 \]  .................................................. (9)
The simultaneous equation from equations (6) and (9) was derived to get equation (12) as follows.

\[ 148033.71 Y + 14736.348 Z = 4061988.0 \] .................................................. (10)

\[ 148033.71 Y + 14133.872 Z = 4028525.5 \] .................................................. (11)

\[ \text{----------------------------------------} \]

\[ 602.476 Z = 33462.5 \] .................................................. (12)

Therefore, \( Z = 55.5 \) .................................................. (13)

Substituting the value of \( Z \) from equation (13) in equation (9), the equation (14) was obtained as follows.

\[ 39.8 Y + 3.8 \times 55.5 = 1083.1 \] .................................................. (14)

Therefore, \( Y = 21.9 \) .................................................. (15)

Substituting the value of \( Z \) from equation (13) and that of \( Y \) from equation (15) in equation (3), the equation (16) was obtained as follows.

\[ X + 21.9 + 55.5 = 84.46 \] .................................................. (16)

Therefore, \( X = 7.06 \) .................................................. (17)

Thus, the formula for the control ice cream mix (by weight) included 7.06% SMP, 21.9% cream, 55.5% milk, 15.0% sucrose, 0.05% stabiliser/emulsifier and 0.04% vanilla flavour.

2. Calculation for the mix, in which 25% of sucrose was substituted with HLNC

*Weight equation for this mix*

For this formulation, there were two sources of sugar, sucrose and HLNC. HLNC substituted 25% of sucrose in this formulation and the proportion of sucrose was 75% of the weight of total sugar desired for the formulation. The granular sucrose used for the
formulation was assumed to have 100% total solids. The HLNC contained 15% total solids. Other ingredients used for this formulation were those used for the control mix. Accordingly the weight equation for the 25% sucrose replaced mix was as follows.

\[
\begin{array}{ccc}
75 & 25 & 100 \\
X + Y + Z + 15 \times \frac{X}{100} + (15 \times \frac{X}{100} + \frac{0.5}{100} + \frac{0.04}{15}) & = 100.0 \\
\end{array}
\]

*This 15 is the weight of the total sugar in the mix

**This 15 is the total solid content of HLNC

The above equation was rearranged as follows.

\[
X + Y + Z + 11.25 + 25.0 + 0.5 + 0.04 = 100.0
\]

Or, \[X + Y + Z = 63.21 \] .................................................. (18)

The simultaneous equation from equations (1) and (18) was derived as follows to get equation (21).

\[
0.2 \times X + 40.0 \times Y + 4.0 \times Z = 1100 \] .............................................. (19)

\[
\begin{array}{c}
0.2 \times X + 0.2 \times Y + 0.2 \times Z = 12.642 \\
- \hline \hline
39.8 \times Y + 3.8 \times Z = 1087.3
\end{array}
\] .................................................. (21)

The simultaneous equation from equations (6) and (21) was derived as follows to get equation (24).

\[
148033.71 \times Y + 14736.348 \times Z = 4061988.0 \] .............................................. (22)

\[
\begin{array}{c}
148033.71 \times Y + 14133.872 \times Z = 4044147.1 \\
- \hline \hline
602.476 \times Z = 17840.9
\end{array}
\] .................................................. (24)
Therefore, \( Z = 29.6 \) .................................................. (25)

Substituting the value of \( Z \) from equation (25) in equation (21), the resultant equation was as follows.

\[ 39.8Y + 3.8 \times 29.6 = 1087.3 \] ........................................ (26)

Therefore, \( Y = 24.5 \) ................................................. (27)

Substituting the value of \( Y \) from (27) and that of \( Z \) from (25) in (18), the equation obtained was as follows.

\[ X + 24.5 + 63.21 \] ...................................................... (28)

Therefore, \( X = 9.11 \) ................................................ (29)

Thus, the formula for the mix, in which 25% of sucrose was substituted with HLNC (by \%weight) included 9.11\% SMP, 24.5\% cream, 29.6\% milk, 11.25\% sucrose, 25\% HLNC, 0.5\% stabiliser/emulsifier and 0.04\% vanilla flavour.

3. Calculation for the formulation of mix, in which 50\% of sucrose was substituted with HLNC

*The weight equation for this mix*

\[
\begin{align*}
50 & \quad 50 & \quad 100 \\
X + Y + Z + 15 & \quad \times \quad + (15^{*} \quad \times \quad x \quad \text{-----}) + 0.5 + 0.04 = 100 \\
100 & \quad 100 & \quad 15^{**}
\end{align*}
\]

* This 15 is the weight of total sugar in the mix

** This 15 is the total solid content of HLNC

The above equation was rewritten as follows.

\[ X + Y + Z + 7.5 + 50 + 0.5 + 0.04 = 100 \]
Or, $X + Y + Z = 41.96$ .................................................. (30)

From equations (1) and (30), the simultaneous equation derived was as follows to get equation (33).

$0.2X + 40.0Y + 4.0Z = 1100$ ........................................... (31)

$0.2X + 0.2Y + 0.2Z = 8.392$ .......................................... (32)


$39.8Y + 3.8Z = 1091.6$ ............................................... (33)

From equations (6) and (33), the simultaneous equation derived was as follows to get equation (36).

$148033.71Y + 14736.348Z = 4061988.0$ ......................... (34)

$148033.71Y + 14133.872Z = 4060140.7$ ......................... (35)


$602.476Z = 1847.3$ .................................................. (36)

Therefore, $Z = 3.06$ ...................................................... (37)

Substituting the value of $Z$ from equation (37) in equation (33), the resultant equation was as follows.

$39.8Y + 3.8 \times 3.06 = 1091.6$ .......................................... (38)

Therefore, $Y = 27.1$ ...................................................... (39)

Substituting the value of $Y$ from equation (39) and that of $Z$ from equation (37), the resultant equation was as follows.

$X + 27.1 + 3.06 = 41.96$ .................................................. (40)
Therefore, $X = 11.8$ ................................................................. (41)

Thus, the formula for the mix, in which 50% of sucrose was substituted with HLNC (by weight) included 11.8% SMP, 27.1% cream, 3.06% milk, 7.5% sucrose, 50% HLNC, 0.5% stabiliser/emulsifier and 0.04% vanilla flavour.
APPENDIX 2

Sensory evaluation sheet for ice cream
Sensory evaluation of ice cream

You are presented with 6 samples of ice cream (one at a time). Please evaluate these samples for the 5 attributes indicated below, by encircling a vertical line of your choice on the rating scale (horizontal line).

(Example: ⠠⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠠⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤)

Name: __________________________ Sex (M or F): __________________________

Age: __________________________ Sample code number: __________________________

1. Flavour

⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤
Dislike  Dislike  Dislike  Dislike  Neither  Like  Like  Like  Like
extremely very much moderately slightly like nor slightly moderately very much extremely dislike

2. Sweetness

⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤
None  Slightly sweet  Moderately sweet  Very sweet  Extremely sweet

3. Saltiness

⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤
None  Slightly salty  Moderately salty  Very salty  Extremely salty

4. Grittiness (The amount of crystals felt against the tongue)

⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤
None  Slightly gritty  Moderately gritty  Very gritty  Extremely gritty

5. Overall acceptability

⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤ ⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤⠤stå

Thank you very much for your co-operation.
APPENDIX 3

Statistical analyses of data using Student’s t test

1. Independent samples

Let us assume that \( \sigma_1 = \sigma_2 = \sigma \), where \( \sigma \) is the population standard deviation,
\( \sigma_1 \) is the std. deviation of first population, and
\( \sigma_2 \) is the std. deviation of second population.

Then, our estimate for \( \sigma \) is a pooled standard deviation, defined as,

\[
Sp = \sqrt{\frac{(n_1-1) \times s_1^2 + (n_2-1) \times s_2^2}{(n_1+n_2-2)}},
\]
where \( s_1 \) is the standard deviation of the first sample of size \( n_1 \), and
\( s_2 \) is the standard deviation of the second sample of size \( n_2 \).

