ICE CRYSTALLIZATION AND RECRYSTALLIZATION IN FROZEN MODEL SOLUTIONS AND ICE CREAM AS AFFECTED BY POLYSACCHARIDE GUMS

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ABSTRACT

ICE CRYSTALLIZATION AND RECRYSTALLIZATION IN FROZEN MODEL SOLUTIONS AND ICE CREAM AS AFFECTED BY POLYSACCHARIDE GUMS

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Ice crystal size distributions of dynamically frozen model solutions and ice cream were determined through image analysis using low temperature scanning electron microscopy. The mathematical approach used to describe the distributions (logistic dose response model) showed that there was a good correlation between average crystal sizes and calculated ice content. Low constant temperature storage (-30°C) had no effect in the overall ice crystal size, whereas storage at a higher temperature (-16°C) showed clear evidence of crystallization, possibly through Ostwald ripening and accretion. Prolonged temperature cycle profiles (9 cycles) had a greater impact on the recrystallization behaviour than that caused by the increased in the average temperature. The recrystallization mechanism in later stages would involve melt-refreeze in which case stabilizers exerted a measurable effect, retarding or preventing crystal growth. The alteration of the diffusion properties of the unfrozen phase by promoting recrystallization within a stabilizer matrix was hypothesized as a possible mechanism of the stabilizer action.
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1. INTRODUCTION

Ice cream and frozen desserts may often present a coarse or icy texture after storage, a defect commonly attributed to the presence of large ice crystals. This deteriorative effect is a result of ice recrystallization and is more prevalent in products stored at higher temperature or that undergo temperature fluctuations (heat shock) during storage and distribution (Marshall and Arbuckle, 1996). An accurate characterization of particle size distributions is important in the determination of the physical properties of a material (Langton and Hermansson, 1993). Therefore, ice crystal size becomes a critical attribute to the textural properties of ice cream and other frozen products. A reliable and accurate method to quantify the ice crystal population would be also useful for quality control, product development, and basic research application (Donhowe et al., 1991).

In ice cream making, stabilizers have historically been used to prevent recrystallization but their effectiveness has not been consistently proved and their exact mechanism has not been clearly explained. Stabilizers are also used in ice cream to produce smoothness in body and texture, provide uniformity of product and resistance to melting (Marshall and Arbuckle, 1996). Their ability to retard or reduce ice crystal growth during storage has tentatively been explained by their effect on the flow properties of the serum phase, the diffusion properties of the latter or by a weak adsorption onto the ice crystal surface. However, as mentioned before, none of these mechanisms have been conclusively demonstrated.

This study follows a series of studies in this laboratory on the effect of stabilizers in ice crystallization. Caldwell et al. (1992a;b) developed a methodology and studied the microstructure of model systems and ice cream through image analysis using low-temperature scanning electron microscopy (SEM). The effect of abusive storage conditions on the ice crystal size of ice cream were compared to sensory evaluation of the samples. The effect of
stabilizers on the freezing process was also studied in this laboratory. Ice crystallization temperatures and their relationship to thermal events during warming were determined through differential scanning calorimetry (DSC) and thermomechanical analysis (TMA) (Carrington et al., 1994). The influence of stabilizers on the glass transition has been thoroughly studied as well (Goff et al., 1993; Sahagian and Goff, 1994; Carrington et al., 1996). Further studies on this subject were carried out to determine the effect of freezing rate, annealing, physical aging and polysachharide addition on the glass transition and other thermal events during warming of frozen carbohydrate solutions (Sahagian and Goff, 1995a; b).

The overall objective of this research was to accurately characterize the ice crystal population in model solutions and ice cream obtained from image analysis, in order to study the effect of stabilizers in recrystallization during various storage conditions. Thus, a preliminary objective involved determining appropriate sampling parameters for image analysis of crystal distributions obtained using the freeze-etching technique for scanning electron microscopy. This also involved the discussion of different mathematical approaches to characterize ice crystal size distributions.

The objective of studying model solutions was to determine the impact of the different ingredients of ice cream mixes on ice crystal size distributions. Other processing variables, such as rheological behaviour of the cold mixes and overrun of the frozen product, were also studied to determine their effect on the distributions. However, the focus of the study was ice crystal formation, growth and recrystallization characteristics of ice cream as affected by the presence of stabilizers.
2. LITERATURE REVIEW

2.1 Principles of freezing

The removal of heat from any material containing water, e.g., a food, and consequent reduction of temperature to below 0°C is loosely termed freezing. It is a pre-requisite for freezing but, indeed, freezing does not always occur under such conditions. The removal of heat from the liquid will reduce its temperature to below 0°C but it will not necessarily freeze. Freezing is the crystallization of liquid water into the solid form of water known as ice (Reid, 1983).

The freezing rate according to the official definition published by the International Institute of Refrigeration (IIR) in 1971 is (Heldman, 1983):

"The freezing rate of a food mass is the ratio between the minimum distance from the surface to the thermal center and the time elapsed between the surface reaching 0°C and the thermal center reaching 5°C colder than the temperature of initial ice formation at the thermal center; where the depth is measured in cm and the time in hr, the freezing rate will be expressed as cm/hr."

Freezing has always been considered an excellent method for preservation of taste, texture and nutritional value in food products. Most deteriorative processes are temperature dependent so the control of temperatures during distribution, storage and retail plays a crucial role in the quality attributes of the frozen product. Quality cannot be gained but easily lost; therefore, aspects of the freezing process are critical to preserve the integrity of the product throughout the cold chain (George, 1993).

2.1.1 Freezing process
2.1.1.1 Undercooling

Cooling is the reduction of temperature due to the removal of sensible heat. The removal of sensible heat will reduce the temperature of the system below its initial freezing point before crystallization or a phase change is
observed. This process is called supercooling, or preferably undercooling, which results in a thermodynamic unstable state of the system that will eventually lead to the liquid-solid transformation. Thus, the degree of undercooling is dictated by the onset of ice nucleation (Franks, 1985).

2.1.1.2 Nucleation

According to the classic nucleation theory, the formation of stable nuclei is a sequence of processes in which atoms in liquid phase join a growing cluster or embryo. This process can be explained in terms of changes in the free energy of the system during undercooling and can be expressed according to the equation:

\[ \Delta G_{I \rightarrow S} = \frac{4}{3} \pi r^3 \Delta G_v + 4 \pi r^2 \gamma \]  
(Eq.1)

where \( \Delta G_{I \rightarrow S} \) is the net free energy of formation, \( r \) is the radius of the particle, \( \Delta G_v \) is the difference between the solid and aqueous phase free energies and \( \gamma \) being the interfacial free energy per unit area between the ice and unfrozen phase (Sahagian and Goff, 1996).

The first term on the right side of the equation reflects the contribution of volume energy to the equation and has a negative value during undercooling, therefore favouring nucleation. The second term corresponds to the surface energy which constitutes a barrier to growth due to the large surface-to-volume ratio of a small cluster size. Eventually, the increase of the cluster radius allows for the negative value of the volume term to overcome the surface effect and a critical size is achieved. The net free energy of formation reaches a maximum and with the addition of more molecules to the cluster the total energy of formation is reduced thereafter (Sahagian and Goff, 1996; Fennema, 1973).

Once the cluster becomes an active nucleus or a stable seed (acting as a foundation), the process leads to crystal growth. Depending on the composition of the liquid, two types of nucleation may take place:
Homogeneous nucleation

According to the nucleation theory, homogeneous nucleation will occur only in pure systems due to random fluctuations of density (Sahagian and Goff, 1996). Clusters of molecules arise spontaneously due to such density fluctuations. Since the critical radius falls as the temperature is reduced, the probability of nucleation in a pure system (no foreign particles) does not reach unity until a low temperature of approximately -41°C is reached (Blanshard and Franks, 1987). Even though heterogeneous nucleation is more likely to occur in real systems, homogeneous nucleation is more easily accessible for theoretical analysis yet restricted to its idealism (Blond and Colas, 1993).

Heterogeneous nucleation

Heterogeneous nucleation occurs in the presence of a nucleating agent such as the wall of a container, foreign bodies or insoluble material. Water aggregates assemble on the agent which increases the cluster stability and facilitates the nucleation process. Thus, the activation energy required is less at any temperature than in the case of homogeneous nucleation (Sahagian and Goff, 1996).

Certain materials are found to be more effective heterogeneous nucleants of ice. The nucleation behavior of such materials indicate that the probability of a freezing nucleus being present increases significantly with a reduction of temperature. However, the effectiveness of such nucleus will also involve a time-dependent probability (Reid, 1983).

The mechanism of nucleating agents in lowering the activation energy is not fully understood, but the geometry and structure of the material, particularly its surface properties, may explain such effect (Sahagian and Goff, 1996). Carrington et al. (1994) reported crystallization temperatures for carbohydrate solutions of different molecular weights, showing a reduction on the
crystallization temperature as a result of an increase on the freezing point depression. This suggests that colligative properties influence heterogeneous nucleation. Charoenrein et al., (1991) studied the effect of the type and concentration of solute on the nucleation temperature of seeded solutions. They concluded that the effect of the solutes depended on the type of nucleating agent (seed). Therefore, predicting the behaviour of real systems becomes rather difficult due to the complexity of these systems (Sahagian and Goff, 1996).

Secondary nucleation

Nucleation can also be instigated by physical disturbances which will enhance the rate of nucleation or allowing it to take place at a higher temperature. Comparatively, a water sample that is homogeneously nucleated at -41°C, heterogeneously at -4°C, can be nucleated at -0.5°C by secondary nucleation (Fennema, 1973).

The term secondary refers to the formation of secondary nuclei by attrition between existing ones and the walls or impellers of a nucleation vessel (e.g., scraped-surface heat exchanger) causing the seed crystal to break. This explains the increase on the temperature at which nucleation and propagation takes place compared to the other mechanisms (Sahagian and Goff, 1996).

This type of nucleation is also referred to as dynamic nucleation and can be of two types: dynamic true nucleation and pseudo-dynamic nucleation. The first is caused by physical disturbances such as friction, ultrasonic vibration, or a single pressure pulse. The second type is believed to occur as a result of milder disturbances such as stirring, swirling and low-energy ultrasonic treatments (Fennema, 1973).
2.1.1.3 Crystal growth

Crystal growth will occur when the number of water molecules successfully attached to the nucleus is greater than those departing. The rate of attachment will depend on the morphology of the nucleus, i.e., the amount of irregularities (defects) of the lattice which provide favourable sites for new molecules to attach (Fennema, 1973).

Crystal growth is possible once nucleation has taken place. However, this process requires less degrees of undercooling (e.g., 0.01 K) and will occur as long as a stable crystal is present. The rate of growth is mandated by the rate of heat removal which will determine the morphology of the frozen system. Rapid freezing tends to form smaller crystals and, because of the increased freezing rate, nucleation will dominate over propagation. This results in the formation of an increased number of crystals with the concomitant reduction in size. On the other hand, slow freezing will allow the formation of larger crystals because of the opposite reason. The growth will also depend on the direction of heat removal and the medium in which it is being frozen (Reid, 1983).

According to Muhr and Blanshard (1986), two important potential rate determining steps for crystal growth are mass transfer and heat transfer. Since water molecules are small and highly mobile it is unlikely that mass transfer can be a limiting factor during the first stages of freezing. However, during the final stages the viscosity of the unfrozen phase is high, the temperature is low and little water is left, thereby reducing mass transfer and crystal growth consequently. On the other hand, heat transfer can be a reasonable limiting factor just considering the large latent heat of crystallization of water. The crystal growth rate declines as the rate of heat removal is decreased (Fennema, 1973).

2.1.1.4 Recrystallization

Ice crystals formed after freezing are unstable and might undergo changes in time if they have not been stabilized. Recrystallization is the process
of changes in the number, size and shape of crystals during frozen storage. Although the amount of ice stays constant throughout this process, it can alter and damage the structure and stability of frozen food systems (Sahagian and Goff, 1996). Recrystallization basically involves small crystals disappearing (melting), large crystals growing and crystals fusing together (Hartel, 1998).

Recrystallization occurs due to the tendency of the system to reach a lower free energy level, thus moving towards a state of equilibrium (Fennema, 1973). A thermodynamic basis that can be used to explain the recrystallization phenomenon is the Kelvin equation (or Gibbs-Thomson equation) and it can be written for a sphere as:

\[
\Delta T = T^\infty - T(r) = \frac{2 \cdot \sigma \cdot T^\infty}{\rho \cdot \Delta H_f \cdot r}
\]

(Eq. 2)

where \(\Delta T\) is the difference between the melting point for a crystal of infinite size \((T^\infty)\) and that of a crystal of size \(r\) \((T(r))\), \(\sigma\) is the interfacial tension between crystal and solution, \(\rho\) is the density of the crystal and \(\Delta H_f\) is the latent heat of fusion (Hartel, 1998). According to this relationship, small crystals have lower equilibrium melting points (or greater solubilities) than large crystals. The term \(\Delta T\) may also be regarded as the difference between the solid-liquid equilibrium temperature (for a planar interface) \((T_E)\) and the temperature of the sample \((T)\); thus, small crystals have a higher equilibrium melting point \((T_E)\) than large crystals. More simply, the radii of curvature of small crystals cannot bind their surface molecules as firmly as a large crystal; therefore, they show a greater tendency to melt compared to larger crystals (Fennema, 1973; Hartel, 1998).

Given that the constants of this equation \((\sigma, \rho, \Delta H_f)\) do not change with temperature, \(\Delta T\) would correspond to the undercooling due to the radius of curvature effect, which decreases as the size of the crystal increases. The degree of undercooling of smaller crystals and/or the differences between large and small crystals can be used to define the driving forces that explain some of
the recrystallization mechanism from a thermodynamic viewpoint (Hartel, 1998). The recrystallization phenomena can be classified as follows:

**Migratory recrystallization**

Migratory recrystallization refers to the tendency of larger crystals to grow at the expense of smaller crystals, classically called Ostwald ripening. Possible mechanisms involved in this type of recrystallization are melting-diffusion-refreezing or sublimation-diffusion-condensation. At constant temperature and pressure, differences in surface energies will cause smaller crystals to disappear (melt) and larger crystals to grow (refreeze). This occurs at significant rates in systems containing large amounts of crystals with diameters less than 2μm, which is unlikely for a food system (Fennema, 1973).

According to Hartel (1998), in any crystal population the difference between the undercooling of a large crystal and that for a small crystal would result in a driving force \( \Delta T_{\text{small crystal}} - \Delta T_{\text{large crystal}} > 0 \) for small crystals to melt and large crystals to grow. There would also exist a single (critical) crystal size in equilibrium for the storage temperature, thus crystals smaller than the critical size would tend to melt, while larger ones would tend to grow. However, these differences are small in pure water and growth rates in concentrated solutions are even lower (Fennema, 1973). Thus, in a system such as ice cream, crystals larger than 5 μm would not be expected to grow significantly through migratory recrystallization during typical storage temperature and distribution (Hartel, 1998).

However, migratory recrystallization is greatly enhanced by temperature fluctuations (heat shock) and associated vapour pressure gradients. In areas of higher vapour pressure (higher temperature) the smaller crystals may completely disappear and favour the growth of crystals in lower vapour pressure areas (lower temperature). Once the smaller crystals have disappeared they cannot
reappear because of the energy barrier imposed by nucleation (Fennema, 1973).

Melt-refreeze can be considered as a separate category since it can also cause significant changes in ice crystal size distributions. This type of recrystallization occurs specifically due to temperature oscillations and it involves the increase and decrease in size due to the partial melting of the crystal. Melt-refreeze should occur to a greater extent at higher temperatures and more rapidly for smaller crystals (Hartel, 1998).

Iso-mass recrystallization

This type of recrystallization refers to changes in surface or internal structure so that crystals with irregular shapes and large surface-to-volume ratios assume a more compact structure (Fennema, 1973). The driving force can also be explained through the Kelvin equation (Equation 2), if \( r \) is thought of as a local radius of a protrusion from the surface of the crystal. In other words, sharper surfaces are less stable than flatter ones and will show a tendency to become smoother over time (Hartel, 1998).

In ice this is normally referred to as “rounding off” and surface diffusion is thought to play a major role. Another result of iso-mass recrystallization is a reduction of defects within crystals (Fennema, 1973).

Accretive recrystallization

Crystals in contact will naturally fuse together since this results in a reduction on the surface energy of the crystalline phase. Two crystals in close proximity may join since the concentration gradients in the areas between them are high. Thus, material is transported to the point of contact between crystals and a neck is formed. Further “rounding off” of the neck is also explained by the Kelvin equation (Equation 2) since this high curvature surface would have a natural tendency to become planar (Hartel, 1998).
This type of recrystallization is likely to occur in food systems since they normally contain large amounts of ice with ample contact between crystals. The mechanism that likely explains this type of recrystallization is surface diffusion (Fennema, 1973).

Irruptive recrystallization

Aqueous systems will solidify in a partially non-crystalline state under appropriate freezing conditions. If a critical temperature is reached (determined by the solutes present) during warming of the specimen, crystallization will resume abruptly. This type of recrystallization behaviour has also been referred to as “devitrification”. However, this terminology assumes that the frozen specimen has reached a vitreous state upon freezing (Fennema, 1973).

Another possible crystallization phenomenon during storage is due to the increased concentration of the liquid phase, which may lead to co-crystallization of one or more components. However, the viscosity of this unfrozen phase also increases (due to reduction of liquid water) and as crystallization requires appropriate nucleation of the solute, it may not occur at the expected eutectic temperature (Reid, 1983).

2.1.2 Freezing operation

Two aspects must be considered when studying freezing, i.e., the thermodynamic factors which define the equilibrium, and the kinetics of the process which will describe how we approach this equilibrium (Reid, 1993).

An important kinetic factor in the freezing process is the freezing rate. As mentioned earlier, slow rates (<1°C/min.) will induce the formation of larger crystals and in tissue systems most likely at the extracellular level, whereas rapid freezing will result in smaller crystals and uniformly located throughout the product (Reid, 1990). Due to the high rate of heat removal during rapid freezing,
the frequency of nucleation is favoured comparatively to the propagation of crystals (growth). This results in an increased number of crystals with the corresponding decrease of size (George, 1993). Slow rates will lead to maximum dislocation of water, a shrunken appearance of cells in the frozen state, drip loss and tissue shrinkage during thawing. Therefore, the overall food quality is jeopardized by slow freezing rates, opposite to rapid freezing that leads to a higher quality product (Reid, 1990).

Sahagian and Goff (1994) showed that the thermal and mechanical properties of a frozen sucrose solution depended upon the freezing rate. Two thermal events can be defined during warming of the frozen solutions according to changes in the heat capacity, namely $T_{\text{Tr1}}$ and $T_{\text{Tr2}}$. The first transition, $T_{\text{Tr1}}$, corresponds to the glass transition of the freeze concentrated phase (Roos and Karel, 1991). However, the second transition, $T_{\text{Tr2}}$, has not been clearly defined but it is thought to be associated with some type of ordering of water molecules or formation of a critical ice content. Solutions rapidly frozen (quench frozen in liquid nitrogen) had a low $T_{\text{Tr1}}$, measurable divitrification and a reduced mechanical resistance to structural flow; whereas slow freezing (2°C/min) resulted in higher $T_{\text{Tr1}}$, smaller divitrification peaks and higher resistance to structural flow. According to the author, the freezing rate has a dramatic effect on the crystallization process; and, therefore, it would have great implications on the stability of frozen systems (Sahagian and Goff, 1994).

Sahagian and Goff (1995b) also studied the influence of the freezing rate on the stress relaxation behaviour of frozen sucrose solutions using a thermomechanical analyzer (TMA). The calculated relaxation times ($\tau$) of the slowly frozen systems were substantially larger within the apparent glassy range (-55°C up to -40°C) and a considerable shift (5°C) in the spectrum was found when compared to rapid freezing samples. Within the rubbery state (above $T_g$), the dissipation of mechanical energy of rapidly frozen systems occurred over a much shorter time than in slowly frozen systems. These differences suggested that the freezing rate influenced the structural makeup of the frozen system in
terms of an apparent viscous component. This clearly demonstrated the dependency of the rheological response of the frozen system on the freezing rate.

In order to incorporate the freezing rate into the process design it is necessary to express it as a freezing time. The freezing time will determine the freezing capacity of the system according to the residence time in the medium. Some of the factors that need to be considered when estimating the freezing time are the product size, latent heat of fusion, product density, freezing medium temperature, thermal conductivity and the convective heat transfer coefficient (Heldman, 1983).

The quality of frozen products not only depends on the properties of the food product but also on the efficiency of heat transfer from the freezing medium. Slow freezers (e.g. still-air freezers and cold stores) necessitate long holding times due to their low heat transfer coefficient and ultimately resulting in a significant loss of quality. However, an advantage of such a system is their temperature stability to help minimize recrystallization. Quick freezers (e.g., air blast, tunnel or plate freezers) are preferred in industrial scale because of their flexibility, ease of operation and economy for large production volumes. Rapid freezers (e.g., fluidized bed, immersion and scraped-surface freezers) offer an improved quality of the product due to the combination of efficient heat transfer and small size of the product, resulting in rapid ice formation throughout the product. The advantages of immersion freezers (using a cryogen like liquid nitrogen) include rapid freezing, dry processing and uniform temperature distribution (George, 1993).

2.1.3 Stability of frozen food at sub-zero temperatures

At sub-zero temperatures, foods contain a significant amount of unfrozen water that can be responsible for many deteriorative reactions, thus limiting the shelf life (Reid, 1990). Also, freezing and thawing affects the properties of this
unfrozen phase including its pH, titratable acidity, ionic strength, viscosity, freezing point, surface tension, and redox potential (Franks, 1985).

During storage, fluctuations in temperatures may cause important textural defects in ice cream, such as coarse or grainy texture, due to changes in size, shape and number or crystals. This is often the result of migratory recrystallization at constant temperature (Ostwald ripening) or due to temperature fluctuations, in which smaller crystals melt as the temperature rises but do not renucleate as the temperature decreases. This phenomenon can have minimum effects if a low and constant storage temperature is maintained (Goff, 1992b).

Cryostabilization of frozen food has been based on the recognition and manipulation of the glassy state in the unfrozen phase formed by freeze-concentration around the ice crystals (Levine and Slade, 1988). A glass can be characterized as a liquid with an extremely high viscosity \(10^{12}-10^{14} \text{ Pa s}\). A “glassy” or vitreous state corresponds to an amorphous or non-crystalline metastable state of solids. The glass/rubber transition is a kinetic phenomenon that is dependent on temperature and type and concentration of the solute (Slade and Levine, 1991). The glass transition temperature of a polymer (Tg) is affected by the molecular weight, chemical structure, plasticizers (e.g., low molecular additives), branching, cross-linking, and entanglement of the polymer chains (Goff, 1992b).

Above the glass transition temperature a solution can be in the liquid form or it can reach a rubbery state from an increased viscosity due to high concentration of solutes. In this state the system is unstable and reactive. Ice will form as the temperature is lowered during freezing and freeze-concentration of the unfrozen phase will take place. In an “equilibrium” freezing process the system would reach a point of maximum freeze-concentration of the unfrozen phase at \(T=T_g'\) and the glass transition will be achieved with a minimum amount of unfrozen water. However, when foods are subjected to rapid freezing the
system reaches the glass transition line at a temperature lower than Tg' and more glass will form since the unfrozen phase has not been maximally freeze-concentrated. As the glass transition is reached during cooling, the unfrozen phase will reach the glassy state and become completely stable and unreactive as the unfrozen water is kinetically immobilized (Levine and Slade, 1988). This immobilization has been explained as a result of the extremely high viscosity (10^{14} \text{ Pa-s}) of the amorphous phase at temperatures below Tg', hindering diffusion of water molecules to the ice interface. This would ultimately prevent crystal growth and/or recrystallization (Franks, 1985). However, there is evidence from NMR and ESR studies that unfrozen water is fairly mobile probably as a result of inhomogeneity within the sample containing domains with different degrees of mobility. Thus, movement of water should be considered as a factor affecting frozen food stability since it may occur at appreciable rates (Goff and Sahagian, 1996b).

Storage of foods below the Tg' or formulation that raises Tg' would allow cryostabilization during storage and therefore, better stability would be ensured (Goff, 1992b). The importance of Tg resides in the stability that frozen systems achieve when the freeze-concentrated phase is in the glassy state: the structure remains the same as that of the undercooled liquid but the changes in position are almost stopped. However, the relationship between Tg and stability cannot be generalized (Blond, 1994).

