PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ESCUELA DE INGENIERIA

CONSIDERATIONS OF ICE MORPHOLOGY AND DRIVING FORCES IN FREEZE CONCENTRATION

GUILLERMO PETZOLD MALDONADO

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Advisor:

JOSÉ M. AGUILERA

Santiago de Chile, December 2013

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Dedicado a mi esposa Paula, mis hijos Matías, Cristóbal y Constanza, a mis padres y hermanos.

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Guillermo

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ABSTRACT

Ice morphology (size and shape) influence decisively in sensory appreciation, texture and quality of many frozen foods. Ice morphology is also important in some technological processes such as freeze drying and freeze concentration, which influences the efficiency of these processes. The overall objective of this thesis was to increase our knowledge about the control on morphology of the ice phase in freezing food and related processes such as freeze concentration. Freezing in food and solutions, as well as the methods of observation and measurement of ice morphology were analyzed, and the role of ice morphology in some technological applications was discussed. Special attention was put in the architecture or spatial arrangement of elements in the structure of the frozen phase and the role in the efficiency of the freeze concentration process. When an ice nucleus begins to grow in a solution, solutes are rejected from the ice phase and accumulate at the solid–liquid interphase, forming a hydraulic system in the frozen matrix (ice phase) with veins or channels between the ice crystals occluding the concentrated solution. Assisted techniques (such as ultrasound or ice nucleation agents) have proven to be important in providing a morphology of the frozen phase that improves the efficiency of freeze concentration. Other alternative are the use of external driving forces such as vacuum or centrifugation in the recovery of concentrated solutions Propose two novel techniques (vacuum or centrifugation) in order to use conveniently this hydraulic system to improve efficiency in the separation of solutes in freeze concentration processes, using sucrose aqueous solutions as model samples. By applying vacuum (80 kPa), the efficiency of the freeze concentration was significantly

improved over atmospheric conditions showing a higher recovery of solute, approximately 0.5 kg of sucrose obtained per 1 kg of initial sucrose compared to recovery values ranging from 0.16 to 0.3 kg/kg for atmospheric treatments. On the other hand, by applying centrifugation, a high recovery of solutes is achieved, reaching approximately 0.73 kg of sucrose obtained per 1 kg of initial sucrose at 1600 relative centrifuge force (RCF), compared to recovery 0.5 kg/kg using a less centrifuge speed (800 RCF). The high separation efficiency of solutes using assisted techniques for freeze concentration is a consequence of using an external driving force that improves the natural separation of gravitational thawing and taking advantage of the microstructural features of the frozen phase. We can conclude that vacuum or centrifugation in freeze concentration is an effective assisted technique to enhance the separation efficiency.

Keywords: ice morphology, freezing, freeze concentration, vacuum, centrifugation

CONSIDERACIONES DE LA MORFOLOGIA DEL HIELO Y FUERZAS MOTRICES EN LA CONCENTRACION POR CONGELACION

Tesis enviada a la Dirección de Investigación y Postgrado en cumplimiento parcial de los requisitos para el grado de Doctor en Ciencias de la Ingeniería.

GUILLERMO PETZOLD

RESUMEN

La morfología del hielo (tamaño y forma) influye en forma decisiva en la apreciación sensorial, textura y calidad de numerosos alimentos congelados. También es importante la morfología del hielo en algunos procesos tecnológicos como es la liofilización y la concentración por congelación, donde influye en la eficiencia de estos procesos. El objetivo general de esta tesis fue incrementar el conocimiento acerca del control de la morfología de la fase hielo en la congelación de alimentos y otros procesos relacionados como la concentración por congelación. La congelación en alimentos y en soluciones, así como los métodos de observación y medición de la morfología del hielo fueron analizados, y el rol de la morfología del hielo en algunas aplicaciones tecnológicas fue discutido. Especial atención fue puesta en la arquitectura o arreglo espacial de elementos en la estructura de la fase congelada y el rol en la eficiencia de los procesos de concentración por congelación. Cuando un núcleo de hielo comienza a crecer en una solución, los solutos son rechazados desde la fase hielo y se acumulan en la interfase sólido-líquido, formando un sistema hidráulico en la matriz congelada (fase hielo) con venas o canales entre los cristales de hielo ocluyendo la solución concentrada. Técnicas asistidas (tal como el ultrasonido o agentes de nucleación del hielo) han probado ser importantes en proveer una morfología de la fase congelada que mejora la eficiencia de la concentración por congelación. Otras alternativas son el uso de fuerzas impulsoras externas tales como el vacío o la centrifugación en la recuperación de soluciones concentradas. Se propusieron dos novedosas técnicas (vacío o centrifugación) con el fin de utilizar convenientemente este sistema hidráulico para mejorar la eficiencia en la

separación de solutos en los procesos de concentración por congelación, utilizando soluciones acuosas de sacarosa como muestras modelo. Mediante la aplicación de vacío (80 kPa), la eficiencia de la concentración por congelación fue significativamente mejorada por sobre las condiciones atmosféricas mostrando una mayor recuperación de soluto, aproximadamente 0,5 kg de sacarosa obtenidos por 1 kg de sacarosa inicial en comparación con los valores de recuperación entre 0,16 y 0,3 kg / kg para los tratamientos atmosféricos. Por otra parte, mediante la aplicación de centrifugación, se obtuvo una alta recuperación de solutos, llegando a aproximadamente 0,73 kg de sacarosa obtenidos por 1 kg de sacarosa inicial con 1600 de fuerza centrífuga relativa (FCR), comparado al valor de recuperación 0.5 kg/kg usando una menor velocidad de centrifugación (800 FCR). La alta eficiencia de solutos usando técnicas asistidas en concentración por congelación es una consecuencia de usar una fuerza impulsora externa que mejora la separación natural en el descongelado gravitacional y toma ventaja de las características microestructurales de la fase congelada. Podemos concluir que el vacío o la centrifugación en concentración por congelación es una efectiva técnica asistida para mejorar la eficiencia de separación.

Palabras Claves: Morfología del hielo, congelación, concentración por congelación, vacío, centrifugación

LIST OF PAPERS

This thesis is based on the following Papers, referred to in the text by their respective Roman numerals (I-IV).

Chapter II: Ice morphology: fundamentals and technological applications in foods. Petzold, G. and Aguilera, J.M. Food Biophysics, 4, 378-396 (2009).

Chapter III: Vacuum-assisted freeze concentration of sucrose solutions. Petzold, G., Niranjan, K. and Aguilera, J.M. Journal of Food Engineering, 115, 357-361 (2013).

Chapter IV: Centrifugal freeze concentration. Petzold, G. and Aguilera, J.M. Innovative Food Science and Emerging Technologies, 20, 253-258 (2013).

Proceedings

Parts of the work have also been presented at one international congress under the following reference:

Petzold, G. and Aguilera, J.M. Ice crystal morphology in some model food solutions. In: Proceedings of the 7th Latin American Congress of Food Engineering (CIBIA VII), September 6-9, Bogota, Colombia (2009).

1. INTRODUCTION

1.1 Freezing in food products and solutions

Freezing is the process of ice crystallization from supercooled water. It is an efficient process of food preservation because in the frozen state water is immobilized as ice and the rates of deterioration are much slower than at higher temperatures. Freezing of solutions comprises two related processes: (1) lowering the temperature below the equilibrium freezing point, and (2) a change of phase from liquid to solid (formation of ice). Both processes are accompanied by a reduction in the heat content of the material. Lowering the temperature will *per se* reduce the rates of reactions, hence extending the storage life of a product. More relevant is that during commercial freezing of foods (i.e., lowering the temperature between −18 °C and −30 °C), the phase change immobilizes a significant portion of free water in the solid state, thus making it unavailable as solvent to mobilize reactants (Reid, 1993).

In practice, freezing of food products and solutions consists of three stages, i.e., cooling the product to its freezing point (pre-cooling or chilling stage), removing the latent heat of crystallization (phase transition stage) and finally cooling the product to the final storage temperature (tempering stage) (Kianni and Sun, 2011).

On the other hand, water crystallizes in structures that have been accurately elucidated by studies involving X-ray, neutron and electron diffraction, as well as infrared and Raman spectroscopy (Fennema, 2007). Stable ice I or Ih is the equilibrium state of water at 0 °C and 1 atm and it is one of nine known crystalline polymorphic structures of ice, each of which is stable in a certain temperature and pressure range (Belitz et al., 2004).

1.2 Concept of ice morphology

Morphology relates to the physical form and structure of a material. In the case of ice in nature, morphology means characterizing the structure at scales ranging from crystal lattices to supra-crystalline structures such as needles, plates and columns, and macrostructures such as floes (sheets of floating ice) and glaciers. In the context of this

thesis, “ice morphology” will be understood as those parameters that allow characterization of the forms adopted by ice in liquid and solid foods that are relevant to their properties (Petzold and Aguilera, 2009). The final ice crystal morphology depends on the conditions under which the crystal was formed and grown as well as the rate of crystal growth, temperature, and the presence of solutes (Hartel, 2001).

1.3 Role of ice morphology in the quality of frozen foods

Ice morphology plays an important role in the sensorial properties of foods that are consumed in the frozen state. For example, the texture of ice cream is derived, in part, from a large number of small ice crystals (<50 μm in size) present in the product which are not perceived by the palate (Hartel, 2001).

Ice morphology formed and the final quality in a frozen food is a direct consequence of freezing rate and possible temperature fluctuations during frozen storage. Freezing damage, generally associated with a slow freezing rate, has an important adverse consequence in tissue foods which is manifested after thawing. In opposition, the positive effect of quick freezing on the final texture of several fresh products has been documented (Alvarez et al., 1997; Roy et al., 2001; Martí and Aguilera, 1991; Haiying et al., 2007). Temperature fluctuations during frozen storage cause recrystallization (changes in number, size and shape of ice crystals). If the temperature during the frozen storage increases, some of the ice crystals, particularly the smaller ones, melt, and consequently, the amount of unfrozen water increase (Gormley et al., 2002). Conversely, in the period where temperature decreases, no further nucleation will take place and free water will refreeze on the surface of large crystals, so the net result is that the total number of crystals diminishes and the mean crystal size increases. Temperature fluctuations are common in frozen storage as a result of the cyclic nature of refrigeration systems and the need for automatic defrosts. However, mishandling of product is probably the biggest culprit of changes in ice morphology (Petzold and Aguilera, 2009).

1.4 Role of ice morphology in technological applications

Ice morphology is important in freeze related processes as such freeze drying and freeze concentration. In freeze drying, ice morphology is important because it influences the time of sublimation (Kochs et al., 1991), several structural characteristics of the freeze-dried material, and the biological activity and stability of dry bioactives (Hottot et al., 2004, 2006). In freeze concentration, ice morphology affects the efficiency of the separation step between ice and concentrate, with a mash of large ice crystals of uniform size resulting in fewer losses of entrained juice concentrate (Deshpande et al., 1982; Bruin and Jongen, 2003).

On the other hand, ice morphology provides opportunities for new structuring technologies (freeze texturization, formation of sponges, etc) and new techniques are becoming available to control the ice crystallization process (ultrasound, high-pressure freezing) and ice morphology (use of ice nucleation agents and antifreeze proteins, among others).

1.5 Ice: segregator of solutes.

Ice is an interesting material on many levels. Its molecular structure and morphologies inspire a multitude of disciplines from glaciology to astrophysical science. In particular, in the same form of most materials, important redistribution of solutes occurs during crystallization of water (Bartels-Rausch et al., 2012). When an ice nucleus begins to grow in a solution, solutes are rejected from the ice phase and accumulate at the solid–liquid interphase during freezing process. Thus, the impurities or solutes become segregated on the frozen interphase to increase their concentration compared to that in the original solution. This exclusion phenomenon of ice is not only common to a solution (including both inorganic and organic) but also to suspensions and that is a major principle of the freeze concentration techniques (Nakagawa et al., 2010).

Freeze concentration, also known as cryoconcentration, has long been recognized as one of the best concentration techniques. As compared to evaporation and membrane technology, freeze concentration has some significant potential advantages for

producing a concentrate with high quality because the process occurs at low temperatures where no vapor/liquid interface exists, resulting in minimal loss of volatiles (Morison and Hartel, 2007). Additionally, the energy consumption of freeze concentration is lower than in the vaporization method due to the lower latent heat of water solidification (0.33 kJ/g) than that of vaporization (2.26 kJ/g) (Ramaswamy and Marcotte, 2006).