\( \sigma \) can be estimated by \( s_1 \) or \( s_2 \) alone, but then we will not be able to use all the
information available to us. Using \( Sp \) as our estimate of the standard deviation of the two
populations, the estimate of the standard deviation of the difference between the two
sample means works out to

\[
Sp\sqrt{(1/n_1+1/n_2)},
\]
And finally, when \( \sigma \) is replaced by \( Sp \), the distribution of

\[
\frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{Sp\sqrt{(1/n_1+1/n_2)}},
\]
where \( \bar{x}_1 \) and \( \bar{x}_2 \) are the sample means, and
\( \mu_1 \) and \( \mu_2 \) are the population means

is a t distribution with \((n_1+n_2-2)\) degree of freedom (d.f.).

Thus, a test procedure can be developed using the t distribution with \((n_1+n_2-2)\) d.f. For
that we first state the null and the alternative hypotheses as follows:

\[
H_0: \mu_1 \geq \mu_2 \quad \text{i.e. } \mu_1 - \mu_2 \geq 0
\]
\[
H_1: \mu_1 < \mu_2 \quad \text{i.e. } \mu_1 - \mu_2 < 0
\]

The test statistic will again be \((\bar{x}_1 - \bar{x}_2)\), and if the populations are normally distributed,

then \((\bar{x}_1 - \bar{x}_2)\) will also have a normal distribution with its mean as \((\mu_1 - \mu_2)\), and a
standard deviation, which can be estimated by the pooled standard deviation, \(Sp\).

The probability of type I error (i.e. Reject \( H_0 \)) is the highest when \((\mu_1 - \mu_2)\) is at the
breakpoint value between \( H_0 \) and \( H_1 \) i.e. when \((\mu_1 - \mu_2) = 0\), and so from t tables, at 5%
level of significance and with \((n_1+n_2-2)\) d.f., the cut-off value of \((\bar{x}_1 - \bar{x}_2)\) will be given by

\[
(\mu_1 - \mu_2) - t \text{ value } \cdot Sp\sqrt{(1/n_1+1/n_2)}, \quad \text{when } (\mu_1 - \mu_2) = 0
\]
Finally, the test procedure will be summarised as:

Reject H0, if the observed value of \((\bar{x}_1 - \bar{x}_2) \leq\) cut-off value, and

Accept H0, if the observed value of \((\bar{x}_1 - \bar{x}_2) >\) cut-off value.

(i) Figures 4 & 5

(A) Comparison of the flux at 30 min (MCR 1.73) with that at 5 min (MCR 1.2)

We have \(n_1 = 2\) (Two values of flux at 30 min)
\(n_2 = 2\) (Two values of flux at 5 min)
\(s_1 = 0.7\) (Standard deviation of flux at 30 min)
\(s_2 = 0.21\) (Standard deviation of flux at 5 min)

Now, the pooled standard deviation,

\[Sp = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}}\]

\[= \sqrt{\frac{(2-1)(0.7)^2 + (2-1)(0.21)^2}{(2+2)-2}}\]

\[= 0.516\]

Now, we state the null and the alternative hypothesis as follows:

\(H_0: \mu_1 \geq \mu_2\) i.e. \(\mu_1 - \mu_2 \geq 0\)
\(H_1: \mu_1 < \mu_2\) i.e. \(\mu_1 - \mu_2 < 0\), where \(\mu_1\) and \(\mu_2\) are the population means.

From the \(t\) table, at 5% level of significance, with \((n_1+n_2-2)\) degree of freedom (d.f.), the cut-off value of the difference between two sample means will be given by

\[(\mu_1 - \mu_2) - 2.92 \times Sp \sqrt{(1/n_1 + 1/n_2)}, \text{ when } \mu_1 - \mu_2 = 0\]

\[= 0 - 2.92 \times 0.516 \times \sqrt{(1/2 + 1/2)}\]

\[= -1.50\]

The observed value of the difference between two sample means (i.e. the difference between the mean of flux rates at 30 min and that at 5 min) is 8.7-16.35 = -7.65.

Thus, as the observed value is lower than the cut-off value, the H0 is REJECTED, which means that the flux at 30 min was significantly lower than that at 5 min of nanofiltration.
(B) Comparison of the flux at 30 min (MCR 1.73) and that at 40 min (MCR 2.09).

We have, $n_1 = 2$ (Two values of flux at 30 min)
$n_2 = 2$ (Two values of flux at 40 min)
$s_1 = 0.7$ (Std. deviation of flux at 30 min)
$s_2 = 2.12$ (Std. deviation of flux at 40 min)

The pooled std. deviation,

$S_p = \sqrt{\{(2-1)(0.7)^2 + (2-1)(2.12)^2\}/(2+2-2)}$

$= 1.57$

From t table, at 5% level of significance, with $(2+2-2)$ d.f., the cut-off value of the difference between two sample means (i.e. mean flux at 30 min - mean flux at 40 min) would be as follows:

$0 - 2.92 \times 1.57 \sqrt{1/2 + 1/2}$

$= - 4.58$

The observed value of the difference between the flux at 30 min and that 40 min is $8.7 - 9.5 = -0.8$

Thus, as the observed value is higher than the cut-off value, the H0 is ACCEPTED, which means that there was no significant difference between the flux at 30 min and that at 40 min.

(C) Comparison of the flux at 40 min (MCR 2.09) with that at 45 min (MCR 2.40)

We have, $n_1 = 2$ (Two values of flux at 40 min)
$n_2 = 2$ (Two values of flux at 45 min)
$s_1 = 2.12$ (Standard deviation of flux at 40 min)
$s_2 = 0.00$ (Standard deviation of flux at 45 min).

Therefore, $S_p = \sqrt{\{(2-1)(2.12)^2 + (2-1)(0)^2\}/(2+2-2)}$

$= 2.24$

From t tables, the cut-off value at 5% level of significance, with 2 d.f., will be as follows:

$0 - 2.92 \times 2.24 \times \sqrt{1/2 + 1/2}$

$= - 6.54$

The observed value of the difference between flux at 40 min and that at 45 min is $9.5 - 10.8 = -1.3$
Thus, as the observed value is higher than the cut-off value, the H0 is ACCEPTED, which means that there was no significant difference between the flux at 40 min and that at 45 min of nanofiltration.

(D) Comparison of the flux at 50 min (MCR 2.64) with that at 45 min (MCR 2.40)

We have, \( n_1 = 2 \) (Two values of flux at 50 min)
\( n_2 = 2 \) (Two values of flux at 45 min)
\( s_1 = 0.56 \) (Standard deviation of flux at 50 min)
\( s_2 = 0.00 \) (Standard deviation of flux at 45 min)

Therefore, \( S_p = \sqrt{\{(2-1)(0.56)^2 + (2-1)(0)^2\}/(2+2-2)} \)
\[ = 0.15 \]

The cut-off value at 5% level of significance with 2 d.f. will be
\[ 0 - 2.92 \times 0.15 \times \sqrt{1/2 + 1/2} \]
\[ = -0.43 \]

The observed value of difference between flux at 50 min and that at 45 min is 6.4 - 10.8
\[ = -4.4 \]

Thus, as the observed value is lower than the cut-off value, the H0 is REJECTED, which means that the flux at 50 min was significantly lower than that at 45 min of nanofiltration.

(E) Comparison of the flux at 50 min (MCR 2.64) with that at 55 min (MCR 3.01)

We have, \( n_1 = 2 \) (Two values of flux at 50 min)
\( n_2 = 2 \) (Two values of flux at 55 min)
\( s_1 = 0.56 \) (Standard deviation of flux at 50 min)
\( s_2 = 1.83 \) (Standard deviation of flux at 55 min)

Therefore, \( S_p = \sqrt{\{(2-1)(0.56)^2 + (2-1)(1.83)^2\}/(2+2-2)} \)
\[ = 1.83 \]

The cut-off value at 5% level of significance with 2 d.f., using t tables will be
\[ 0 - 2.92 \times 1.83 \sqrt{1/2 + 1/2} \]
\[ = -5.34 \]

The observed value of the difference between the flux at 50 min and that at 55 min is 6.4 - 8.9 = -2.5.