2.2 Ice cream
2.2.1 Definition

Frozen dairy products can be frozen to increase their shelf life and thawed for consumption, or they are frozen to develop desired structure and texture, and are consumed frozen. Ice cream, ice milk, sherbet and frozen yogurt fall into the latter category (Goff and Sahagian, 1996a).
The concept of ice cream apparently originated in China and was brought to Europe in 1300 by Marco Polo (Berger, 1990). Ice cream is a palatable, nutritious, healthful and relatively inexpensive food that contains proteins, minerals (e.g., calcium, phosphorus, iron) and vitamins (e.g., vitamin A, thiamin, riboflavin) (Dickinson, 1992). It is made by freezing a pasteurized mix with agitation to incorporate air and to ensure uniformity and consistency (Arbuckle, 1986). It has been described as a partially frozen foam and emulsion in a viscous macromolecular aqueous solution held together by de-emulsified fat and an ice crystal network (Dickinson, 1992). Therefore, it is both a foam and an emulsion.

2.2.2 Ingredients

Ice cream is a mixture of food materials such as milkfat, milk solids-non-fat products (MSN), sweeteners, stabilizers, emulsifiers and flavors. Most of dairy ingredients (Table 2.1) also supply minor amounts of other components, thus a detailed calculation of ingredients is necessary to formulate a recipe.

Table 2.1. Ingredients of ice cream mix and components supplied

<table>
<thead>
<tr>
<th>Component</th>
<th>Ingredient (also supplies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milkfat</td>
<td>Cream (SNF, water)</td>
</tr>
<tr>
<td></td>
<td>Butter (SNF, water)</td>
</tr>
<tr>
<td>MSNF</td>
<td>Skim powder (water)</td>
</tr>
<tr>
<td></td>
<td>Condensed skim (water)</td>
</tr>
<tr>
<td></td>
<td>Condensed milk (water, fat)</td>
</tr>
<tr>
<td></td>
<td>Whey powder (water)</td>
</tr>
<tr>
<td>Water</td>
<td>Skim milk (MSN)</td>
</tr>
<tr>
<td></td>
<td>Milk (fat, MSN)</td>
</tr>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Sweetener</td>
<td>Sucrose</td>
</tr>
<tr>
<td></td>
<td>Corn syrup solids</td>
</tr>
<tr>
<td></td>
<td>Liquid sugar (water)</td>
</tr>
</tbody>
</table>

Source: Goff and Sahagian, 1996a
In North America, the composition of ice cream is standardized and regulated in terms of minimum fat content, total solids content and weight per volume. A typical compositional range for components is listed in Table 2.2.

Table 2.2 Typical compositional range of ice cream mixes

<table>
<thead>
<tr>
<th>Component</th>
<th>Range (% by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milkfat</td>
<td>10-16</td>
</tr>
<tr>
<td>MSNF</td>
<td>9-12</td>
</tr>
<tr>
<td>Sucrose</td>
<td>9-12</td>
</tr>
<tr>
<td>Corn syrup solids</td>
<td>4-6</td>
</tr>
<tr>
<td>Stabilizers / Emulsifiers</td>
<td>0-0.5</td>
</tr>
<tr>
<td>Total Solids</td>
<td>36-45</td>
</tr>
<tr>
<td>Water</td>
<td>55-64</td>
</tr>
</tbody>
</table>

Source: Goff and Sahagian, 1996a

2.2.2.1 Milk fat

The fat fraction of milk consists mainly of triglycerides of fatty acids and exists as an emulsion with a droplet size in the range of 0.5 to 2.0 μm. The fat globule (after secretion) is coated with a membrane comprised of proteins and phospholipids which is broken up as a result of homogenization (Goff and Sahagian, 1996a). Milkfat is liquid above 40°C and usually completely solidified below -40°C. Therefore, during freezing the fat structure is a mixture of crystals and oil (Thomas, 1981).

Milkfat is an ingredient of major importance because it imparts the desired richness of flavor, smoothes the texture by lubrication of the palate, gives body and provides the desirable melting properties in ice cream. Milkfat also provides lubrication in the freezer barrel during manufacture (Goff and Sahagian, 1996a). The best source of milkfat is fresh cream, however there are several other sources such as frozen cream, plastic cream, butter, butter oil and condensed
milk blends. Milk fat does not lower the freezing point and retards the rate of whipping (Arbuckle, 1986).

2.2.2.2 Milk Solids-Non-Fat

Milk solids-non-fat is the solids of skim milk and is comprised of proteins (36.7%), milk sugars (lactose: 55.5%) and minerals (7.8%) (Arbuckle, 1986). Milk proteins, both casein micelles and whey proteins, act as the primary emulsifying agent in the mix by coating the surface of fat globules during homogenization. They also play an important role in the aeration of the mix during freezing avoiding the snowy or flaky texture associated with high amounts of air (Keeney, 1982; Westerbeek, 1996). Goff et al. (1989) concluded that enhanced concentrations of whey proteins led to a desirable increase in fat destabilization during freezing. Proteins contribute to the overall structure of ice cream by improving the whipping characteristics (smaller air bubbles) and imparting texture and body by enhancing viscosity (water-holding capacity) (Goff and Sahagian, 1996a). Lactose, a disaccharide of glucose and galactose, does not contribute much to the sweetness of the mix. It is relatively insoluble and can crystallize producing the defect known as sandiness (Goff and Sahagian, 1996a). However, it is a useful bulking agent (Westerbeek, 1996).

The best sources for these solids are fresh concentrated skim milk or spray-dried skim milk powder. Other sources include (sweetened) condensed milk, (sweetened) condensed skim milk, condensed or powder buttermilk, and dried or condensed whey (Goff, 1992a).

2.2.2.3 Sweeteners

The sweeteners provide the desired palatability, texture and enhancement of flavor, but they also have an important role in the depression of the freezing point. As ice crystals form, the unfrozen aqueous phase increases its concentration progressively lowering the freezing point of that phase. Sugars are the main reason for this effect since they do not dissociate in solution
(ionize), so the freezing point depression will be inversely proportional to the molecular weight of the sugar (Marshall and Arbuckle, 1996).

The most common sweetener used in ice cream is sucrose or a blend of sucrose and corn starch hydrolysates. Corn sweeteners are available as refined corn sugar (dextrose), dried corn syrup or corn syrup solids, and as a liquid corn syrup. They contain dextrose and maltose together with dextrins of varying chain length (Marshall and Arbuckle, 1996). They are obtained from the hydrolysis of starch and are classified according to their total reducing power or “dextrose equivalent”, normally ranging between 28 to 68 DE (Berger, 1990). The higher the D.E. the greater the ratio low to high molecular weight sugars (Keeney and Josephson, 1961).

The use of hydrolysis products (corn syrups or glucose solids) in ice cream provides a firmer and more chewy body, better meltdown characteristics, and reduces the heat shock potential (Goff et al., 1990; Keeney and Josephson, 1961). Their impact on the freezing point depression is less than sucrose since this property is related to molecular weight (Baer and Keating, 1987). They slightly increase the viscosity of the mix due to their higher hydrating capability compared to simple sugars (Goff et al., 1990; Glazier and Mack, 1941).

2.2.2.4 Emulsifiers

An emulsifier is a substance that will aid in the formation of an emulsion of two liquids which do not naturally mix by reducing the surface tension of the system (Frandsen and Arbuckle, 1961). An emulsion is thermodynamically unstable and emulsifiers with polar and apolar properties orient at the oil/water surface stabilizing it. The surfactants used in frozen products can be divided into the following categories: monoglycerides; lecithins; polysorbates; stearoyl lactylates; and sorbitan, sugar, polyglycerol and other esters of fatty acids. In ice cream, they are typically a blend of mono- and diglycerides, and polysorbates (Keeney, 1982).
Emulsifiers in ice cream concentrate on the fat surface with their polar groups at the interface promoting the desired degree of destabilization of the fat emulsion during whipping and freezing (Berger, 1990). They may also act as a stabilizing agent of the foam matrix. More air can be incorporated, and more finely comminuted rendering a more stable foam structure (Keeney, 1982, Arbuckle, 1986). Structural measurements show that they promote somewhat smaller ice crystals and more evenly distributed as well (Arbuckle, 1986). Thus, they impart a smoother texture and stiffer body, they reduce the whipping time and they give a uniform whipping quality (Arbuckle, 1986).

2.2.2.5 Stabilizers

Gelatin was the first stabilizer used in ice cream. Many polysaccharide stabilizers have been used since then, including sodium carboxymethyl cellulose (CMC), microcrystalline cellulose, sodium alginate, carrageenan, furcelleran, agar, pectin, xanthan gum, locust bean gum (carob) and guar gum (Berger, 1990). Guar gum and carrageenan are normally used in ice cream formulation, but CMC, locust bean gum and alginate are frequently found on package labels (Keeney, 1982).

The stabilizers are added in relatively small amounts (0-0.5%). They increase the viscosity but have no effect significant on the freezing point. They have a high water holding capacity which is effective in smoothing the texture and giving body to the product, but their major role is to prevent crystal growth as temperature fluctuates during storage. In summary, stabilizers (1) improve smoothness of body, (2) retard or reduce recrystallization of ice and lactose crystals during storage, (3) slow down moisture migration from the product to the package or the air, (4) give uniformity of product and hold flavouring compounds in dispersion (e.g., ripple sauces), and (5) give desired resistance to melting (Goff and Caldwell, 1991; Arbuckle, 1986).

In addition, some stabilizers (especially carrageenan) are traditionally used as a secondary stabilizer. The primary stabilizers can interact with milk
proteins and cause precipitation of the reacted complex, normally called wheying off. Carrageenan is an efficient stabilizer against the precipitation of proteins due to interactions with other polysaccharides (Goff and Caldwell, 1991). The reactivity of some stabilizers with milk proteins has been studied by Schmidt and Smith (1992a). In this study, guar gum, xanthan gum and κ-carrageenan showed some degree of reactivity with casein and whey proteins depending on their concentration and heat treatment. The combination of polysaccharide-protein rendered higher viscosities compared to their counter part without milk proteins (Schmidt and Smith, 1992a,b). Wittinger and Smith (1986), also showed the interaction between corn syrup (36 DE) and guar gum to increase viscosity and chewiness of ice cream.

However, some of the limitations on the use of stabilizers include undesirable melting characteristics of the product (slow melting and gummy texture), excessive mix viscosity, and contribution to a heavy, soggy body (Goff and Sahagian, 1996a).

2.2.3 Processing

The steps involved in ice cream making are: mixing, pasteurization, homogenization, cooling, aging, freezing, hardening and storage. The first 5 steps can be considered as the mix preparation for freezing and they strongly determine the behaviour of the mix during the most critical step, freezing.

2.2.3.1 Mix preparation

The liquid and solid ingredients are normally blended and dispersed into a jacketed tank with strong agitation. Dispersion and activation of the stabilizer is essential since it determines the viscosity of the mix (Doan and Keeney, 1965). In modern facilities the dry ingredients are injected through a pumping system and the process is completely automated (Goff and Sahagian, 1996a).

The purpose of pasteurization is to render a product with minimum amounts (if none) of harmful bacteria. However, it also aids in the blending and uniformity of the liquid mix (Desrosier and Desrosier, 1977). Pasteurization can
be carried out as a batch process or in continuous pasteurizers, such as HTST (high temperature, short time) systems. Legal regulations require for a batch process to be done at least at 69°C and held for 30 minutes. HTST pasteurization requires a minimum of 25 seconds at 80°C. Different time-temperature combinations can be used to comply with legal regulations (Goff and Sahagian, 1996a). Special attention should be paid to the increased viscosity of stabilized systems when considering holding times in HTST systems (Goff et al., 1994).

During homogenization the mix is converted into a true emulsion with fat globule sizes of less than 2μm. Homogenization also allows a thorough blending of the mix; disperses the fat globules, thus preventing churning during freezing; improves the texture and palatability of the ice cream; reduces ageing, aids incorporating air; and produces a more uniform mix (Desrosier and Desrosier, 1977). Variations on the homogenization pressures may also influence the viscosity of the mix, the destabilization of the fat during freezing and the gloss of the products (Schmidt and Smith, 1989). Typical homogenization pressure are 15.5-18.9 MPa (2000-3000 psig) for a first stage and 3.4 MPa (500 psig) if a second stage is used (Goff and Sahagian, 1996a).

The mix is then cooled to 4°C as rapidly as possible to prevent bacterial growth. The mix is then allowed to age from 4 to 24 hours to allow the hydration of the stabilizer. As a result, the fat begins crystallizing, proteins and emulsifiers continue to adsorb onto the fat globules, viscosity increases and mix ingredients become more stable (Desrosier and Desrosier, 1977; Marshall and Arbuckle, 1996). The ageing of the mix allows for a complete hydration of the milk proteins, especially if dried milk products were used (Thomas, 1981). This step is believed to be necessary particularly when gelatin is used as a stabilizer. Sodium carboxymethyl cellulose and guar gum (like most vegetable stabilizers) set up immediately upon cooling. However, carrageenan (used to prevent whey separation during long-term storage) hydrates slowly when compared to other
stabilizers. In general, 4 hours ageing is considered ample but many plants age mixes overnight (Desrosier and Desrosier, 1977; Marshall and Arbuckle, 1996).

It has been demonstrated that emulsifiers are capable of displacing some of the amphiphilic proteins (e.g., caseins) from the interface adsorbed after homogenization, i.e., during ageing. This leads to a reduction of the interfacial tension with a concomitant increase in fat destabilization during freezing (Goff and Jordan, 1989).

2.2.3.2 Freezing and hardening

As mentioned earlier, freezing is the most important step for upon it depends the quality, palatability and yield of the finished product. The dynamic freezing process consists of two stages: the freezing of a portion of the water; and the incorporation of air into the system. As the temperature in the scraped-surface heat exchanger is lowered, the freezing point of the mix is reached (usually about -2.5°C due to the total solids), ice starts forming and extensive nucleation (particularly secondary nucleation) is promoted by the high shear in the barrel of the heat exchanger. Removing liquid water as ice from the system further concentrates the serum phase increasing the freezing point depression of the mixture. Therefore, only 33-67% of the water is frozen in this step of the freezing process, depending on the drawing temperature and composition of the mixture (Arbuckle, 1986).

In a typical commercial scraped-surface freezer, ice crystals are formed near the walls of the barrel and most likely grow in the shape of dendrites inwards (towards the center of the barrel). The scraper blade clips off these crystals and disperses them into the center of the freezer. Due to the temperature gradient across the barrel (from the wall to the center), the temperature of the center is higher, allowing for crystals to ripen into block shaped ice crystals. The dasher type and speed may also influence the crystallization phenomenon. A low displacement dasher promotes the ripening of the crystals (longer residence time), hence larger ice crystal mean sizes would
be expected. The inverse effect would be expected from a higher displacement dasher (Hartel, 1996).

The dynamic whipping incorporates air into the mixture and is also responsible for creating a fat network: as air is incorporated part of the protein layer around the fat globules preferably adsorbs onto the new air cell surface. As a result, the fat globules spread onto the air surface as well. The high shear of the system continuously breaks up the air cells causing partial coalescence of the fat and thus creating a support network for the foam structure. The emulsifiers also play an important role in the fat destabilization by replacing part of the proteins around the small fat globules after homogenization (Goff and Jordan, 1989; Goff and Sahagian, 1996a). Fat agglomeration and solidification occurs within the first seconds of freezing but the degree of churning will not only depend on the type of fat but also on the freezer temperature (Sherman, 1965).

The whipping action increases the frequency of collision of the fat particles as well as their rate of separation (Sherman, 1965), but the mechanical action also allows for the incorporation of air. As a result, the product expands to approximately 190% of its original volume (Desrosier and Desrosier, 1977). This is termed the overrun and is calculated as:

\[
\% \text{ Overrun} = \frac{\text{Vol. ice cream} - \text{Vol. mix used}}{\text{Vol. mix used}} \times 100\%
\]  
(Eq. 3)

The temperature of extrusion determines the amount of ice formed in ice cream. In a continuous system, the temperature can be adjusted according to the capacity of the freezer and the flow rate of the product. In a batch process, the dwell time can also be adjusted to obtain desired overrun and fat destabilization, yet the refrigeration system has a limited capacity imposing a restriction to the drawing temperature. The effect of the extrusion temperature on the ice content of ice cream (36% solids) is shown in Table 2.3.

Table 2.3. Influence of temperature on the percentage of
water as ice in ice cream (36% solids)

<table>
<thead>
<tr>
<th>Extrusion temp.</th>
<th>Water frozen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(°F) / (°C)</td>
<td></td>
</tr>
<tr>
<td>25 / -3.9</td>
<td>33</td>
</tr>
<tr>
<td>24 / -4.4</td>
<td>31</td>
</tr>
<tr>
<td>23 / -5.0</td>
<td>47</td>
</tr>
<tr>
<td>22 / -5.6</td>
<td>52</td>
</tr>
<tr>
<td>21 / -6.1</td>
<td>56</td>
</tr>
<tr>
<td>20 / -6.7</td>
<td>59</td>
</tr>
</tbody>
</table>


The type and concentration of sweetener also plays an important role in the ice phase behaviour during freezing. The freezing point depression of the mix due to the presence of sweeteners affects the ice phase volume at a given temperature. Thus, a higher concentration of sweetener results in a lower ice phase volume and average ice crystal size (Hartel, 1996). The freeze concentration process gradually increases the concentration of the sweetener in the unfrozen phase, further reducing the freezing point of this phase, thus limiting the amount of water frozen.

In fact, all ingredients may affect the ice phase volume beyond the reduction in the total water content. An equation (Equation 3) currently used to predict the ice phase volume for mixes at any given temperature considers the binding coefficients ($BC_0$, $BC_1$) of each solid as an estimation of the effect on the freezing point depression (Ablett, 1998). Therefore, it should be noted that this equation is applicable only to those solids that may contribute to the freezing point depression. The generic equation for any number of solids can be written as:
\[ IC(T) = \text{Water Content} - \sum_i \left( \frac{\text{Weight}_i \times \%}{100} \right) \left( BC_0 + \frac{BC_i}{T} \right) \left( 100 - \text{Moisture}_i \% \right) \]  
(Eq. 4)

where \( IC(T) \) is the ice content at temperature \( T \) (°C) (w/w), the water content corresponds to the total content before freezing, \( i \) represents each ingredient, according to their concentration in the mix, moisture content and respective binding coefficients, \( BC_0 \) and \( BC_1 \). The following is a list of the binding coefficients and typical moisture content of some ingredients in ice cream (Ablett, 1998). It should also be noted that due to their low concentrations and low impact on the freezing point depression, stabilizers and emulsifiers are not considered for their binding coefficients.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>( BC_0 )</th>
<th>( BC_1 )</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk powder</td>
<td>0.1308</td>
<td>-4.8288</td>
<td>3.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1939</td>
<td>-5.6596</td>
<td>0.0</td>
</tr>
<tr>
<td>40 DE CSS</td>
<td>0.1018</td>
<td>-2.6045</td>
<td>5.0</td>
</tr>
</tbody>
</table>

After drawing, ice cream has a semi-solid consistency and is not stiff enough to hold its shape. The freezing process is completed when the frozen aerated product undergoes a hardening process and the product reaches -18°C or lower, preferably -25°C to -30°C. Quick hardening is desirable since it prevents the formation of larger crystals as a result of recrystallization. Factors that affect this rate include temperature differential; conductance properties of the product, container, and the cooling plate (when used); and the distance for heat to travel (Marshall and Arbuckle, 1996).

Common facilities designed for this purpose include cabinets, cold cells within storage rooms, hardening tunnels, and contact plate freezers. Cabinets
are normally used in small size operation in which air is circulated in a closed
environment to speed the heat removal. In the cold cells, chilled air is blown over
newly arrived packages as they are conveyed to the storage area. In some
installations, the same is achieved using a hardening tunnel equipped with
conveyors, trays and belts. In the latter, the product is exposed to very cold air
(-30°C or lower) at very high velocity. Contact plate freezers quickly lower the
temperature of the product by conducting heat from the packages.

During hardening the water will continue to freeze and crystal growing
takes over from nucleation. This step can be accountable for an additional 23-
57% of increase of the ice phase depending on the drawing temperature
(Aruckle, 1986). The low temperature of this stage of the process does not
allow for new ice crystals to form so the increase of the ice phase volume results
in growth of the present crystals. Typically, ice crystals in ice cream exit the
freezer with a mean size of -30-35 μm, increasing to -45-50 μm after hardening
(Hartel, 1996). However, according to the recrystallization mechanisms
previously described, recrystallization may take place immediately after draw.
Thus, rapid hardening is critical to maintain the number of crystals (small
crystals) obtained from the initial freezing process. On the other hand, an
extremely rapid hardening rate may be detrimental to the product since a non-
equilibrium phase volume may be achieved (vitrification). As the temperature of
the product is raised (devitrification), an increase in the ice phase volume
causes existing crystals to grow larger (Hartel, 1998). Thus, crystal growth or
recrystallization may not be prevented by an extremely high hardening rate.

2.2.4 Ice cream structure

2.2.4.1 Physical structure

The physical structure of ice cream is a complicated physico-chemical
system affected primarily by the presence of unfrozen material, de-emulsified fat,
ing crystals and air cells (Aruckle, 1960). Structurally, ice cream is regarded as
a partly frozen foam with an air content of 40 to 50% by volume (Shama and Sherman, 1966). Air cells are dispersed in a continuous liquid phase with ice crystals embedded in it. This continuous phase contains solidified fat globules, soluble milk salts, lactose and added sugars, and stabilizers (Arbuckle, 1960; Shama and Sherman, 1966). Thus, ice cream contains three phases: liquid (unfrozen phase), gaseous (air) and solid (ice). However, ice cream is typically referred to as a four phase system if one considers fat as a separate phase since it is a discrete component of the microstructure.

Flocculation and coalescence of fat globules have a profound effect on the structure of ice cream (Keeney, 1982). Air cells may be excessively ruptured if large fat clumps are present initially or formed in early stages of the freezing process. However, if the process is properly controlled, as the viscosity increases air cells become less vulnerable to rupturing and the fat structure formed aids in the incorporation and formation of small air cells. Emulsifiers, if properly used, also have this effect: more air cells are formed with the concomitant increase of surface area (Thomas, 1981).

Shama and Sherman (1966) studied the rheology of ice cream and compared it to pure ice. They showed that the introduction of fat and air changed the rheological properties of the frozen product beyond the effect of the degree of undercooling. According to their creep measurements, they also concluded that the air content caused far greater changes in the rheological parameters of ice cream by comparing the parameters for pure ice and those considering ice cream as an ice crystal network. They reported that with an increasing overrun the air cells, the ice crystals and fat agglomerates become smaller (Sherman, unpublished data).

According to Thomas (1981), an increase in air-cell dispersion causes a reduction in the thickness of the unfrozen phase. This would result in limiting the size of ice crystals by mechanical hindrance of the numerous air cells and the fat structure. Hartel (1996) stated that low overrun induce the formation of coarser
ice crystals in ice cream compared to an equal formulation made at higher overrun. During freezing in a scrape-surface freezer air cells may provide a physical impediment to ice crystallization (Hartel, 1996).

Goff et al. (1995a) studied the impact of overrun in the rheological properties of ice cream. Their studies indicated that air contributes to the elastic component of the frozen product. Increasing overrun (20-60%) had a significant effect on the storage modulus ($G'$) yet less effect on the loss modulus ($G''$). Further incorporation of air (60-100%) had no impact on these moduli indicating that a critical air volume had been achieved. According to the author, most likely at such point air bubbles are tightly packed, begin to interact with each other through the fat network, and the increase in overrun causes a volume expansion but has less impact on its structure. Samples had the same size, thus at higher overruns they would contain less ice per unit volume, which would be reflected in the viscoelastic behaviour of the sample. Therefore, the air bubble/fat network structure would be responsible for this elastic response. In this same study, large-scale deformation testing of ice cream showed that puncture force decreased as a result of an increased overrun. Thus, ice cream with a higher air content was more easily deformed under stress.

Microscopical examination of ice cream by different workers has allowed the determination of the typical average sizes of the structural items (Table 2.5). Arbuckle (1960) used light microscopy to study thin sections embedded in an immersion liquid on a microscope slide. This methodology required the equipment to be kept in the hardening room, imposing uncomfortable conditions to carry out these measurements. Similar procedures were utilized to obtain those values compiled by Berger and White (1979). Caldwell et al. (1992a) used a scanning electron microscopy (SEM) technique to study ice cream microstructure. The freeze-etching technique using cryo-SEM allows for the preparation of the frozen samples to be carried out by immersion in a cryogenic liquid, simplifying the sampling procedure and microscopical examination. The
latter also allows for a more accurate determination by the increased number of samples and measurements that can be performed.