Recently, the industrial future of freeze concentration has been associated more with developments in the configuration of one-step systems (block freeze concentration or progressive freeze concentration) than conventional freeze concentration systems (suspension crystallization), because of the simpler separation step (Miyawaki et al., 2012; Petzold & Aguilera, 2009; Sánchez et al., 2009, 2010). An additional advantage of these one-step systems is their simplicity in terms of both the construction and operation of the equipment (Sánchez et al., 2009).

Assisted techniques that improve the efficiency of processing in one-step configuration of freeze concentration are important in achieved commercial viability. Examples of assisted techniques are the use of ultrasound to control ice nucleation (Matsuda and Kawasaki, 1997; Matsuda et al., 1999; Kawasaki and Matsuda, 2004) or the use of ice nucleation agents to suppress the initial supercooling (Liu et al., 1997). Other alternatives are the uses of external forces such as vacuum or centrifugation, which are similar to the principle used by children to suck the sugar solution containing colorants from popsicles. The process takes advantage of the hydraulic system existing in the frozen matrix formed by veins (or channels) between the ice crystals occluding the concentrated solution (Martel, 2000).

The existence of microchannels between ice crystals has been confirmed by Luo et al. (2010) in thawing assays of frozen brackish water (where the solutes were the total dissolved solid in water) and recently by Miyawaki et al. (2012) in progressive freeze concentration by partial melting of sucrose solutions. In these thawing assays, the concentration of the ice-melted in first stages was much higher than that of later stages and decreased with time, and Nakagawa et al. (2009, 2010) suggested that this

phenomenon (ice-melted concentration in a freeze-thawing process) is not governed by the inter-phase equilibrium as for conventional freeze concentration but by the kinetics of mass transfer during the phase transition. In addition, these experiments showed similar results to the natural phenomena of the ice thawing process in a frozen river, where the initial fraction of melting solutions were concentrated in impurities while the last fractions of the melting solutions had much lower concentration (Leung & Carmichael, 1984), and this matrix in a frozen solution (microchannels) is responsible for differences in the concentration of impurities in ancient polar ice, where solutes migrated through the microchannels between the ice crystals under the pressure of upper ice layers (Rempel et al., 2001).

1.1. Scope and objectives of the thesis

From the above presentation, it is clear that the subject of ice morphology is an important parameter in the quality and stability of frozen and frozen-thawed foods, and additionally ice morphology plays an important role in freeze-related process as such freeze drying and freeze concentration. In freeze concentration, configurations of one-step systems will increasingly attract the attention of researchers because of the simpler separation step than conventional freeze concentration configurations, and more research will be needed on assisted techniques to improve the process.

The hypothesis of the present thesis was: "the use of different driving forces (vacuum or centrifugation) improves the separation in one-step freeze concentration configurations, taking advantage of microstructural characteristics of the frozen phase".

Therefore, the overall objective was to increase our knowledge about the control on morphology of the ice phase in food freezing and related process such as freeze concentration with the aim of studying assisted techniques to improve freeze concentration process in one step configuration. In order to achieve this aim, the thesis was divided into the following specific objectives:

- Review the fundamentals of freezing, methods of observation and measurement of ice morphology, and the role of ice morphology in frozen and partly frozen systems.

Also to analyze the importance of ice morphology in technological applications such as freeze drying, freeze concentration, and freeze texturization (Chapter II).

- Study the effect of vacuum as an assisted technique in one-step freeze concentration (Chapter III).
- Analyze the use of centrifugation as an external driving force in freeze concentration assays (Chapter IV).

A summary of the content of the chapters II to IV is schematically presented in Fig. 1.1

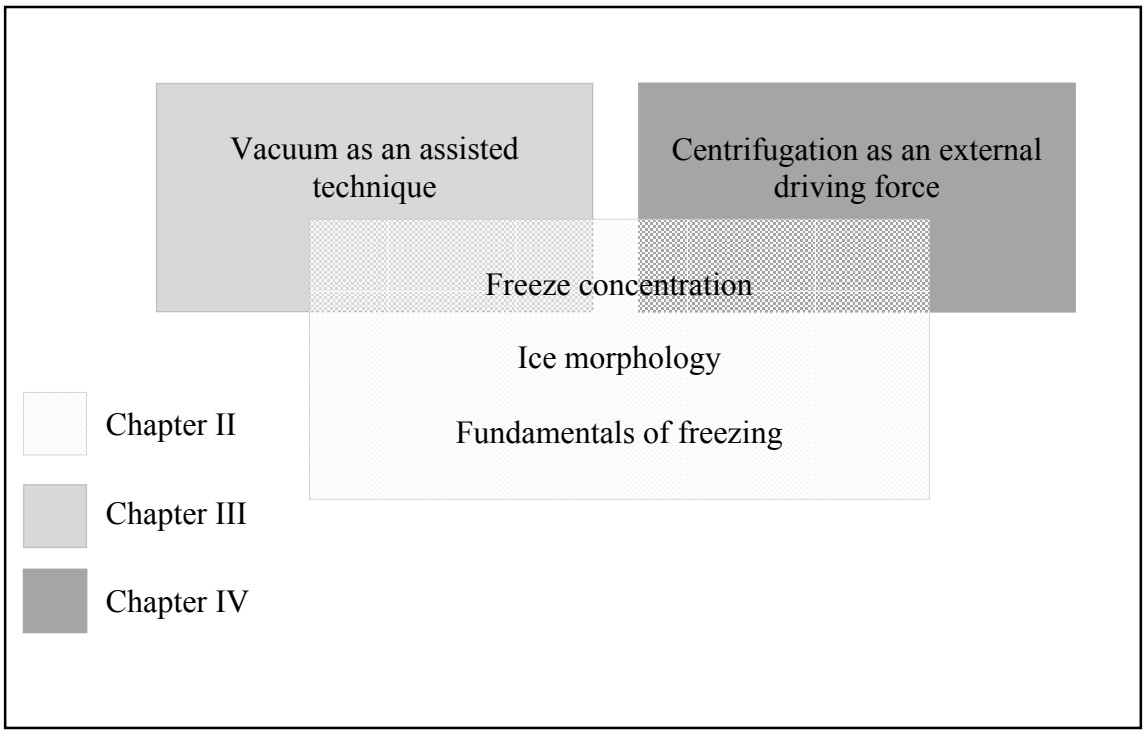

Figure 1.1. Overview of the studies comprising this thesis.

2. ICE MORPHOLOGY: FUNDAMENTALS AND TECHNOLOGICAL APPLICATIONS IN FOODS

2.1 Introduction

Freezing is the process of ice crystallization from supercooled water. It is an efficient process of food preservation because in the frozen state, water is immobilized as ice and the rates of deterioration are much slower than at higher temperatures (Reid, 1993). Ice morphology (e.g., the size and shape of crystals) is important in the quality of frozen foods as well as in freeze-related processes such as freeze concentration and freeze drying (Aguilera, 2005; Miyawaki, 2001).

The morphology of ice crystals plays an important role in the sensorial properties of foods that are consumed in the frozen state. For example, the texture of ice cream is derived, in part, from a large number of small ice crystals (<50 μm in size) present in the product which are not perceived by the palate. Also, the shape of ice crystals is important since smooth and rounded crystals slide past one another easily, giving ice cream a smooth texture, whereas crystals with jagged edges and rough surfaces (as in popsicles) flow unevenly as the product is sheared during consumption (Hartel, 2001). Ice morphology, which develops in the freezing stage, is also important in freeze drying because it influences the time of sublimation (Kochs et al., 1991), several structural characteristics of the freeze-dried material, and the biological activity and stability of dry bioactives (Hottot et al., 2004, 2006). In freeze concentration, ice morphology affects the efficiency of the separation step between ice and concentrate, with a mash of large ice crystals of uniform size resulting in fewer losses of entrained juice concentrate (Deshpande et al., 1982; Bruin and Jongen, 2003).

Nucleation is probably the most important step to control the crystal size distribution during crystallization (Hartel, 2001). The freezing rate is usually the parameter used for controlling the size and size distribution of ice crystals in frozen and partly frozen systems. Lately, the use of nucleation agents, antifreeze proteins, ultrasound, and pressure freezing methods, known by the generic name of “freezing assisting

techniques," have been proposed to control nucleation and ice morphology (Li and Sun, 2002a). The aim of this part of the thesis was to discuss the role of ice morphology in frozen and partly frozen systems, in processes involving freezing, as well as to describe methods to characterize the morphology of ice crystals.

2.2 Freezing of foods

Freezing of foods is a traditional preservation technology that stands on two basic prerequisites to deliver high quality products:

1. Rapid freezing rates: The rate of freezing is extremely important because it determines the size and location of ice crystals within a product. In the case of tissue foods, slow freezing generally causes ice crystals to grow in extracellular locations, resulting in large crystals, maximum dislocation of water, shrunken appearance of cells in the frozen state, and, as a consequence, in poor food quality. On the other hand, rapid cooling produces small ice crystals formed inside and outside cells, uniform crystallization, and a product quality superior to that of a slowly frozen food (Aguilera and Stanley, 1999; de Man, 1999; Zaritzky, 2006; Fennema, 2007).

2. Frozen storage at a low and constant subfreezing temperature: The storage temperature conditions influence the quality of frozen foods in a significant manner. Any elevation in temperature above the designed storage temperature tends to reduce the quality of frozen foods, and fluctuations in storage temperature tend to be even more detrimental to product quality (Singh and Heldman, 2001).

Freezing damage, generally associated with a slow freezing rate, has an important adverse consequence in tissue foods which is manifested after thawing. The effects of freezing damage in plant tissues include disruption of metabolic systems, dislocation of enzymes, and loss of turgor due to damage to cell walls and cell membranes, resulting in the permanent transfer of intracellular water to the extracellular fluid which cannot be reversed after thawing (see Figure 2.1). The positive effect of quick freezing on the final texture of several fresh produce has been documented for potatoes (Alvarez et al., 1997), carrots (Roy et al., 2001), cranberries and blackberries (Martí and Aguilera, 1991),

mushrooms, green cauliflower, navy beans, and peas (Haiying et al., 2007), among others. On the other hand, a slow freezing rate (and fluctuations in storage temperature) causes drip losses in the product after thawing, as documented, for example, for pork (Ngapo et al., 1999), leeks (Pukszta and Palich, 2006), and strawberries (Pukszta and Palich, 2007).

Dehydrofreezing is a technology to preserve fruits and vegetables by removing part of the water (e.g., the "free water") by drying prior to freezing. Partial dehydration reduces the mass of water to be frozen, which thus lowers the refrigeration load during freezing. In addition, due to the reduced weight and possibly volume, dehydrofrozen products have lower packaging and transport costs than frozen products (Li and Sun, 2002a). Some studies on dehydrofreezing of fruits (Garrote and Bertone, 1989; Onishi and Miyawaki, 2005; Marani et al., 2007) cite the beneficial effect of a reduced drip loss after thawing. Small foods such as berries, shrimps, and diced pieces can be frozen as distinct units by a process known as individually quick freezing (IQF). IQF is possible when there is no barrier to heat transfer between the refrigerant and the product, and the rate of freezing is not limited by internal heat transfer due to the small size. The refrigerants used in these systems may be low-temperature air at high speeds (or under fluidization conditions), as well as liquid cryogens that undergo phase change in contact with the product surface, removing heat from the product (Singh and Heldman, 2001). In opposition, some products such as animal carcasses or sizeable containers have geometric configurations or large sizes that do not allow rapid freezing in the central portions (Singh and Heldman, 2001). Freezing of large pieces normally occurs under internal thermal gradients with high freezing rates in the external regions in contact with the refrigerant medium and decreasing rates toward the thermal center of the sample (Zaritzky, 2006), often inducing mechanical stresses in the outer layers of the frozen product that lead to surface cracking (Kondratowicz and Matusevi*ć*ius, 2002).