Thus, as the observed value is higher than the cut-off value, the H0 is ACCEPTED, which means that there was no significant difference between the flux at 50 min and that at 55 min.
(ii) Table 7

(A) Comparison between the titratable acidity (T.A.) of milk UF permeate and its 3 fold nano-concentrate

We have, n1 = 2 (Two values of T.A. of milk UF permeate)
  n2 = 2 (Two values of T.A. of 3 fold nano-concentrate)
s1 = 0 (Standard deviation of T.A. of milk UF permeate)
s2 = 0.02 (Standard deviation of T.A. of 3 fold nano-concentrate)

Therefore, \[ Sp = \sqrt{(2-1)(0)^2 + (2-1)(0.02)^2}/(2+2-2) \]
  \[ = 0.0002 \]

The cut-off value at 5% level of significance, with 2 d.f., using t tables, will be
  \[ 0 - 2.92 \times 0.0002 \sqrt{1/2 + 1/2} \]
  \[ = -0.00058 \]

The observed value of difference between the T.A. of milk UF permeate and that of its 3 fold nano-concentrate is 0.16 - 0.38 = -0.22.

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the T.A. of 3 fold nano-concentrate is significantly higher than that of milk UF permeate.

(B) Comparison between the pH of milk UF permeate and that of its 3 fold nano-concentrate

We have, n1 = 2 (Two values of pH for 3 fold nano-concentrate)
  n2 = 2 (Two values of pH for milk UF permeate)
s1 = 0 (Standard deviation of pH for nano-concentrate)
s2 = 0 (Standard deviation of pH for milk UF permeate)

Therefore, \[ Sp = \sqrt{(2-1)(0)^2 + (2-1)(0)^2}/(2+2-2) \]
  \[ = 0 \]

The cut-off value at 5% level of significance, with 2 d.f., using t tables will be
  \[ 0 - 2.92 \times 0 \sqrt{1/2 + 1/2} \]
  \[ = 0 \]

The observed value of difference between the pH of 3 fold nano-concentrate and the milk permeate is 6.60 - 6.74 = -0.14.

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the pH of milk UF permeate significantly reduced after its 3 fold nanofiltration.
(iii) Table 8

Comparison between the yellow colour intensity of milk UF permeate and its 3 fold nano-concentrate

We have, \( n_1 = 4 \) (Four values of colour intensity at 1.2 fold nanofiltration)
\( n_2 = 4 \) (Four values of colour intensity after 3 fold nanofiltration)
\( s_1 = 0 \) (Standard deviation of colour intensity at 1.2 fold nanofiltration)
\( s_2 = 4.24 \) (Standard deviation of colour intensity after 3 fold nanofiltration)

Therefore, \( Sp = \sqrt{\{(4-1)(0)^2 + (4-1)(4.24)^2\}/(4+4-2)} \)
\[ = 8.98 \]

The cut-off value at 5% level of significance, with 6 d.f, using t tables will be
\[ 0 - 1.943 \times 8.98\sqrt{(1/4 + 1/4)} \]
\[ = - 12.3 \]

The observed value of difference between the colour intensity at 1.2 fold and that at 3 fold nanofiltration is 20 - 49 = -29

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the intensity of yellow colour at 3 fold was significantly higher than the 1.2 fold nanofiltration.

(iv) Table 11

(A) Comparison between the \( pH \) of nano-concentrate before and after hydrolysis

We have, \( n_1 = 2 \) (Two values of \( pH \) after hydrolysis)
\( n_2 = 2 \) (Two values of \( pH \) before hydrolysis)
\( s_1 = 0.014 \) (Standard deviation of \( pH \) after hydrolysis)
\( s_2 = 0 \) (Standard deviation of \( pH \) before hydrolysis)

Therefore, \( Sp = \sqrt{\{(2-1)(0.014)^2 + (2-1)(0)^2\}/(2+2-2)} \)
\[ = 0.000098 \]

The cut-off value at 5% level of significance, with 2 d.f., using t tables, will be
\[ 0 - 2.92 \times 0.000098\sqrt{(1/2 + 1/2)} \]
\[ = - 0.00028 \]

The observed value of difference between the \( pH \) of nano-concentrate after and before hydrolysis is 6.5 - 6.6 = - 0.1

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that hydrolysis significantly reduced the \( pH \) of nano-concentrate.
(B) Comparison between the colour intensity of nano-concentrate before and after hydrolysis

We have, \( n_1 = 4 \) (Four values of colour intensity after hydrolysis)  
\( n_2 = 4 \) (Four values of colour intensity before hydrolysis)  
\( s_1 = 0 \) (Standard deviation of colour intensity after hydrolysis)  
\( s_2 = 0 \) (Standard deviation of colour intensity before hydrolysis)  

Therefore,  
\[
Sp = \sqrt{\{(4-1)(0)^2 + (4-1)(0)^2\}/(4+4-2)} = 0
\]

The cut-off value at 5% level of significance, with 6 d.f., using \( t \) Tables will be  
\( 0 - 1.943 \times 0 \sqrt{(1/4 + 1/4)} = 0 \)

The observed value of difference between the colour intensity after and before hydrolysis is 41 - 49 = -8

Thus, as the observed value is lower than the cut-off value, \( H_0 \) is REJECTED, which means that hydrolysis significantly reduced the colour intensity of nano-concentrate.

(v) Table 13

(A) Comparison between the colour intensity of control and the 25% HLNC ice cream

We have, \( n_1 = 4 \) (Four values of control ice cream)  
\( n_2 = 4 \) (Four values of 25% HLNC ice cream)  
\( s_1 = 0 \) (Std. deviation of control ice cream)  
\( s_2 = 0.31 \) (Std. deviation of 25% HLNC ice cream)  

Therefore,  
\[
Sp = 0.219
\]

The cut-off value at 5% level of confidence, with 6 d.f., using \( t \) tables = -0.30

The observed value = 14 - 14 = 0

Thus, as the observed value is higher than the cut-off value, \( H_0 \) is ACCEPTED, which means that the colour intensity of control and the 25% HLNC ice cream was not significantly different from each other.

(B) Comparison between the colour intensity of control and the 50% HLNC ice cream

We have, \( n_1 = 4 \) (Four values of control ice cream)  
\( n_2 = 4 \) (Four values of 50% HLNC ice cream)
\[ s_1 = 0 \text{ (Std. deviation of control ice cream)} \]
\[ s_2 = 0 \text{ (Std. deviation of 50\% HLNC ice cream)} \]

Therefore, \( Sp = 0 \)

The cut-off value at 5\% level of significance, with 6 d.f., using \( t \) tables = 0

The observed value = 14.0 - 17.0 = -3.0

Thus, as the observed value is lower than the cut-off value, \( H_0 \) is REJECTED, which means that the colour intensity of 50\% HLNC ice cream was significantly higher than the control ice cream.

(C) Comparison between the colour intensity of 25\% and 50\% HLNC ice cream

We have, \( n_1 = 4 \) (Four values of 25\% HLNC ice cream)
\[ n_2 = 4 \] (Four values of 50\% HLNC ice cream)
\[ s_1 = 0.31 \text{ (Std. deviation for 25\% HLNC ice cream)} \]
\[ s_2 = 0 \text{ (Std. deviation for 50\% HLNC ice cream)} \]

Therefore, \( Sp = 0.219 \)

The cut-off value at 5\% level of significance, with 6 d.f., using \( t \) tables = -0.30

The observed value = 14.0 - 17.0 = -3.0

Thus, as the observed value is lower than the cut-off value, \( H_0 \) is REJECTED, which means that the colour intensity of 50\% HLNC ice cream was significantly higher than the 25\% HLNC ice cream.