Table 2.5. Average sizes of structural items in ice cream

<table>
<thead>
<tr>
<th>Item</th>
<th>Arbuckle, 1960</th>
<th>Berger and White, 1979</th>
<th>Caldwell et al., 1992a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat globules</td>
<td>0.2-2</td>
<td>0.05-2.00</td>
<td>0.15-1.5</td>
</tr>
<tr>
<td>(range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice crystals</td>
<td>45-55</td>
<td>44.3</td>
<td>40</td>
</tr>
<tr>
<td>(average diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air cells</td>
<td>110-185</td>
<td>30</td>
<td>10-60*</td>
</tr>
<tr>
<td>(average diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfrozen Material</td>
<td>6-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(average thickness)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Range

The values obtained for the fat globules and ice crystals by the different authors are comparable. However, there is a noticeable discrepancy in the air cell average diameter obtained by Arbuckle (1960).

Other researchers have also developed other methodologies to study the microstructure of frozen systems. Donhowe and Hartel (1996a) studied ice crystal distributions of frozen desserts using an optical microscope housed in a glove box insulated with styrofoam and cooled with a Freon-12 refrigeration system. Sutton et al. (1994) studied ice crystal distributions of sugar/stabilizer solutions also using an optical microscope equipped with a cold stage. In the latter, products were transferred from the cold stage to a cryostat held at -20°C and a small sample (monolayer of ice crystals) was spread onto a microscope slide. White spirit was used to render the air and fat phase invisible (similar refractive index), a cover slip was placed on top of the slide and transferred to the microscope using a cooled (cold nitrogen gas) and insulated conveyor
system. Samples were viewed under brightfield with crossed polars and a lambda plate.

2.2.4.2 Microscopical examination of ice crystal distributions using low-temperature scanning electron (LT-SEM) microscopy and image analysis

Internal structure using microscopical techniques dates back to 1915. It was originally intended as a tool to illustrate or for visual comparison. However, it has grown into a technique capable of determining size and distribution of ice crystals and air cells (Arbuckle, 1960). Many authors have reviewed the different techniques that have been used to determine ice cream structure (Arbuckle, 1960; Berger and White, 1979; Berger, 1990), such as embedding thin slices of ice cream in liquids with particular refractive index for light microscopy, using microslides and polarized light to determine ice crystal sizes of a squash mount, fixing ice cream or melted ice cream with reagents for electron microscopy, freeze replication for transmission electron microscopy (TEM), etc.

LT-SEM represents a suitable technique to study the structure of a frozen sample. The specimens are immersed in a cryogenic liquid, such as liquid nitrogen (-210°C), resulting in a fully hydrated and intact sample. This avoids any structural damage because of the microscopical technique or the introduction of artifacts due to fixation chemicals (Caldwell et al., 1992a). Thus, it can provide valuable information about the air cells and ice crystals, their sizes and distributions, without the possible structural impact that other techniques may impose.

The great advantage of microscopical determination of particle size is that it provides a permanent picture of the sample allowing to identify particulate matter and discern its origin. It provides the size, shape and morphology of particles within a specimen, and a direct observation of the sample which gives a certain degree of credibility and confidence in the results (Yamate and Stockham, 1979). With an appropriate image analysis procedure, a large
number of data can be generated for an accurate determination of the ice crystal distribution.

Most of the research published regarding ice crystal sizes consider the average diameter as the descriptor of the distribution (Caldwell et al., 1992b; Moorty and Balachandran, 1994; Donhowe and Hartel, 1996a; Hagiwara and Hartel, 1996; Miller-Livney and Hartel, 1997; Sutton et al., 1997). However, even though the average diameter is the hypothetical particle that represents a distribution and the standard deviation can be used as a measurement of the dispersion (Stockham, 1979), it is only an adequate descriptor of the distribution if the latter corresponds to a normal or Gaussian distribution. Some researchers have reported normality for ice crystal distributions. Smith and Schwartzberger (1985) measured the ice crystal distribution in sugar solutions and showed that the number of crystals of a given diameter follow a Gaussian distribution. Caldwell et al. (1992b) also reported normality for the ice crystal distribution of ice cream samples.

However, most fine particle systems have particle size distributions described by the log-normal distribution function (Stockham, 1979). Donhowe et al. (1991), studying ice crystals in frozen desserts, reported that the data fit the log-normal distribution best when compared to the normal (Gaussian) or gamma distributions. According to these researchers, the fitting of a model to these distributions can be a useful tool to study the crystallization phenomena by calculating relevant distribution parameters.

2.3 Polysaccharide hydrocolloids (Gums)
2.3.1 Definition

The word "gum" is derived from the Egyptian term quernai or kami and it refers to the exudation of the Acanthus plant. Gums are not colloids but rather polymers of colloid size (10Å to 1000Å) that can be divided in 2 categories: water soluble and water insoluble. A more scientific designation often used for
these polymers is "hydrophilic colloid", preferably contracted to "hydrocolloid" (Glicksman, 1982a).

Gums used as food additives come from a variety of sources including plant materials (e.g., seaweed, seeds and tree exudates), as a product of microbial biosynthesis, or by chemical modification of naturally occurring polysaccharides (Anonymous, 1991).

Most hydrocolloids happen to be polysaccharides. In the solid state, all polysaccharides have amorphous regions and therefore, have numerous hydrogen bonding positions that can easily hydrate. When placed in water, the water molecules quickly penetrate these amorphous regions binding to available polymer sites. Thus, segments of the polysaccharide chain become fully solvated and move away by kinetic action breaking up more interpolysaccharide bonds. Soluble polysaccharides continue to hydrate until the polymer is monodispersed. However, many polysaccharides do not hydrate beyond an intermediate gel state and remain as incompletely dispersed molecules (Glicksman, 1982a). For effective dispersion and hydration, an adequate separation of the gum particles in the dry powder is necessary. There are many options to prevent the agglomeration of the particles when incorporated into an aqueous system, an efficient mixer being the most important. Gums can be pre-blended with other dry ingredients of the formulation and sugar is particularly useful in this respect (Sanderson, 1996).

These long chain polymers find their importance in food products due to their thickening or viscosity-producing effect. The degree of thickening depends upon the type of gum but generally small amounts are required (well below 1%) to render high viscosities. A few gums also have the ability to form gels by cross-linking their chains to form a three-dimensional continuous network that immobilizes water, a structure resistant to flow under pressure (Glicksman, 1982a).

A food stabilizer can be defined as a material that extends the useful storage life of a food product. Such material reduces the rate at which certain
types of physical changes occur during storage, transport and normal handling of the food product. Most of the stabilizers in food are gums, because they are capable of inhibiting undesired physical processes such as crystallization, gravitational separation, coalescence, syneresis in gels, etc. (Walker, 1984). By inhibiting these processes gums can stabilize dispersions, emulsions and foams and are sometimes referred as suspending agents. However, for the same reason they can be mistakenly referred to as emulsifiers and foaming agents (Sanderson, 1996).

Gums have functional properties in food in addition to their functionality as stabilizers. They can have subtle effects on the perception of flavour and texture by consumers. Adhesive, thickening and whipping agents are added primarily because of their effect on textural perception (Walker, 1984). Furthermore, when gums are used to impart viscosity in a system they also provide texture, body and mouthfeel (Sanderson, 1996). Thus, it is fair to state that the functionality of gums includes the stabilization and perception of texture (Walker, 1984).

These stabilizers can also impart freeze/thaw stability through their ability to bind water. However they do not bring about changes in the water activity of the system. Water activity is a colligative property and, therefore, dependent on the number of molecules. Stabilizers having a high molecular weight and used in low levels provide relatively few molecules to the aqueous phase (Sanderson, 1996).

2.3.2 Rheology of hydrocolloid solutions

As mentioned earlier, one of the main applications of gums in food products is as a thickening agent. Hydrocolloid solutions can be characterized according to their rheological behaviour, which in turn can be related to organoleptic acceptance, i.e., mouthfeel. The addition of hydrocolloids to an aqueous system will most likely render a non-Newtonian behaviour. Non-Newtonian flow implies that the shear stress is not directly proportional to the
shear rate, in other words, the viscosity value depends on the shear rate applied (Glicksman, 1982b). This non-Newtonian behaviour in macromolecules in general is caused by the increased orientation of asymmetric molecules and/or changes in the shape of flexible molecules with shear rate (Sharma, 1981). Furthermore, most hydrocolloid solutions exhibit a shear thinning behaviour, regarded as pseudoplastic. In this type of flow, the system flows more readily as the shear rate increases, i.e., the viscosity decreases with increasing shear rate (Glicksman, 1982b).

The general relationship between shear rate and shear stress is best described by the general power law equation which covers all types of flow behaviour:

\[ \tau = \tau_0 + K \gamma^n \]  

(Eq. 5)

where \( \tau \) = shear stress, \( \tau_0 \) = yield stress, \( K \) = consistency index, \( \gamma \) = shear rate, and \( n \) = flow behaviour constant (Glicksman, 1982b). A particular model generally used to describe the rheological behaviour of gums in solution is the Herschel-Bulkley model (Abdelrahim et. al, 1994). This model considers the existence of the yield stress (\( \tau_0 > 0 \)) and flow behaviour constants between 0 and 1, characteristic values for pseudoplastic behaviour. The Newtonian equivalent for this model is the Bingham plastic model since it considers a flow behaviour constant of 1, as well as the existence of a yield stress. However, because of this yield stress, the viscosity becomes dependent on the shear rate and therefore should not be considered a Newtonian behaviour by definition. In both cases, the apparent viscosity would be described according to equation 6 (Steffe, 1992).

\[ \eta_{app} = \frac{\tau}{\gamma} = \tau_0/\gamma + K \gamma^{n-1} \]  

(Eq. 6)

2.3.3 Gums of interest
2.3.3.1 Carboxymethyl cellulose (CMC)
Cellulose is the principal structural component of cell walls in plants, a homoglanucan composed of linear chains of (1→3)-β-D-glycopyranosyl units (Whistler and Daniel, 1990). Cellulose gums are a family of products derived from chemical modification of cellulose. The backbone of cellulose is comprised of cellobiose units which can be converted into an ether by an alkaline treatment. The length, number and nature of these ethers influence the solubility, gelling and thickening properties. Sodium carboxymethyl cellulose commonly called CMC is one example of these compounds (Anonymous, 1991). A schematic of the structure of CMC as obtained from chemical modification is shown in Figure 2.1.

CMC is normally used to thicken, suspend, and stabilize solutions and can even be used as a bulking agent because of its high water binding capacity (Sanderson, 1981). The negatively charged carboxyl groups confer solubility and at the same time are in part responsible for its thickening ability due to repulsion between these groups. A distinctive advantage of using CMC as a multipurpose thickener is that it produces clear solutions (Sanderson, 1996). It dissolves in water to form a non-Newtonian solution and helps solubilize common food proteins such as gelatin, casein and soy protein (Whistler and Daniel, 1990).

2.3.3.2 Guar Gum

Guar gum is obtained from the ground endosperm of the guar plant, Cyamopsis tetragonolobus, native to India and Pakistan. Guar gum consists of a β,1,4-linked chain of D-mannopyranose units, with side chains of a single α,1,6-linked D-galactopyranose units in the ratio of one galactose per every two mannose units (Biswas et al., 1975; Anonymous, 1991). Due to this high degree of substitution it is soluble in cold water (Sanderson, 1996). A schematic of the chemical structure of this molecule is shown in Figure 2.2.
Figure 2.1 Chemical structure of sodium carboxymethyl cellulose (Source: Anonymous, 1991).

Figure 2.2 Chemical structure of guar gum (Source: Caldwell, 1992)

Figure 2.3 Chemical structure of xanthan gum (Source: Caldwell, 1992)
This galactomannan gives a highly viscous solution at low concentrations so its wide-ranging applications render it the most widely used food thickener. This is also because of its relatively low cost (Sanderson, 1996). Ice cream, fruit drinks and salad dressings are examples of products commonly stabilized using guar gum. It is also a very efficient water binder used in products such as canned meat, pet food and icings (Sanderson, 1981). Since it is nonionic it is very stable from pH 4 to 10. Guar gum is used to impart creaminess because it tends to be slightly slimy in the mouth (Anonymous, 1991).

A similar galactomannan frequently used in ice cream formulations is locust bean gum, also called carob seed gum. It also corresponds to a neutral polysaccharide and, like guar gum, is made up of a mannose backbone and a single-unit galactose side chain. However, it presents a lower substitution level (mannose/galactose, 4:1) rendering it insoluble in cold water and must be heated to be dissolved. It does not form a gel by itself yet it can bring about gelation when combined with xanthan gum or carrageenan (Anonymous, 1991; Sanderson, 1981; 1996).

2.3.3.3 Xanthan Gum

Xanthan gum is a high molecular weight extracellular polysaccharide produced by the organism Xanthomonas campestris. It is comprised of a cellulosic backbone with charged side chains so it corresponds to an anionic polyelectrolyte (Launay et al., 1984). Because of this particular molecular architecture (Figure 2.3), the molecule in solution exists as a rigid rod stabilized by interactions between the backbone and the side chains (Sanderson, 1996).

Xanthan gum was the first microbial polysaccharide to be approved for foods (Sanderson, 1996). It is readily dispersed in water due to its cellulosic nature so high viscosity can be obtained rapidly in hot or cold systems. Thus, this gum finds its application in products such as milkshakes, bakery fillings, sauces, beverages and desserts. Other applications rely on the unusual rheological behaviour such as high pseudoplasticity (highly shear thinning) and
yield value as a result of intermolecular associations. Xanthan gum solutions thin under shear in the mouth and hence flavor release is excellent (Sanderson, 1981).

2.3.4 Effect of stabilizers in ice crystallization

The following is a compilation of studies regarding the effect of stabilizers on the crystallization of water in sugar systems and ice cream, particularly those related to crystal growth and recrystallization of ice cream.

2.3.4.1 Properties of frozen systems

The freezing point of a solvent is the temperature at which solid and liquid coexist in equilibrium. Addition of solutes lowers the freezing point depending on the amount of solute initially dissolved and the amount of crystallized solvent (Fennema, 1973). According to Raoult's law, the depression of the chemical potential of the ideal solution is proportional to the molar fraction of the solute. In non-ideal solution, the depression has shown to be greater than the one predicted by Raoult's law. According to Blond (1985), the addition of polysaccharides, such as carboxymethyl cellulose (CMC) and methoxyl pectin, resulted in a depression of the freezing point, effect that increased as the concentration of the polysaccharide increased.

Shipe et al. (1963) studied the effect of a number of stabilizers (gelatin, sodium alginate, agar, guar gum and Irish moss) on the freezing characteristics of aqueous systems, namely water and milk. They reported an increase in the freezing point depression (0.01 to 0.1°C) caused by gelatin in both systems. Similar results were reported by Budiaman and Fennema (1987a) on the freezing point depression of aqueous solutions of different stabilizers (CMC, guar gum, locust bean gum, sodium alginate, tragacanth gum, carrageenan and gelatin). Only a slight increase of 0.01°C was observed for hydrocolloids in concentrations up to 2.85%.
Shipe et al. (1963) also reported that the addition of stabilizers, in general, decreased the initial rate of rise in temperature, increased the length of the freezing point plateau and decreased the final rate of fall in the two systems. The length of the freezing point plateau was related to the concentration of stabilizers and therefore to the viscosity of the system. The authors proposed that such effects would ultimately affect the rate of crystallization and heat transfer via resistance to thermal changes, i.e., resistance to convection currents.

Miller-Livney and Hartel (1997) reported an unexpected decrease on the \( T_f \) of different combinations of sweeteners and stabilizers in ice cream and compared them with calculated values. A large deviation from the predicted values was found in mixes containing high fructose corn syrup (HFCS) and explained by the limited validity of such calculation, applicable to diluted systems. This is particularly true for ice cream since the high concentration of solutes deviates the behaviour from being ideal and the influence of non-soluble material (e.g., fat) will exert significant differences on the freezing characteristics (Heldman, 1974).

Biswas et al. (1975) studied the effect of carbohydrates on the heat of fusion of water. Their results showed that carbohydrates such as glucose and sucrose lower the molar enthalpy of fusion (\( \Delta H_f \)) in a lesser degree than high molecular weight polysaccharides such as guar gum and CMC. According to these researchers, this effect cannot be attributed to impurities or a change in colligative properties but to solute/solvent interactions. Buyong and Fennema (1988) arrived at the same conclusion when studying similar hydrocolloid suspensions. Considering that \( \Delta H_f \) is due only to water, these results would imply that water associated with the stabilizers ("bound water") with a concomitant reduction on the amount of freezable water. This is particularly true for high molecular weight stabilizers. Their molecular structure allows them to bind more water than an equal amount of a monosaccharide (Biswas et al., 1975). However, Muhr and Blanshard (1984) reported no effect on the addition
of manucol (sodium alginate with mannanuronate) to sucrose solutions on the $\Delta H_f$ of water, concluding that stabilizers in themselves cannot significantly reduce the amount of ice formed in frozen confectionery at a given temperature. Similar conclusions were drawn by Buyong and Fennema (1988) since the addition of gelatin to ice cream showed no difference in the $\Delta H_f$ of the mix.

Budiaman and Fennema (1987b), using a U-tube, studied the effect of the viscosity of hydrocolloid suspensions on the linear rate of crystallization (LRC) at $-3^\circ$C. Viscosities of aqueous solutions of different stabilizers (CMC, guar gum, locust bean gum, sodium alginate, and tragacanth gum) and combinations of them, and stabilizer/sucrose systems were measured at $0^\circ$C. In all instances, increased viscosity decreased the LRC and the extent of the effect was dependent on the type and combination of solutes, CMC and guar gum combined with sucrose having the greater impact. Viscosities in the high range (3 Pa·s, closest to the high viscosity of the unfrozen phase) also demonstrated that molecular size was not a factor in the reduction of the LRC. CMC having a larger molecular weight than guar gum, locust bean gum and alginate, was less efficient in retarding crystallization. Also, sucrose proved to be a far more effective crystallization retardant than any stabilizer. The fact that the LRC was not comparable for similar viscosities of different stabilizers led to the conclusion that resistance to crystallization may depend on molecular associations (intrapolymers, water/polymer) that are not necessarily reflected in the viscosity value, i.e., within the hydration sphere of a single hydrocolloid molecule.

Cottrel et al. (1979b) had also studied the effect of commonly used stabilizers (guar gum, locust bean gum, carrageenan and sodium carboxymethyl cellulose) in the viscosity of ice cream mixes. The increased amount of stabilizer rendered a non-linear increase in the apparent viscosity of the mix. The neutral gums (i.e., locust bean gum and guar gum) had a greater impact in the non-Newtonian behaviour compared to the anionic gums. A comparison with previous work on stabilizers in water and milk systems, showed that the intrinsic
viscosity in such systems is higher than in ice cream. This suggested that the higher complexity of the systems (presence of other solutes) imposed a restriction to the hydration of the gum molecules and, therefore, having a less extended configuration.

However, gums may also interact with some of the components in ice cream having a synergistic effect on the viscosity of the mixes. Schmidt and Smith (1992a) demonstrated that interactions between casein and whey proteins with carrageenan, guar gum and xanthan gum in aqueous solutions rendered higher viscosities. According to Whistler and Daniel (1990), CMC helps solubilize proteins (e.g., casein) by forming complexes which also results in an increased viscosity.

Blond (1994), using dynamic thermal mechanical analysis (DTMA), concluded that the rheology of the frozen product is affected by the presence of added polysaccharides. The addition of different molecular polymers (maltodextrin, dextran and guar gum) enhanced the rigidity (G') of the sample during warming, particularly in the case of guar gum.

Goff et al. (1995a) studied the viscoelastic behaviour of frozen samples through dynamic rheological measurements. They reported all parameters (storage modulus, loss modulus and tan(δ)) decreased as the temperature at which they were measured increased (-16°C to -5°C). However, samples with stabilizer showed lower storage (G') and loss (G'') moduli, and a decrease in tan(δ) compared to unstabilized samples. This was hypothesized to be due to the differing ice crystal size distributions, with smaller sizes from the effect of the stabilizer leading to lower moduli. The role of these compounds in imparting elasticity to the frozen mix was demonstrated by a greater increase in G' (elastic component) with decreasing temperature than G'' (viscous component). In this study, the author also showed that the freeze-concentration process may lead to attaining the critical concentration (c*) of the stabilizer. The latter is the concentration of a polymer at which the volume occupied equals the volume of
the solvent which corresponds to the onset of coil overlap between polymer chains. The freeze-concentration process was simulated using ice cream formulations with diminishing water content. The rheological measurements led to the conclusion that stabilizers effect on the ice phase (reduction of crystal size and arrangement) reduces the viscoelastic moduli while it enhances them on the unfrozen phase, with the former dictating the rheological behaviour below a critical temperature.

Blond (1994), using thermomechanical analysis (TMA), concluded that the addition of small amounts of polysaccharides (maltodextrin, dextran and guar gum) did not affect the glass transition temperature of the freeze concentrated amorphous fraction. However, high concentrations of dextran (over 20%) shifted the position of the peak in the thermograms. Carrington et al. (1996) reported significantly lower $T_g$ for slow freezing/annealing experiments in fructose solutions containing CMC. According to the researchers, this would suggest that CMC interfered in the attainment of maximal freeze concentration due to the enhanced viscosity and hindered diffusion. No differences were found in rapidly frozen solutions. Goff et al. (1993) and Sahagian and Goff (1994), studying glass transition temperatures by differential scanning calorimetry (DSC) in model solutions for ice cream, concluded that stabilizers are not capable of altering the $T_g$ due to their low concentration in the system. These results were later confirmed by Sahagian and Goff (1995b) when studying the stress relaxation behaviour of freeze concentrated sucrose solutions, i.e., stabilizers (gelatin, guar gum and xanthan gum) did not alter the onset temperatures of the phase transitions.

Results from the latter study also indicated that the addition of stabilizers influence the mechanism to dissipate mechanical stress. Within the glassy state of these solutions (-55°C to -40°C), the presence of stabilizers increased the calculated relaxation times ($\tau$) indicating changes in the behaviour of the amorphous state. Further exploration of the data revealed that the xanthan gum and gelatin systems appeared to behave more like a viscoelastic solid than a
liquid. Above the Tg (rubbery state), the behaviour of the systems depended on the freezing rate at which solutions were frozen, however, the combined effect of slow freezing and the presence of stabilizer had the greatest effect on the rheological response of the system. Stabilized systems clearly showed a more elastic behaviour compared to the unstabilized system (sucrose) but also depended on the type of stabilizer. As a result of the freeze-concentration process, gelatin and xanthan gum appeared to provide stronger elasticity possibly because of polymer-polymer interactions compared to the side chain entanglements of guar gum. It was hypothesized that the physical entanglements of overlapping coils and side chains resulted in a limitation to molecular reorientation (stabilizers themselves) and kinetics necessary to reduce the internal stress. Such increase in the kinetics of the viscous component may relate to reduced diffusion kinetics of the unfrozen phase.

Increased mechanical resistance of frozen polymer/sucrose systems above Tg were previously reported (Sahagian and Goff, 1995a). Strong resistance to thermal deformation was observed in stabilized samples after rapid freezing, xanthan having the largest effect. The increased resistance to structural rearrangement and viscous flow of the latter and of gelatin systems, strongly suggested that the molecular structure of the polymer affects the mechanical stability above Tg. Moreover, viscosity profiles measured through thermomechanical analysis (TMA) also suggested such dependency on the rheological response of the system. DSC data also showed that the enthalpy of melt (ΔH_m) were comparable between stabilized and unstabilized systems, thus, stabilizers did not affect the ice phase volume achieved by rapid or slow freezing rates. Proton (¹H) NMR measurements were also performed to determine the effect of guar gum in the proton rotational mobility of sucrose solutions cooled at 5°C/min. These measurements showed relatively smaller relaxation times (t₂) for systems containing guar gum between the transition regions T_{Tr1} and T_{Tr2} (refer to section 2.1.2), and was attributed to the possible influence of the stabilizer on the proton exchange rates between different sites. Above T_{Tr2}, guar gum reduced
the relaxation times importantly. According to the author, and considering no differences between the ice phase volume of the two systems, these results would denote changes in the self-diffusion properties of detectable protons, or less likely a reduction in the proton mobility and mass transfer properties due to an increased intrinsic viscosity. It was hypothesized that stabilizers may alter the ordering and resulting kinetics of the water molecules of the unfrozen phase. Thus, recrystallization would be encouraged to occur within the dispersed polymer rather than diffusion to existing crystals.

2.3.4.2 Nucleation and crystal growth

Shirai et al. (1985) reported a decrease in the nucleation rate in polymer solutions compared to water. The polymers included different polysaccharides (dextran, CMC, ficoll) and proteins (fraction of skim milk, ovalbumin). The effect of polymers on kinetic parameters in the calculation of induction times was proposed to be due to a decrease in the nucleation rate since small effects on freezing point depression were observed. The secondary nucleation of these polymer solutions was also studied and the addition of high molecular weight compounds also decreased the nucleation rate. According to the author, the increase in viscosity may have decreased the frequency of collision between crystals and walls or impellers, reducing the nucleation rate.