2.3 Ice crystal size and morphology in ice cream

Ice crystallization in ice cream determines its final quality; consequently, it has been extensively studied (Hartel, 2001; Adapa et al., 2000; Goff, 2008). Ice crystals are formed in the freezer barrel during the initial freezing process and then grow in size during storage. The size of the ice crystals is very critical in determining the quality of the ice cream. Smaller size crystals are preferred because large crystals results in an icy

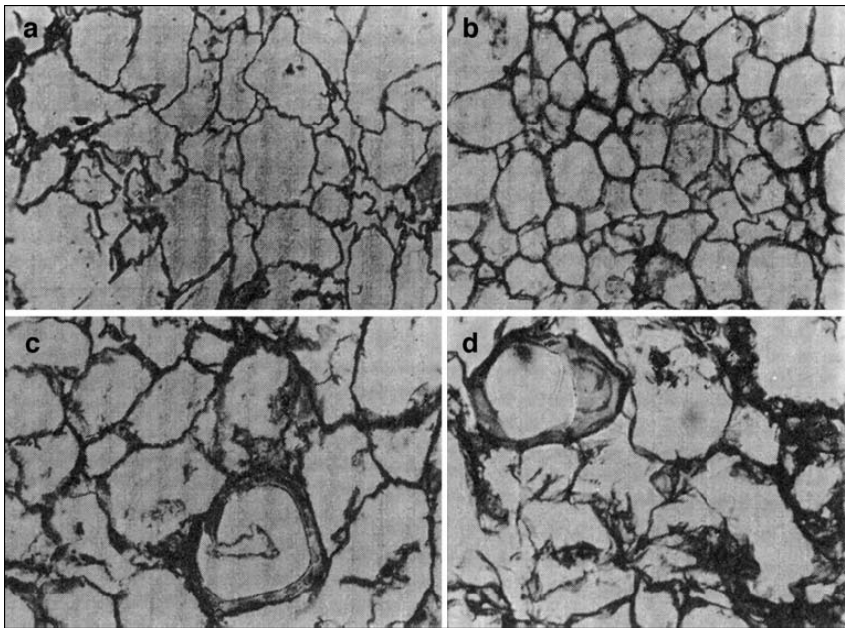

Figure 2.1. Effect of freezing rate on the microstructure of blueberries. Photomicrographs of parenquimatic tissue. a) Fresh; b) frozen by immersion in liquid nitrogen; c) after cold plate freezing; and d) after static freezing at −18 °C. Fast freezing methods (b, c) produced almost no damage in cell walls, while in contrast, a significant damage is observed by the slow freezing method (d). Source: Martí and Aguilera, 1991 (with permission).

texture (Hartel, 2001). The composition of the ice cream mix and temperature during hardening play an important role in determining the final quality. Sugars influence the crystallization by decreasing the freezing point of the mix, leading to more unfrozen water during the initial freezing process (during storage, unfrozen water will readily take

part in recrystallization of ice). Several stabilizers, usually a combination of two or more, are used to control the size of the ice crystals (Goff, 2008). Commonly used stabilizers to control ice recrystallization are hydrocolloids (locust bean gum, guar gum, carrageenan, xanthan gum, etc.) added to the ice cream formulation (Sutton and Wilcox, 1998a; Sutton and Wilcox, 1998b; Flores and Goff, 1999a; Flores and Goff, 1999b; Regand and Goff, 2003; Bolliger et al., 2000). The specific action mechanism of the different stabilizers on ice recrystallization is still under study (Zaritzky, 2006). Stabilizers seem to have little or no impact on the initial size distribution of ice crystals in ice cream as it comes out from the scraped surface heat exchanger and also little or no impact on initial ice growth during quiescent freezing and hardening. However, they do limit the rate of growth of ice crystals during recrystallization. Additionally, stabilizers have no effect on the freezing properties of an ice cream mix, e.g., freezing point depression, amount of freezable water or enthalpy of melting, or on heterogeneous nucleation, and thus, it may not be expected to affect the initial ice crystallization processes (Goff and Hartel, 2004). However, some studies have reported beneficial effects of added stabilizers particularly in relating ice cream mix viscoelasticity and ice crystal growth as a function of stabilizer addition (Bolliger et al., 2000). Similarly, novel ingredients are being suggested to help control ice recrystallization, among them are the antifreeze proteins that can be extracted from natural sources (Regand and Goff, 2006) and the use of gelatin hydrolyzates (Damodaran, 2007). Recently, Hagiwara et al. (2006) indicated that the self-diffusion coefficient of water in a freeze concentrated matrix is a useful parameter for predicting and controlling the recrystallization rate.

2.4 Fundamentals

2.4.1. Freezing and formation of ice from solutions

Freezing of water comprises two related processes: (1) lowering the temperature below the equilibrium freezing point and (2) a change of phase from liquid to solid (formation of ice). Both processes are accompanied by a reduction in the heat content of the material. Lowering the temperature will per se reduce the rates of reactions, hence

extending the storage life of a product. More relevant is that during commercial freezing of foods (i.e., lowering the temperature between −18 °C and −30 °C), the phase change immobilizes a significant portion of free water in the solid state, thus making it unavailable as solvent to mobilize reactants (Reid, 1993). Further reduction of the temperature (e.g., below Tg′) will vitrify the unfrozen concentrated liquid phase, further reducing the mobility of the system (Roos and Karel, 1991).

In practice, crystallization of water involves three phenomena: (1) nucleation or the formation of a minuscule crystalline lattice structure from solution, (2) crystal growth which corresponds to a subsequent growth from nuclei until a crystal in equilibrium is attained, and (3) recrystallization or the reorganization of the crystalline structure to a lower energy state (Hartel, 2001).

Before the crystallization process occurs at the equilibrium freezing point (melting point of ice), a significant energy barrier must be surmounted by generating a large driving force. The existence of an energy barrier is demonstrated by the continuous withdrawal of sensible heat below 0 °C without the occurrence of a phase change. This process, called undercooling or supercooling, results in a thermodynamic unstable state until submicron water aggregates form, leading to a suitable interface (nucleus, embryo, or seed) necessary for a massive liquid-to-solid transformation. The degree of undercooling is dictated by the onset of ice nucleation. Without a stable nucleus, phase change is not possible since molecules of liquid do not easily align themselves in the configuration of a crystal. Therefore, nucleation serves as the initial process of freezing and can be considered the critical step preceding complete solidification. In general, the nucleation involves creating a stable interface via a foreign particle (heterogeneous nucleation) or through a process by which enough water molecules become ordered spontaneously due to intrinsic fluctuations (homogeneous nucleation) (Shagian and Goff, 1996).

Matsutomo et al. (2002), using molecular dynamic simulation, reported how a nucleus forms and grows in supercooled pure water. At the microsecond timescale, ice nucleation occurs once a sufficient number of relatively long-lived hydrogen bonds develop spontaneously at the same location to form a fairly compact nucleus. Once

stable ice nuclei are formed, crystal growth is possible by the addition of molecules to the interface. Growth is controlled by the rate of latent heat released during the phase change as well as by the rate of mass transfer (diffusion of water molecules from the solution to the crystal lattice and counter-diffusion of solutes away from the growing crystal surface). The rate of crystal growth (G) is also a function of the supercooling (ΔT_s) reached by the specimen according to the phenomenological expression (Zaritzky, 2006):

$$G = \beta(\Delta T_s)^n \quad (2.1)$$

where β and n are experimental constants. Recently, the group of Jia (Wathen et al., 2004) has used a Monte Carlo simulation technique for the investigation of both ice crystal growth and ice crystal growth inhibition and also extended the technique from simulating 2D surfaces to whole 3D crystals. Recrystallization, in turn, involves the enlargement of large crystals at the expense of smaller ones, and during frozen storage, this is probably the most important change leading to loss of quality in frozen foods (Aguilera and Stanley, 1999; Zaritzky, 2006).

2.4.2. Temperature fluctuations and ice morphology

During frozen storage, ice crystals are relatively unstable and undergo changes in number, size, and shape, a phenomenon known collectively as recrystallization. Some recrystallization occurs naturally at constant temperatures because water vapor will tend to transfer from regions of high vapor pressure (i.e., in the surface of round small crystals) to regions of lower vapor pressure (in larger crystals), a phenomenon known as Ostwald ripening, but by far, the majority of problems are created as a result of temperature fluctuations. If the temperature during the frozen storage increases, some of the ice crystals, particularly the smaller ones, melt, and consequently, the amount of unfrozen water increases (Gormley et al., 2002). Conversely, in the period where temperature decreases, no further nucleation will take place and free water will refreeze on the surface of large crystals, so the net result is that the total number of crystals

diminishes and the mean crystal size increases. Temperature fluctuations are common in frozen storage as a result of the cyclic nature of refrigeration systems and the need for automatic defrosts. However, mishandling of product is probably the biggest culprit of changes in ice morphology. The effect of temperature fluctuations on the quality of several frozen foods such as dough (Phimolsiripol et al., 2008), potato (Alvarez and Canet, 2000), salmon, strawberries, broccoli, and pork (Gormley et al., 2002) has been documented.

2.4.3. Structure of ice and polymorphism

Water crystallizes in structures that have been accurately elucidated by studies involving X-ray, neutron and electron diffraction, as well as infrared and Raman spectroscopy (Fennema, 2007). Stable ice I or Ih is the equilibrium state of water at 0 °C and 1 atm. It is one of nine known crystalline polymorphic structures of ice, each of which is stable in a certain temperature and pressure range (see Figure 2.2) (Belitz et al., 2004). Most substances become denser as they are cooled, so they have a higher density in the frozen (solid) state than when liquid. When water is cooled below +4 °C, it begins to expand so that when water freezes, ice becomes less dense than water (Akyurt et al., 2002). This expansion of ice (ice I) formed at atmospheric pressure (about 9% in volume at 0 °C and approximately 13% at −20 °C) causes tissue damage in freezing of cellular foods. However, at high pressures, several forms of ice (ice II to ice IX) are formed with densities greater than that of water (because in the phase transition expansion in volume does not occur). This may reduce tissue damage, and frozen preservation of food would take full advantage of the phase transition of water (Li and Sun, 2002a).

2.4.4. Ice crystal morphology

Morphology relates to the physical form and structure of a material. The term includes a wide range of characteristics, extending from dimensions of a crystal lattice to the external size and shape of large objects. In the case of ice in nature, morphology means characterizing the structure at scales ranging from crystal lattices to supra-crystalline

structures such as needles, plates and columns, and macrostructures such as floes (sheets of floating ice) and glaciers. In the context of this article, "ice morphology" will be understood as those parameters that allow characterization of the forms adopted by ice in liquid and solid foods that are relevant to their properties. However, to get an appreciation of the nomenclature of ice crystal structures and the variables influencing their formation, a short description of snow crystal morphology follows.

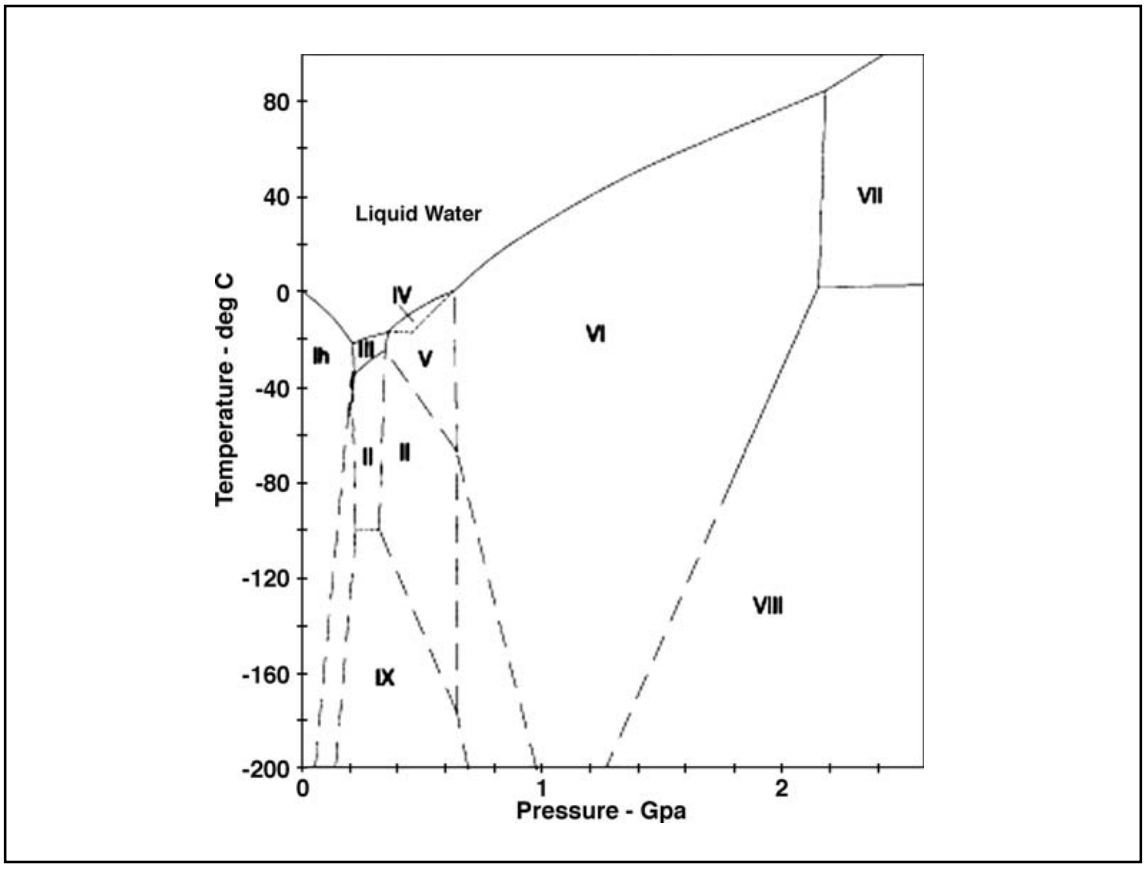

Figure 2.2. Phase diagram of ice. I–IX ice polymorphic structures. Source: Akyurt et al. (2002) (with permission).