(vi) Table 17

(A) Comparison between the ash content of the control and the 25\% HLNC ice cream

We have, \( n_1 = 2 \) (Two values of ash content of control ice cream)
\[ n_2 = 2 \] (Two values of ash content of 25\% HLNC ice cream)
\[ s_1 = 0.02 \text{ (Standard deviation of ash content of control ice cream)} \]
\[ s_2 = 0.01 \text{ (Standard deviation of ash content of 25\% HLNC ice cream)} \]

Therefore, \( Sp = \sqrt{\{2-1\}(0.02)^2 + (2-1)(0.01)^2\}}/(2+2-2) \]
\[ = 0.015 \]

The cut-off value at 5\% level of significance, with 2 d.f., using \( t \) tables, will be
\[ O - 2.92 \times 0.015 \sqrt{1/2 + 1/2} = -0.04 \]
The observed value of the difference between the ash content of control and the 25% HLNC ice cream is 1.04 - 1.23 = - 0.19

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the ash content of 25% HLNC ice cream was significantly higher than that of the control ice cream.

(B) Comparison between the ash content of control and the 50% HLNC ice cream

We have, n1 = 2 (Two values of ash content of control ice cream)
   n2 = 2 (Two values of ash content of 50% HLNC ice cream)
   s1 = 0.02 (Standard deviation of ash content of control ice cream)
   s2 = 0.04 (Standard deviation of ash content of 50% HLNC ice cream)

Therefore, \(Sp = \sqrt{\{(2-1)0.02^2 + (2-1)0.04^2\}/(2+2-2)}\)
   = 0.03

The cut-off value at 5% level of significance, with 2 d.f., using t tables, will be
0 - 2.92 x 0.03 \(\sqrt{(1/2 + 1/2)}\) = - 0.08

The observed value of the difference between the ash content of control and 50% HLNC ice cream is 1.04 - 1.39 = - 0.35

Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the ash content of 50% HLNC ice cream was significantly higher than the control ice cream.

(C) Comparison between the ash content of 25% and 50% HLNC ice cream

We have, n1 = 2 (Two values of ash content of 25% HLNC ice cream)
   n2 = 2 (Two values of ash content of 50% HLNC ice cream)
   s1 = 0.01 (Standard deviation of ash content of 25% HLNC ice cream)
   s2 = 0.04 (Standard deviation of ash content of 50% HLNC ice cream)

Therefore, \(Sp = \sqrt{\{(2-1)0.01^2 + (2-1)0.04^2\}/(2+2-2)}\)
   = 0.029

The cut-off value at 5% level of significance, with 2 d.f., using t tables, will be
0 - 2.92 x 0.029 \(\sqrt{(1/2 + 1/2)}\) = - 0.08

The observed value of the difference between the ash content of 25% and 50% HLNC ice cream is 1.23 - 1.39 = - 0.16
Thus, as the observed value is lower than the cut-off value, H0 is REJECTED, which means that the ash content of 50% HLNC ice cream was significantly higher than that of 25% HLNC ice cream.

2. Dependant samples

The comparison between two variables can also be established by designing the t test based on dependant samples. Using the same symbols as used in case of independant samples, we introduce one more random variable, \( d \), defined as

\[ d = x_1 - x_2 \]

where \( d \) is the difference between the two variables being compared.

If the expected value of \( d \) is represented by \( \mu d \), then

\[ \mu d = \mu 1 - \mu 2 \]

Let us define our null and alternative hypotheses as before

- \( H_0: \mu 1 - \mu 2 \geq 0 \) i.e. \( \mu d \geq 0 \)
- \( H_1: \mu 1 - \mu 2 < 0 \) i.e. \( \mu d < 0 \)

This is a test concerning the population mean \( \mu d \) when we have a sample of \( d \) values. We use the sample mean \( \bar{d} \) as the test statistic. If we assume that the \( d \) values are normally distributed, then by knowing the sample standard deviation, \( S_d \), the cut-off value can be easily obtained from the t tables with \( (n - 1) \) d.f.

\[ \bar{d} = \mu d - (t \text{ value} \times S_d / \sqrt{n}) \text{, when } \mu d = 0 \]

and,

\[ S_d = \sqrt{\sum (x_i - \bar{x})^2 / (n - 1)} \]

Finally, the test procedure will be summarised as follows:

Reject \( H_0 \), if the observed value of \( \bar{d} \) ≤ the cut-off value

Accept \( H_0 \), if the observed value of \( \bar{d} \) > the cut-off value

(ii) Figure 6

(A) Effect of rinsing of fouled NF membrane (with water) on the water flux

The water flux (25°C) at various TMP can be arranged as per the table given below. According to the table, \( n = 12 \).
<table>
<thead>
<tr>
<th>TMP (KPa)</th>
<th>500 T1</th>
<th>800 T1</th>
<th>1000 T1</th>
<th>1200 T1</th>
<th>1500 T1</th>
<th>2000 T1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
</tr>
<tr>
<td>Water flux after rinsing (LPH)</td>
<td>54.4 60.0</td>
<td>87.2 73.6</td>
<td>97.7 88.0</td>
<td>112.7 105.2</td>
<td>126.2 128.6</td>
<td>151.3 152.3</td>
</tr>
<tr>
<td>Water flux at the beginning</td>
<td>64.02 66.22</td>
<td>95.43 86.03</td>
<td>112.1 103.0</td>
<td>118.3 118.5</td>
<td>146.9 139.9</td>
<td>179.7 181.7</td>
</tr>
<tr>
<td>Difference (d)</td>
<td>-9.62 -6.22</td>
<td>-8.23 -12.43</td>
<td>-14.4 -15.01</td>
<td>-5.6 -13.3</td>
<td>-20.7 -11.3</td>
<td>-28.4 -29.4</td>
</tr>
</tbody>
</table>

N.B. T1 represents trial 1 and T2 represents trial 2

We have, 
\[ d.f. = n - 1 = 11, \]

Observed value of \( \bar{d} = -14.55 \), and

\[ S_d = 7.86 \]

The cut-off value (from t tables, at 5% level of significance) of
\[ \bar{d} = 0 - (1.796 \times 7.86 / \sqrt{12}) = -4.07 \]

Thus, as the observed value is less than the cut-off value, H0 is REJECTED, which means that the water flux after rinsing the fouled NF membrane with water was significantly lower than that before the beginning of the nanofiltration of milk permeate.

(B) Effect of cleaning of fouled NF membrane on the water flux.

The water flux (25°C) at various TMP can be arranged as per the following table. According to this table, \( n = 12 \).

<table>
<thead>
<tr>
<th>TMP (KPa)</th>
<th>500 T1</th>
<th>800 T1</th>
<th>1000 T1</th>
<th>1200 T1</th>
<th>1500 T1</th>
<th>2000 T1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
<td>T2</td>
</tr>
<tr>
<td>Water flux after cleaning (LPH)</td>
<td>63.2 65.6</td>
<td>89.9 89.9</td>
<td>111.1 107.5</td>
<td>120.2 120.8</td>
<td>141.9 140.5</td>
<td>174.4 181.0</td>
</tr>
<tr>
<td>Water flux at the beginning (LPH)</td>
<td>64.02 66.22</td>
<td>95.43 86.03</td>
<td>112.1 103.0</td>
<td>118.3 118.5</td>
<td>146.9 139.9</td>
<td>179.7 181.7</td>
</tr>
<tr>
<td>Difference (d)</td>
<td>-0.82 -0.62</td>
<td>-5.53 3.87</td>
<td>-1.0 4.49</td>
<td>1.9 2.3</td>
<td>-5.0 0.6</td>
<td>-5.3 -0.7</td>
</tr>
</tbody>
</table>

N.B. T1 represents trial 1 and T2 represents trial 2

We have, \( d.f. = n - 1 = 12 - 1 = 11 \)
The observed value of $\bar{d} = -0.48$

$$S_d = 3.26$$

The cut-off value (from t tables, at 5% level of confidence) of

$$\bar{d} = 0 - (1.796 \times 3.26 / \sqrt{12}) = -1.69$$

Thus, as the observed value is more than the cut-off value, H0 is ACCEPTED, which means that there was no significant difference between the water flux obtained before the beginning of nanofiltration of milk permeate and that obtained after cleaning the fouled NF membrane with enzyme cleaner ultrasil-56.