Carrington et al. (1996) reported that the addition of CMC to fructose solutions enhanced ice nucleation and/or reduced ice crystallization of rapidly frozen samples. Edge-section cryo-SEM micrographs revealed that solutions containing CMC had smaller ice crystals. This coincides with the effect of this polysaccharide in the ice-crystallization temperatures of fructose and glucose solutions as determined by thermomechanical analysis (Carrington et al., 1994). It was concluded from these studies that the stabilizer in the frozen systems enhanced nucleation and/or reduced crystallization, most likely by altering the diffusion or free water within the system (Carrington et al., 1996).
Blond (1986) found that food biopolymers (gelatin and CMC) raised the temperature of heterogeneous nucleation ($T_H$). According to this author the two opposite effects to be considered are: addition of macromolecules depresses the $T_H$ of water, as in any solution; the addition of macromolecules raises the $T_H$ as a result of the polymer structure promoting nucleation. The latter would overcome the former in the case of these biopolymers. In this study, the viscosity of the solutions did not show any correlation to the increase of the $T_H$ observed.

Muhr et al. (1986) showed that methyl celluloses (Celacols) and xanthan gum exerted minimal effect on heterogeneous nucleation. The alginates seemed to be the only polysaccharides that contributed significantly to heterogeneous nucleation, however, it was not clear if this was a direct effect of the polysaccharide or the presence of impurities. They concluded that stabilizers have nucleating abilities yet they do not affect nucleation in a considerable rate. Muhr and Blanshard (1984) also found minimal effect on the nucleation behaviour of stabilized samples. The slight inhibitory effect of certain compounds (celacols and xanthan) was attributed to solid particles present in solution, i.e., no consistent effect of the stabilizer itself was found. However, stabilizers showed a small but significant effect on ice propagation, retarding crystal growth.

The propagation rate of the ice front in U-tube experiments showed that the presence of CMC greatly decreased this rate but was not dependent on the concentration and/or viscosity of the solution (Blond, 1985; 1988). An increase in the molecular weight of the solute significantly decreased the rate of propagation up to a limit after which the effect was not observed. These researchers concluded that this cannot be attributed to a reduced diffusivity since the mobility of small solutes by electron spin resonance showed to be as high as in pure water. They stated that the inhibitory effect of such polymer on crystal growth is not due to modified thermodynamic or kinetic properties of water, but to a mechanical hindrance exerted by the macromolecular system on the crystallization interface.
Using a similar apparatus, Budiaman and Fennema (1987a) reported that all samples containing hydrocolloids (CMC, guar gum, locust bean gum, sodium alginate, tragacanth gum, carrageenan and gelatin) showed an increase in the linear rate of crystallization when the initial supercooling temperature or the concentration were decreased. Guar gum and locust bean gum showed to be most effective in retarding the ice crystal growth under these conditions, while carrageenan and sodium alginate proved to be the least. A possible explanation stated was the ability of the galactomannans to hydrate and possibly hinder water crystallization due to their galactose side branching units, whereas carrageenan and alginate correspond to linear polysaccharides. However, these differences were rather unimpressive when compared to the effect exerted by sucrose alone.

Muhr and Blanshard (1986) also studied the effect of stabilizers in sucrose solutions and gels using the tube and flask experiment for ice crystal growth rates. Methyl celluloses (celacols) and alginates (manucols) at different concentrations reduced the linear velocity of crystallization (tube experiment) of the solutions in a small but significant amount. The effect of manucols were influenced by their molecular weight and the amount of sucrose in solution, i.e., a lower concentration of sucrose rendered less reduction of the linear velocity. The flask experiment was less descriptive of these differences due to the turbidity of the system, however, it was apparent that all stabilizers retarded the ice growth depending on the undercooling they exerted. Guar gum showed to have the most substantial effect. These researchers concluded that stabilizers used at concentrations of their commercial use retarded ice crystal growth by a factor of one-third to two-thirds, yet not affecting the crystal morphology.

In ice cream manufacturing, the ice crystal population is largely determined by fracture due to the continuous scraping of the refrigerated wall. Therefore, only a massive effect on the nucleation would affect the overall process (Muhr et al., 1986). Furthermore, parameters like the linear velocity on a tube experiment measures the invasion rate of ice crystals into a solution and does not attend to the ice crystal size or morphology (Blond and Colas, 1993).
2.3.4.3 Recrystallization

In studies of model solutions of frozen desserts, Harper and Shoemaker (1983) examined the impact of locust bean gum (LBG) and selected sweeteners on the recrystallization rates. Their results showed that viscosity of the solutions was not related to the recrystallization behaviour and that LBG was not an effective inhibitor of recrystallization. However, the different sweeteners had an important impact in accord with their effect on the freezing point. Thus, sucrose caused the greatest reduction in the rate of change of crystal sizes, followed by corn syrup solids and finally fructose. They also reported that the effect of temperature fluctuations was quantitatively greater than recrystallization at constant storage temperature. The methodology used showed that migratory recrystallization was the predominant mechanism observed.

Min et al. (1994) studied recrystallization in sucrose solutions with different polymers added (sodium alginate, CMC, gelatin, carrageenan and LBG). They concluded that stabilizers do not inhibit but delay recrystallization and that the differences in recrystallization between stabilizers are a result of the structure which are likely to influence water molecule migration. They reported that the temperature also determines the effect of the stabilizer against recrystallization.

Sutton et al. (1994) studied the recrystallization behaviour of fructose solutions (model solutions of the unfrozen phase), ice waters and ice cream. They developed mathematical models to predict the ice crystal coarsening and found good agreement of their predictions to experimental data. The results showed that viscosity of the mixes was not a factor on the recrystallization rates for water ices or ice cream. In the case of ice cream, more difficulties were encountered as the complexity of the system requires careful consideration of the microstructure. These researchers concluded that the rate of crystallization is not controlled solely by diffusion to and from the interface. In similar studies by the author (Sutton et al. 1996a), the recrystallization rate of the model fructose
solutions showed to be dependent on the ice phase volume and the temperature at which samples were kept, i.e., a decrease in the ice phase volume or temperature decreased the rates. Accretion was the dominant mechanism at the beginning of recrystallization, however as crystals grew and became less, migratory recrystallization was predominant. This suggested that recrystallization in the later stages was driven by a nonrandom diffusion process. Further studies on the effect of locust bean gum in this system (Sutton et al., 1996b) showed that the rates were importantly decreased by LBG and independently of the temperature, contrary to what was reported by Min et al. (1994). Concentrations up to 0.2 to 0.3% reduced the recrystallization rate yet addition of further LBG had no further effect. The authors concluded that LBG and probably other stabilizers weakly adsorb onto the crystal surface impeding its growth.

Sutton et al. (1997) compared the effect of different concentrations of locust bean gum (LBG) and guar gum in recrystallization rates of fructose solutions at -15°C. They found that LBG at a low concentration (0.3%) did not affect the recrystallization rate of the 40% fructose solution. As the concentration was increased, LBG decreased the rate down to a minimum value which stayed constant for concentrations higher than 0.3%. Guar gum reduced the recrystallization rate for low concentrations, reaching a similar minimum value as LBG at 0.7%, yet increasing after a concentration of 0.8%, thus promoting recrystallization. The viscosity of calculated unfrozen fractions were also tested and showed no correlation with the recrystallization behaviour proving that macromolecular viscosity was not responsible for this behaviour. The different degree of branching between the two gums was proposed as a possible cause. Later studies with enzyme-modified guar gum and pectins confirmed the plateau effect of stabilizers in recrystallization rate-concentration curve previously reported (Sutton and Wilcox, 1998a).

In a recent study, Sutton and Wilcox (1998b) showed that the dependence of the recrystallization rate in the ice phase volume of model fructose solutions previously reported (Sutton et al. 1996b) was not observed in
ice cream. However, the addition of LBG to ice cream reduced the rate of crystal growth during heat shock and constant temperature storage. They also reported some evidence in the plateau effect in the recrystallization rate as the concentration of LBG was increased.

Donhowe and Hartel (1996a) studied recrystallization in ice cream for steady temperatures of -5, -7, -10 and -15°C, and for sinusoidal fluctuations of 1°C from those temperatures. Crystals had a typical oblong shape and iso-mass recrystallization (rounding off) was evident in all cases but particularly dramatic at high, constant temperature (e.g., -5°C). Migratory recrystallization was rarely observed whereas accretion was predominant in all cases. Melt-refreeze recrystallization was apparent during fluctuations but could not be distinguished from the effects of accretion due to the proximity of the crystals. Further experiments indicated that a higher amplitude renders an increased recrystallization rate and that low storage temperature (-20°C) does not cause changes in crystal size. Donhowe and Hartel (1996b) also reported the effect of temperature fluctuations on the crystal distribution between the interior and surface in bulk samples. These differences were not observed in constant temperature experiments. They concluded that part of the effect of storage temperature on recrystallization is due to higher effective thermal mass (more unfrozen phase) of a sample at higher temperatures. However, the amount of frozen water does not change during constant temperature experiments and recrystallization was still evident.

Moorty and Balachandran (1994) studied recrystallization in low fat ice cream containing sodium alginate and a blend of stabilizers. At a constant temperature of -15°C, the stabilized samples did not show any important improvement in the recrystallization rates. They concluded that stabilizers exert only a minimal effect on crystal growth.

Hagiwara and Hartel (1996) reported smaller ice crystals in stabilized ice cream for all combinations of sweetener and storage temperature. However,
inhibition of the recrystallization rate was only statistically significant for stabilized samples containing HFCS. The rest of the combinations also rendered lower rates but due to the variability of the measurements (standard deviations) no conclusions could be drawn. Further studies (Miller-Livney and Hartel, 1997) showed that the effect of stabilizers was negligible compared to that exerted by the different sweeteners during storage at -15°C. Significant differences were observed in particular cases such as xanthan gum and gelatin when combined with sucrose or HFCS, whereas sucrose/LBG or sucrose/carrageenan combinations retarded crystallization when stored at -9.5°C.

Microscopic examination of stabilized (carrageenan and guar gum) and unstabilized ice cream showed that the former had smaller ice crystals both initially and after heat shock (Caldwell et al., 1992b). Initially unstabilized samples showed slightly larger crystals but differences were evident after 24 weeks of storage. The fusion of crystals was more predominant in unstabilized samples attesting for the increase of recrystallization during thermal abuse. Stabilized samples also showed more serum phase between crystals and more air distributed in the fracture. The air bubbles in this case contained a larger number of ruptures compared to unstabilized samples. However, more air crevices were observed in unstabilized samples coinciding with shrinkage observed in the packages. Therefore, stabilizers seemed to hold the air once the air bubble was broken.

2.3.4.4 Textural properties

Cottrell et al. (1979a) investigated the effect of 18 different stabilizers in ice cream. The sensory evaluation of these samples showed that the majority of the stabilizers were acceptable after manufacture, however some of them deteriorated after storage. The stabilizers under study showed no satisfactory control of ice crystallization.
Moore and Shoemaker (1981) compared ice cream stabilized with CMC at different concentrations to an unstabilized sample. A higher degree of iciness was reported for samples with no CMC or a low concentration (0.05), compared to those with higher concentrations (0.15 and 0.2%). Interestingly, the duration of the perception of iciness increased with the increase in concentration of CMC. However, this response can be attributed to the melting characteristics of the frozen mixes and not necessarily to the ice crystal sizes.

The storage temperature can have the most important effect on the textural characteristics of ice cream. Ahvenainen and Malkki (1985) reported that independent of the type of package used, ice cream stored at -12°C was icy and its appearance lumpy. Comparatively, those stored at -18°C were doughy, tough, thick and fatty and at the end of the storage period seemed icy. Changes for the higher storage temperature (-12°C) using microscopical examination were evident after only 2 weeks, whereas for the lower temperature (-18°C) crystals began to grow after 8 weeks. Fluctuating temperatures were even more detrimental showing iciness within the first 3 weeks (oscillations from an average of -15°C).

Earl and Tracy (1960) also studied the impact of storage temperature on textural properties. Their research showed that there was little effect on samples stored at -26°C. However, the body, texture and flavor of samples stored at -13°C became progressively worse as the storage time was prolonged. Similar results were found for those samples that were heat shocked (temperature fluctuations). Also of importance, results indicated that ice cream with a higher overrun (110%) were more susceptible to loss of body, texture and flavour quality than those with a lower overrun (80%).

Caldwell et al. (1992b) concluded that stabilized ice cream held its structure better during storage and after heat shock. The unstabilized samples ranked lower in sensorial analysis for both cases compared to the stabilized samples as corroborated by microscopical examination.
2.3.4.5 Summary

According to the different studies, stabilizers do not significantly affect the freezing characteristics of an ice cream mix. The freezing point depression is negligible when compared to the effect of the sweeteners and the heat of fusion of water in ice cream is not significantly reduced by the presence of stabilizers. They do exert some effect on the nucleating abilities (heterogeneous nucleation) of the mixes, thus encouraging the formation of smaller crystals and/or reducing crystallization during the initial freezing process and most likely by the increase in viscosity of the mix. Linear rate of crystallization studies support this assumption. However, the viscosity of the mix has no effect on the recrystallization rate during storage conditions.

Stabilizers play an important role in the rheological properties of the frozen product. They increase the elastic component of the rheological response, imparting a more viscoelastic solid behaviour to stabilized systems. It has been reported that this behaviour reduces the structural damage of ice cream by preventing the air structure from collapsing. However, this also affects the melting characteristics of ice cream increasing the perception (duration) of iciness due to heat shock.

According to the results from thermomechanical analysis and differential scanning calorimetry, stabilizers, in their typical concentration in frozen desserts, do not affect the glass transition temperature of the frozen product, nor do they affect the amount of ice formed during rapid or slow freezing. However, they affect the mechanism to dissipate mechanical stress leading to the presumption that they may alter the reorientation of their molecules and the kinetics involved to reduce internal stress. NMR studies have shown that stabilizers may also influence the proton exchange rates in the unfrozen phase. The latter can be related to changes in the ordering of the unfrozen water molecules, ultimately affecting the diffusion kinetics of such phase.
In recrystallization studies, temperature fluctuations have shown to exert a greater impact on ice crystal distributions compared to constant storage temperature. The recrystallization process occurred by different mechanisms at different stages of the process involving Ostwald ripening and accretion in earlier stages and melt-refreeze in later stages. However, the effect of stabilizers in recrystallization has not been consistent and also depended on the storage condition. In ice cream they typically rendered lower ice crystal sizes after the initial freezing process and reduced the recrystallization rate at higher constant storage temperature or during temperature fluctuations. Some of the proposed mechanisms are the viscosity enhancement of the unfrozen phase, weak adsorption onto the ice surface and the reduction in the diffusion kinetics of the unfrozen phase.
3. MATERIALS AND METHODS

3.1. Model solution design

The different model solutions were calculated based on a standard ice cream formulation (Table 3.1). According to the approximated sweetener/water ratio for each sweetener component, the total sweetener/water ratio in a typical ice cream mix can be approximated to 0.34 (Table 3.2).

Table 3.1 Standard ice cream formulation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>10.0</td>
</tr>
<tr>
<td>MSNF (6.1% lactose)</td>
<td>11.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn syrup solids</td>
<td>5.0</td>
</tr>
<tr>
<td>Stabilizer</td>
<td>0.2</td>
</tr>
<tr>
<td>Emulsifier</td>
<td>0.15</td>
</tr>
<tr>
<td>Total Sweeteners</td>
<td>Sugars + Oligosaccharides 21.1</td>
</tr>
<tr>
<td>Total Water</td>
<td>Water 63.7</td>
</tr>
</tbody>
</table>

Table 3.2 Equivalent solutions according to sweetener/water ratio

<table>
<thead>
<tr>
<th>Type of sweetener</th>
<th>Calculated Ratio</th>
<th>Approximated Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose / water</td>
<td>10.0 / 63.7 = 0.1569</td>
<td>0.16</td>
</tr>
<tr>
<td>Lactose / water</td>
<td>6.1 / 63.7 = 0.0957</td>
<td>0.10</td>
</tr>
<tr>
<td>Corn SS/ water</td>
<td>5.0 / 63.7 = 0.0784</td>
<td>0.08</td>
</tr>
<tr>
<td>Sweetener/water</td>
<td></td>
<td>0.34</td>
</tr>
</tbody>
</table>
According to the sweetener/water ratio, an equivalent solution would correspond to a 25.4% w/w solution (34/(100+34)). Thus, considering this concentration as a base of calculation (base solution) and the relative amount of each sweetener (percentage of presence from the total of sweeteners), the sweetener equivalent solutions can be estimated as shown in Table 3.3.

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Percentage of presence (%)</th>
<th>Sweetener conc. in base solution (%)</th>
<th>Equivalent solution conc. (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>47.3 (10.0 / 21.1)</td>
<td>25.4*0.47 = 11.9</td>
<td>12.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>28.9 (6.1 / 21.1)</td>
<td>25.4*0.29 = 7.4</td>
<td>8.0</td>
</tr>
<tr>
<td>CSS</td>
<td>23.7 (5.0 / 21.1)</td>
<td>25.4*0.24 = 6.1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

An initial study considered a sucrose solution containing the equivalent amounts of sucrose and lactose (20%) (Table 3.3). Lactose was replaced with sucrose because of its poor solubility. The ratio sweetener/water thus obtained was 0.25. This formulation (F0) was used to determine the effects of stabilizer concentration and storage time on ice crystal size distributions. Samples were prepared at 2 levels of stabilizer concentration: 0.2 and 0.4% w/w; and ice crystal sizes measured after drawing (fresh), and after 2 and 4 weeks of constant temperature storage at -30°C.

According to the sum of equivalent solution concentrations in Table 3.3, a second solution (F1) was prepared containing 26% sucrose to determine the ratio effect on the crystal sizes. The ratio for this formulation corresponded to 0.35 which is comparable to the sweetener/water ratio in ice cream. For the following formulation (F2), corn syrup solids were incorporated according to their equivalent percentage to study the effect of the type of sweetener on the ice crystal distribution. Therefore, 6% of the sucrose content of the previous formulation was replaced with 42 D.E. corn syrup solids (CSS).
The next effect studied was the addition of the milk solids non-fat (MSNF) to the formulation (F3). In this case, lactose was incorporated along with these solids so the sucrose and CSS concentrations were adjusted accordingly. Finally, a full formulation was prepared (F4) to be compared to the prior model solutions.

In all cases, the stabilizers were added according to the stabilizer/sucrose ratio for the initial 20% sucrose formulation and 0.2% stabilizer (ratio = 0.01). This ratio was kept constant for all formulations and also applies to the standard amount in ice cream formulation. Also, formulations F2, F3, and F4 were prepared with and without emulsifier to determine if there was an effect of the emulsifier on the crystal size distribution. Due to their low values, the addition of emulsifier or stabilizer was not considered in the calculation of percentages of the other ingredients. The moisture content of stabilizers, sugars and oligosaccharides, and skim powder were also ignored. A summary of all formulations is presented in Table 3.4.

Table 3.4 Summary of formulations

<table>
<thead>
<tr>
<th>Effect</th>
<th>Other ingredients</th>
<th>Stabilizer</th>
<th>Emulsifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>Initial 20% sucrose 80% water</td>
<td>0, 0.2 and 0.4%</td>
<td>-</td>
</tr>
<tr>
<td>F1</td>
<td>Ratio 26% sucrose 74% water</td>
<td>0 and 0.26%</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>CSS 20% sucrose 6% CSS 74% water</td>
<td>0 and 0.26%</td>
<td>0 and 0.15%</td>
</tr>
<tr>
<td>F3</td>
<td>MSNF 13.6% skim powder 11.3% sucrose 5.6% CSS 69.5% water</td>
<td>0 and 0.26%</td>
<td>0 and 0.15%</td>
</tr>
<tr>
<td>F4</td>
<td>Ice cream see Table 3.1</td>
<td>0 and 0.2%</td>
<td>0 and 0.15%</td>
</tr>
</tbody>
</table>
3.2 Preparation of mixes

Three stabilizers were used in this study: carboxymethyl-cellulose (CMC), guar gum and xanthan gum (Germantown Canada Inc., Scarborough, Ont.). When added, stabilizers were pre-blended with sucrose (Red Path, Toronto, Ont.), 42 D.E. glucose solids (Casco Inc., Etobicoke, Ont.), and the emulsifier blend (Food Specialties, Halton Hills, Ont.) according to the formulation. The emulsifier blend consisted of a mix of mono- and diglycerides (80%) and polysorbate 80 (20%). The source for milk solids non-fat was instant pasteurized powdered skim milk (Gay Lea Foods, Weston, Ont.), and fresh cream (41.01% fat) (Gay Lea Foods, Weston, Ont.) was used as the source of fat.

The mixes were prepared by addition of the dry mix of ingredients to pre-heated water (65-70°C), pasteurized at 74°C (15 min) and homogenized at 17.2 MPa (2500 psig), 3.4 MPa (500 psig) second stage on a Gaulin V15-8T homogenizer (Gaulin Corporation, Mass.). The mixes were then stored overnight under refrigeration (4°C) to allow for the hydration of the stabilizer.

3.3 Rheology of mixes

Samples of the mixes, after preparation (2-3 hours at 4°C) and after 24 hours of cool storage (4°C), were tested for their rheological properties. Measurements were carried out using the Carri-Med controlled stress rheometer CSL 100 (Carri-Med Dorking, Surrey, UK). Shear stress sweeps were performed at 4°C using the Mooney-Ewart (stainless steel) concentric cylinder, stator inner radius 25 mm, rotor outer radius 24 mm, cylinder immersed height 30 mm and measurement system gap 77μm. Shear stress ranges were determined according to the rheological behaviour of the mixes: (1) Newtonian behaviour was tested for a range of 0.5 to 10 Pa·s; (2) non-Newtonian for a range of 0.5 to 35 Pa·s. The statistical package of the Carri-Med software was used to fit the data to the Bingham model and the Herschel-Bulkley model for Newtonian and
non-Newtonian behaviour respectively. These measurements were performed in duplicates for each sample.

3.4 Freezing and storage of mixes

Aliquots of 2L were frozen in the Taylor Batch Freezer model B733-32 (Tekni-Craft, Rockton, IL). The refrigeration system was turned off once the mixes had reached -5°C (22°F) and samples were drawn after a total of 15 minutes of continuous whipping. The overrun was measured according to equation 3 by weighing a known volume (stainless steel cup) of frozen mix and the equivalent of the aged mix. These measurements were performed twice before drawing samples for storage. Samples of approximately 50 g were extruded into covered plastic cups and stored at -30°C (walk-in freezer) for the hardening period (24 h).

As mentioned earlier, formulation F0 was studied at constant storage temperature after 2 and 4 weeks at -30°C. In this particular case, the time 0 samples (fresh) were taken right after drawing to study the ice crystal distribution before hardening. The rest of the formulations (F1, F2, F3 and F4) were sampled after hardening and after 4 weeks of storage at -30°C. Formulation F4 (ice cream with and without stabilizer) was also stored at -16°C after hardening (-30°C, 24 hours) in a tempering cabinet for 4 weeks to study the effect of a high storage temperature. Samples from the ice cream formulation (F4e) from both storage temperatures were also cycled after the storage period in the Low Profile Freezer model 85-21 (ScienTemp™, Adrian, MI) using a Watlow Series 942 ramping control (Watlow Controls, Winona, MN). Table 3.5 shows the parameters of the cycles performed.
Table 3.5 Temperature cycle programs for ice cream

<table>
<thead>
<tr>
<th>Set</th>
<th>Previous Storage Temp. (°C)</th>
<th>Temp. cycle Temp. (°C)</th>
<th>Cycle time length (h)</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>-16°C</td>
<td>-15°C ± 2°C</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Set 2</td>
<td>-30°C</td>
<td>-15°C ± 2°C</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Set 3</td>
<td>-30°C</td>
<td>-15°C ± 5°C</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Set 4</td>
<td>-30°C</td>
<td>-15°C ± 5°C</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

Temperatures were monitored during the cycles by inserting the probe of a thermocouple thermometer model 8112-10 (Cole Parmer Instrument Company, Taiwan) into the core of the sample. Cycle times were determined experimentally to allow the core temperature to reach the freezer temperature and hold this temperature for not less than 10 minutes. Samples previously stored at -30°C were allowed to reach the higher limit of the temperature cycle (freezer setting) before setting the freezer controller to cycle.