2.4.5. Snow crystal morphology: ice crystals from vapor

The morphology of snow crystals (i.e., ice crystals growing from supersatured water vapor) exhibits a complex and puzzling dependence of temperature and supersaturation

(Figure 2.3). Snow crystal growth is typically dominated by the kinetics of molecular attachment in combination with the already mentioned transport effects: mass diffusion, which carries water molecules to the growing crystal, and heat diffusion, which removes the heat generated by solidification. The interplay of these three processes is ultimately responsible for the vast diversity of snow crystal morphologies. However, the specific physical mechanisms responsible for the unusual temperature dependence of the morphology of growing ice crystals is still not well understood (Libbrecht, 2005).

Some authors have classified the snow crystal morphology into primary and secondary habits of single crystals. The primary habit of single crystals depends on the aspect radio Γ between the maximum length $2c$ and the maximum width $2a$ along the axis ($\Gamma=c/a$; see Figure 2.4). The secondary habit corresponds to the finer details of shape, such as the amount of hollowing, and the number and shape of branches or needles. Except at the lowest supersaturation, the primary habit in an inert gas atmosphere depends only on temperature, whereas the secondary habit changes with time, temperature, supersaturation, crystal size, vapor mean free path, and thermal conductivity of the air. Both habits are also sensitive to small concentrations of gaseous impurities (Nelson, 2001).

2.4.6. Ice crystal morphology by water and solutions

Polymorphism of ice occurs not only at the atomic level, i.e., as previously explained, but also by "macroscopic polymorphism"—a multiplicity of macroscopic shapes or patterns of crystals which grow under highly nonequilibrium conditions yielding a specific structure (Shibkov et al., 2003). The final ice crystal morphology depends on the conditions under which the crystal was formed and grown as well as the rate of crystal growth, temperature, and the presence of solutes (Hartel, 2001). Of particular importance is the fact that the oriented nature of ice Ih leads to unequal growth rates on different crystal surfaces (termed anisotropic growth). In particular, growth is much more rapid in both the primary and secondary prism plane directions than in the basal plane direction. Therefore, at low degree of supercooling, this leads to ice crystals with

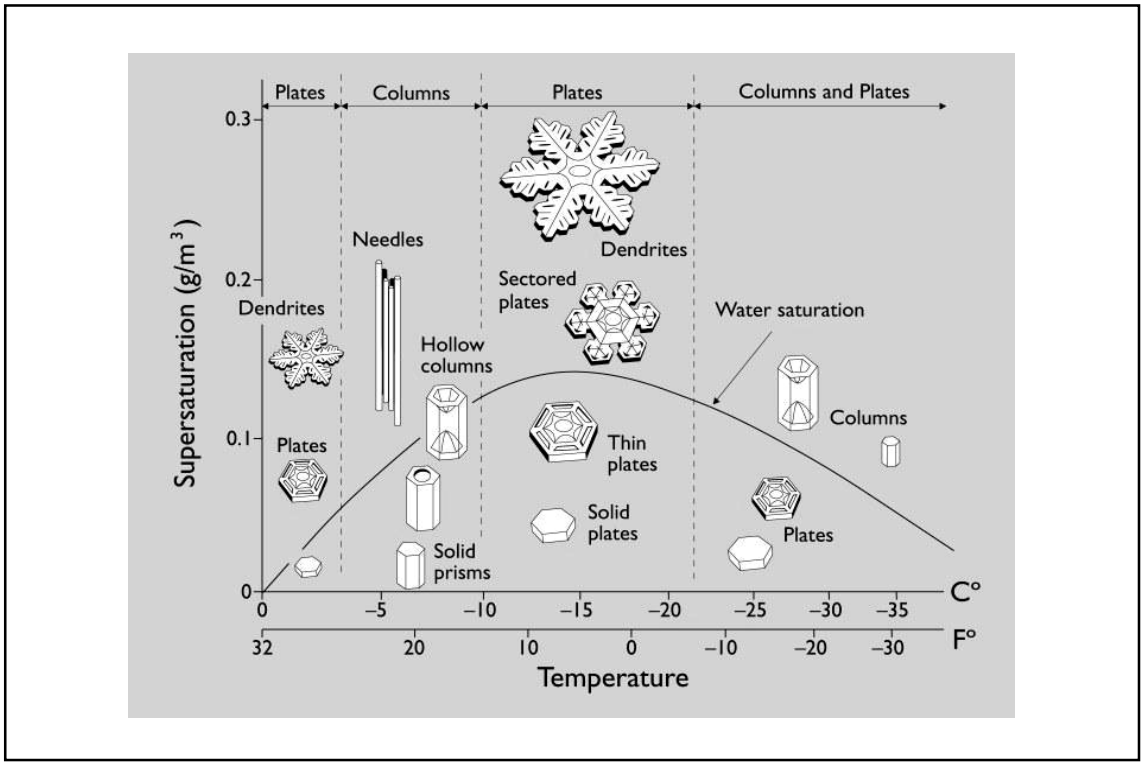

Figure 2.3. The snow crystal morphology diagram. Different types of snow ice single crystals that grow in air at atmospheric pressure as a function of temperature and water vapor supersaturation. Source: Libbretch (2005) (with permission).

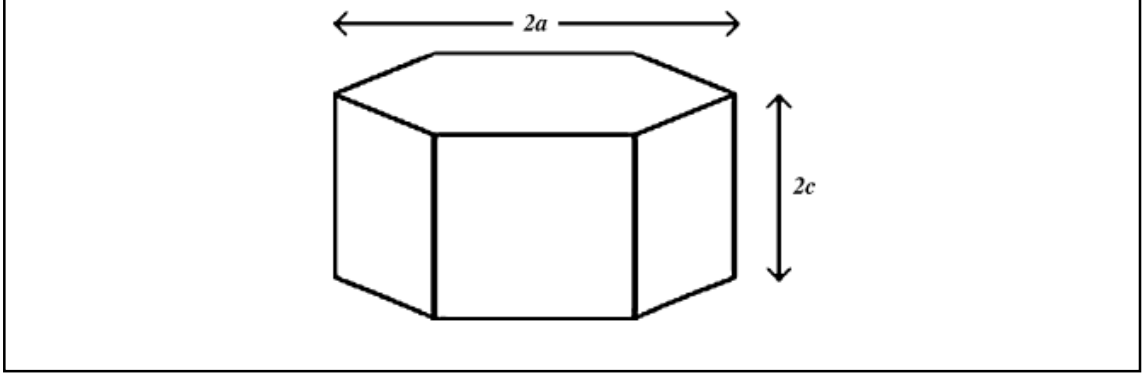

Figure 2.4. Primary habits of single snow ice crystals. Habits depends on the aspect ratio $\Gamma = c/a$: tabular ($\Gamma<1$), isomeric ($\Gamma\approx1$), and columnar ($\Gamma>1$). Adapted from Nelson (2001).

disk morphologies (Wathen et al., 2004). As mentioned before, an important condition under which the ice crystal is formed is supercooling. Shibkov et al. (2003a, 2003b) investigated the morphology of ice crystals freely grown from supercooled water finding that when supercooling increased from 0.1 °C to about 30 °C, the different structures of growing ice changed sequentially from disk, to perturbed disk, to a dense-branching morphology due to splitting of the fingertips, to dendrite, to stable needle, to fractal needled branch, to compact needled branch, and, finally, to platelet. In solutions, three patterns of growth of ice crystals around nuclei may arise (Luyet, 1968): (1) if the molecules of water are given enough time, they arrange themselves regularly into hexagonal crystallization units called dendrites (see Figure 2.5); (2) if they become incorporated at a fast rate into the crystal at odd places, they construct units called "irregular dendrites" or axial columns that originate from the center of crystallization; and (3) at higher cooling rates, many ice spears originate from the center without side branches; these units are called spherulites. However, the only ice crystal form of importance in most foods is the hexagonal crystallization unit or "regular dendrite" (Fennema, 2007).

Dendrite morphology is defined by sinusoidal perturbations at the solid–liquid interface. The wavelength of these perturbations is dependent on the rate of crystalline growth, the temperature gradient in frozen region, and the degree of supercooling. There is a threshold wavelength which leads to the formation of stable dendrites; below this critical wavelength, the perturbations disappear. This limit is known as the limit of morphological stability and is used to define the crystal size in relation to freezing kinetics (Pardo et al., 2002).

2.4.7. Effect of solutes on ice morphology

When an ice nucleus begins to grow in a solution, solutes are rejected from the ice phase and accumulate at the solid–liquid interphase. This situation leads to a solute concentration gradient in the liquid surrounding the ice front, giving rise to a in the solid–liquid equilibrium temperatures. Equilibrium temperatures decrease with

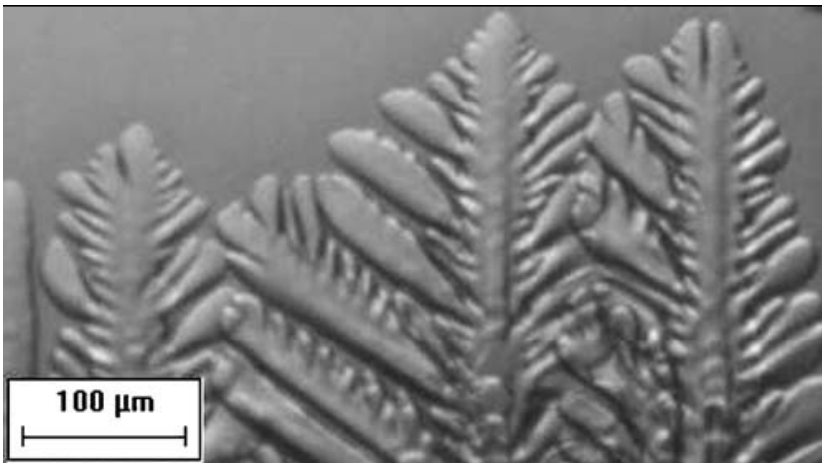

Figure 2.5. Typical dendritic ice structure. Formed in a droplet of a 30% sucrose solution during cooling in a Linkam® thermally controlled microscopy stage kept at −13 °C.

increasing solute concentration; thus, a zone where supercooling increases in front of the interphase can be generated, and this is denominated constitutional supercooling. The existence of constitutional supercooling results in an unstable condition for ice crystal growth since a maximum for supercooling occurs just before the interface and the planar ice front becomes susceptible to small perturbations. If an ice protrusion of the interface advances just slightly ahead of the plane, then its growth rate will increase in the supercooled zone. Such instability will grow through the supercooled region; ice cells will grow adjacent to each other with segregation of solute between them. The cells exclude solute to the sides as well as in front, while the regions between cells will contain concentrated solute. Growth of ice crystals is produced from the border toward the interior forming columns where those oriented closest to the direction of the thermal gradient predominate. If the conditions leading to cellular growth are particularly pronounced (morphological stability), then the cells may turn to dendrites, which are protuberances with side branches (Figure 2.5) (Zaritzky, 2006).