(ii) Table 9

Comparison between the production of Glucose and Galactose during lactose hydrolysis

The percent production of glucose and galactose and the difference between them can be arranged as per the following table. According to the table, n = 12.

<table>
<thead>
<tr>
<th>Time of hydrolysis (Min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>1080</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Galactose (%)</td>
<td>13.7</td>
<td>15.6</td>
<td>17.1</td>
<td>20.5</td>
<td>20.0</td>
<td>24.3</td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>28.2</td>
<td>26.4</td>
<td>35.8</td>
<td>32.6</td>
<td>40.7</td>
<td>34.4</td>
</tr>
<tr>
<td>Difference (d)</td>
<td>-14.5</td>
<td>-10.8</td>
<td>-18.7</td>
<td>-12.1</td>
<td>-20.7</td>
<td>-10.1</td>
</tr>
</tbody>
</table>

N.B. T1 represents trial 1 and T2 represents trial 2.

We have, d.f. = n - 1 = 12 - 1 = 11

The observed value of $\bar{d} = -12.9$, and

$$S_d = 6.71$$

The cut-off value (from t tables, at 5% level of confidence) of

$$\bar{d} = 0 - (1.796 \times 6.71/ \sqrt{12}) = -3.47$$

Thus, as the observed value is less than the cut-off value, H0 is REJECTED, which means that the production of glucose was significantly higher than that of galactose during the lactose hydrolysis of nano-concentrate.
(iii) Table 10

Effect of oligosaccharides on the calculation of the degree of hydrolysis.

The difference between the degree of hydrolysis (without the consideration of OS as the hydrolytic product) and the amount (%) of lactose converted to hydrolytic products including the OS, has been arranged in the table below. According to the table, n = 12.

<table>
<thead>
<tr>
<th>Time of hydrolysis (Min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>1080</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Degree of hydrolysis (%)</td>
<td>42.0</td>
<td>42.0</td>
<td>52.9</td>
<td>53.1</td>
<td>60.7</td>
<td>59.8</td>
</tr>
<tr>
<td>Lactose conversion (%)</td>
<td>49.6</td>
<td>48.8</td>
<td>62.5</td>
<td>62.9</td>
<td>70.6</td>
<td>69.6</td>
</tr>
<tr>
<td>Difference (d)</td>
<td>-7.6</td>
<td>-6.8</td>
<td>-9.6</td>
<td>-9.8</td>
<td>-9.9</td>
<td>-9.8</td>
</tr>
</tbody>
</table>

N.B. T1 represents trial 1 and T2 represents trial 2.

We have, d.f. = n - 1 = 12 - 1 = 11

The observed value of \( \bar{d} = -10.24 \)

\[ S_d = 4.59 \]

The cut-off value (from t tables, at 5% level of significance, with 11 d.f.) of

\[ \bar{d} = 0 - (1.796 \times 4.59 / \sqrt{12}) = -2.38 \]

Thus, as the observed value is less than the cut-off value, H0 is REJECTED, which means that the OS have significant effect on the calculation of the degree of hydrolysis.
APPENDIX 4

Student’s t distribution table
## t DISTRIBUTION
Areas in Both Tails Combined for Student's t Distribution

![Image of a t-distribution curve with critical values]

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>.10</th>
<th>.05</th>
<th>.02</th>
<th>.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.314</td>
<td>12.706</td>
<td>31.821</td>
<td>63.657</td>
</tr>
<tr>
<td>2</td>
<td>2.920</td>
<td>4.303</td>
<td>6.965</td>
<td>9.295</td>
</tr>
<tr>
<td>3</td>
<td>2.353</td>
<td>3.182</td>
<td>4.541</td>
<td>5.841</td>
</tr>
<tr>
<td>4</td>
<td>2.132</td>
<td>2.776</td>
<td>3.747</td>
<td>4.604</td>
</tr>
<tr>
<td>5</td>
<td>2.015</td>
<td>2.571</td>
<td>3.365</td>
<td>4.032</td>
</tr>
<tr>
<td>6</td>
<td>1.943</td>
<td>2.447</td>
<td>3.143</td>
<td>3.707</td>
</tr>
<tr>
<td>7</td>
<td>1.895</td>
<td>2.365</td>
<td>2.998</td>
<td>3.499</td>
</tr>
<tr>
<td>8</td>
<td>1.860</td>
<td>2.306</td>
<td>2.896</td>
<td>3.355</td>
</tr>
<tr>
<td>9</td>
<td>1.833</td>
<td>2.262</td>
<td>2.821</td>
<td>3.250</td>
</tr>
<tr>
<td>10</td>
<td>1.812</td>
<td>2.228</td>
<td>2.764</td>
<td>3.169</td>
</tr>
<tr>
<td>11</td>
<td>1.796</td>
<td>2.201</td>
<td>2.718</td>
<td>3.106</td>
</tr>
<tr>
<td>12</td>
<td>1.782</td>
<td>2.179</td>
<td>2.681</td>
<td>3.055</td>
</tr>
<tr>
<td>13</td>
<td>1.771</td>
<td>2.160</td>
<td>2.659</td>
<td>3.012</td>
</tr>
<tr>
<td>14</td>
<td>1.761</td>
<td>2.145</td>
<td>2.624</td>
<td>2.977</td>
</tr>
<tr>
<td>15</td>
<td>1.753</td>
<td>2.131</td>
<td>2.602</td>
<td>2.947</td>
</tr>
<tr>
<td>16</td>
<td>1.746</td>
<td>2.120</td>
<td>2.583</td>
<td>2.921</td>
</tr>
<tr>
<td>17</td>
<td>1.740</td>
<td>2.110</td>
<td>2.567</td>
<td>2.898</td>
</tr>
<tr>
<td>18</td>
<td>1.734</td>
<td>2.101</td>
<td>2.552</td>
<td>2.878</td>
</tr>
<tr>
<td>19</td>
<td>1.729</td>
<td>2.093</td>
<td>2.539</td>
<td>2.861</td>
</tr>
<tr>
<td>20</td>
<td>1.725</td>
<td>2.086</td>
<td>2.528</td>
<td>2.845</td>
</tr>
<tr>
<td>21</td>
<td>1.721</td>
<td>2.080</td>
<td>2.518</td>
<td>2.831</td>
</tr>
<tr>
<td>22</td>
<td>1.717</td>
<td>2.074</td>
<td>2.508</td>
<td>2.819</td>
</tr>
<tr>
<td>23</td>
<td>1.714</td>
<td>2.069</td>
<td>2.500</td>
<td>2.807</td>
</tr>
<tr>
<td>24</td>
<td>1.711</td>
<td>2.064</td>
<td>2.492</td>
<td>2.797</td>
</tr>
<tr>
<td>25</td>
<td>1.708</td>
<td>2.060</td>
<td>2.485</td>
<td>2.787</td>
</tr>
<tr>
<td>26</td>
<td>1.706</td>
<td>2.056</td>
<td>2.479</td>
<td>2.779</td>
</tr>
<tr>
<td>27</td>
<td>1.703</td>
<td>2.052</td>
<td>2.473</td>
<td>2.771</td>
</tr>
<tr>
<td>28</td>
<td>1.701</td>
<td>2.048</td>
<td>2.467</td>
<td>2.763</td>
</tr>
<tr>
<td>29</td>
<td>1.699</td>
<td>2.045</td>
<td>2.462</td>
<td>2.756</td>
</tr>
<tr>
<td>30</td>
<td>1.697</td>
<td>2.042</td>
<td>2.457</td>
<td>2.750</td>
</tr>
<tr>
<td>40</td>
<td>1.684</td>
<td>2.021</td>
<td>2.423</td>
<td>2.704</td>
</tr>
<tr>
<td>60</td>
<td>1.671</td>
<td>2.000</td>
<td>2.390</td>
<td>2.660</td>
</tr>
<tr>
<td>120</td>
<td>1.658</td>
<td>1.980</td>
<td>2.358</td>
<td>2.617</td>
</tr>
</tbody>
</table>