After the different storage times and conditions, samples were collected for cryo-SEM by immersing the plastic container in liquid nitrogen (-150°C) and allowing the sample (block of ~50 g) to equilibrate with the medium (once bubbling had stopped). The block was carefully fractured using a hammer (samples were brittle) while still immersed in liquid nitrogen and reduced to smaller pieces. Small chips were then collected and stored in cryo-vials in liquid nitrogen until preparation for the cold stage of the Hitachi S-570 SEM (Hitachi Ltd., Tokyo, Japan). This procedure provided an unbiased sampling procedure as to the position of the specimens (chips) within the sample. However, considering the sample size, no important differences in the ice crystal distribution would be expected.
3.5 Specimen preparation for SEM

The sample stub for cryo-SEM consists of a copper base with a gorged area along the stub and 2 spring loaded supports. Two specimens (= 2-3 mm cubes) of each frozen mix were placed in the stub while immersed in liquid nitrogen slush. The stub was then transferred under vacuum (1x10^-2 torr) to the preparation chamber (Emscope SP2000A Sputter-Cryo Cryogenic preparation System, Emscope Ltd., Kent, U.K.) using the transfer device. The specimens were fractured using the blade in the preparation chamber and then transferred to the cold stage of the microscope for sublimation.

Changes during sublimation were monitored from the onset (-80°C) until signs of destruction of the ice crystal sockets were observed. In this case, specimens were viewed at 5 kV accelerating voltage and the images were collected using the Voyager Acquisition System (Noran Instruments, Middleton, WI). This kV is preferred over a high voltage because the latter will cause charging of the uncoated specimen (Nei, 1974). An average sublimation time was determined that allowed a clear differentiation of the crystal sockets (ca., 15-20 min). After etching, the stubs were transferred back to the preparation chamber for gold sputter-coating (2.5 minutes). Specimens were viewed at 10 or 15 kV accelerating voltage with an objective lens aperture of 50μm. Digital images were collected using the Voyager Acquisition System. Photographs were also collected using the Mamiya ProSD-120 (Camera Co. Ltd., Tokyo, Japan) and Ilford FP4-125 film.

3.6 Image Analysis

Sample sizes of not less than 100 crystals were obtained from at least 2 different locations (fields) of each specimen. The magnification was adjusted according to the type of sample to ensure a clear view of the ice crystals in each field. The magnifications typically used were 150 and 250 times. The Mocha™ Image Analysis Software (Jandel Scientific, Sausalito, CA) was used to calculate
the cross section area and the shape factor of the ice crystals by manual circumvention of the perimeter of the sockets exposed. The shape factor (adimensional) is a measurement of how circular an object is, its value ranges from 0 to 1, and is defined according to the formula:

\[
\text{Shape factor} = \frac{4\pi \cdot \text{Area}}{(\text{perimeter})^2}
\]

(Eq.7)

The individual particles approach was used (Langton and Hermansson, 1993), separating slightly fused crystals into their components. This distinction was applied to a cluster only when clear evidence of the original crystals was found, *i.e.*, inflections in the curvature and/or residues of the interface. This criterion was applied only if at least 2/3 of the perimeter of each crystal could still be differentiated.

3.7 Crystal size and statistical analysis

The crystal size is presented as the equivalent diameter calculated from the measured cross-section area of each crystal. Several authors have used this approach to represent the crystal size (Harper and Shoemaker, 1983; Donhowe *et al.*, 1991; Goff *et al.*, 1993; Hagiwara and Hartel, 1996; Miller-Livney and Hartel, 1997). The images were calibrated by a 3 point calibration of a 49μm squared grid. The equivalent diameter distribution was presented according to 3 mathematical approaches.

The first model to be considered was the arithmetic average and the standard deviation as a measure of the span of the distribution. These parameters are good descriptors of the distribution assuming normality of the latter, however it is commonly used to characterize the overall ice crystal size. This average was compared to the log-normal average and the calculated standard deviation of the values from the mean. In this case, the assumption is the log-normal behaviour of the population.
For a third model, histograms of the equivalent diameters were constructed for the cumulative percentage of presence considering a bin size of 2.5 or 5μm according to the sample. This allows the consideration of the values as a continuous function of the distribution and reduces the noise of the regular percentage distribution. The mathematical model used to describe this distribution was the Logistic Dose Response model (Equation 8) and it was considered for its simplicity. Its equation is:

\[
y = \frac{a}{1 + \left(\frac{x}{b}\right)^n}
\]  
(Eq.8)

where a is the asymptotic value (100%), b is the inflection point and 50% of the distribution; and n is a parameter related to the slope of the distribution.

For statistical purposes the different formulations were replicated once and the measurements of the crystal sockets were performed for two specimen of each replicate and treated independently. Statistical analysis of the data was carried out using the Statistical Analysis System package (SAS), (SAS version 6.0, SAS Institute Inc., Cary, NC). The Analysis of Variance Procedure (ANOVA) and the Ryan-Einot-Gabriel-Welsch Multiple F-test (REGWF) were used to determine the statistical significance (α= 0.05) of differences between the means (see appendix 8.1)
4. RESULTS AND DISCUSSION

4.1 Rheological behaviour of mixes

4.1.1 Models

The addition of the stabilizers had the expected effect on the rheology of the mixes: stabilizers showed a non-Newtonian behaviour compared to the Newtonian behaviour of unstabilized samples. Different models were examined to determine the best fit in each case. Similar to what was reported by Abdelrahim et al. (1994), the power law and Herschel-Bulkley models showed good adjustment to the rheological behaviour of stabilized samples. However, the Herschel-Bulkley model showed a better fit to the data in all cases by considering a yield stress ($\tau_0$). The apparent viscosities were calculated from the model parameters at a shear rate of 50s$^{-1}$ according to previous studies on ice cream mixes (Goff et al., 1995a). High shear rates are common in industrial settings; however, lower shear rates may be more appropriate when studying interaction phenomena (Schmidt and Smith, 1992a).

The Newtonian behaviour of unstabilized samples also found better fit in a model considering a yield stress. The Bingham model was fit to the data of the unstabilized samples, however, the viscosity values were taken directly from the consistency index (coefficient of viscosity in this case) since the shear dependent term ($\tau_0\gamma$) was negligible.

4.1.2 Ageing of the mixes

The statistical analysis showed no significant differences ($p>0.05$) for the apparent viscosities between the fresh mixes and those aged overnight. During ageing of the mix the stabilizer hydrates, milk proteins also hydrate and fat solidifies when present, so an increase in viscosity is expected. The ageing of the model solutions or the ice cream formulation showed no effect on the rheological parameters. However, stabilizers such as guar gum and CMC readily set up upon cooling (Marshall and Arbuckle, 1996), thus the time elapsed
between the preparation of the mixes, the initial cooling time and the rheological measurements (ca., 2-3 hours) might have been enough for changes in the viscosity to occur. A similar behaviour was observed for xanthan gum. Therefore, stabilizers probably hydrated within the first 2 hours of cooling, hence no changes in the rheological properties of the mixes for an extended ageing time (24 hours) were observed.

According to these results, the (apparent) viscosities were calculated as the average of all viscosity values for each formulation.

4.1.3 (Apparent) viscosities of the mixes

The yield stress ($\tau_0$) and consistency index (coefficient of viscosity) values are presented in Table 4.1, according to the Bingham model considered for the unstabilized samples. As previously mentioned, considering the negligible effect of the shear stress dependent term, the consistency index ($K$) was regarded as the viscosity value of the mixes.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Yield Stress ($\tau_0$) (mPa)</th>
<th>Viscosity $(\text{mPa-s})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.20</td>
<td>4.54 ± 0.45</td>
</tr>
<tr>
<td>F2</td>
<td>0.15</td>
<td>4.87 ± 0.18</td>
</tr>
<tr>
<td>F2 e</td>
<td>0.24</td>
<td>4.86 ± 0.36</td>
</tr>
<tr>
<td>F3</td>
<td>0.01</td>
<td>9.62 ± 0.90</td>
</tr>
<tr>
<td>F3 e</td>
<td>0.06</td>
<td>9.55 ± 0.43</td>
</tr>
<tr>
<td>F4</td>
<td>0.01</td>
<td>25.05 ± 2.06</td>
</tr>
<tr>
<td>F4 e</td>
<td>0.02</td>
<td>22.27 ± 1.33</td>
</tr>
</tbody>
</table>

The variance in the viscosity values was mostly due to differences between replicates and not to the ageing of the mix as previously discussed. As shown in Table 4.1, the addition of different types of sweetener (F1 and F2) did
not affect the viscosity of the unstabilized solutions showing similar yield stress and viscosity. However, the incorporation of MSNF (F3) increased the viscosity to approximately twice the value of the sweetener solutions. This can be explained by the presence of proteins that denature during pasteurization. Denaturation of whey proteins happens in a range of temperatures as low as 70°C (Schmidt and Smith, 1992a). As they denature, they unfold increasing the viscosity of the solution. The yield stress of these solutions was even lower than that of the sweetener solutions. This may imply that the yield stress observed in the latter may be an artifact caused by sensitivity limitations of the apparatus at low shear stress for such viscosity profiles, and/or the mathematical approximation performed by the software to the data. This also suggests that the yield stress values should be disregarded for the viscosity calculation of unstabilized samples.

An even greater effect in viscosity was observed with the incorporation of fat into the formulation (Table 4.1). The effect of fat on the viscosity of the solution can be explained by a bulking effect (filler), particularly in the case of milk fat which is partially crystallized at the temperature of the experiment (4°C). No significant effect was exerted by the presence of emulsifier in the formulations.

The same trend is observed for the stabilized solutions (Table 4.2): higher apparent viscosities in the ice cream formulations compared to those containing merely milk solids-non-fat and/or sweeteners. However, according to the different rheological behaviour of these samples (compared to unstabilized samples), the yield stress values were consistent with the type of formulation and stabilizer used.

The apparent viscosity reported for our ice cream formulations were within range (100-150 mPa·s) of a typical mix viscosity (Goff et al., 1989). However, the effect of the type of formulation (type of ingredient) is smaller compared to that of unstabilized mixes since the rheological behaviour is dictated by the presence of the stabilizer.
Table 4.2 Herschel Bulkley parameters and apparent viscosities at 50 s$^{-1}$ for stabilized samples at 4°C.

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>Formul.</th>
<th>Yield Stress($\tau_0$) (mPa)</th>
<th>Consistency Index (K) (mPa s$^n$)</th>
<th>Flow Behaviour Constant (n)</th>
<th>Apparent Viscosity (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>F1</td>
<td>0.06</td>
<td>0.34</td>
<td>0.70</td>
<td>102.59 ± 5.84</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0.00</td>
<td>0.16</td>
<td>0.79</td>
<td>70.90 ± 6.12</td>
</tr>
<tr>
<td></td>
<td>F2e</td>
<td>0.00</td>
<td>0.11</td>
<td>0.81</td>
<td>53.53 ± 9.06</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>0.20</td>
<td>0.29</td>
<td>0.75</td>
<td>106.37 ± 10.64</td>
</tr>
<tr>
<td></td>
<td>F3e</td>
<td>0.12</td>
<td>0.30</td>
<td>0.73</td>
<td>100.93 ± 21.62</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>0.31</td>
<td>0.32</td>
<td>0.77</td>
<td>127.02 ± 13.33</td>
</tr>
<tr>
<td></td>
<td>F4e</td>
<td>0.31</td>
<td>0.35</td>
<td>0.76</td>
<td>134.36 ± 13.07</td>
</tr>
<tr>
<td>Guar</td>
<td>F1</td>
<td>0.00</td>
<td>0.31</td>
<td>0.65</td>
<td>79.23 ± 2.55</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0.16</td>
<td>0.33</td>
<td>0.65</td>
<td>82.41 ± 6.79</td>
</tr>
<tr>
<td></td>
<td>F2e</td>
<td>0.00</td>
<td>0.32</td>
<td>0.67</td>
<td>89.36 ± 3.99</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>0.08</td>
<td>0.27</td>
<td>0.72</td>
<td>90.76 ± 9.33</td>
</tr>
<tr>
<td></td>
<td>F3e</td>
<td>0.15</td>
<td>0.33</td>
<td>0.72</td>
<td>105.52 ± 10.05</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>0.22</td>
<td>0.28</td>
<td>0.78</td>
<td>118.13 ± 6.92</td>
</tr>
<tr>
<td></td>
<td>F4e</td>
<td>0.25</td>
<td>0.35</td>
<td>0.75</td>
<td>129.90 ± 11.95</td>
</tr>
<tr>
<td>Xanthan</td>
<td>F1</td>
<td>1.31</td>
<td>0.31</td>
<td>0.65</td>
<td>41.19 ± 2.42</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>1.29</td>
<td>0.17</td>
<td>0.65</td>
<td>41.82 ± 4.46</td>
</tr>
<tr>
<td></td>
<td>F2e</td>
<td>1.20</td>
<td>0.15</td>
<td>0.66</td>
<td>37.88 ± 2.84</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>1.84</td>
<td>0.20</td>
<td>0.74</td>
<td>70.49 ± 9.97</td>
</tr>
<tr>
<td></td>
<td>F3e</td>
<td>1.80</td>
<td>0.16</td>
<td>0.73</td>
<td>54.23 ± 4.23</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>1.94</td>
<td>0.19</td>
<td>0.79</td>
<td>82.38 ± 3.19</td>
</tr>
<tr>
<td></td>
<td>F4e</td>
<td>1.99</td>
<td>0.23</td>
<td>0.76</td>
<td>91.81 ± 7.99</td>
</tr>
</tbody>
</table>

An important aspect to consider is the reactivity of the stabilizer with milk proteins. Schmidt and Smith (1992b) reported an interaction between casein and whey proteins with carrageenan, guar gum and xanthan gum in aqueous
solutions. Stabilizers rendered higher viscosities when the proteins were present in the system independent of the heat treatment. Furthermore, according to Whistler and Daniel (1990), CMC helps solubilize proteins (e.g., casein) by forming complexes that are observable by an increase in viscosity.

However, as shown by the flow behaviour constants (n), the stabilizers had a high impact on the non-Newtonian behaviour (lower n values) for the sweetener solutions but their effect subsequently decreased as other solids were present. Therefore, despite the possible viscosity enhancement effect of interactions between the gums and proteins, the restriction on the hydration of the gum molecules and therefore their less extended configuration plays a more important role on the rheological response (Cottrell *et al.*, 1979b).

When comparing the stabilizer type, according to the statistical analysis CMC and guar gum had comparable results on their apparent viscosity values whereas xanthan gum rendered lower viscosities in all cases. Similar differences in apparent viscosities between guar gum and xanthan were reported by Schmidt and Smith (1992a) for heat treated solutions (batch process) containing whey protein concentrate. However, considering the pseudoplastic behaviour, while CMC and guar gum exhibited approximately the same consistency index (K), CMC had a lower pseudoplastic behaviour according to the flow behaviour constant (n). Xanthan gum was characterized by a high yield stress compared to the others gums yet a flow behaviour index comparable to that of guar gum. The molecular weight and particularly the molecular structure is responsible for these differences. Side-branching is a factor in rheological properties, thus CMC being a linear type of molecule would be expected to render a different behaviour than guar gum. However, the branch-associated entanglements are more sensitive (weaker) to shear than main chain entanglements. The rod-like behaviour (linearity) of xanthan gum is reflected in its apparent viscosity and a consequence of having a marked shear thinning behaviour (Whistler and Daniel, 1990). Nevertheless, its molecular structure, spatially more complex than the other stabilizers, renders a higher yield stress. Such effect is comparable to the
increase in yield stress of mixes containing crystallized fat acting as a bulking agent at initial stress.

4.2 Overrun

The statistical analysis showed that replication of the mixes was an influential factor on the variation of the overrun measurement. However, averages between replicates allowed for the detection of statistical differences between the different formulations.

As can be seen from Tables 4.3 and 4.4, the mixes exhibited marked differences in overrun depending on the type of formulation. The ability of sweetener solutions to incorporate air is limited and the volume gain of the frozen mixes is mostly due to ice phase volume. Only a minimal amount of air bubbles were present in these specimens as observed by microscopical examination. In ice cream, the ice phase volume would account for approximately 9% of the increase in volume of ice cream at a drawing temperature of -5°C considering the amount of water in a typical formulation (64%) (Goff et al., 1995b). However, for these model solutions the total amount of ice formed is higher due to a higher water content (Table 3.4). Therefore, the overrun values for a drawing temperature of -5°C are a result of a higher ice phase volume for this type of formulation. An important experimental observation was the chewiness of the frozen samples containing CSS as reported in other studies (Goff et al., 1990; Keeney and Josephson, 1961). Therefore, these oligosaccharides have an important impact on the rheology of the frozen mix due to their molecular characteristics which can also reflect the ability to incorporate and/or stabilize more air under these freezing conditions.

The emulsifier effect on stabilization of the air phase in the CSS/sugar formulation (F2e) is evident from these results (Table 4.3). According to the properties of an emulsifier blend mentioned earlier, this ingredient allows more air to be incorporated and more finely dispersed independent of the presence of stabilizers. These results were also evident from the images collected.
Table 4.3 Overrun measurements (%) for sweetener formulations (F1, F2)

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>F1</th>
<th>F2</th>
<th>F2e</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stab.</td>
<td>14.01 ± 6.11</td>
<td>22.80 ± 6.36</td>
<td>35.12 ± 4.13</td>
</tr>
<tr>
<td>CMC</td>
<td>16.40 ± 6.11</td>
<td>32.24 ± 2.64</td>
<td>46.12 ± 5.50</td>
</tr>
<tr>
<td>Guar</td>
<td>32.86 ± 1.17</td>
<td>45.87 ± 4.12</td>
<td>72.75 ± 5.54</td>
</tr>
<tr>
<td>Xanthan</td>
<td>18.09 ± 6.36</td>
<td>30.65 ± 9.32</td>
<td>41.79 ± 2.17</td>
</tr>
</tbody>
</table>

Values with same letter in the same column are not significantly different (α= 0.05)

Table 4.4 Overrun measurements (%) for formulations F3 and F4

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>F3</th>
<th>F3e</th>
<th>F4</th>
<th>F4e</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stab.</td>
<td>113.7 ± 12.3</td>
<td>73.1 ± 1.2</td>
<td>75.8 ± 7.4</td>
<td>87.4 ± 9.1</td>
</tr>
<tr>
<td>CMC</td>
<td>121.0 ± 16.1</td>
<td>99.3 ± 8.9</td>
<td>83.4 ± 16.4</td>
<td>79.4 ± 2.4</td>
</tr>
<tr>
<td>Guar</td>
<td>147.5 ± 7.7</td>
<td>120.8 ± 9.1</td>
<td>89.5 ± 3.1</td>
<td>99.8 ± 3.8</td>
</tr>
<tr>
<td>Xanthan</td>
<td>111.4 ± 5.4</td>
<td>85.1 ± 7.4</td>
<td>80.5 ± 5.8</td>
<td>82.8 ± 8.2</td>
</tr>
</tbody>
</table>

Values with same letter in the same column are not significantly different (α= 0.05)

The incorporation of MSNF (F3) had a marked influence on the overrun of the frozen mixes (Table 4.4). Milk proteins contain a variety of amphiphilic proteins (caseins) which contain regions of hydrophobicity that provides them with important whipping capabilities (Goff et al., 1989). This effect would be similar in its nature to that exerted by the emulsifier blend on the CSS/sugar formulation yet proteins can have a larger impact merely considering their higher concentration in the system. However, a reduction on the overrun was observed when both MSNF and emulsifier were present (F3e). This shows evidence of interactions between these ingredients, reducing the overall amount of air incorporated. It would be expected that the air cells would be more finely dispersed. However, the images (and magnification chosen) did not allow to conclusively determine this effect.
A substantial decrease in the overrun can be observed as fat is incorporated into the formulation when emulsifier is not present (F3 vs. F4). During freezing, the air interface is comprised of proteins, and, in ice cream, the presence of agglomerating fat. The fat, while having an overall stabilizing effect, will decrease the total volume of air incorporated when compared to only the protein (F3). It has been suggested that liquid fat may spread onto the air/mix interface acting as a foam depressant (Marshall and Arbuckle, 1996). No statistical differences were found between the overrun of ice cream samples with or without emulsifier. In this case, emulsifier is preferentially adsorbed onto the fat interface and the presence of the emulsifier would have an effect on the desired fat destabilization during freezing (leading to a more stable foam), rather than in the total amount of air.

According to the type of stabilizer, guar gum rendered the larger overrun in all formulations. CMC and xanthan gum had comparable overruns, whereas the unstabilized sample had a slightly lower overrun. These differences were particularly evident in the sweetener formulations (F1, F2 and F2e) yet less noticeable as the milk solids and fat were added. These results are in accord with those published by Cottrell et al. (1979a) where guar gum exhibited the highest overrun (168%) compared to other stabilizers including CMC (128%). The general trend reported by the author was that a higher overrun was observed with higher viscosities of the mixes. According to the rheological behaviour, guar gum had a greater pseudoplastic behaviour (lower n and greater K) despite the similarities in the apparent viscosity value to the CMC formulations. However, the pronounced differences in rheology of the unstabilized sample and CMC or xanthan gum did not seem to affect the overrun significantly. Goff et al. (1989) also reported that the overrun of ice cream did not always follow the viscosity behaviour of the mix. In this study the viscosity of the mixes increased due to the presence of certain proteins, however, in some cases (depending on the protein) there was a detrimental effect on overrun despite the increased viscosity.
Despite the fact that overrun does not necessarily follow the rheology of the mixes, the increased microviscosity may be responsible for the behaviour observed in guar gum, i.e., the increase in viscosity of the unfrozen phase as a result of freeze-concentration. According to Goff et al. (1995), the effect of stabilizers on the ice phase enhances the viscoelastic moduli imparting elasticity to the unfrozen phase. Therefore, a more stable foam can be achieved when stabilizers are present, similar to the effect observed in whipped cream (Anderson and Brooker, 1988). Thus, air bubbles are indirectly stabilized by a more elastic unfrozen phase surrounding them, also preventing drainage of serum from the air cells as the frozen mix is extruded. According to the higher pseudoplastic behaviour of guar gum and possibly its behaviour during freeze-concentration (possible onset of coil overlap between polymer chains), such effect would be more pronounced than for the other stabilizers.

4.3 Comparison of mathematical models

As described in Materials and Methods, two specimens for each replicate were analyzed and sample sizes of no less than 100 crystals were considered for each specimen. The equivalent diameters were calculated from the circumvented areas assuming a circular shape. The crystals showed a typical oblong shape as described by their shape factor (0.7-0.8); therefore, the equivalent diameter provides a fair characterization of the ice crystal size.

No significant statistical difference between replicates was found in the majority of the formulations (70% of the data) nor between specimens. However, a conservative approach was considered and each specimen was analyzed independently. Thus, each formulation is represented by 4 independent sets of equivalent diameters for a total of not less than 400 ice crystals.

The arithmetic average of the equivalent diameter was initially considered to describe the distributions mainly due to its common use amongst researchers. As mentioned earlier, the standard deviation may be used to describe the span of the distribution. However, these parameters would only represent the
distribution under the assumption of normality (Gaussian distribution) for the percentage of presence. The Log-Normal distribution is a better model and has proved to have a better fit to these distributions. According to Donhowe et al. (1991), log mean diameters are more appropriate descriptors than the arithmetic averages, however, it requires additional calculation to determine the standard deviation, i.e., to determine the span of the distribution.

In Table 4.5, a comparison between the arithmetic and log average diameters is presented for two distinctive distributions obtained from formulations F0 and F4 for the CMC stabilizer. The correlation coefficients ($R^2$) presented in this table represent the curve fit for the Gaussian and Log-Normal equations as calculated by the TableCurve™ 2D V3 software (Jandel Scientific, Sausalito, CA).

<table>
<thead>
<tr>
<th></th>
<th>Arithmetic</th>
<th>Logarithmic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ($\mu$m)</td>
<td>Span ($\mu$m)</td>
</tr>
<tr>
<td>F0</td>
<td>51.83</td>
<td>22.02</td>
</tr>
<tr>
<td>F4</td>
<td>13.48</td>
<td>9.84</td>
</tr>
</tbody>
</table>

The skewness of the distribution is reflected by the better fit of the log-normal distribution according to their correlation coefficients. Due to the type of skewness (towards the y axis), the arithmetic average would overestimate the average value of the distribution and under estimate the span. The skewness is more pronounced in the case of the ice cream formulation (F4) which also has a markedly smaller average size. As a result, a mathematical inconsistency can be seen from the calculated spans (standard deviations from the means): the span of the distribution surpasses the average value, thus, theoretically there would be negative diameters in the distribution.
The situation can be clearly observed through the plots of the distributions (Figures 4.1 and 4.2). Formulation F0 has a wide distribution with a noticeable skewness towards the y axis which is compensated by adjusting the data to a Log-Normal distribution. For the case of formulation F4, the greater skewness cannot be compensated by the model and the higher range of diameters is importantly underestimated. This increases the standard deviation from the mean creating the inconsistency previously described.

Despite the fact that equivalent diameters may not have a normal distribution, the arithmetic average could still be used as a descriptor of the ice crystal size since the focus of the study is the comparison between formulations. In all cases, the different models were capable of detecting and following the same major trends between the different formulations and storage conditions. However, the parameters (average and deviation) are limited in the description of the crystal behaviour.