The presence of a solute, especially one with a high molecular weight and a low diffusion coefficient, will markedly affect the nucleation of ice. In general, the nucleation rate is reduced by such solutes because, for instance, the volume fraction of water has been reduced (very small effect); the radius of the critical nucleus is increased, which may possibly be accompanied by a change in surface tension; or the value of the standard Gibbs free energy to be employed relates to the slowest diffusing species in the mixture; and so on. As a cluster grows, it becomes surrounded by a region depleted in water; that is, the chemical potential of water increases so that quite apart from the potential barrier associated with a phase change, the crossing of a diffusion layer is an additional factor that retards nucleation. On the other hand, the anomalous divergence of the physical properties of water is suppressed by high solute concentrations, and much larger degrees of supercooling can be achieved than are possible in pure water. Secondary nucleation may be increased by the adsorption of macromolecules (e.g., gelatine inhibits the rate of ice crystal growth in sugar solutions) to growing crystal surfaces (→ influence on σ!), so crystal size will be decreased consequently (Leloux, 1999).

The nature of particles present in a solution or suspension is a very important factor in ice morphology resultant, as demonstrated by Zhang et al. (2005) in directional freezing of solutions and colloidal suspensions of polymers and nanoparticles. These authors explained the reasons why different particles may yield different sample morphologies (i.e., different freeze-dried structures obtained by sublimation of different ice morphologies) even if all other variables (for example, concentration, freezing rate) are kept constant. First, particles of different sizes—either from sample to sample or in a single polydisperse sample—will induce different instability wavelengths in the ice front. Second, particle–particle interactions may cause agglomerates, effectively altering the particle shape, size, and possibly aspect ratio. Third, large differences in $\Delta\sigma$ (balance of the surface forces at the ice/solution/particle boundary) can be achieved using different particles. Fourth, the surface tension of ice is anisotropic: as such, different

particle–ice interactions are possible, and hence, a range of structures could coexist in the same sample if multiple crystal grains were formed.

Another effect of solutes on ice morphology is the hydrophobic hydration: the introduction of apolar molecules, or apolar residues on otherwise polar molecules into water leads to a reduction of the degrees of freedom of the neighboring water molecules, resulting in the formation of a "framework." This framework is of interest in relation to cooled aqueous solutions containing hydrocarbons and slightly polar molecules in which the solid phase that separates out does not consist of ice but of a so-called clathrate hydrate (Leloux, 1999). Structural match of ice with solutes can also occur by interaction with ice nucleation agents (see "Ice Nucleation Agents") or by adsorption of macromolecules such as proteins (see "Antifreeze Proteins").

2.5. Observing and measuring ice morphology

Ice morphology has been observed and measured by several techniques that are summarized in Table 2.1. Complementary details are in the review by Diller (2005). Using the classical light cryomicroscope, the ice front may advance in a single direction and at a specific rate by imposing a controlled thermal gradient oriented either perpendicular to the optical axis of the microscope (microscope stage for directional solidification, MSDS) (Körber, 1988) or to monitor the freezing process. Fluorescence microscopy was instrumental in highlighting along the optical axis (modified MSDS) (Neils and Diller, 2004). Several microscopy techniques have been used the ice–liquid interface as a fluorescent dye became segregated into the liquid phase, leaving the ice dark (Neils and Diller, 2004). Optical microscopy with episcopic coaxial lighting, a method originally developed by physicists studying the polar ice structure (Arnaud et al., 1998), was modified to characterize the frozen structure of ice cream with and without overrun (Faydi et al., 2001; Caillet et al., 2003). The technique is based on the differences in light flux reflected by the interfaces within the material, and its foremost advantages are that it keeps the original microstructure of the food and has low running costs.

Digital image processing and image analysis are important assisting techniques to derive quantitative information from photomicrographs obtained in freezing assays (Russ, 2004). For example, in modified MSDS experiments, 3D images of freezing solutions were obtained by digital reconstruction of serial images of sections (Neils and Diller, 2004) and the quantification of solute concentration estimated using the brilliant color of sodium permanganate (Kourosh et al., 1990a, 1990b). Hagiwara et al. (2002) using fractal analysis described the ice morphology (shape) and the morphological changes of ice crystal particles (recrystallization) in frozen soybean curd (tofu). As storage time increased, the values of the fractal dimension (dp) of the perimeter of ice crystal in samples stored at different temperatures tended to decrease, meaning that their contours became less irregular. Similarly, fractal analysis has been used to describe changes in ice crystals during frozen storage of fish (Hagiwara et al., 2003) and in a sucrose solution being frozen in the presence of stabilizers (pectin and xanthan gum) (Koshiro et al, 2006). In the Micro- Slicer Image Processing System (MSIPS), the 3D reconstruction is effected by multi-slicing a frozen sample and capturing images of individual slices with a CCD camera coupled to a fluorescent microscope (Ueno et al., 2004; Do et al., 2004). Nondestructive MRI techniques allow the visualization of the freezing process and generation of 3D images by following the spatial distribution of non-frozen water (Mahdjoub et al., 2006; Hindmarsh et al., 2004). In Mach- Zender optical interferometry, small relative changes in refractive index are accurately measured and subsequently related to changes in the solution concentration and in the morphology of the ice–solution interface (Butler, 2001). This optical method has been employed to obtain the 3D morphology and the solute concentration field around the dendritic tip for various solutes at different concentration (Butler, 2002) as well as to study the ice crystal growth in supercooled pure water (Teraoka et al., 2004). Fluorescence coupled with confocal laser scanning microscopy provides a 3D view of ice growth in real time and has been applied to study ice formation around red blood cells suspended in an acrydine orange solution (Ishiguro and Koike, 1998).

Traditional indirect methods of sample preparation such as freeze substitution, freeze fixation, and freeze drying techniques have the disadvantage of being invasive in the preparation steps prior to observation and may, therefore, introduce artifacts. Freeze substitution consists of replacing ice by a solvent (methanol, for example), evaporating it and observing the sample directly or after embedding (Bevilacqua et al., 1979). In freeze fixation, the sample is immersed into a fixative solution (glutaraldehyde) which migrates into the sample and creates links between proteins to fix the sample structure (Miyawaki et al., 1992). In freeze drying, it is assumed that pores appearing after the sublimation step at low pressure are "phantoms" of ice crystals in the frozen sample; this technique has the advantage of being easy to carry out (Kochs et al., 1993).

Ice crystallization studies in biology have used thin water/ice layers that overcome the limitations of fixation and sectioning in transmitted light microscopy. Measured thickness of specimens is in the order of 100 μm for solutions and suspensions in flat glass capillary tubes (Schoof et al., 2000).

The primary disadvantage of using thin samples for transmission microscopy is that confining the sample in capillaries potentially alters the morphology of the interface of ice crystals (Neils and Diller, 2004). An added drawback of this technique in foods is that it is limited to low-viscosity liquid samples. Another important technique to help visualization and quantification of ice crystallization is the so-called double oil layer micro-sized crystallization, which is performed in a micro-sized water droplet suspended between two layers of immiscible oil in a circular quartz cell. This technique could minimize the influence of the walls of the container and dust particles on ice nucleation and has been used to examine quantitatively the effect of antifreeze proteins on ice nucleation kinetics using a polarized optical microscope with a freezing stage (Du and Li, 2002, 2003).

Table 2.1 Some techniques used to observe and measure ice morphology at the microscopic level

Observation technique	***Fundament or principle***	***Advantage/ Disadvantage***	***References***
Microscope stage for directional solidification (MSDS)	Ice front is forced to grow in a specific direction and at a specific rate by imposing a controlled thermal gradient	Observing phase front morphology and cell entrapment in growing ice	Körber, 1988
Modification of MSDS: direction of solidification along the optical axis		Quantification of chemical fields during freezing. 3-D images by digital reconstruction	Kourosh et al., 1990a, 1990b Neils and Diller, 2004
Optical microscopy with episcopic coaxial lighting	Light flux differences reflected by the various interfaces of the material	Direct observation of the frozen original structure of ice cream samples	Faydi et al, 2001; Caillet et al., 2003
Micro-slicer image processing system (MSIPS)	Reconstruction of images obtained by multi-slicing a frozen sample	3-D morphology of ice crystals	Ueno et al., 2004; Do et al., 2004
Brightfield microscopy	Exclusion of solutes by crystalline ice segregates dye into the liquid and leaves the ice dark	3-D view of ice growth around red blood cells by confocal laser scanning microscopy	Ishiguro and Koike, 1998
Mach-Zender optical interferometry	Measure changes in concentration by changes in refractive index and monitored the ice-liquid interface	3-D view of morphology and the solute concentration field around the dendritic tip	Butler, 2001, 2002; Teraoka et al., 2004
Fractal analysis coupled with freeze-fixation method	Degree of irregularity applied in image analysis of ice crystals	Shape of ice crystals can be described numerically by fractal analysis	Hagiwara et al.,2002, 2003; Koshiro et al., 2006
Magnetic resonance imaging (MRI)	Visualization of the freezing process following the spatial distribution of non-frozen water.	Images of the freezing process and final microstructure after the solution becomes opaque due to ice formation	Mahdjoub et al., 2006; Hindmarsh et al., 2004
Indirect methods: freeze-substitution, freeze-fixation and freeze-drying	Replacing ice by solvents or air and observation of the space occupied by ice crystals.	May introduce artefacts; simple	King, 1971; Ratti, 2001; Aguilera and Flink, 1974

2.6. Technological implications of ice morphology

2.6.1. Freeze drying

Freeze drying or lyophilization is the process of removing water from a product by sublimation and desorption. Sublimation is the transformation of ice directly into gas without passing through a liquid phase. Sublimation occurs when the vapor pressure and the temperature of the ice surface are below those of the triple point of water (4.58 mmHg, 0 °C) Welti-Chanes et al., 2004).

Freeze drying is a dehydration process widely used in the biotechnology, fine chemicals, food, and pharmaceutical fields at the laboratory and industrial levels. Although high-quality dried products can be obtained by freeze drying, the process is multistage, relatively slow, and expensive (King, 1971; Ratti, 2001). Freezing is a major step in the freeze drying process and significantly affects the performance of the overall freeze drying process (Aguilera and Flink, 1974). Specifically, the freezing step dictates the morphology of ice crystals and crystal size distribution, which in turn influence several critical parameters (Figure 2.6).

The freezing step fixes the ice crystal structure (size, shape, dimensions) and, consequently, the sublimation time the major time of the whole process (Kochs et al., 1991). Therefore, slow freezing rates allow the growth of large ice crystals, leading to larger pores, higher mass flow, and, thus, to shorter freeze drying times (Welti-Chanes et al., 2004).

2.6.1.1. Role of ice morphology in freeze drying of pharmaceutical formulations

In pharmaceutical formulations, the freezing step determines the final morphological characteristics of the freezedried material which are related to the functional properties (e.g., solubility), biological activity, and stability of the bioactive components (Hottot et al., 2004, 2006). Searles et al. (2001) pointed out that the temperature of ice nucleation fixed the final ice morphology of the frozen material (ice and cryo-concentrated phase), and it was strongly correlated to the rate of the primary drying step. Sadikoglu et al.

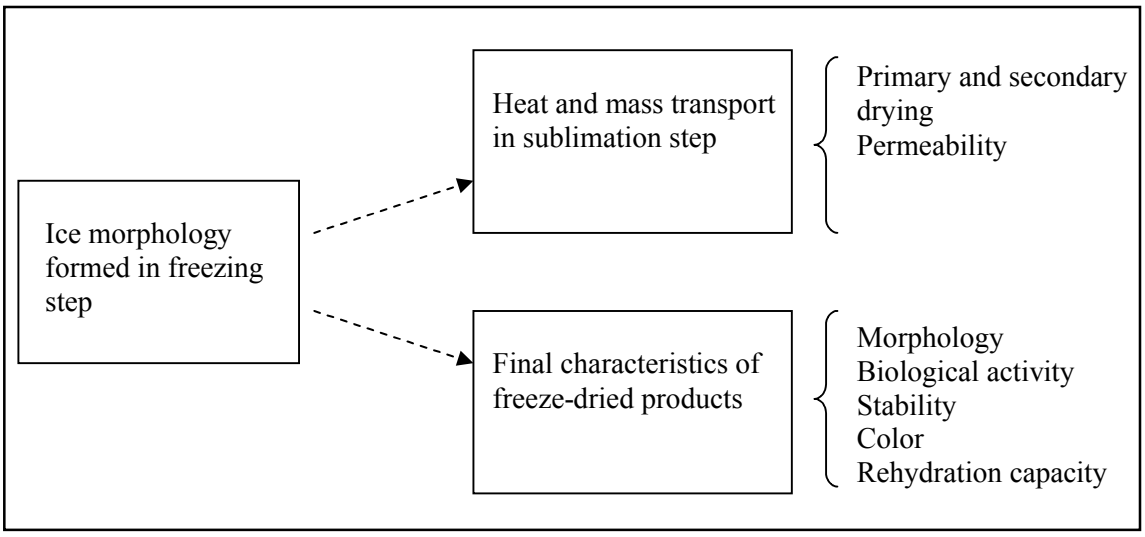

Figure 2.6. Importance of ice morphology in freeze drying. Ice morphology (formed in the freezing step) determines the heat and mass transport during sublimation and the final characteristics of freeze-dried products.