Normal Distribution

<table>
<thead>
<tr>
<th>.10</th>
<th>.05</th>
<th>.02</th>
<th>.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.645</td>
<td>1.960</td>
<td>2.326</td>
<td>2.576</td>
</tr>
</tbody>
</table>
APPENDIX 5

Statistical analyses (ANOVA and Duncan’s multiple range test) of ice cream data.

adh represents adhesiveness
chew represents chewiness
cohesiveness
f.p. represents freezing point
f.t. represents freezing time
gum represents gumminess
hard represents hardness
o.r. represents overrun
ph represents pH
springiness
represents springiness
tg represents glass transition temperature
tm represents melting temperature
visc represents viscosity
MACEPT represents overall acceptability
MSWEET represents sweetness
MSALT represents saltiness
MCOOKED represents cooked flavour
MICI represents iciness
TRT 1 represents control ice cream
TRT 2 represents 25% HLNC ice cream
TRT 3 represents 50% HLNC ice cream
Analysis of Variance Procedure
Class Level Information

TRT  3  1 2 3

Number of observations in by group = 12

Dependent Variable: VAL

Source    DF    Sum of Squares    Mean Square    F Value    Pr > F
Model    2    449295.8590    224647.9295    2.38    0.1481
Error    9    849682.2260    94409.1362
Corrected Total    11    1298978.0850

R-Square    C.V.    Root MSE    VAL Mean
0.345884    -52.90085    307.2607    -580.8238

The SAS System
10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

Dependent Variable: VAL

Source    DF    Anova SS    Mean Square    F Value    Pr > F
TRT    2    449295.8590    224647.9295    2.38    0.1481

The SAS System
10:47 Tuesday, February 25, 1997
Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

\[ \text{Alpha} = 0.05 \quad \text{df} = 9 \quad \text{MSE} = 94409.14 \]

Number of Means \( 2 \quad 3 \)
Critical Range \( 490.7 \quad 512.5 \)

Means with the same letter are not significantly different.

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-------------------------------- ID = adh --------------------------------

Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-352.7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>-564.0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>-825.8</td>
<td>4</td>
<td>1</td>
</tr>
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</table>

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-------------------------------- ID = chew --------------------------------

Analysis of Variance Procedure

Class Level Information

<table>
<thead>
<tr>
<th>TRT</th>
<th>1 2 3</th>
</tr>
</thead>
</table>

Number of observations in by group = 12

The SAS System

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-------------------------------- ID = chew --------------------------------

Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>36575.18959</td>
<td>18287.59480</td>
<td>2.91</td>
<td>0.1061</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>56604.33807</td>
<td>6289.37090</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Corrected Total 11 93179.52766

R-Square C.V. Root MSE VAL Mean
0.392524 42.74377 79.30555 181.2957

The SAS System 28
10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

Dependent Variable: VAL

Source DF Anova SS Mean Square F Value Pr > F
TRT 2 36575.18959 18287.59480 2.91 0.1061

The SAS System 29
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not
the experimentwise error rate

Alpha = 0.05 df = 9 MSE = 6289.371

Number of Means 2 3
Critical Range 126.7 132.3

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

Duncan Grouping Mean N TRT
A 226.86 4 1
A
A 213.42 4 2
A
A 103.61 4 3

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Analysis of Variance Procedure
Class Level Information

TRT 3 1 2 3

Number of observations in by group = 12

The SAS System 32
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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.00227850</td>
<td>0.00113925</td>
<td>0.59</td>
<td>0.5765</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.01749975</td>
<td>0.00194442</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>11</td>
<td>0.01977825</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square 0.115202  C.V. 21.64198  Root MSE 0.044096

The SAS System 33
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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>0.00227850</td>
<td>0.00113925</td>
<td>0.59</td>
<td>0.5765</td>
</tr>
</tbody>
</table>

The SAS System 34
10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL
NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate.

\[ \text{Alpha} = 0.05 \quad \text{df} = 9 \quad \text{MSE} = 0.001944 \]

Number of Means 2 3
Critical Range .07042 .07355

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.21600</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>0.21075</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>0.18450</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

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Analysis of Variance Procedure

Class Level Information

<table>
<thead>
<tr>
<th>TRT</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

Number of observations in by group = 12

The SAS System

10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.27521667</td>
<td>0.13760833</td>
<td>29.58</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.04187500</td>
<td>0.00465278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>11</td>
<td>0.31709167</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
R-Square | C.V.     | Root MSE | VAL Mean
---------|---------|----------|--------
0.867940 | -3.812461 | 0.068211 | -1.789167

The SAS System
10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>0.27521667</td>
<td>0.13760833</td>
<td>29.58</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The SAS System
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05 df = 9 MSE = 0.004653

Number of Means 2 3
Critical Range .1089 .1138

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

Duncan Grouping | Mean | N | TRT
-----------------|------|---|-----
A                | -1.62000 | 4 | 1
B                | -1.76000 | 4 | 2
C                | -1.98750 | 4 | 3
Analysis of Variance Procedure
Class Level Information

TRT 3 1 2 3

Number of observations in by group = 6

The SAS System 42
10:47 Tuesday, February 25, 1997

----------------------------- ID=f.t -----------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>2233.333333</td>
<td>1116.666667</td>
<td>33.50</td>
<td>0.0089</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>100.000000</td>
<td>33.333333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>2333.333333</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square 0.957143 C.V. 2.793630 Root MSE 5.773503 VAL Mean 206.6667

The SAS System 43
10:47 Tuesday, February 25, 1997

----------------------------- ID=f.t -----------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
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<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>2233.333333</td>
<td>1116.666667</td>
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<td>0.0089</td>
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</tbody>
</table>

The SAS System 44
10:47 Tuesday, February 25, 1997

----------------------------- ID=f.t -----------------------------

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate
Alpha = 0.05  df = 3  MSE= 33.3333

Number of Means  2  3
Critical Range  18.35 18.42

Means with the same letter are not significantly different.

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----------------------------------- ID=f1 -----------------------------------

Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>225.000</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>215.000</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>180.000</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

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----------------------------------- ID=gum -----------------------------------

Analysis of Variance Procedure
Class Level Information

TRT  3 1 2 3

Number of observations in by group = 12

The SAS System 47
10:47 Tuesday, February 25, 1997

----------------------------------- ID=gum -----------------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>46328.16182</td>
<td>23164.08091</td>
<td>3.78</td>
<td>0.0641</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>55081.39966</td>
<td>6120.15552</td>
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<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>11</td>
<td>101409.56148</td>
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<td></td>
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</tr>
</tbody>
</table>

R-Square  C.V.  Root MSE  VAL Mean
Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>46328.16182</td>
<td>23164.08091</td>
<td>3.78</td>
<td>0.0641</td>
</tr>
</tbody>
</table>

Analysis of Variance Procedure

Duncan’s Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05  df = 9  MSE = 6120.156

Number of Means  2  3
Critical Range  124.9 130.5

Means with the same letter are not significantly different.