The logistic dose response model considers a cumulative function for the distributions. One of the advantages of using a cumulative function is that the distributions become a continuous function, thus the bin sizes of the discrete function do not impose a restriction when analyzing (or reading) parameters or actual values of the distribution. Another important advantage is that by considering the cumulative percentage of presence (percent less than size), deviated values for a particular bin have less impact on the overall distribution. This is clearly observed by the greater correlation coefficient for the distributions previously discussed (Table 4.6). Despite the normality assumption of this function, the model always showed an excellent fit to the data ($R^2$ higher than 0.98). Nevertheless, a restriction of this model has to be noted. Due to the symmetry of the model around the inflection point and considering the skewness of the distribution, this model tends to underestimate the population below this point and overestimate the opposite range. The latter effect helps to explain the better fit of the model to the raw data considering the underestimation of such
Figure 4.1 Histograms for model solution F0 containing CMC. Gaussian and Log-Normal distribution fit.

Figure 4.2 Histograms for ice cream formulation (F4) containing CMC. Gaussian and Log-Normal distribution fit.
range of the other models. Despite the symmetry assumption imposed by the model, the residuals of the model did not show a marked pattern between the two portions of the fit but rather the usual oscillatory pattern of a curve fit to raw data.

Table 4.6 Logistic model parameters for CMC formulations

<table>
<thead>
<tr>
<th>Logistic Model</th>
<th>b (X_{50}) (μm)</th>
<th>n</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>49.05 ± 4.35</td>
<td>-3.56 ± 0.18</td>
<td>0.9978</td>
</tr>
<tr>
<td>F4</td>
<td>10.58 ± 1.04</td>
<td>-2.35 ± 0.32</td>
<td>0.9924</td>
</tr>
</tbody>
</table>

According to this model, the inflection point (b or X_{50}) represents 50% of the population and therefore is comparable to the arithmetic average. However, these values were always lower than the arithmetic average somehow compensating for the overestimation of the latter values. Also, the width (span) of the distribution is not only affected by the n value but also by the B value. Therefore, the slope at the inflection point is a better estimation of the span considering the S-shape of the cumulative percentages. According to the equation of the logistic model and its first derivative evaluated at X=b, the slope can be expressed as:

\[
\text{slope} = -\frac{a-n}{4b} \quad \text{(Eq. 9)}
\]

From this expression it can be seen that the slope depends on all parameters so comparisons using the n values would have to be carefully carried out. However, this parameter can be a direct indication of the span of the population when comparing similar averages. For simplicity, only the slopes at the inflection point will be reported as one of the parameters of the distributions.
and parameter $b$ will be referred to as $X_{50}$ or the average (equivalent) diameter throughout the text.

The graphical comparison between the formulations allows for a clear understanding of the crystal distributions (Figure 4.3). This visual effect is important when studying recrystallization since the curves would not only reflect the overall crystal size growth (through an increase in $X_{50}$), but also valuable information as to how the ice crystals are growing according to changes in the shape of the curve.

Figure 4.3 Logistic model fit. Comparison between formulation F0 and F4.
4.4 Determination of stabilizer concentration and storage time

An initial study was conducted with a 20% sucrose solution (F0) and two levels of stabilizer concentration, namely 0.2 and 0.4%. As mentioned in Materials and Methods, the samples in this case were immersed in liquid nitrogen right after being drawn from the freezer to study the initial ice crystal distribution and crystal growth during a prolonged hardening period. As can be seen from the parameters of the model (Table 4.7) and as corroborated by the statistical analysis, the stabilizers had no measurable effect on the initial crystal size distribution at the time of draw. In general, distributions were characterized by a broad ice crystal population of sizes ranging between 5-160\mu m.

<table>
<thead>
<tr>
<th>Table 4.7 Logistic model parameters for formulation F0 before hardening (fresh), and after 2 and 4 weeks of storage at -30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
| No stab.  
  fresh | 45.74$^a$ | 2.25$^a$ | 49.05$^{aw}$ | 1.97$^a$ |
| 2 weeks  | 51.85$^a$ | 1.75$^a$ | 52.98$^a$ | 1.85$^a$ |
| 4 weeks  | 53.86$^a$ | 2.00$^a$ | 57.17$^a$ | 1.63$^a$ |
| CMC  
  fresh | 52.98$^a$ | 1.85$^a$ | 52.78$^a$ | 1.81$^a$ |
| 2 weeks  | 57.17$^a$ | 1.63$^a$ | 52.55$^a$ | 1.92$^a$ |
| 4 weeks  | 51.94$^b$ | 2.23$^b$ | 48.82$^b$ | 2.01$^b$ |
| Guar  
  fresh | 46.82$^{aw}$ | 2.11$^a$ | 39.32$^{aq}$ | 2.56$^a$ |
| 2 weeks  | 53.57$^b$ | 1.82$^a$ | 47.59$^b$ | 2.15$^b$ |
| 4 weeks  | 51.94$^b$ | 2.23$^b$ | 48.82$^b$ | 2.01$^b$ |
| Xanthan  
  fresh | 44.60$^{aw}$ | 2.48$^a$ | 47.89$^{aw}$ | 2.08$^a$ |
| 2 weeks  | 52.99$^{ab}$ | 1.79$^{ab}$ | 47.62$^{ab}$ | 2.12$^a$ |
| 4 weeks  | 48.47$^{b}$ | 2.12$^{ab}$ | 49.77$^{b}$ | 1.99$^a$ |

$^1$No concentration value is applicable to this case

Values with same letter within each stabilizer set are not significantly different ($\alpha = 0.05$)

Values with the same symbol (between stabilizers) are not significantly different ($\alpha = 0.05$)
For the first stabilizer concentration (0.2%), there is a clear trend between fresh samples and after 2 weeks of storage accounting for crystal growth (Table 4.7). It would be expected that the crystal growth during hardening would have had a higher impact on the average crystal size. Considering the sucrose concentration and water content, according to Equation 4, at the drawing temperature (-5°C) of these samples only 53.5% of the water is in the solid state increasing to a 72.4% during hardening (-30°C). Nevertheless, the increase in diameter observed was approximately 10% of the original size and according to the changes of the n values, the slopes decreased resulting in a broader distribution. These changes are comparable to those typically in ice cream, exiting the freezer with a mean size of ~30-35 μm, increasing to ~45-50 μm after hardening (Hartel, 1996). A different trend can be observed when comparing the differences between 2 and 4 weeks of storage. In this case, the average crystal size remained fairly constant throughout storage, however the changes on the n coefficient indicate an increase in the slopes at the inflection point. The variability of the parameters between specimens did not allow for these differences to be statistically significant in most cases.

The differences between fresh frozen mixes and after 2 weeks of storage are mostly due to the increase of the ice phase volume caused by the lower storage temperature and as reflected by the overall growth of crystals. As observed from Figure 4.4, smaller crystals may have also disappeared by a migratory recrystallization mechanism, increasing the size of larger crystals and therefore the average equivalent diameter of the distribution. Accretion may also have been responsible for some of the changes observed according to the closely packed arrangement of the unfrozen phase. As a result, the plots (Figure 4.4) showed a parallel shift of the distribution (between fresh and 2 weeks storage) implying an overall growth of the ice crystals as the ice phase volume increased. However, as previously stated, recrystallization can also be responsible for these changes as it takes place readily after drawing (Hartel,
Comparatively, the differences between 2 and 4 weeks are caused mostly by an accretive recrystallization mechanism in which the ice phase volume stays constant yet crystals continue to join together and the total number of crystals is reduced. The extent of recrystallization is fairly low so only a minor change in the width of the distribution can be observed, thus the slope (or n) decreased accordingly showing evidence of larger crystals growing and broadening the distribution. This type of mechanism is likely to be responsible considering the constant temperature and the microstructure observed in these samples. That is, the matrix consisted of a large number of ice crystals of different sizes closely packed together (Figure 4.5). The disappearance of smaller crystals by a mechanism such as Ostwald ripening was not evident in these distributions. Even though it would be expected at constant temperature storage, the smaller range of sizes had already disappeared after 2 weeks of hardening and larger crystals are less likely to undergo migratory recrystallization.
Figure 4.5 The microstructure of formulation F0 before (fresh) and after hardening: a) No stabilizer, fresh, b) 0.2% CMC after 2 weeks of storage at -30°C, c) and d) 0.2% and 0.4% guar gum respectively, after 4 weeks of storage at -30°C. Bar = 100 μm (images collected at a magnification of 150x).
Differences between the two concentrations of stabilizer were not significant and the trends between the storage times were similar to those for the lower concentration. Although the rheology of the mixes was not measured in this initial study, it would be expected that the higher concentration would increase the viscosity of the mixes. These changes in the viscosity would have not affected the overall ice crystal structure (Figure 4.5) nor their distribution. According to these results, the following model solutions were prepared according to the lower stabilizer concentration and the ice crystal distributions were determined after hardening and after 4 weeks of constant temperature storage.

4.5 Ice crystallization in model and ice cream formulations

4.5.1 Freezing properties of formulations

The freezing point depression for the different formulations was calculated according to the procedure described by Bradley and Smith (1983). In this procedure the sweeteners are considered according to their equivalence factor, which can be expressed as a sucrose equivalent concentration (Table 4.8). The freezing point depression of the milk salts was also considered. Baer and Keating (1987) reported that the values thus calculated are a good estimation compared to those measured by an osmometer. The percentage of water as ice was calculated according to Equation 4 considering the percentages of the ingredients presented in Table 3.4, and the binding coefficients and moisture content presented in Table 2.4. The following table (Table 4.8) shows some of the parameters related to the freezing properties of the formulations.

In general, an increase of total solids reduces the total amount of water. The less water content, the less amount of freezable water (water available for freezing), the less amount of latent heat to be removed, resulting in a faster
temperature drop in the initial stages of freezing. This may result in an overall faster freezing of the mix promoting the formation of smaller ice crystals. However, once the system reaches the freezing point (faster if there is less amount of water to be frozen), water is removed in the form of ice causing further concentration of the unfrozen phase (freeze-concentration). At this stage, a higher solid content reduces the rate of removal of water as ice depending on the type of solids present and their effect on the freezing point depression.

According to the last column of Table 4.8, the amount of ice formed would decrease as the water content decreases for the different formulations. However, formulation F2 has a higher ice content than formulation F1 (despite the same total solids) as a result of the replacement of sucrose with CSS. This relates to the freezing point depression effect of the type of sweetener in the formulation: replacing sucrose by CSS increases the initial freezing point of the mix (second last column) and more importantly that of the freeze-concentrated unfrozen phase. As a result, the percentage of water frozen is higher for those formulations containing CSS.

According to the initial freezing point depression, the only significant differences are those between formulation F0 and the rest of the model solutions including ice cream, basically due to the differences in the amounts of sweeteners present. It is important to mention that, despite the fact that
stabilizers are polysaccharides, they do not influence the freezing point depression significantly (Shipe et al., 1963; Budiaman and Fennema, 1987a).

Experimentally, it was found that for the first formulation (F0) the frozen mix reached the drawing temperature after approximately 7-8 minutes. A similar profile was seen for the other sweetener formulations (F1, F2) despite the differences in the freezing point depression, whereas formulation F3 had a slightly higher freezing time (10-11 min). Also, those formulations containing emulsifier consistently showed a more prolonged freezing time (13-14 min), particularly with guar gum. This shows the impact of the incorporation of air on the freezing properties of the mixes. The thermal conductivity of air is much lower than that of water and ice at freezing temperature (Table 4.9), therefore exerting an insulating effect and imposing a barrier to the freezing of the mix.

<table>
<thead>
<tr>
<th></th>
<th>$k^1$ (W/(m °K))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.024</td>
</tr>
<tr>
<td>Ice</td>
<td>2.250</td>
</tr>
<tr>
<td>Liquid water</td>
<td>0.594</td>
</tr>
</tbody>
</table>

*Table 4.9 Thermal conductivity at atmospheric pressure for water, ice and air*


4.5.2 Sweetener formulations

As shown in Table 4.10, there was no significant differences ($p > 0.05$) for samples with or without stabilizer in formulations F1 and F2. The type of sweetener did not importantly affect the model parameters of the ice crystal size distribution after hardening (average size and span). However, formulation F2 rendered slightly higher average diameters in most cases. As shown in Figure 4.6, the distributions had an overall increase of the size of the crystal population for formulation F2 (sucrose/CSS) compared to the population for F1. This is in
Table 4.10 Logistic model parameters for formulations F1, F2 and F2e after hardening

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th></th>
<th>F2</th>
<th></th>
<th>F2e</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_{50}$</td>
<td>slope$^1$</td>
<td>$X_{50}$</td>
<td>slope</td>
<td>$X_{50}$</td>
<td>slope</td>
</tr>
<tr>
<td>No stab.</td>
<td>30.31$^{aw}$</td>
<td>3.63</td>
<td>33.35$^{aw}$</td>
<td>2.94</td>
<td>31.50$^{aw}$</td>
<td>3.32</td>
</tr>
<tr>
<td>CMC</td>
<td>28.47$^{aw}$</td>
<td>3.44</td>
<td>32.51$^{aw}$</td>
<td>3.26</td>
<td>32.84$^{aw}$</td>
<td>2.97</td>
</tr>
<tr>
<td>Guar</td>
<td>30.29$^{aw}$</td>
<td>3.13</td>
<td>31.48$^{aw}$</td>
<td>3.08</td>
<td>21.67$^{bc}$</td>
<td>2.98</td>
</tr>
<tr>
<td>Xanthan</td>
<td>33.03$^{aw}$</td>
<td>2.98</td>
<td>30.87$^{aw}$</td>
<td>3.10</td>
<td>31.63$^{aw}$</td>
<td>3.33</td>
</tr>
</tbody>
</table>

$^1$ No significant differences were found between the $n$ values or slopes
Values with same letter in the same column are not significantly different ($a=0.05$)
Values with the same symbol in the same row are not significantly different ($a=0.05$)

![Logistic Model Distributions](image)

**Figure 4.6** Comparison of distributions between formulation F1 and F2 for CMC after hardening
accordance with the differences in the ice phase content for these formulations after drawing (43.9% and 39.5% for formulations F2 and F1 respectively) and after hardening (65.37% and 64.1% for formulations F2 and F1 respectively), as calculated from Equation 4.

As shown in Figure 4.6 and similarly to the curves in formulation F0 (Figure 4.4, fresh vs. 2 weeks hardening), an increase in the ice phase volume would result in a parallel shift of the curves. Thus, considering that the freezing conditions would render the same number of crystals (Hartel, 1996), the differences between the ice crystal distributions (F1 vs. F2) would clearly indicate an overall increase of the size of the ice crystals formed. These differences would also indicate that the increase in the freezing point of the mix exerted by the presence of CSS (freezing temperature achieved faster) is overcome by the increase in the ice phase volume due to the lower freezing point depression of the unfrozen phase.

Comparing these formulations (F1 and F2) to formulation F0, there was a substantial reduction of the average crystal size, as well as an increase of the slopes at the inflection point, therefore a reduction of the span of the distributions. This is a result of the reduction in the amount of water and the increased amount of sweeteners (increase in sweetener/water ratio), factors that reduce the ice phase volume. Sutton and Wilcox (1998a) have previously discussed the effect of changes in the crystal size according to changes of the ice phase volume. That is, a higher ice phase volume renders larger ice crystals in sweetener model solutions. According to the constant freezer parameters, the number of crystals formed are approximately the same, thus the increase in the amount of freezable water would be reflected in a larger crystal size.

The addition of emulsifier to formulation F2 did not affect the average crystal size distribution ($X_{50}$) except in the case of guar gum. The formulations with and without emulsifier for this stabilizer are identical in their freezing properties; however, according to the overrun measurements, they differed in the amount of air incorporated during freezing. Air certainly affects the thermal
properties of the frozen mix (lower thermal conductivity) but it would be expected that all guar formulations would exhibit a proportional reduction in their crystal size according to their higher overruns. Low amounts of air do not importantly influence the overall microstructure, thus a certain amount of air was necessary to have a measurable impact on the ice crystal size. This indicates that air does not only affect the thermal properties but may also act as a physical barrier during freezing. Well dispersed air cells contribute to reduce the probability of collisions between ice crystals as well as dispersing or "stretching" the unfrozen phase around them. As mentioned by Thomas (1981), reduction of the thickness of the unfrozen phase limits the sizes of crystals by mechanical hindrance exerted by the air/fat structure.

Microscopical examination showed that the presence of a high amount of air bubbles finely dispersed, confines the unfrozen phase to thinner and more isolated portions during freezing (Figure 4.7). However, this ability to initially reduce the ice crystal size does not necessarily translate in an effective physical barrier during crystal growth or recrystallization. Air bubbles can easily be deformed or burst by growing ice crystals during storage.

4.5.3 Milk solids-non fat formulations

The incorporation of milk solids-non-fat (MSNF) into the formulation (F3) also reduced ice crystal sizes according to the reduction of the amount of water (reduction of the ice phase volume) and the increase in the overrun (Table 4.11). According to the statistical analysis, no significant differences were found between any of the stabilizers or the unstabilized mixes in formulation F3 despite the differences in their overruns. This is probably due to the extremely high overrun of these mixes, incorporating an excessive amount of air and not properly dispersing or stabilizing it. The weaker structure thus formed may also collapse more easily promoting accretion between the existing crystals over the size reduction effect exerted by the air bubbles. According to rheological measurements in ice cream reported by Goff et al. (1995a), the volume of air
Figure 4.7 Microstructure of formulation F2 with guar gum after hardening: a) no emulsifier, b) 0.15% emulsifier blend. Bar = 50 μm (Images collected at a magnification of 250x).
likely reached a critical volume (over 80%) at which point, increasing overrun causes a volume expansion but has less impact on the air structure and, therefore on the overall microstructure.

The addition of emulsifier slightly reduced the average ice crystal size ($X_{50}$) and the span of the distributions in most cases as shown by the slopes at the inflection point. Considering the similarities between emulsifier and no emulsifier formulations in their freezing characteristics, this could only be attributed to the way the air was incorporated; although less in the emulsifier formulation, more finely dispersed (Figure 4.8). Again, in this case the higher overruns in guar gum and CMC formulations exhibited a statistically significant lower average ice crystal size compared to xanthan gum and no stabilizer. According to these differences observed in the emulsified formulations, it can be proposed that not only the amount but also the way the air is dispersed may affect the ice crystal distribution importantly.

4.5.4 Milkfat formulations (ice cream)

The addition of milkfat (cream) also reduced the ice crystal size according to the reduction of the water content (reduction of ice phase volume). The
Figure 4.8 Microstructure of formulations F3(e) and F4(e) with xanthan gum after hardening. a) and b) formulation F3, without and with emulsifier, respectively; c) and d) formulation F4, without and with emulsifier, respectively. Bar = 50 μm (Images collected at a magnification of 250x).
addition of stabilizers or emulsifiers did not affect the average crystal size (Table 4.12). However, the slope of the distribution of xanthan gum was significantly higher than the other stabilizers, thus rendering a somewhat narrower population.

Table 4.12 Logistic model parameters for formulations F4 and F4e after hardening.

<table>
<thead>
<tr>
<th></th>
<th>F4</th>
<th>F4e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_{50}$</td>
<td>slope $^a$</td>
</tr>
<tr>
<td></td>
<td>(µm)</td>
<td>(%/µm)</td>
</tr>
<tr>
<td>No stab.</td>
<td>9.56 $^a$</td>
<td>5.46</td>
</tr>
<tr>
<td>CMC</td>
<td>10.58 $^a$</td>
<td>5.66</td>
</tr>
<tr>
<td>Guar</td>
<td>9.91 $^a$</td>
<td>5.44</td>
</tr>
<tr>
<td>Xanthan</td>
<td>10.40 $^a$</td>
<td>6.47</td>
</tr>
</tbody>
</table>

$^a$ No significant differences were found between the n values or slopes. Values with same letter in the same column are not significantly different ($\alpha = 0.05$)

Considering the comparatively similar overruns of these samples no significant differences would be expected in the ice crystal according to the similar amount of air incorporated (similar microstructure). The emulsifiers most likely did not have a measurable impact on the way the air was incorporated in this case due to presence of fat acting as the main air stabilizer. Emulsifiers would preferably be involved in fat destabilization (adsorption onto fat globules during ageing) than directly involved in the stabilization of air cells during freezing.

The average diameter ($X_{50}$) of the ice cream formulations was surprisingly small compared to values reported in other studies. However, other studies have considered the average diameter (overestimates a log-normal distribution) or the major length of the ice crystal as the descriptor of the ice crystal size (Caldwell et al., 1992b). A probable cause for these smaller values is the criterion used when considering accreted crystals. Thus, according to the individual particle approach, more crystals are considered and therefore smaller sizes would be
measured. However, all samples were treated equally and the degree of accretion of crystals were similar independent of the type of samples. Also, the consistency of the measurements would rule out the possibility of an artifact and allow for comparisons to be meaningful.

4.5.5 Comparison of formulations after hardening

A graphical comparison of the ice crystal distributions for model solutions and ice cream can be observed in Figure 4.9. This plot shows the distributions according to the parameters obtained for the logistic model for CMC formulations. The characteristic reduction of the ice crystal size and the span of the distribution is a direct result of the reduction of the water content and/or the increase in total solids and the effect on the ice phase content after drawing (Table 4.8) and after hardening (Table 4.13). (Ice contents in these tables were calculated according to Equation 4 for the two different temperatures). However, it should be noted that the deviation of formulation F2 from this behaviour is due to the effect of the type of solids (CSS) in the amount of frozen water after drawing and after hardening.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Total Solids (% w/w)</th>
<th>Ice Content at -30°C (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>20.0</td>
<td>72.4</td>
</tr>
<tr>
<td>F1</td>
<td>26.0</td>
<td>64.1</td>
</tr>
<tr>
<td>F2</td>
<td>26.0</td>
<td>65.3</td>
</tr>
<tr>
<td>F3</td>
<td>30.5</td>
<td>60.4</td>
</tr>
<tr>
<td>F4</td>
<td>36.0</td>
<td>56.2</td>
</tr>
</tbody>
</table>
The differences in the distributions between model solutions and ice cream are not only in their average size but also in the width (span) of the distributions (Figure 4.9). This is directly related to the microstructure of the samples. Thus, simplified models, such as those containing only sweeteners, do not contain air and the unfrozen matrix is dispersed around crystals of a wider range and larger sizes. It was also noticeable that the unfrozen phase of sweetener formulations was unevenly distributed around the crystals (areas of higher thickness) compared to those containing air. It should also be noted that formulation F3 and F4 exhibited an important percentage of small crystals (below 10μm), whereas for the different sweetener formulations this range was almost non-existent. This might greatly influence the rate of recrystallization during storage, particularly when dictated by the migratory mechanism.

Linear regressions were performed between the total solid content (TS) of the different formulations and the average diameter ($X_{50}$), and the ice content
(IC) after hardening of the different formulations and the average diameter \(X_{50}\) for each stabilizer and no stabilizer formulations (Table 4.14). The total solids were calculated considering (or not) the emulsifier content, hence the regressions were accordingly performed for the average diameters obtained for formulations with and without emulsifiers.

Table 4.14 Correlation coefficients of linear regression for total solids (TS) and ice content (IC) vs. average diameter \(X_{50}\) for frozen mixes after hardening

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficient ((R^2))</th>
<th>(TS) vs. (X_{50})</th>
<th>(IC) vs (X_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Emulsifier</td>
<td>Emulsifier</td>
<td>No emulsifier</td>
</tr>
<tr>
<td>No stab.</td>
<td>0.9691</td>
<td>0.9738</td>
<td>0.9970</td>
</tr>
<tr>
<td>CMC</td>
<td>0.9523</td>
<td>0.9046</td>
<td>0.9762</td>
</tr>
<tr>
<td>Guar</td>
<td>0.9236</td>
<td>0.8474</td>
<td>0.9894</td>
</tr>
<tr>
<td>Xanthan</td>
<td>0.9616</td>
<td>0.9703</td>
<td>0.9802</td>
</tr>
</tbody>
</table>

In general, as shown in Table 4.14, the correlation coefficients from the linear regression analysis were fairly high for both parameters (TS and IC). This shows that there is an acceptable correlation between the amount of solids and the ice crystal size (characterized as an average of the population) since it is directly related to the amount of water present. However, as mentioned earlier, the type of solids and their influence on the freezing properties of the mixes are also important. Higher correlation coefficients were observed for the ice content regressions compared to those for total solids. According to this, using an estimate for the frozen content of water (IC calculated from Equation 4) would be a better parameter to predict the average ice crystal size since it considers the direct effect of the type of solids on the ice phase content. A good correlation between the total ice content (representing the volume) and a unidimensional measurement (diameter considering a round shape) would be expected. Also of
great importance, these results show consistency in the ice crystal size measurements and characterization, as well as for the ice phase content approximation.