(2006) pointed out that the ice crystals formed during the freezing stage determined the size and shape of pores, the pore size distribution, and the pore connectivity of the porous matrix, thus affecting heat and mass transfer during the primary and secondary drying stages. Recently, Nakagawa et al. (2007), using a mathematical model for the freezing process of a standard pharmaceutical formulation (mannitol and bovine serum albumin-based), confirmed that the mass transfer parameters in freeze drying were strongly dependant on the morphological parameters of the frozen phase and, consequently, on the nucleation temperatures. Nevertheless, large dendritic ice crystals (formed at lower freezing rates) that result in a higher mass transfer rate are not always appropriate for all products undergoing freeze drying. For pharmaceutical formulations, large ice crystals can damage some nutrients and biological materials and can cause phase separation, a subject of active research these days (Sadikoglu et al., 2006; Tang and Pikal, 2004). Moreover, large pores means more occluded air in the matrix and the possibility of oxidation reactions to occur in the final product.

2.6.1.2. Role of ice morphology in freeze drying of food matrices

In freeze drying of liquid foods, Sagara (2001) demonstrated the importance of the structure fixed during freezing on mass transport properties. A linear relationship was found between vapor permeability and ice crystallization time during the freezing step for mashed apple samples, behaviour attributed to the larger ice crystals formed in the samples as the freezing rate decreased. This would suggest that liquid materials should be slowly frozen in a manner that straight ice columns are formed parallel to the direction of heat and mass transport. If a higher permeability coefficient is obtained by controlling the freezing method, the drying rate will be probably limited by the heat transfer rate across the dried layer to the frozen core. In freeze drying of coffee extracts, the freezing rate is a major factor affecting the final color of the product due to the interaction of light with the surface pores. Barnett (1973) gathered microscopic and macroscopic evidence showing the need for relatively slow freezing, leading to growth of large ice crystals that produce a desired dark freeze-dried product. Similarly, Katz and Dwyer (1976) determined that after sublimation of large, nonordered dendritic ice structures formed by slow freezing, a darker coffee-like color is obtained.

2.6.2. Freeze concentration and ice morphology

Freeze concentration is a concentration technique that involves lowering the temperature of an aqueous solution sufficiently to partially freeze the water, resulting in a slurry of ice crystals dispersed in a concentrated solution (Hartel, 1992). Basically, there are two methods of freeze concentration (Figure 2.7). In the conventional method of suspension crystallization (folded chain single crystal, FCSC; Figure 2.7a), individual ice crystals are formed that are enlarged in size by Ostwald ripening (Huige and Thijssen, 1972). Nevertheless, separation between ice crystals and the mother liquid is quite complicated and results in losses of concentrate trapped in a mash of solution and crystals (Miyawaki, 2001; Deshpande et al., 1982). Progressive freeze concentration (PFC; Figure 2.7b) is based on a completely different concept because a large ice mass is

formed and grown on the cooling surface so that the separation from the mother solution is relatively easy (Bae et al., 1994; Liu et al., 1997).

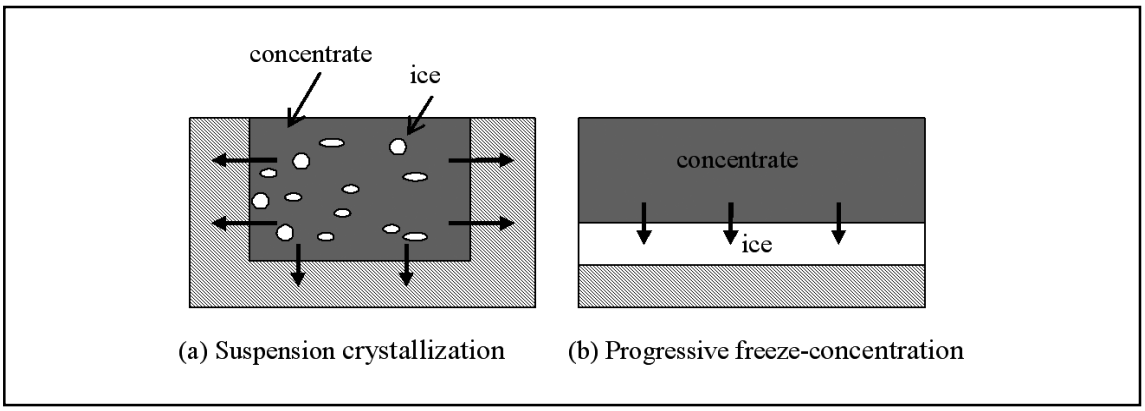

Figure 2.7. Methods for freeze concentration. a) Suspension crystallization: Many small ice crystals are formed and enlarged in size by Ostwald ripening. b) Progressive freeze concentration: A large single ice crystal is grown from a cooling surface in a crystallization vessel. The direction of arrows represented heat transfer.

In FCSC, research has focused on how to control the crystallization step to obtain ice crystals as large as possible. Large ice crystals are favorable to ease the separation of ice crystals from the concentrated mother solution (Miyawaki, 2001). These crystals, preferably of uniform size, present a low specific surface (i.e., low surface area per unit mass), and therefore, losses of entrained juice concentrate are minimized (Deshpande et al., 1982; Bruin and Jongen, 2003). An efficient way to control crystallization in FCSC is the use of low levels of subcooling. It has been reported that for sugar solutions and liquid foods and at low levels of subcooling (about 0.1°K), the growth of ice crystals takes the form of disks, in contrast to the dendrites produced at higher degrees of subcooling (Huige, 1972; Omran and King, 1974; Stocking and King, 1976). This change in morphology is in itself a sufficient reason to operate at lower subcooling since the surface-to-volume ratio of the crystals is much improved. Another important factor is the viscosity of the concentrate because the capacity of ice separators is inversely proportional to the viscosity and directly proportional to the square of the mean diameter of the crystals, as expressed by

$$Q = \frac{\Delta P g d_e^2}{0.2 \mu l} * \frac{\varepsilon^3}{(1-\varepsilon)^2} \quad (2.2)$$

where Q is the draining rate from the crystal bed (cm^3/cm^2s); ΔP is the pressure difference exerted over the bed by compression or by centrifugal or pressure drop of the filtrate (kg/cm^2); d_e is the diameter of the crystals (cm); μ is the viscosity of the liquid (poise); l is the thickness of the bed (cm); g is the gravity acceleration (cm/s^2); and ε is the volume fraction of pores in the bed filled by the liquid phase (Welti-Chanes et al., 2004).

In PFC, the effective partition coefficient of a solute between the ice and the liquid phases at the ice-liquid interface is the most important parameter. The value of the partition coefficient K changes between 0 (ideal freeze concentration) and 1 (no concentration) and decreases (giving a higher ice purity) with a decrease in the advance rate of the ice front (u) and/or an increase in the stirring rate N (see equations 2.3-2.5) (Miyawaki, 2001; Gu et al., 005).

$$K = K_0/[K_0 + (1 + K_0)\exp(u/k)] \quad (2.3)$$

$$K = C_S / C_L \quad (2.4)$$

$$k = aN^b \quad (2.5)$$

where, C_S, solute concentration in ice-liquid interface; C_L, solute concentration in solution phase, K_0 is limiting partition coefficient at the ice-liquid interface, u is advance rate of ice front, k is mass transfer coefficient at the interface, and a and b are constants experimentally determined. Noted that these equations (equations 2.3-2.5) are valid only if K is constant during the concentration process (*i.e.* quasi-steady state), condition reached if the volume of solution is sufficient large (Butler, 2002).

When PFC was applied to concentration of tomato juice of 4.3 up to 18.8 wt.%, no substantial differences were observed in acidity, vitamin C content, or color compared with a sample before freeze concentration (Liu et al., 1999). Recently, PFC was applied to Andes berry (*Rubus glaucus* Benth) pulp preserving the flavor (with a total volatiles loss near 20%), and sensorial analyses show that PFC did not change the sensorial properties of fresh pulp (Ramos et al., 2005). In addition, to avoid impurities in the ice phase, it is recommended to suppress the initial supercooling (when it occurs before the initial crystallization at the bottom of the sample vessel) with ice nucleation agents (Liu et al., 1997). Although PFC is proven to be effective for a high-quality concentration of liquid foods, its productivity is much lower when compared with FCSC. To improve this factor, a configuration that increases the heat transfer surface using a tube system was proposed for scaling up (Shirai et al., 1999; Miyawaki et al., 2005). In this modification, ice crystal grows on the inside surface of a pipe being externally cooled by a refrigerant, and numbers of pipes can be bundled together and interconnected in series to further increase the cooling surface area. The future of freeze concentration applications seems to be associated more with developments in the configuration of PFC systems than advances in FCSC because of the simpler separation step. Furthermore, the efficiency of operation may be improved by the implementation of some freezing assisting techniques (see below).

2.6.3. Freeze texturization

A major goal of food technology is creating structures that are attractive and palatable (Aguilera, 2005; Aguilera, 1992; Aguilera and Lillford, 2008). In particular, the uniform texture associated with protein gels may be changed into the anisotropic structures of meat and fish by freeze texturization. In Japan, freeze texturization is an old process to produce kori-tofu, a porous, spongelike product from soybean curd (Lillford, 1985). Chewable fibrillar structures can be induced in protein solutions via the controlled growth and orientation of the ice phase by unidirectional freezing and later fixed through protein–protein interactions. Cooling only one surface of the protein system will

generally cause the crystals to grow aligned and perpendicular to that surface. As the ice crystals grow from the surface as "spears" into the slurry, they force the proteinaceous material out of the space occupied by the advancing crystals and draw water molecules out of the slurry to bind onto the ice crystal surface. This action concentrates the proteinaceous material in the interstitial spaces between the ice crystals and the branches of each crystal. Upon melting of the ice, the proteinaceous material remains compacted in the former interstitial spaces and long, thin, parallel voids are formed where the crystals had existed (Lawrence et al., 1986). Figure 2.8 shows the main stages in the freeze texturization process.

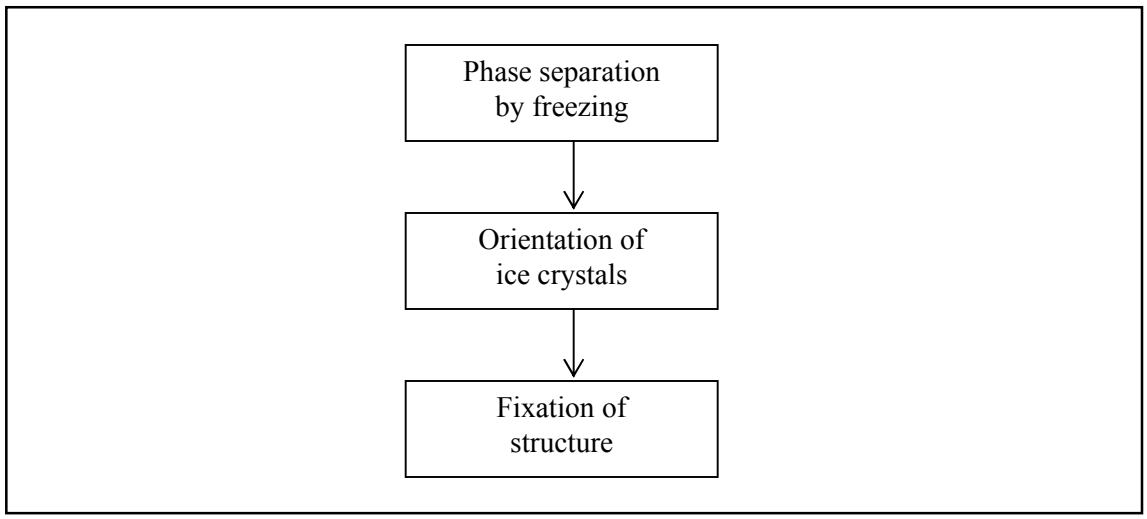

Figure 2.8. Stages in freeze texturization. Source: Aguilera (1992) (with permission).