Analysis of Variance Procedure

Duncan Grouping  Mean  N  TRT

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>270.01</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>180.25</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>118.69</td>
<td>4</td>
<td>3</td>
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</tbody>
</table>

Analysis of Variance Procedure

ID = hard
Class Level Information

TRT  3 1 2 3

Number of observations in by group = 12

The SAS System
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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>1962193.722</td>
<td>981096.861</td>
<td>2.77</td>
<td>0.1151</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>3182169.448</td>
<td>353574.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>11</td>
<td>5144363.169</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square    C.V.    Root MSE    VAL Mean
0.381426    58.51947   594.6212    1016.108

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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>1962193.722</td>
<td>981096.861</td>
<td>2.77</td>
<td>0.1151</td>
</tr>
</tbody>
</table>

The SAS System
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Analysis of Variance Procedure

Duncan’s Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate
Alpha = 0.05  df = 9  MSE = 353574.4

Number of Means  2  3
Critical Range  949.6  991.8

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

Duncan Grouping

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>1565.9</td>
<td>4</td>
<td>1</td>
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<tr>
<td>A</td>
<td>877.4</td>
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<tr>
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<td>605.0</td>
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Analysis of Variance Procedure

Class Level Information

<table>
<thead>
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<th>TRT</th>
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<th>2</th>
<th>3</th>
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</table>

Number of observations in by group = 12

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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>282.16666667</td>
<td>141.0833333</td>
<td>7.15</td>
<td>0.0138</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>177.50000000</td>
<td>19.72222222</td>
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</tbody>
</table>

R-Square  C.V.  Root MSE  VAL Mean

11
Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
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<td>282.1566667</td>
<td>141.0833333</td>
<td>7.15</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 9 MSE= 19.72222

Number of Means 2 3
Critical Range 7.092 7.408

Means with the same letter are not significantly different.

Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>63.750</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>62.750</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>53.000</td>
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Class Level Information

TRT 3 1 2 3

Number of observations in by group = 9

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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.0088888889</td>
<td>0.004444444</td>
<td>3.36</td>
<td>0.1049</td>
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<tr>
<td>Error</td>
<td>6</td>
<td>0.007933333</td>
<td>0.00132222</td>
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</tr>
<tr>
<td>Corrected Total</td>
<td>8</td>
<td>0.01682222</td>
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</tr>
</tbody>
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R-Square 0.528402  C.V. 0.543985  Root MSE 0.036362  VAL Mean 6.684444

The SAS System 63
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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
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<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>0.0088888889</td>
<td>0.004444444</td>
<td>3.36</td>
<td>0.1049</td>
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</table>

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Analysis of Variance Procedure

Duncan’s Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate.
Alpha = 0.05  df = 6  MSE = 0.001322

Number of Means 2 3
Critical Range .07265 .07530

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

Duncan Grouping  Mean  N  TRT

A  6.70667  3  1
A
A  6.70667  3  2
A
A  6.64000  3  3

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Analysis of Variance Procedure
Class Level Information

TRT 3 1 2 3

Number of observations in by group = 12

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Analysis of Variance Procedure
Dependent Variable: VAL

Source    DF    Sum of Squares    Mean Square    F Value    Pr > F
Model 2 0.13655617 0.06826808 0.46 0.6461
Error 9 1.33934350 0.14881594
Corrected Total 11 1.47587967

R-Square C.V. Root MSE VAL Mean
Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>0.13653617</td>
<td>0.06826808</td>
<td>0.46</td>
<td>0.6461</td>
</tr>
</tbody>
</table>

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05  df = 9  MSE = 0.148816

Number of Means  2  3
Critical Range .6161 .6435

Means with the same letter are not significantly different.

Analysis of Variance Procedure

Duncan Grouping  | Mean  | N  | TRT |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.1410</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>0.9168</td>
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<td>3</td>
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<tr>
<td>A</td>
<td>0.9128</td>
<td>4</td>
<td>1</td>
</tr>
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</table>

Analysis of Variance Procedure
Class Level Information

TRT  3 1 2 3

Number of observations in by group = 12

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------------------------------- ID=tg -----------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>67.5200000000</td>
<td>33.760000000</td>
<td>4.31</td>
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<td>Error</td>
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<td>70.5600000000</td>
<td>7.840000000</td>
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<tr>
<td>Corrected Total</td>
<td>11</td>
<td>138.080000000</td>
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</tbody>
</table>

R-Square  C.V.  Root MSE  VAL, Mean
0.488992  -4.123711  2.800000  -67.90000

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------------------------------- ID=tg -----------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Anova SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>67.5200000000</td>
<td>33.760000000</td>
<td>4.31</td>
<td>0.0487</td>
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------------------------------- ID=tg -----------------------------

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate
Alpha = 0.05  df = 9  MSE = 7.84

Number of Means 2 3
Critical Range 4.472 4.670

Means with the same letter are not significantly different.

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------------------------------- ID = tg -----------------------------

Analysis of Variance Procedure

Duncan Grouping  | Mean  | N  | TRT
-----------------|-------|---|-----
A                | -64.900 | 4 | 1
A                | -64.900 | 4 | 2
B                | -68.100 | 4 | 2
E                | -70.700 | 4 | 3

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------------------------------- ID = tm -----------------------------

Analysis of Variance Procedure

Class Level Information

TRT  | 3 1 2 3

Number of observations in by group = 12

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------------------------------- ID = tm -----------------------------

Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>10.59963267</td>
<td>5.29981633</td>
<td>115.07</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.41451900</td>
<td>0.04605767</td>
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<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>11</td>
<td>11.01415167</td>
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</tbody>
</table>

R-Square  | C.V.  | Root MSE | VAL Mean | 17
0.962365  -18.97249  0.214610  -1.131167
The SAS System
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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
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<tbody>
<tr>
<td>TRT</td>
<td>2</td>
<td>10.59963267</td>
<td>5.29981633</td>
<td>115.07</td>
<td>0.0001</td>
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</tbody>
</table>

The SAS System
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: VAL.

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05  df = 9  MSE = 0.046058

Number of Means  2  3
Critical Range  .3427  .3580

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.2500</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>-0.7100</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>-2.4335</td>
<td>4</td>
<td>3</td>
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</table>

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Analysis of Variance Procedure
Class Level Information

TRT  3 1 2 3

Number of observations in by group = 9

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Analysis of Variance Procedure

<table>
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<tr>
<th>Source</th>
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<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>14.908888889</td>
<td>7.454444444</td>
<td>27.95</td>
<td>0.0009</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>1.600000000</td>
<td>0.266666667</td>
<td></td>
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<tr>
<td>Corrected Total</td>
<td>8</td>
<td>16.508888889</td>
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</tbody>
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R-Square  C.V.  Root MSE  VAL Mean
0.903083  2.217357  0.516398  23.28889

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Analysis of Variance Procedure

Dependent Variable: VAL

<table>
<thead>
<tr>
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<th>Mean Square</th>
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<td>TRT</td>
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<td>0.0009</td>
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</table>

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Analysis of Variance Procedure

Duncan’s Multiple Range Test for variable: VAL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

19
Alpha = 0.05  df = 6  MSE = 0.266667

Number of Means 2 3
Critical Range 1.032 1.069

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>TRT</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>25.0333</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>22.8667</td>
<td>3</td>
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<tr>
<td>B</td>
<td>21.9667</td>
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File: "Over.1st"

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Analysis of Variance Procedure
Class Level Information

<table>
<thead>
<tr>
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<th>Levels</th>
<th>Values</th>
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</thead>
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<tr>
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<td>1 2 3</td>
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Number of observations in data set = 72
Analysis of Variance Procedure

Dependent Variable: MACEPT

<table>
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<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>6161.896319</td>
<td>3080.948160</td>
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<td>0.0013</td>
</tr>
<tr>
<td>Error</td>
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<td>28856.927813</td>
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<tr>
<td>Corrected Total</td>
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</table>

R-Square 0.175960  C.V. 36.30551  Root MSE 20.45034  MACEPT Mean 56.32847

The SAS System
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Analysis of Variance Procedure

Dependent Variable: MACEPT

<table>
<thead>
<tr>
<th>Source</th>
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<tbody>
<tr>
<td>TRT</td>
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The SAS System
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Analysis of Variance Procedure

Dependent Variable: MSWEET

<table>
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<tr>
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<th>Mean Square</th>
<th>F Value</th>
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<tbody>
<tr>
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<td>23668.570833</td>
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R-Square 0.135015  C.V. 37.06440  Root MSE 18.52087  MSWEET Mean 49.96944

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Analysis of Variance Procedure

Dependent Variable: MSWEET
### Analysis of Variance Procedure

**Dependent Variable: MSALTI**

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<thead>
<tr>
<th>Source</th>
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<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
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<td>1847.205972</td>
<td>5.39</td>
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The SAS System
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<table>
<thead>
<tr>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>Model</td>
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The SAS System
10:47 Tuesday, February 25, 1997

**Analysis of Variance Procedure**

**Dependent Variable: MSALTI**

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The SAS System
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Dependent Variable: MCOOKED

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Analysis of Variance Procedure

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The SAS System 169
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Analysis of Variance Procedure

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The SAS System 170
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MACEPT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05  df = 69  MSE = 418.2163

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Critical Range 11.79 12.39

Means with the same letter are not significantly different.