Comparing the different stabilizers and no stabilizer formulations (Table 4.14), the correlation coefficients were fairly high for no stabilizer, CMC and xanthan gum formulations whereas guar gum showed a considerably lower coefficient, particularly for the emulsifier formulations. This can in part be explained by the deviated values (lower average diameters than those estimated by the linear correlation) due to the presence of air in the microstructure. The low correlation coefficients for guar gum coincide with the noticeable impact of higher overruns in lowering the average ice crystal size of certain formulations (e.g., F2e). Therefore, air may cause a deviation in the linear dependence found between total solids or the amount of ice formed, and the characteristic crystal size (average equivalent diameter). However, no effect on the amount of frozen water (ice volume) would be expected by the presence of air, thus the effect could only be attributed to differences in the microstructure of the unfrozen phase affecting the characteristic crystal size.

An interesting result from the regression analysis (Table 4.14) was the better fit observed for the no stabilizer compared to the stabilizers formulations. The effect of air in the microstructure as a result of higher viscosities of stabilizer formulations was not responsible for this behaviour considering that in most cases, xanthan gum had comparable amounts of air incorporated to the no stabilizer. Therefore, this might be an indication that the presence of stabilizers may also deviate the linear correlation by affecting the microstructure or the characteristic of the unfrozen phase, hence affecting the crystal size distributions. However, these differences were very small and no consistent conclusions can be drawn from these results.
4.5.6 Discussion of the effect of stabilizers on ice crystallization

As shown by the results in model and ice cream formulations, stabilizers did not directly affect the ice crystal size distribution or morphology of the frozen mixes after hardening. According to several studies, they do not consistently affect the freezing properties of the mixes (freezing point depression, ΔHf) (Muhr and Blanshard, 1984; Buyong and Fennema, 1988), the nucleation process (Muhr and Blanshard, 1986) or the thermodynamic properties of the mix or the unfrozen phase (Goff et al., 1993; Blond, 1994; Goff and Sahagian, 1996b; Hagiwara and Hartel, 1996). According to Muhr et al. (1986), only a massive effect on the nucleation would affect the product considering the importance of secondary nucleation in a continuously agitated (scraped) system. Therefore, it would not be expected for stabilizers to directly affect the initial ice crystal size distribution in this type of system. Miller-Livney and Hartel (1997) using a scraped-surface heat exchanger (batch process) reported no difference in ice crystal size distribution between unstabilized and stabilized mixes (carrageenan, gelatin, LBG and xanthan).

Goff et al. (1993) reported smaller ice crystals in ice cream after hardening containing a blend of stabilizers (locust bean gum and carrageenan). However, in this study the samples were frozen using a continuous freezer in which the freezing conditions are much different than in a batch process. In the former, the flow rate (residence time) is adjusted to obtain the extrusion temperature, therefore, there is no dwell time of the mixes at the extrusion temperature. This variable may exert an important effect on the initial ice crystal distribution as shown by the rounding off of crystals in the batch process with longer dwelling time (discussed further in the text). Also, and more importantly, the continuous process is carried out under pressure (~5 atm) so as to reduce the effect of the air phase volume on thermal conductivity (Table 4.9). As the product is extruded and released to atmospheric pressure, the air expands possibly altering the microstructure of the unfrozen phase. Under these conditions, stabilizers may affect the initial ice crystal distributions by reinforcing
the unfrozen phase as shown by the increase of the elastic component \((G')\) of this phase (Goff et al., 1995a). This may help to protect against accretion as the air expands and ice crystals are forced together. Also considering that recrystallization takes place at early stages of the freezing process in the barrel of a scrape-surface heat exchanger (Hartel, 1996), if stabilizers are capable of controlling recrystallization to some degree at this stage, smaller ice crystal distributions could be expected after extrusion.

Sutton and Wilcox (1998b), according to earlier studies in model solutions of the unfrozen phase, had reported no effect of locust bean gum or guar gum on the initial average ice crystal diameter of frozen mixes; however, in ice cream studies they reported larger ice crystals as a result of the addition of locust bean gum, contrary to what was expected. As established from our model solutions, only a reduced amount of air is incorporated when sweeteners like sucrose are used. Their model solutions were formulated using fructose (comparable to sucrose), thus despite using a continuous freezer the amount of air incorporated would be expected to be fairly low (overrun values not mentioned). Therefore, the impact of the expansion of air (low amount) may not have affected the microstructure of the model solution. This would be comparable (despite the differences in the type of freezers) with the results observed in our sweetener model solutions (F0, F1) according to a low content of air. On the contrary, in an ice cream formulation a considerable amount of air can be incorporated during freezing (set at 90% in their studies). However, as seen from our results an important variable is the way the air is incorporated, i.e., the effect that emulsifiers and stabilizers might have on the air cells. Emulsifiers were not used in their ice cream formulation, therefore it is possible that the stabilizer (LBG) might have somehow affected the way air was incorporated (LBG would have a similar behaviour as guar gum) affecting the unfrozen phase microstructure as it expanded.

Blond (1986), using a U-shape tube studied the effect of macromolecules (CMC, gelatin and pectins) on the linear velocity of crystallization. They
concluded that the viscosity of the solutions had little effect on crystallization. The propagation rate was only slightly reduced for a viscosity increase of as much as 500 times. However, Budiaman and Fennema (1987b) using a similar system reported that an increase in viscosity reduced the linear rate of crystallization in hydrocolloid suspensions. The limitations of the methodology used in this study (U tube), may not allow to extrapolate such effects to a mechanically agitated system in which secondary nucleation controls the crystallization process. Thus, conclusions from a linear velocity measurement may not be applicable to our system. As seen from our results, no relationship between the apparent viscosities of the stabilized mixes or the viscosity of the unstabilized mix and the ice crystal distribution was found. As has been reported in other studies (Sutton et al., 1994; Miller-Livney and Hartel, 1997), the macroscopic viscosity does not affect the ice crystal size distribution directly. However, depending on the freezing conditions, (e.g., the freezer type, pressure in the barrel) the rheological behaviour of the partially frozen mix would affect the whipping characteristics of the mix or the microviscosity of the unfrozen phase during extrusion rendering differences in the ice crystals size distributions.

4.6 Recrystallization in ice cream formulations for different storage conditions
4.6.1 Effect of low temperature storage in model and ice cream formulations

Storage at a low constant temperature (-30°C) did not importantly affect the ice crystal distributions of the different formulations (Appendix 8.2) when compared to those after hardening (Tables 4.10, 4.11 and 4.12). The glass transition temperature of ice cream can range from -24 to -43°C according to the formulation (Levine and Slade, 1990; Goff et al., 1993). Considering the concentrations of sweeteners in the formulations under study, the glass transition temperature would certainly range between those values. Therefore, it is not surprising that the storage time at this low temperature did not affect the overall ice crystal size of the samples. Stabilizers do not affect the glass
transition temperature of ice cream (Goff et al., 1993), particularly considering the different studies in model solutions of the amorphous unfrozen phase (Blond, 1994; Sahagian and Goff, 1995b). Also, the reduction on the ice crystal size due to the presence of higher amounts of air did not affect the distributions after low constant temperature storage.

The shape factor of the crystals (roundness) for formulations F0, F1, F2 (with and without emulsifier) after the low temperature storage period (-30°C, 4 weeks) did not show statistically significant differences (p>0.05) (Table 4.15).

Table 4.15 Shape factor after hardening and after 4 weeks of storage (-30°C) for model sweetener solutions (F0,F1,F2)

<table>
<thead>
<tr>
<th></th>
<th>Shape Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0</td>
</tr>
<tr>
<td>0 weeks</td>
<td>No stab</td>
</tr>
<tr>
<td></td>
<td>CMC</td>
</tr>
<tr>
<td></td>
<td>Guar</td>
</tr>
<tr>
<td></td>
<td>Xanthan</td>
</tr>
<tr>
<td>4 weeks</td>
<td>No stab</td>
</tr>
<tr>
<td></td>
<td>CMC</td>
</tr>
<tr>
<td></td>
<td>Guar</td>
</tr>
<tr>
<td></td>
<td>Xanthan</td>
</tr>
</tbody>
</table>

Values with same letter for the same stabilizer in the same column are not significantly different (α= 0.05)

However, the shape factor increased for the rest of the formulations (F3 and F4) after the storage period, except for formulation F4e (Table 4.16), coinciding with the higher overruns of these samples, namely F3, F3e and F4. Although the average crystal size was fairly similar, this effect (increase in the shape factor) could also be seen as a statistically significant decrease in the slope of the distributions (Appendix 8.2) denoting a certain degree of
recrystallization during storage. This can be attributed to the rounding off effect of iso-mass recrystallization. This recrystallization mechanism at a constant storage temperature was also reported by Donhowe and Hartel (1996a) in ice cream samples. The greater effect in samples containing higher overrun could be related to the microstructure of the samples. A greater deformability of the matrix would be expected by the presence of air cells in the microstructure, allowing ice crystals to reshape more easily. This is particularly true considering that air bubbles can be easily distorted by growing ice crystals (Caldwell et al., 1992b).

Table 4.16 Shape factor after hardening and after 4 weeks of storage (-30°C) for model solution F3 and ice cream (F4)

<table>
<thead>
<tr>
<th>Shape Factor</th>
<th>F3</th>
<th>F3e</th>
<th>F4</th>
<th>F4e</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No stab</td>
<td>0.71⁰</td>
<td>0.72⁰</td>
<td>0.79⁰</td>
<td>0.80⁰</td>
</tr>
<tr>
<td>CMC</td>
<td>0.69⁰</td>
<td>0.70⁰</td>
<td>0.75⁰</td>
<td>0.78⁰</td>
</tr>
<tr>
<td>Guar</td>
<td>0.71⁰</td>
<td>0.71⁰</td>
<td>0.77⁰</td>
<td>0.76⁰</td>
</tr>
<tr>
<td>Xanthan</td>
<td>0.68⁰</td>
<td>0.70⁰</td>
<td>0.77⁰</td>
<td>0.78⁰</td>
</tr>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No stab</td>
<td>0.74⁰</td>
<td>0.76⁰</td>
<td>0.78⁰</td>
<td>0.79⁰</td>
</tr>
<tr>
<td>CMC</td>
<td>0.73⁰</td>
<td>0.78⁰</td>
<td>0.79⁰</td>
<td>0.77⁰</td>
</tr>
<tr>
<td>Guar</td>
<td>0.76⁰</td>
<td>0.74⁰</td>
<td>0.80⁰</td>
<td>0.76⁰</td>
</tr>
<tr>
<td>Xanthan</td>
<td>0.72⁰</td>
<td>0.76⁰</td>
<td>0.74⁰</td>
<td>0.76⁰</td>
</tr>
</tbody>
</table>

Values with same letter for the same stabilizer in the same column are not significantly different (α= 0.05)

Goff et al. (1995a), demonstrated through rheological measurements that increased amounts of air contributed importantly to an increase of the elastic component (G', solid-like behaviour) of ice cream. However, through large-scale deformation testing, higher overruns led to ice cream that was more easily deformed. The author stated that despite the apparent incongruity of these
results, this represents the difference between recovery from a nominal stress
deformation (oscillatory stress) and a permanent deformation (puncture). Crystal
growth during storage could be more easily regarded as a permanent
deformation of the matrix since crystals are capable of deforming the air cells
and even rupturing them as they grow (Caldwell et al., 1992b). Thus, the
presence of air leads to a microstructure that can be more easily deformed
(permanently) under the stress of crystal growth.

It can also be noted that F0 exhibited a higher shape factor (0.76)
compared to F1 (0.72). This could be attributed to the freezing profile of these
formulations. F0 had a longer overall whipping time at drawing temperature (as
discussed in section 4.5.1) compared to F1, favouring the rounding off of
crystals. In the latter, the time to reach the freezing temperature of the mix was
comparable to that of F0, however crystal growth during freezing would occur at
a slower rate in a formulation with higher solid content (F1) due to the barrier
imposed by the freeze-concentration of an unfrozen phase with a progressively
lower freezing point. This results in a longer freezing profile and a reduction of
whipping time after crystal growth (after the equilibrium ice phase volume has
been achieved). This freezing profile is comparable to that of formulation F2 and
so was the shape factor. Formulation F3 rendered a slightly lower shape factor
(0.70), which can be directly related to the increase in the freezing time due to
the incorporation of air, i.e., the increase of the thermal mass increasing the time
to reach the freezing point and crystal growth. Therefore, despite the reduction
in the water content, the overall freezing time increased, reducing whipping time
after crystal growth. The shape factor of formulation F4 was higher (0.76) than
F3 possibly due to a better distributed and less overall amount of air
incorporated and the concomitant lower freezing time. Therefore, a relationship
could be established between the dwelling time at temperature of the batch
process and the degree of rounding off (iso-mass recrystallization) of the
crystals after hardening.
In addition to isomass recrystallization, migratory recrystallization would also be expected to be responsible for part of these changes considering the high percentage of smaller crystals present after hardening. Ostwald ripening would dictate that the smallest crystals would continue to disappear and the largest crystals would continue to grow as the system progresses towards the attainment of equilibrium. However, it might also be expected that as the driving force for this process decreases, the system will become more stable once the smallest crystals have disappeared, and further migratory recrystallization becomes less probable. These observations also reassure the importance of considering the whole distribution of ice crystals and not only the average sizes since slight changes can go undetected by such rough representation of the distribution.

4.6.2 Effect of high storage temperature in ice cream

As discussed in the previous section, the low storage temperature (-30°C) had little effect on the average ice crystal size \(X_{50}\) after storage in ice cream and no significant differences were found between formulations with and without emulsifier (F4 and F4e) after this storage condition (Table 4.17). However, the impact of a higher storage temperature (-16°C) was reflected by an increase in the average size and a broader distribution of crystals (lower slopes) (Table 4.17). Samples were initially hardened (30°C, 24 hours) and then transferred to the tempering cabinet (-16°C) for the rest of the storage period (4 weeks). Thus, samples most likely attained the 56.2% ice content after hardening (Table 4.13), later being reduced to 52.7% (ice content at -16°C) as the samples were transferred to the tempering cabinet. Such changes of the ice phase volume would result in smaller crystals disappearing (melting) due to their lower melting points (or greater solubilities) (Fennema, 1973). This effect alone would explain the increase in the average diameter observed. However, the broadening of the distribution (lower slopes) implies that larger crystals also increased in size, ultimately showing evidence of recrystallization. Migratory
recrystallization (Ostwald ripening) was most likely the most important recrystallization mechanism involved during warming and throughout storage. These samples contained large amounts of small crystals after hardening (typically 15% between 0 and 5μm) which disappeared after the storage time at -16°C (typically 2% between 0 and 5μm). An example of these changes are shown in Figure 4.10 in which the raw data for CMC and no stabilizer formulations were plotted after 4 weeks of storage at the different temperatures.

Table 4.17 Logistic model parameters for formulations F4 and F4e after 4 weeks of storage at different temperatures

<table>
<thead>
<tr>
<th></th>
<th>F4</th>
<th>F4e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_{50}$</td>
<td>slope (%)</td>
</tr>
<tr>
<td></td>
<td>(μm)</td>
<td>(μm)</td>
</tr>
<tr>
<td>-30°C No stab.</td>
<td>10.49$^a$</td>
<td>4.59$^a$</td>
</tr>
<tr>
<td>CMC</td>
<td>9.80$^a$</td>
<td>5.35$^a$</td>
</tr>
<tr>
<td>Guar</td>
<td>9.67$^a$</td>
<td>5.13$^a$</td>
</tr>
<tr>
<td>Xanthan</td>
<td>12.24$^a$</td>
<td>4.98$^a$</td>
</tr>
<tr>
<td>-16°C No stab.</td>
<td>21.51$^a$</td>
<td>2.06$^a$</td>
</tr>
<tr>
<td>CMC</td>
<td>20.87$^a$</td>
<td>2.66$^b$</td>
</tr>
<tr>
<td>Guar</td>
<td>20.29$^a$</td>
<td>2.39$^{a,b}$</td>
</tr>
<tr>
<td>Xanthan</td>
<td>18.66$^a$</td>
<td>2.96$^b$</td>
</tr>
</tbody>
</table>

Values with same letter in the same column for a particular temperature are not significantly different ($\alpha=0.05$)
Accretion is another mechanism most likely to be involved in the ice crystal size changes observed. The initial microstructure of the samples exhibited crystals closely packed and confined to a thinner and more unevenly distributed (isolated areas) unfrozen phase with a large amount of crystals in contact. The melting of smaller crystals would also promote the accretion of larger crystals as they might participate in the “neck” formation between two crystals. According to Sutton et al. (1996a) the first stages of recrystallization of model solutions at constant storage temperature (-15°C) involved smaller crystals mainly disappearing through this accretive mechanism. Therefore, it is feasible to assume that some of the melting small crystals may have joined larger crystals as well as participation in the rounding off of already accreted crystals.

As shown by the statistical analysis, stabilizers did not prevent the recrystallization phenomenon observed in ice cream during constant
temperature storage. According to the mechanisms explained (i.e., migratory recrystallization also promoting accretion), stabilizers would not be capable of controlling or reducing crystal migration, particularly during warming. As shown by Sahagian and Goff (1995b), according to proton ($^1$H) NMR, water mobility is not hindered by the presence of stabilizers even at temperatures below $T_g$. Accordingly, stabilizers are not capable of reducing water diffusion during warming and recrystallization is not impeded.

It should also be noted that in some cases the correlation coefficients of the fit to the logistic model were slightly lower for the higher storage temperature (-16°C) than those normally observed (0.97 compared to a usual value of 0.99). This was observed mostly in the case of no stabilizer or high overrun (guar gum) in which there was an uneven growth of certain sizes of the population. Figure 4.10 compares the raw distributions between the no stabilizer and CMC formulations showing the deviation from the S-shape curves after storage at -16°C. This behaviour coincided with a larger extent of damage to the air bubbles (larger crevices) after the storage period (Figure 4.11). The collapse of a weaker matrix may induce irregular changes in ice crystal sizes by promoting accretion of the crystals. In such event, a stabilized sample with a high overrun (like guar gum) would apparently have poor control over recrystallization. However, the structure deformation (shrinkage) would be responsible for this occurrence, rather than a low effectiveness of the stabilizer. According to Goff et al. (1995b), the increased viscosity of the serum, contributed by the presence of stabilizers, helps to control shrinkage (volume loss) in ice cream during storage. However, as previously mentioned, such effect may be overcome by the impact of a high content of air (high overrun) as observed for the guar gum formulation.

The latter observation is one of the advantages of using low-temperature SEM microscopy. This technique allows to obtain images of the structure of ice cream and its components (air cells, lamella, crystals, etc.), providing a complete overview of the structural changes and, particularly, the effect of one structural component on the overall microstructure.
Figure 4.11 Microstructure of ice cream after low (-30°C) and high (-16°C) storage temperatures. a) and c) No stabilizer and CMC, respectively, stored at -30°C; b) and d) No stabilizer and CMC, respectively, stored at -16°C. Bar = 50 μm (Images collected at a magnification of 250x).
4.6.3 Effect of temperature cycles in ice cream formulations

The following experiments were performed only for ice cream formulations with emulsifier (full ice cream formulation). Samples previously stored at -16°C for 4 weeks were further stored at this temperature before the temperature cycle was performed. The extension of the storage period to 6 weeks did not importantly affect the average diameter ($X_{50}$) except for the case of the no stabilizer formulation (Table 4.18). In this case, a noticeable increase in the average diameter as well as the slope was observed. A narrower distribution (increase of the slope) may be explained by a massive disappearance of small crystals and a less important growth of larger crystals. However, the variance within the replicates did not allow for the differences be statistically significant.

Table 4.18  Logistic model parameters for 4 and 6 weeks of constant storage temperature (-16°C), and for a low amplitude temperature cycle (Set 1)

<table>
<thead>
<tr>
<th></th>
<th>4 weeks (constant)</th>
<th>6 weeks (constant)</th>
<th>Set 1 (-15°C ± 2°C / 3 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_{50}$ (µm)</td>
<td>slope ($%/µm$)</td>
<td>$X_{50}$ (µm)</td>
</tr>
<tr>
<td>No stab.</td>
<td>16.91 $^a$V</td>
<td>2.57</td>
<td>19.99 $^a$V</td>
</tr>
<tr>
<td>CMC</td>
<td>21.40 $^a$V</td>
<td>3.14</td>
<td>22.78 $^a$V</td>
</tr>
<tr>
<td>Guar</td>
<td>19.49 $^a$V</td>
<td>2.43</td>
<td>18.10 $^a$V</td>
</tr>
<tr>
<td>Xanthan</td>
<td>18.94 $^a$V</td>
<td>3.39</td>
<td>22.32 $^a$V</td>
</tr>
</tbody>
</table>

$^a$No significant differences were found between the $n$ values or slopes
Values with same letter in the same column are not significantly different ($α= 0.05$)
Values with the same symbol in the same row are not significantly different ($α= 0.05$)

The temperature cycling (3 cycles of 15 ± 2°C) did not affect the ice crystal size distribution (Table 4.18). The amplitude of the temperature fluctuation (± 2°C), length of each cycle (3 h) or number of cycles (3) did not allow for the samples to undergo a significant recrystallization. The high
temperature storage period induced a massive effect on ice crystal sizes and distribution, and the disappearance of most of the smaller crystals. Such crystals are most likely to be affected by a temperature fluctuation of such magnitude and time length. Thus, the extent of the ice phase volume changes in this case (2.2%, difference between the ice content at -13°C and -17°C) did not exert an effect on the overall sizes of the distributions. However, it is possible that an increase in the time length of each cycle would influence the recrystallization behaviour. No particular effect by the presence of stabilizers was observed.

Temperature fluctuations would be expected to have a greater impact on ice crystal sizes and distributions for samples previously stored at -30°C. Accordingly, the differences between the parameters of the latter (Table 4.17) and those for even the less severe set of cycles (Set 2: -15°C ± 2°C / 3 cycles) (Table 4.19) were statistically significant (p≤0.05). However, an increase in amplitude of the temperature fluctuation (Set 3: -15°C ± 5°C / 3 cycles) had a similar effect on the ice crystal distributions as the previous set (Set 2) (Table 4.19). Thus, the increase in ice content changes (2.2% and 6.0% for Set 2 and 3 respectively) did not seem to affect the recrystallization behaviour after 3 cycles for the respective cycle times (i.e., 3 and 4 h for Set 2 and 3 respectively). However, an increase in the number of cycles (Set 4: 15°C ± 5°C / 9 cycles) showed a considerable increase in the average diameter with an increase of the slope at the inflection point (Table 4.19).

A graphical comparison for one of the stabilizers (CMC) helps understand the changes in the distributions as a result of the temperature cycles after the storage period (Figure 4.12). As shown by the distributions according to the logistic parameters, the result of 3 cycles (Set 2 and Set 3) was an increase in the average crystal size and a reduction of the slope compared to the original distributions (storage at -30°C). The decrease in slope implies that the greater
Table 4.19 Logistic model parameters for temperature cycles of samples previously stored at -30°C

<table>
<thead>
<tr>
<th></th>
<th>Set 2 (-15°C ± 2°C / 3 cycles)</th>
<th>Set 3 (-15°C ± 5°C / 3 cycles)</th>
<th>Set 4 (-15°C ± 5°C / 9 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₅₀ (µm)</td>
<td>slope¹ (%/µm)</td>
<td>X₅₀ (µm)</td>
</tr>
<tr>
<td>No stab.</td>
<td>20.68ᵃ</td>
<td>2.80</td>
<td>20.76ᵃ</td>
</tr>
<tr>
<td>CMC</td>
<td>17.92ᵃ</td>
<td>3.70</td>
<td>17.45ᵃ</td>
</tr>
<tr>
<td>Guar</td>
<td>17.24ᵃ</td>
<td>3.93</td>
<td>15.41ᵃ</td>
</tr>
<tr>
<td>Xanthan</td>
<td>16.36ᵃ</td>
<td>3.83</td>
<td>15.42ᵃ</td>
</tr>
</tbody>
</table>

¹ No significant differences were found between the n values or slopes. Values with same letter in the same column are not significantly different (α = 0.05)

Logistic Model Distributions for CMC formulations

Figure 4.12 Comparison of model distributions for CMC formulations after constant storage temperatures and temperature cycles.
effect was the disappearance of smaller crystals by recrystallizing onto larger crystals (Ostwald ripening), rather than the parallel shift observed by an increase in ice volume (Figure 4.6) or as a possible result of accretion (reduction of total number of crystals, no distinction according to size). It is also clear from this plot that there was no difference between sets 2 and 3 (amplitude effect). The average diameter of samples stored at -16°C (no effect of the cycling in these samples) were comparable to those for sets 2 and 3. However, the differences between the slope revealed an increased number and size of larger crystals after the storage temperature. Again, this is an indication of migratory recrystallization but could also be a result of a combined effect of the latter with an accretive mechanism. Accretion would most likely be induced and/or promoted by the melting of smaller crystals, considering their relative amount in the distribution and their disappearance after the cycles (Set 2 and 3) or the constant storage temperature (-16°C). It is important to clarify that the trends observed in the distribution according to the logistic model parameters between these sets of samples (Set 2 or 3, and storage at -16°C) were clearly observed in the raw data, thus the model proved to be fairly accurate predicting these differences in the shape of the curves.