In the review by Lillford (1985), several patents on freeze texturization are discussed. The freezing rate is critical to determine whether a spongy or fibrous structure is formed. Contrary to freeze preservation of foods, freeze texturization relies on slow freezing so that the rate of crystal growth exceeds the rate of nucleation, since achieving a homogeneous structure requires control over the freezing process. This is difficult to achieve because heat transfer rates and solute concentrations change as the freezing front advances. To alleviate this problem and force fiber formation, use of mechanical means

to break ice crystals and induce alignment of the protein phase has been proposed in some patents (Aguilera, 1992). Kolakowski et al. (1997) have proposed the use of a mild trypsin proteolysis pretreatment to accelerate later the fixation of structure by transglutaminase in freeze texturization of minced fish.

2.6.4. Technologies to control ice nucleation

As previously mentioned, the most important step in the crystallization process for controlling the crystal size and crystal size distribution is nucleation. By controlling nucleation, a desired crystalline microstructure and ice morphology may be attained (Hartel, 2001). In practice, the freezing rate, agitation, and seeding are the preferred variables for controlling the size and distribution of ice crystals (see "Fundamentals"). However, recent investigations are opening new possibilities to control nucleation (and crystal growth) using ice nucleation agents and antifreeze proteins, as well as physical methods like ultrasound and pressure freezing (Sun and Zheng, 2006).

2.6.4.1 Ice Nucleation Agents

Insoluble materials, of biogenic or non-biogenic origin, that dramatically influence ice nucleation are called ice nucleation agents (INAs). The use of INAs leads to a heterogeneous ice nucleation process that always occurs at a temperature higher than that of homogeneous ice nucleation. Incidentally, a review on substances that inhibit ice nucleation is presented by Holt (2003).

2.6.4.1.1. Non-biogenic Ice Nucleation Agents

The most studied non-biogenic INA is silver iodine, the material used for cloud seeding. Aliphatic alcohols have been shown to be very potent ice nucleators when they are arranged as monolayers at the surface of water drops (Gavish et al., 1990). However, both types of INAs are not suited for food applications.

2.6.4.1.2. Biogenic ice nucleation agents

INAs of biogenic origin have been used to reduce the degree of supercooling in the freezing of food by raising the temperature of ice nucleation. These activators change the morphological characteristics and ice formation patterns in frozen foods (Watanabe and Arai, 1994; Li and Lee, 1998) and have shown potential in freeze drying, cryopreservation, and freeze concentration. These substances are present in several biological organisms such as bacteria, fungi, insects, and even frogs (Lundheim, 2002); however, recent studies and technological applications have focused on microbial INAs. The ability of these microbiological agents to reduce supercooling results in shorter freezing times, reduction of freezing costs, and a more efficient ice production (Li and Lee, 1995). The properties of microbiological INAs are summarized in Figure 2.9. At least six species of ice nucleation bacteria have been studied (*Pseudomonas fluorescens*, *P. syringae*, *P. viridiflava*, *Erwinia herbicola*, *E. ananas*, *E. uredovora* and *Xanthomonas campestris*), of which *P. syringae* has been the most widely used. However, because not all natural strains exhibit ice nucleation activity, those which produce INA substances are called Ina+, and those which do not are Ina− (Li and Sun, 2002a). Some strains of *Fusarium* and related genera of fungi are also active in ice nucleation (Cochet and Widehem, 2000; Kawahara, 2002). The physiological role of bacterial INAs is probably an adaptative advantage to induce frost damage to their host, thereby giving the bacteria access to nutrients from the plant (Buttner and Army, 1989). However, in insects, INAs appear to play a different role since they are mainly produced by those that survive freezing (Lundheim, 2002). Bacterial INAs are classified into three chemically distinct classes or structures: A (lipoglycoprotein), B (glycoprotein), and C (only protein) (Turner et al., 1991). Properties of these structures that favor ice nucleation include their similarity to that of the ice crystal lattice, the paucity of the surface charge, and a high hydrophobicity (Kawahara, 2002). However, the beneficial applications of INAs in foods raise concerns with the pathogenic character of bacterium from genera *Pseudomonas* or *Erwinia*. This problem is avoided using non-pathogenic strains of ice nucleation bacteria or by inactivation of the biological activity of cells

(e.g., by thermal killing or entrapment) (Watanabe and Arai, 1994). In a different application, *E. ananas* treated with ultraviolet irradiation was used as an "encapsulating" agent for ice to improve the thermal efficient of a water chilling system (Tsuchiya et al., 2004).

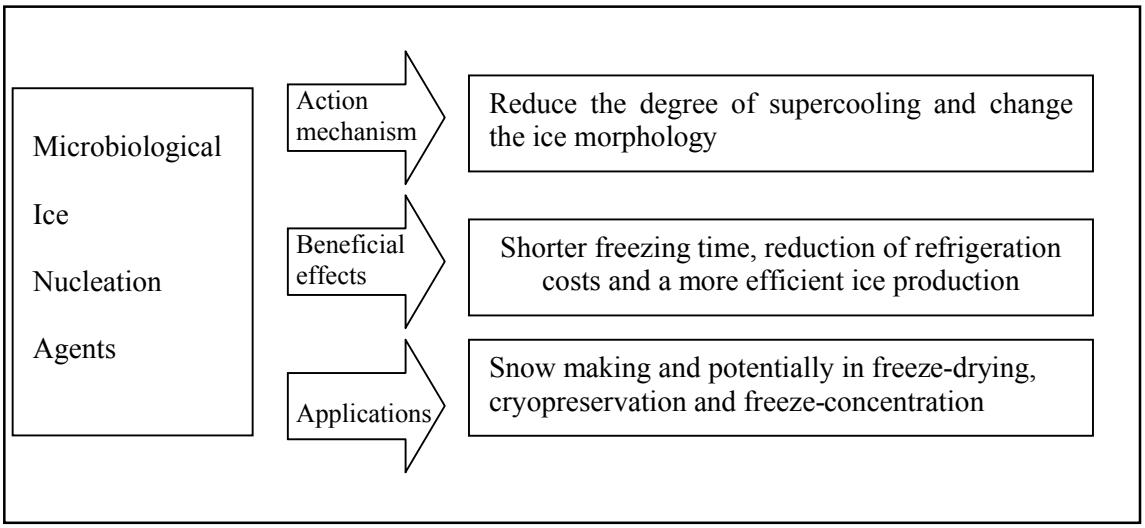

Figure 2.9. Principal properties of microbial ice nucleation agents. Action mechanism, beneficial effects, and applications.

2.6.4.2. Antifreeze proteins

Antifreeze proteins (AFPs) are ice-binding proteins found in some organisms (such as fish, insects, plant, and soil bacteria) that live at temperatures where they encounter freezing conditions (Jia and Davis, 2002). Details are in the review by Venketesh and Dayananda (2008). Based on the presence or absence of carbohydrates, AFPs are classified into two main types: glycoproteins and non-glycoproteins. Antifreeze glycoproteins mainly consist of repeating units of two amino acids in which one of them is glycosylated. For convenience, non-glycoproteins are still called AFPs, which can be further subdivided into four distinct antifreeze subtypes: the alanine-rich AFPs of right eye flounders and sculpins (type I), the cystine-rich AFPs of sea raven smelt and herrins (type II), an AFPs (type III) found in ocean pout and eelpout wolfish, and the glutamine and glutamate-rich AFPs of long horn sculpin (type IV) (Sun and Zheng, 2006).

Several authors have reported the effect of AFPs on the morphology of ice crystals which becomes evident when the supercooling temperature exceeds the level of freezing temperature suppression and the growth morphology differs significantly from that of ice growth in pure water (Raymond and DeVries, 1977; Yeh and Feeney, 1996; Zhang and Laursen, 1999; Strom et al., 2004; Strom et al., 2005). Therefore, when an ice crystal grows in pure water, water molecules add onto the non-basal planes of the ice and the crystal grows along the a-axis. However, when an ice crystal is placed in a solution of AFPs, these proteins absorb onto non-basal planes of ice at the ice–water interface and inhibit its growth. As a consequence of AFPs absorption, the ice grows in the non-preferred direction along the c-axis of the crystal lattice (Griffith and Antikainen, 1993). Under these conditions, bipyramidal crystallites and columnal spicules are formed instead of sheets (Yeh and Feeney, 1996). See Figure 2.10 for different bipyramidal ice morphologies induced by AFPs (DeLuca et al., 1996).

In practical terms, AFPs can completely stop crystal growth of ice in solutions at temperatures below the melting temperature of ice (Jia and Davies, 2002; Yeh and Feeney, 1996). The widely accepted cause of the difference between the melting temperature and nonequilibrium freezing temperature at which ice crystals start to grow (thermal hysteresis) is attributed to the discontinuous adsorption of AFPs molecules onto the ice crystal facets (Raymond and DeVries, 1977). These adsorbed molecules prevent that water molecules be incorporated onto the ice surface at the adsorption sites, thus pinning the crystal growth of ice in the supercooled solution and increasing the solid–liquid interfacial area by producing a microscopically curved ice surface.

2.6.4.2.1. Applications of AFPs in food technology and perspectives

Several potential applications for AFPs have been envisaged in foods. AFPs have been used in ice cream manufacture leading to ice crystals smaller than a control (Warren et al., 1992). In meat products, soaking bovine and ovine muscle in a solution up to 1 mg/ml of AFP prior to freezing at −20 °C showed evidence of reduced ice crystal size (Payne et al., 1994). Preslaughter administration of AFP to lambs (injected

intravenously) reduced ice crystal size and drip loss after thawing (Payne and Young, 1995). AFPs have also been used to preserve the gel-forming functionality of surimi in both chilled and frozen conditions. AFPs remarkably preserved Ca2+ ATPase activity of actiomyosin during storage and provided better protection than conventional cryoprotectants such as sucrose–sorbitol mixtures (Boonsupthip and Lee, 2003). A Unilever patent suggested that fungal hydrophobin AFPs inhibited ice crystal growth during frozen storage (i.e., ice recrystallization) and modified ice crystal shape in aerated and non-aerated frozen food products (Aldred et al., 2006). Although commercial AFPs are currently available, they are mainly for research or special uses because of their high price. Chemical synthesis and genetic engineering may be a solution to produce cost-effective AFPs, hence to promote their applications in frozen food products (Sun and Zheng, 2006).

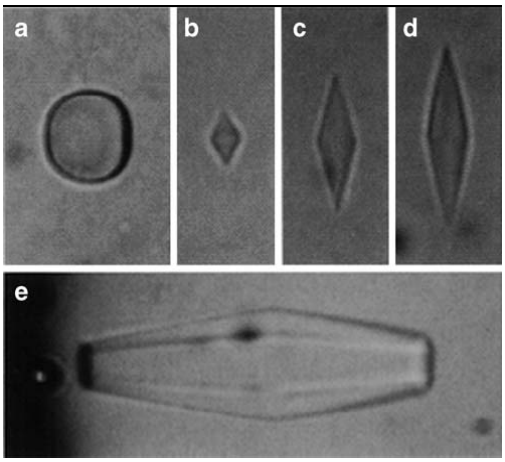

Figure 2.10. Ice crystal morphology in the presence and absence of wildtype and mutant type III AFPs. a) Ice crystal formed in the presence of 0.1 M NH_4HCO_3 (pH 7.9) and photographed after undercooling by 0.1 °C. b–e) Ice crystals formed in the presence of 1 mg/ml of type III AFP in 0.1 M NH_4HCO_3 (pH 7.9) and photographed after 1 min of undercooling by 0.1 °C where the specific AFPs were wild-type (b) and mutant type III AFPs (c–e) (from DeLuca et al. (1996), with permission).

2.6.4.3. Ultrasound

Compared to other freezing assisting methods ultrasound (US) is quite efficient since one or two pulses of ultrasound can accelerate the nucleation of ice. Unlike nucleating agents, it does not require a direct contact with the product, and since it is not chemically invasive, it is unlikely to encounter regulation difficulties (Acton and Morris, 1992). Therefore, US has been recently studied in assisting and/or accelerating freezing during the manufacture of ice cream and sorbets and molded frozen products such as ice lollipops. If proven commercially attractive, these applications could be extended to freezing of high-value foods (Zheng and Sun, 2006).