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Analysis of Variance Procedure

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The SAS System
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MSWEET

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate.

\[ \text{Alpha} = 0.05 \quad \text{df} = 69 \quad \text{MSE} = 343.0228 \]

Number of Means \quad \begin{array}{c} 2 \\ 3 \end{array}
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The SAS System
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Analysis of Variance Procedure

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The SAS System
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MSALTI

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate.

\[ \text{Alpha} = 0.05 \quad \text{df} = 69 \quad \text{MSE} = 351.2509 \]
Number of Means  2  3
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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MCOOKED

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

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The SAS System
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Analysis of Variance Procedure

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Analysis of Variance Procedure
Duncan's Multiple Range Test for variable: MCI

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha = 0.05  df = 69  MSE = 357.0071

Number of Means  2  3
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The SAS System
10:47 Tuesday, February 25, 1997

Analysis of Variance Procedure

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An Evaluation of Nanofiltration and Lactose Hydrolysis of Milk UF Permeate for use in Ice Cream

Thesis submitted in fulfilment of the requirement for the degree of Master of Science (Hons.) in Food Technology

By

Manoja Nand Chaudhary

Thesis Panel

Dr. K. Kailasapathy
Mr. Minh Nguyen

School of Food Science & Technology
Faculty of Science, Technology and Agriculture
University of Western Sydney, Hawkesbury,
Richmond, NSW 2753, Australia

December 1997
PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
To

PRERNAA

(My Inspiration)
DECLARATION

This is to certify that the work presented in this thesis has not been submitted to any other University or Institution for a higher degree

Manoja Nand Chaudhary
ACKNOWLEDGMENTS

I am highly grateful to Dr. K. Kailasapathy, Chair of my supervisory panel, for his critical outlook, professional supervision, and invaluable suggestions, rendered to me during present investigation. I am thankful to him also for instilling in me, a sense of independent approach to research. High gratitude to Mr. Minh Nguyen, member of my supervisory panel, for his friendly behaviour and invaluable suggestions during present investigation.

Compliments to Dr. Jim Bergan, Chairman of the Postgraduate Committee of the Faculty of Science, Technology and Agriculture of the University of Western Sydney, Hawkesbury; and Dr. Jim Hourigan, Head of the School of Food Science & Technology of the University of Western Sydney, Hawkesbury, for their helpful advises.

Thanks to the University of Western Sydney, Hawkesbury and its technical as well as non-technical staff members, for providing quite a congenial atmosphere and the facilities required for a postgraduate research.

Loving gratitude to all of my family members, especially wife Punam for providing a pleasant domestic environment, and the parents and the in-laws for the persistent encouragement.

Appreciations to all of my friends for making my stay in Hawkesbury, a memorable one.

A world of thanks to the AusAid, Government of Australia for providing financial assistance through ADCOS scholarship as well as to the Department of Education, Ministry of Human Resource Development, Government of India, for selecting me from amongst a number of contenders for this coveted scholarship.
ABSTRACT

This study was mainly aimed at obtaining 15% total solids and reduced mineral content in milk UF permeate by nanofiltration; hydrolysing the lactose content of nano-concentrate enzymically; partially substituting sucrose in ice cream formulations with the hydrolysed lactose nano-concentrate; and investigating into the effects of hydrolysed lactose nano-concentrate on the physico-chemical and sensory characteristics of ice cream. The desired 15% total solids in the nano-concentrate was achieved after three fold concentration of milk UF permeate. The applied transmembrane pressure during a 55 min nanofiltration ranged between 800 and 1500 KPa, whereas the temperature during this 55 min run increased from 10 to 30°C. The initial flux of 16.35 L/m²/h (At 13°C) decreased to 8.9 L/m²/h (At 30°C) at the end of nanofiltration. The colour of milk permeate changed from greenish yellow (20% intensity) to yellow (49% intensity). The pH decreased from 6.74 to 6.60. The crude protein content increased from 0.3 to 0.83% and the lactose content increased from 4.46 to 13.0%. The titratable acidity of milk permeate increased from 0.16 to 0.38% L.A. Mineral content was reduced by 42.6%. K⁺ and Na⁺ were reduced by 49.6 and 37.2% respectively. The lactose content of the nano-concentrate was hydrolysed by enzyme lactase, which was used at a rate of 12 mL/L of nano-concentrate (Equivalent to 1 mL per 10.83 g of Lactose) at 40°C for different periods of time. The hydrolysis for 60 min was selected for the production of hydrolysed lactose nano-concentrate (HLNC), which contained 12.0% of the total carbohydrates as oligosaccharides. The degree of lactose hydrolysis obtained during this period was 80.1%. Formation of oligosaccharides affected the calculation of the degree of hydrolysis. The HLNC was used to replace 25 and 50% of sucrose in ice cream formulations. The springiness, cohesiveness, chewiness, adhesiveness, hardness, iciness, pH and colour were not significantly affected by either of the HLNC treatments. The viscosity, freezing point, glass transition temperature, melting temperature, gumminess and sweetness were significantly decreased on HLNC treatments. The freezing time, saltiness and cooked flavour significantly increased on HLNC treatments. The overrun was not significantly affected at 50% level of substitution, but at 25% level it significantly decreased. The overall acceptability of ice cream significantly decreased at 50% level, but was insignificantly affected at 25% level of sucrose replacement. The lower sweetness of
HLNC treated ice creams than the one without HLNC treatment could have been either due to their higher salt content or the incomplete hydrolysis of lactose. However, the overall acceptability of 25% sucrose replaced ice cream indicated that, about one fourth of sucrose could be replaced by HLNC.
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<tr>
<td>α-LA</td>
<td>Alpha-lactalbumin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of official analytical chemists</td>
</tr>
<tr>
<td>Approx</td>
<td>Approximately</td>
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<tr>
<td>β-LG</td>
<td>Beta-lactoglobulin</td>
</tr>
<tr>
<td>BOD</td>
<td>Biological oxygen demand</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>BTU</td>
<td>British thermal unit</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Ca(OH)_2</td>
<td>Calcium hydroxide</td>
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<tr>
<td>CHWP</td>
<td>Concentrated hydrolysed whey permeate</td>
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<tr>
<td>CIE</td>
<td>Commission international de l’eclairage</td>
</tr>
<tr>
<td>Cl</td>
<td>Chloride</td>
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<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
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<tr>
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<td>Dextrose equivalent</td>
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<td>Diafiltration</td>
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<td>Degree of hydrolysis</td>
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<td>Disaccharides</td>
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<td>DMRT</td>
<td>Duncan’s multiple range test</td>
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<td>HLNC</td>
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<td>Hydrolysed lactose syrup</td>
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<tr>
<td>h</td>
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<td>Immunoglobulin G</td>
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<tr>
<td>LMH</td>
<td>Litre per square meter per hour</td>
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<td>L/m²/h</td>
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</tr>
<tr>
<td>μm</td>
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<td>Mass concentration ratio</td>
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<td>Non fat dry milk</td>
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