From these results it cannot be concluded that the temperature fluctuations (or the amplitude) of sets 2 and 3 were solely responsible for the changes in the crystal sizes. The results were most likely caused by the severe increase in temperature (from -30°C to -16°C), i.e., changes in the ice phase volume with the migratory/accretive recrystallization behaviour previously discussed for the high storage temperature (-16°C). The pronounced effect of the prolonged temperature cycle (Set 4) would indicate that in this case the temperature fluctuations were mostly responsible for further changes observed in the average crystal size. The effect of this cycling set (Set 4) with a range midpoint of -15°C was greater than that observed for a comparable constant storage temperature (4 weeks at -16°C) (Figure 4.12). This greater effect of temperature fluctuations compared to constant storage temperature has been
previously reported by other researchers (Harper and Shoemaker, 1983; Donhowe et al., 1996a).

Donhowe and Hartel (1996b) concluded that an important factor affecting recrystallization rates during temperature fluctuations is the extent of the fluctuation, e.g., amplitude and cycle time. They found a lower threshold amplitude (0.25°C) below which oscillation had no effect, an apparent higher limit on the amplitude effect (results at ±1°C were not statistically different to those at ±2°C) and that longer cycle times had a greater impact on the recrystallization rates. An important consideration is that an increase in the amplitude of the cycle also increases the ice phase volume changes and, therefore, the thermal mass and amount of latent heat to be exchanged. This sensitivity of the recrystallization behaviour to the extent of the fluctuation may in part explain observing no measurable differences between Set 2 and 3. As mentioned before, a longer cycle time might have been necessary in order to observe a measurable effect on the recrystallization rate or behaviour after 3 cycles (Set 1, 2 and 3). However, the greater impact of the increase in the average temperature and the resulting recrystallization behaviour was most likely the predominant factor affecting the ice crystal distributions.

The results from sets 2 and 3 showed no statistically significant differences between stabilized and no stabilizer samples. However, the tendency observed was for the latter to render a consistently higher average diameter and lower slope, reflecting an overall larger and broader population of crystals, particularly considering the similarities between the initial distributions after the storage period (Table 4.17). Such results indicate that stabilizers would have a slight control over recrystallization of ice cream under those conditions. The results for an increased number of cycles (Set 4) reinforce this statement. In this case, significant differences (p<0.05) were found between the no stabilizer, and the guar gum and xanthan gum formulations. Thus, stabilizers exerted a measurable control over recrystallization only after a prolonged temperature fluctuation regime. The differences in the distributions after storage at -30°C and
after the prolonged cycling set (Set 4) are shown in Figure 4.13. The first graph (top) in this figure shows that the distributions after the storage period were comparable (if not identical) between stabilizer and no stabilizer formulations. However, in the second graph (bottom), the no stabilizer formulation shows an increase in the size and range (broader distribution) of the ice crystal population. On the contrary, the stabilizer formulations showed a higher percentage of smaller crystals and an overall smaller size of crystals, particularly for the guar gum formulation.

Prolonged temperature cycles most likely would initially induce the disappearance of smaller crystals (Ostwald ripening) as well as further accretion within the samples. Under such conditions, stabilizers may not exert a measurable effect in recrystallization. As the cycling continues, the overall larger population of crystals would most likely undergo a melt-refreeze recrystallization mechanism in which part of the crystal melts and recrystallizes along with the ice phase volume fluctuation (temperature fluctuation). This mechanism would be expected to be present throughout temperature fluctuations; however, it would most likely be predominant as the average crystal size increases after prolonged temperature cycling. Stabilizers seemed to reduce the recrystallization behaviour under these conditions. Again, it is important to emphasize that all mechanisms are most likely to happen concurrently during thermal abuse. However, a certain type might be predominant at different stages of the recrystallization phenomenon, particularly depending on the thermal history of the sample. The images obtained do not allow to determine which mechanisms were predominant throughout the thermal abuse profiles; however, according to the characteristics of the distributions they can be hypothesized.
Figure 4.13 Comparison between no stabilizer and stabilizer ice cream formulations after storage at -30°C (top) and after cycling (Set 4) (bottom)
4.6.4 Discussion of the effect of stabilizers on ice recrystallization

Storage temperature has a large impact on ice crystals sizes. Sutton et al. (1996) showed that the recrystallization rate in fructose model solutions was strongly affected by the storage temperature. Thus, low and constant temperatures importantly reduce recrystallization rates during storage. According to Hagiwara and Hartel (1997), recrystallization rates in ice cream were almost negligible at -20°C. However, iso-mass recrystallization is a predominant mechanism under constant temperature storage conditions even at low temperatures and particularly dramatic at higher temperatures (Donhowe and Hartel, 1996a). Migratory recrystallization is another mechanism that is likely to occur during constant temperature storage considering the large amount of smaller crystals in ice cream after hardening. The extent of this mechanism on the ice crystal sizes would be mostly dictated by the storage temperature. In our study, stabilizers showed no control over iso-mass recrystallization observed after 4 weeks of storage at -30°C. At such low temperature, other mechanisms are less likely to occur due to the closeness to the glass transition temperature of the unfrozen phase. However, increasing the storage temperature (-16°C) after hardening had a large impact on the ice crystal size distribution which could be mainly attributed to migratory recrystallization (Ostwald ripening) in combination with accretion. Stabilizers did not show a measurable control over recrystallization for this storage condition. Moorty and Balachandran (1994), and Buyong and Fennema (1988), concluded that stabilizers exert minimal effect on the recrystallization of ice; however, their studies also considered samples stored at a constant temperature of-15°C. Hagiwara and Hartel (1996), did not find a consistent effect of stabilizers in the recrystallization rate for constant storage temperatures as low as -6.3°C, and their effect mostly depended on the type of sugar used in each case. As has been proposed in this study, stabilizers most likely cannot control iso-mass and/or migratory recrystallization (Ostwald ripening) in combination with accretion, mechanisms that would be expected in the first stages of recrystallization during constant temperature storage.
As mentioned earlier, temperature fluctuations have a greater impact on the crystal size distributions compared to constant temperature storage but the extent of the fluctuation is also important. Stabilizers showed no statistically significant impact on the recrystallization behaviour of samples after the initial cycles (Set 1, 2 and 3), and only a slight tendency to reduce the effect of recrystallization was observed. Harper and Shoemaker (1983), reported no effect of locust bean gum on the recrystallization rate of sugar solutions. However, their temperature cycles (from -23°C to -9°C at 1°C/min; from -23°C to -9°C 7°C/min; 4 times) had similar characteristics as sets 2 and 3. Thus, despite the greater amplitude in their experiments (±7°C), the short cycle time (total of 16 min) and number of cycles would not allow stabilizers to affect the recrystallization behaviour of the samples.

In our study, the extent of the fluctuation of an increased number of cycles showed significant differences between stabilized and unstabilized samples. These results are supported by those reported by Goff et al. (1993) in ice cream after an abusive temperature cycle. Samples with and without stabilizer (LBG and carrageenan) were removed from the freezer at -25°C and placed in a -10°C cabinet for 8 to 10 hours each day for 2 and 24 weeks. Stabilizers showed a considerable reduction on the ice crystal growth compared to the unstabilized samples. Similar results were presented by Sutton and Wilcox (1998b) on the recrystallization rate of ice cream with different ice phase volumes (controlled by the amount of sweeteners) containing locust bean gum and guar gum. The rates were determined after an abusive heat shock treatment as well. Samples were moved between cold stores maintained at -22°C and -10°C every two days. The addition of stabilizers showed a consistent reduction in the recrystallization rate. The extent of the fluctuation of these abusive treatments would clearly allow for stabilizers to exert an effect on the recrystallization rates.

According to experiments in fructose model solutions of the unfrozen phase (Sutton et al., 1997), stabilizers showed a plateau in the recrystallization rates as the stabilizer concentration was increased and surface adsorption of
these molecules onto the ice crystal surface was proposed. However, this trend was not clear in later studies in ice cream. As shown in our study the effect of the various components of ice cream on the microstructure cause significant differences in the ice crystal size distributions. The microstructure of a sweetener (model) solution differs importantly with that of ice cream, and the recrystallization behaviour could be importantly influenced by these differences. Also, if the mechanism was one of adsorption it would be expected that such protective effect would withstand particularly iso-mass recrystallization (rounding off), as well as migratory recrystallization during constant temperature storage fluctuation.

Considering the results from the first set of cycles (Set 2 and 3) and/or the storage at high temperature (-16°C), stabilizers may not be capable of controlling the recrystallization behaviour observed as a result of these storage conditions. That is, the greater impact of such conditions in the disappearance of smaller crystals by Ostwald ripening, as they cannot control the diffusion of water from smaller crystals after melting. Once the smaller population of crystals have disappeared and the population is comprised of larger crystals, the ice phase changes due to temperature fluctuations would induce a melt-refreeze behaviour, in which the crystal does not disappear but shrinks, most likely to occur on the surface of each crystal. This would also promote accretion of crystals with the concomitant effect on the average crystal size. In such case, stabilizers seemed to be capable of reducing the recrystallization rate.

The concept of distinctive recrystallization mechanisms operating at different times has previously been reported and discussed by Sutton et al. (1996a) for recrystallization at constant temperature storage of model solutions. Initially, the predominant mechanism was one of accretion (of smaller crystals) and in a later stage melt-refreeze became predominant. Donhowe and Hartel (1996a) reported different mechanisms during temperature fluctuations in ice cream. Melt-refreeze of crystals (changes in the size of each crystal as the ice phase volume changes) was evident in their experiments. Accretion was another
evident mechanism, always observed in combination with melt-refreeze due to the proximity of the crystals. These studies further reinforce the possibility of different mechanisms acting at different stages throughout the temperature cycle sets. That is, initially, migratory recrystallization in combination with accretion; later on, as the overall population size increased, melt-refreeze combined with accretion. In the latter, stabilizers seemed to affect the recrystallization behaviour.

Furthermore, according to the preventive effect against melt-refreeze recrystallization under temperature fluctuation, a feasible mechanism would be related to the effect of stabilizers in the diffusion properties of the unfrozen phase. Considering stabilizers do not reduce the diffusivity of water molecules (Blond, 1988; Sahagian and Goff, 1995b), changes in the diffusion properties of the unfrozen phase would most likely involve a localized effect within the proximity of the ice crystal surface. As the temperature rises, water dissolves from an ice crystal into the unfrozen phase as dictated by the equilibrium between the crystal and the sugar solution in the unfrozen phase. The presence of a stabilizer matrix around the crystal would not hinder the diffusion of water molecules; however, it may control the proximity of such molecules to the original crystal during melt-refreeze. Proton (\(^1\)H) NMR showed that guar gum appeared to reduce the relaxation times compared to an unstabilized sample (Sahagian and Goff, 1995a). According to the author, it is possible that the polymer influenced the proton rate of exchange between different sites, so that in the case of sucrose alone (unstabilized) the increase in relaxation time is due to an increased proportion of protons available for detection. Thus, the stabilizer matrix may control recrystallization during melt-refreeze by maintaining close proximity of the water molecules to the crystal so that recrystallization may occur within the polysaccharide matrix, promoting redeposition of water onto the same crystal, rather than diffusion of that water through the unfrozen phase for redeposition onto the largest crystal, which would be thermodynamically favoured.
5. CONCLUSIONS

Ice crystal size distributions of dynamically frozen model solutions and ice cream were successfully characterized by the logistic dose response model. This model also provided a descriptive set of parameters that allowed for an easy graphical comparison of distributions as well as a clear understanding of changes in the crystal population. Some of the limitations of other mathematical approaches commonly used were successfully overcome. However, it is important to clarify that all approaches are capable of describing the main tendencies in crystal growth.

The comparison of the ice crystal size between model solutions and ice cream formulation showed that the average crystal size is mainly affected by the total solids. As measured by the $X_{50}$ parameter of the logistic equation, the crystal sizes in model solutions and ice cream had a good linear correlation to the total solids. As a result of increasing the total solids, the reduction in the amount of water directly affects the freezing properties of the mix, reflected as a reduction in the ice crystal size. However, better correlations were found for the calculated ice content since the latter takes into consideration the effect of the type of solid on the freezing point of the mix. The type of solid also affects the freezing properties of the mix by controlling the freezing time and ultimately the ice content of the final product.

The microstructure of the different frozen systems were not comparable particularly between the sweetener model solutions with a highly packed crystal arrangement in close proximity, compared to the thinner lamella (ice crystals and unfrozen phase) with smaller ice crystals also highly packed of formulations containing MSNF and/or milkfat. This may ultimately influence the recrystallization behaviour during storage or temperature abuse when compared to a real system such as ice cream.

The incorporation of air had a great impact on the microstructure as well as in the ice crystal size distribution. Low amounts of air, i.e., overrun below
50%, did not affect the crystal sizes as it did not importantly affect the microstructure. However, the microstructure of samples containing higher amounts of air (overruns higher than 70%) showed a thinner lamella (smaller portion between air bubbles) distributed around the air bubbles. This resulted in a reduction of the crystal size, which was also observed as a deviation from the linear correlation between crystal size and the ice content. The way the air was incorporated was also responsible for some of the differences between ice crystal populations. Thus, more finely dispersed air bubbles as a result of the incorporation of emulsifiers had less impact on the reduction of the ice crystal size despite the increased value in the overrun. Higher overruns (over 80%) and air not finely dispersed, had no measurable effect on crystal size distributions for the increased volume of air incorporated. However, despite the desired initial reduction of the ice crystal size, the incorporation of air may have an adverse effect on the ice crystal size during storage. A weakness of the structure due to the presence of air caused a noticeable structure failure (channeling) leading to further accretion of ice crystals. In such case, stabilized samples with lower overrun seemed to maintain the structure integrity preventing air bubbles from collapsing. Unstabilized sample and that with an excessive overrun (guar gum) showed a certain extent of air collapse altering the microstructure of the samples. This most likely promoted further accretion of the crystals as described by the uneven growth of the crystal population in such cases.

Stabilizers did not directly affect the initial ice crystal size distribution for any of the model solutions or ice cream. Most of the differences observed within each formulation were explained by the different amount of air incorporated according to the type of stabilizer or the ice content as a result of the type of sweetener used. Therefore, stabilizers did not affect ice crystallization during freezing in a scrape-surface heat exchanger (batch process), in accordance with several studies showing no effect on the freezing properties of the mix, such as the freezing point depression, latent heat of fusion of water, and crystallization temperatures.
Constant temperature storage at -30°C did not affect the overall ice crystal size of model solutions and ice cream probably due to the proximity of this temperature to the Tg of the unfrozen phase. Stabilizers did not affect the overall ice crystal size distributions as they do not affect the glass transition temperature. Evidence of iso-mass recrystallization was found in those formulations with a considerable overrun, rendering crystals with higher roundness (shape factor) after the storage period. This effect can be attributed to the rheological characteristics reported for frozen mixes containing a considerable amount of air.

Storage of ice cream at a higher temperature (-16°C) increased the crystal size and span of the distributions showing evidence of recrystallization after the hardening period (-30°C). Several recrystallization mechanisms may be responsible considering the extent of such temperature change. Migratory recrystallization is possible according to the disappearance of the initial amount of small crystals. However, accretion would be expected to have a high impact as well according to the closely packed ice crystal structure of these samples. In general, the presence of stabilizers did not affect the recrystallization behaviour in model solutions or ice cream under constant storage temperatures.

The temperature cycle after storage at -16°C did not affect the crystal distribution. Most of the small crystals had disappeared due to the storage period, therefore the small amplitude and number of cycles did not have a detectable effect. Temperature cycles of samples previously stored at -30°C showed a considerable effect on the ice crystal distributions and the effect was similar to that observed after 4 weeks of storage at -16°C and independent of the amplitude of the fluctuation. Smaller crystals disappeared and the span of the distributions clearly increased as a result of the combined effect of migratory and accretive recrystallization mechanisms. The impact of the temperature changes from the previous storage temperature may have had the greater impact in the recrystallization behaviour, overwhelming the effect of the cycle or the amplitude of fluctuation. An increase in the number of cycles clearly showed the greater
impact of the temperature cycles when compared to constant temperature storage. In this case, the extent of recrystallization demonstrated that temperature fluctuation was responsible for the changes in crystal size distributions. In all cases of temperature cycles, the no stabilizer sample showed a tendency to render higher average diameters, particularly after an extended number of cycles for which differences were statistically significant.

According to these results, stabilizers appear not to be effective at controlling the extent of recrystallization (or mechanisms) as a result of an increase in the storage temperature. In such cases, small crystals melt and refreeze onto larger crystals by migratory recrystallization (Ostwald ripening) also promoting accretion of the crystals. However, they seem to exert a measurable control on recrystallization over prolonged temperature fluctuations. In this case, melt-refreeze is most likely to be the predominant mechanism considering the crystal population comprised of larger crystals after extensive cycling. All mechanisms are most likely to affect the recrystallization behaviour; however, certain mechanisms may be predominant at different stages of this phenomenon. This study did not allow to conclusively determine the type of mechanism involved at the different stages of recrystallization; however, some mechanisms were inferred according to the characteristics of the changes in ice crystal populations. A possible mechanism has been hypothesized involving the alteration of the diffusion kinetics of water molecules exerted by the presence of a polymer matrix around the crystals, possibly promoting recrystallization within itself. This mechanism can only be hypothesized since it cannot be concluded from this study.
6. RECOMMENDATIONS FOR FURTHER STUDIES

An important point of discussion is the application of these models to ice cream manufacturing. The importance of controlling recrystallization resides in preventing crystal growth beyond a certain size, that which can be detected in the mouth. Normalizing the crystal sizes by using percentage of presence of crystals does not allow to effectively isolate the amount, most likely responsible for iciness in ice cream after thermal abuse, of larger crystals. Therefore, using a different unit to characterize the amount of crystals of different sizes would be advisable. Nevertheless, the logistic model allows for a clear understanding of changes in the crystal size distributions and would find wide application in determining crystal populations in realistic conditions.

The effect of stabilizers only after prolonged cycles opens a series of questions. Future studies could focus on the effect of amplitude or length of cycle considering prolonged temperature cycles. Also, considering that sweetener model solutions were comprised by overall larger crystals, the effectiveness of stabilizers in this system during prolonged cycling (no small crystals, less tendency for Ostwald ripening to affect crystal sizes) could be compared to ice cream to isolate the effect of stabilizers according to the relative size of crystals and/or predominant recrystallization mechanism.
REFERENCES


Biswa


ton, J.I (Eds.), NATO ASI Series, pp. 531-542.


Sherman, P. 1965. The texture of ice cream. J. Food Sci. 30: 201-211.


8. APPENDIX

8.1 Statistical Analysis

The statistical analysis was performed using the Statistical Analysis System package (SAS). The data was balanced in all cases, therefore the Analysis of Variance Procedure (ANOVA) was used to calculate the mean square for error (MSE) for further multiple comparison of means using the Ryan-Einot-Gabriel-Welsch Multiple F-test. This test was chosen for better control of the experiment-wise error (MEER), i.e., to minimize the chance of making a wrong decision for each mean. This is attained through a multi-stage test which first tests differences between sets of all \( n \) means, then between sets of \( n-1 \) means, and so on (Schlozthauer, 1987).

In the case of overrun measurements, differences between replicates and differences between stabilizers for each formulation were analyzed. In the case of apparent viscosity, differences between day 1 and day 2 were tested to determine the effect of ageing. Differences between replicates, as well as between stabilizers for each formulation, were also analyzed.

For the ice crystal size distribution, the parameters of each mathematical approach (normalized, log-normal and logistic model) were tested for differences between replicates, stabilizers, formulation, storage times and/or temperature cycles. All models showed the same statistical differences between their respective parameters. In the case of the logistic model a third parameter, slope at the inflection point, was calculated from those of the model. The slopes were also statistically compared for the different formulations, stabilizers, storage time, storage temperature or temperature cycles.

The following is an example of the results from the statistical analysis of the overrun measurement testing the replication and differences between stabilizers according to formulation.
The SAS System
Analysis of Variance Procedure

Class Level Information

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Number of observations in by group = 16

Dependent Variable: OVERRUN

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R-Square 0.929378  C.V. 9.292417  Root MSE 4.5482272  OVERRUN Mean 48.945577

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Analysis of Variance Procedure
Ryan-Einot-Gabriel-Welsch Multiple F Test for variable: OVERRUN

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05  df= 12  MSE= 20.68637
Number of Means 2 3 4
Critical F 6.5183582 3.8852938 3.4902948

Means with the same letter are not significantly different.

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<td>C</td>
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The SAS System
Analysis of Variance Procedure

---------------------------- FORMUL=F2e STABIL=ns ----------------------------

Class Level Information
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STABIL    1            ns
REPLIC    2            rep1 rep2
Number of observations in by group = 4

Dependent Variable: OVERRUN

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<td>Corrected Total</td>
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R-Square        C.V.       Root MSE     OVERRUN Mean
0.000066        14.40267   5.0588737   35.124558

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Analysis of Variance Procedure
Ryan-Einot-Gabriel-Welsch Multiple F Test for variable: OVERRUN

NOTE: This test controls the type I experimentwise error rate.
Alpha= 0.05  df= 2  MSE= 25.5922
Number of Means 2
Critical F 18.512821

Means with the same letter are not significantly different.

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<td>A</td>
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<td>rep1</td>
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8.2 Model parameters for model and ice cream formulations after 4 weeks of storage at -30°C

The following tables correspond to the logistic model parameters and calculated slopes for the different formulation for storage at -30°C. These tables include the standard deviations of the averages of the 4 sets for each parameter.

Table 8.1 Logistic model parameters for sweetener model solutions after 4 weeks storage at -30°C

<table>
<thead>
<tr>
<th></th>
<th>$X_{50}$ (μm)</th>
<th>n</th>
<th>slope (%/μm)</th>
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<tr>
<td>F1</td>
<td></td>
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<tr>
<td>No stab</td>
<td>32.97 ± 0.86</td>
<td>-4.11 ± 0.21</td>
<td>3.16 ± 0.22</td>
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<tr>
<td>CMC</td>
<td>32.92 ± 0.69</td>
<td>-4.10 ± 0.33</td>
<td>3.16 ± 0.25</td>
</tr>
<tr>
<td>Guar</td>
<td>34.30 ± 2.00</td>
<td>-3.99 ± 0.79</td>
<td>2.95 ± 0.43</td>
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<tr>
<td>Xanthan</td>
<td>32.63 ± 2.28</td>
<td>-3.80 ± 0.71</td>
<td>3.02 ± 0.70</td>
</tr>
<tr>
<td>F2</td>
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<tr>
<td>No stab</td>
<td>30.46 ± 2.03</td>
<td>-3.97 ± 0.28</td>
<td>3.32 ± 0.29</td>
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<tr>
<td>CMC</td>
<td>33.80 ± 2.57</td>
<td>-3.90 ± 0.38</td>
<td>2.97 ± 0.49</td>
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<tr>
<td>Guar</td>
<td>32.60 ± 2.17</td>
<td>-3.82 ± 0.58</td>
<td>2.98 ± 0.31</td>
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<tr>
<td>Xanthan</td>
<td>32.59 ± 2.64</td>
<td>-4.21 ± 0.75</td>
<td>3.33 ± 0.76</td>
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<td>F2e</td>
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<tr>
<td>No stab</td>
<td>33.37 ± 2.53</td>
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<tr>
<td>CMC</td>
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<td>Guar</td>
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<td>-3.60 ± 0.43</td>
<td>4.88 ± 1.57</td>
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<td>Xanthan</td>
<td>33.05 ± 3.91</td>
<td>-3.49 ± 0.39</td>
<td>2.71 ± 0.17</td>
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Table 8.2 Logistic model parameters for formulation F3 after 4 weeks storage at -30°C

<table>
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<tr>
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<th>$X_{50}$ (μm)</th>
<th>n</th>
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<td>F3</td>
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<tr>
<td>No stab</td>
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<td>No stab</td>
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<td>CMC</td>
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<tr>
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<td>16.06 ± 2.89</td>
<td>-2.68 ± 0.33</td>
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Table 8.3 Logistic model parameters for ice cream after 4 weeks storage at 30°C

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<td>-2.35 ±</td>
<td>5.55</td>
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