US-assisted freezing of fresh cellular foods is accelerated mainly by the enhancement of heat transfer which leads to faster frozen products (Li and Sun, 2006b; Mason et al., 1996). It is possible that cavitation of bubbles inside the cell contents may induce intracellular nucleation and help to reduce the size of ice crystals, minimize cell dehydration, and maintain the original shape of the product. Another reason is that cavitation of bubbles may also increase the nucleation rate in the extracellular region, which is also favorable for an overall smaller crystal size distribution. Lastly, crystal fragmentation caused by sonication is another possible reason for the reduced crystal size (Zheng and Sun, 2006). Photomicrographs obtained by cryo-SEM indicated that tissue samples of US-assisted frozen potatoes exhibited a better cellular structure than those without acoustic treatments (Sun and Li, 2003). US can be an effective method to control crystal size distribution in the final product by starting ice nucleation at different supercooling temperatures. If US is applied when the system is at a low degree of supercooling, only a few nuclei will form that will be able to grow into large crystals. On the other hand, application of US at higher degrees of supercooling will cause the formation of many nuclei, which can only grow to a limited size, originating many small crystals. These findings have been successfully applied to the freeze drying of a sucrose solution (Acton and Morris, 1992). US treatment of the solution for 5 s at 1 °C of supercooling resulted in the formation of only a few large crystals, while when US was applied at 5 °C of supercooling, nucleation was widespread and the formed crystals were

smaller. After freeze drying, the former sample was found to have larger pores than the latter. Nakagawa et al. (2006) reported the influence of controlled nucleation by US on the ice morphology of a frozen formulation of pharmaceutical proteins prior to freeze drying. The US treatment increased the temperature of nucleation; consequently, the mean ice crystal diameter was augmented and the primary freeze drying rate was accelerated. These authors concluded that US could be applied for the optimization of freeze drying cycles of pharmaceuticals under industrial conditions. Applications of US as assisting technology for food freezing require optimization of the levels of two key parameters in the process: ultrasonic power and duration or pulse time (Li and Sun, 2002b). Recently, Mortazavi and Tabatabaie (2008) showed that the freezing time in ice cream processing could be shortened by about 30% using a 20-min US treatment. The future of this technology in freezing of foods is strongly dependent on the availability of adequate industrial equipment (Zheng and Sun, 2006). Additionally, the practical use of US in freeze concentration has been reported in effluent processing (Botsaris and Qian, 1999).

The application of US in progressive freeze concentration has been studied by the group of Kawasaki to increase the process efficiency by promoting a strong agitation at the freezing interface (see "Freeze concentration and ice morphology"). As a result, US was used as an alternative means of agitation, and the washing operation became quite easy. They found that concentration efficiency and the dissolved air concentration in the liquid were greatly improved by US (without US, the partition coefficient of solutes was about 1.0 for all freezing rates, and with US, this factor was smaller than 1.0 at slow freezing rates and smaller than 0.5 at fast freezing rates) (Matsuda and Kawasaki, 1997; Matsuda et al., 1999; Kawasaki et al., 2004).

2.6.4.4. High-pressure freezing

As stated before, freezing at atmospheric pressure under a substantial degree of supercooling may result in a fine ice structure. This effect is ascribable to the very rapid freezing when supercooling ceases (Miyawaki et al., 1992). However, supercooling is a

non-equilibrium and a thermodynamically unstable situation that is not easily controllable. High-pressure freezing, specifically pressure-shift freezing (PSF), is one alternative to achieve very rapid freezing with a very fine ice structure (Miyawaki, 2001). The PSF process exploits the fact that at elevated pressure, the freezing point of water is depressed, e.g., from 0 °C to −21 °C at about 210 MPa (Bridgman, 1912). Then, in PSF, a sample is cooled under pressure to a temperature just above the melting temperature of ice and pressure is then rapidly released, resulting in supercooling which enhances instantaneous and homogeneous nucleation throughout the cooled sample (Kalichevsky et al., 1995), resulting in a uniform distribution of small ice nuclei (Chevalier et al., 2000). Ice crystal growth is then achieved at atmospheric pressure in a conventional freezer. In addition, about 36% of the total water content can be instantaneously converted into ice during expansion from 200 to 0.1 MPa (Sanz et al., 1997). After pressure release, PSF proceeds like the classical freezing process and the temperature profiles follow the same course as conventional freezing curves (van Buggenhout et al., 2005). In practical terms, the use of high-pressure facilitates supercooling, promotes uniform and rapid ice nucleation, and produces smaller crystals (Kalichevsky et al., 1995), as has been demonstrated for several foods products including gelatine gels (Zhu et al., 2005) and starch gels (Lille and Autio, 2007), meat pieces (Martino et al., 1998), tofu (Fuchigami and Teramoto, 2003), fish (Chevalier et al., 2001; Sequeira-Muñoz et al., 2005; Alizadeh et al., 2007), and fruit and vegetables like carrots (Fuchigami et al., 1997a, 1997b), peach and mango (Otero et al., 2000), and potatoes (Luscher et al., 2005; Urrutia et al., 2006; Urrutia-Benet et al., 2007). These results suggest that high-pressure freezing is the best known method to preserve the microstructure of large pieces of food.

However, the advantage obtained by high-pressure freezing (e.g., smaller ice crystals than in traditional freezing) may be easily lost during frozen storage at atmospheric pressure when several ice recrystallization phenomena start taking place. Only a few studies have reported the consequences of frozen storage at atmospheric pressure of high-pressure frozen foods. While the beneficial effect of high-pressure freezing in

avoiding recrystallization was demonstrated for fish (Chevalier et al., 2001; Sequeira-Muñoz et al., 2005; Alizadeh et al., 2007), in the case of carrots, no differences were observed between classical freezing and high-pressure freezing (van Buggenhout et al., 2005). Future applications of high pressure freezing in foods are associated with high-value products (due to the high capital costs of equipment) and/or premium foods that obligatorily need small sizes of ice crystals (as in ice cream).

2.6.5. Other applications

2.6.5.1. Porous scaffolds

Porous matrices are attractive carriers in foods for delivering nutrients and bioactive components as well as in gastronomic applications, but so far, biomedical applications seem to have a lead, for example, the porous scaffolds used as permanent skin replacement for the treatment of deep dermal burns. In the regeneration process of the skin, the 3D structure, pore sizes, and pore connectivity of the porous scaffolds is very important (Kuberka et al., 2002; O'Brien et al., 2007). In the fabrication of these porous scaffolds, freezing is a critical step, which involves controlled unidirectional freezing prior to a freeze drying process (Deville et al., 2006). Schoof et al. (2000) investigated the modulation of pore size of porous scaffolds (freeze-dried collagen sponges) by controlling the ice morphology. Results show that by varying the freezing parameters (e.g., the advancing rate of the ice front) and the solute concentration (acetic acid and ethanol), the sizes of ice crystals, which later modulate the pore size of the freeze-dried matrix, could be varied between 30 and 50 μm. O'Brien et al. (2004) has shown that a more uniform structure of porous scaffold (pore size) is obtained by keeping a constant freezing rate.

2.6.5.2. Slurry ice

The use of "slurry ice" (a system consisting of small spherical ice crystals surrounded by freshwater or seawater at subzero temperature) has been proposed for the preservation of

fish (Huidobro et al., 2001). Details are in the review by Piñeiro et al. (2004). There are two relevant characteristics of slurry ice: (1) a fast chilling rate which is a consequence of the improved heat transfer capacity and (2) the reduced physical damage caused to food products by the small spherical ice particles (about 20 μm) compared to that elicited by flaked ice. In addition, the slurry completely covers the surface of the food, providing a better protection with respect to oxidation and dehydration and may even contain protecting agents such as ozone and melanosis inhibitors (Huidobro et al., 2002). However, agglomeration and growth of the small ice crystals tend to occur with time that may be prevented by the addition of a small amount of AFPs or by surfactants (Inaba et al., 2005). Recently, an Israeli group developed an innovative ice-making machine (Bubble Slurry™ Ice Machine) to produce minuscule ice crystals and gas bubbles of CO_2 or O_3 suspended in the cooling liquid, which can be kept at a very low temperature without solidifying. The resulting coolant fluid mixture consists of 5-μm ice crystals and equally sized gas bubbles allowing for highly efficient heat transfer (Menin, 2001).

2.7. Conclusions

Although the size and shape of ice crystals is an important parameter in the quality, sensorial characteristics, and stability of frozen foods, the control of ice morphology has not received enough attention in the past. In freeze related processes, the morphology of ice influences the efficiency of the separation step (freeze concentration), the rates of moisture removal and quality of products (freeze drying), and provides opportunities for new structuring technologies (freeze texturization, sponges, etc). New techniques are becoming available to control the ice crystallization process (ultrasound, high-pressure freezing) and ice morphology (use of ice nucleation agents and antifreeze proteins, among others), and improved methodologies allow to follow ice crystal growth in microscopy stages under controlled conditions and to quantify relevant morphological parameters of ice crystal size populations using powerful and fast image analysis techniques. Eventually, results of these fundamental studies may be scaled up to

improve industrial processes, as in the case of new cooling media for direct food preservation. In the future, modulation of ice morphology could permit improvement in the quality of existing frozen products, development of novel food products and porous food matrices, as well as progress in the operation of existing freeze-related processes.

3. VACUUM-ASSISTED FREEZE CONCENTRATION OF SUCROSE SOLUTIONS

3.1 Introduction

Freeze concentration is a method for concentrating a food solute in a solution based on the separation of pure ice crystals from a freeze-concentrated solution. As compared to evaporation and membrane technology, freeze concentration has some significant potential advantages for producing a concentrate with high quality because the process occurs at low temperatures where no vapor/liquid interface exists resulting in no loss of volatiles. Thus, the flavor and quality of freeze-concentrated products are exceptionally high, especially relative to their evaporated counterparts. These benefits make freeze concentration particularly suitable for the concentration of some products, such as fruit juices, coffee and tea extracts, and aroma extracts (Morison and Hartel, 2007). However, freeze concentration has not reached its full potential due to its complicated operation requirements and low yields (Flesland, 1995).

A conventional freeze concentration process (suspension crystallization) requires three fundamental components: (a) a crystallizer or freezer; (b) an ice–liquid separator or a melter–condenser; and (c) a refrigeration unit (Welti-Chanes et al., 2004). However, the industrial future of freeze concentration is associated more with developments in the configuration of one step systems (block freeze concentration or progressive freeze concentration) because of the simpler separation step (Petzold and Aguilera, 2009). An additional advantage of these one-step systems is their simplicity in terms of both the construction and operation of their equipment (Sánchez et al., 2009).

Assisted techniques that improve the efficiency of processing in one-step configurations of freeze concentration are important in achieving commercial viability. An example of an assisted technique is the use of ultrasound to control ice nucleation in progressive freeze concentration (Matsuda and Kawasaki, 1997; Matsuda et al., 1999; Kawasaki and Matsuda, 2004). An alternative for separating the concentrated solution from the ice fraction is the use of a vacuum, which is similar to the principle used by children to suck

the sugar solution containing colorants from popsicles. The process takes advantage of the hydraulic system existing in the frozen matrix formed by veins (or channels) between the ice crystals containing the concentrated solution, but also influence the shape of ice crystals formed on freezing (Martel, 2000). This matrix in a frozen solution is responsible for differences in the concentration of impurities in ancient polar ice (Rempel et al., 2001). This principle has been recently proposed by Hsieh (2008) to obtain drinkable water from sea water exerting an external force (suction by a pump) on frozen sea water to separate salt, thereby converting the ice of sea water into fresh water.
Other recent technique would be the use of thawing as a concentration process. It was quite recent that freeze–thaw process was recognized as a concentration technique for aqueous solution (Yee et al., 2003) and (Yee et al., 2004). They reported that frozen solutions that were subsequently thawed under ambient atmosphere resulted in concentrated solutions recovered from the early melt fractions. Nakagawa et al., (2009, 2010) also reported an example of freeze–thaw concentration for aqueous dye solution and commercial apple juice, respectively.
The aim of this section of the thesis was to study the use of vacuum as a driving force to remove the concentrated solution from the ice matrix in a one-step freeze concentration of sucrose solutions.

3.2 Materials and methods

3.2.1. Materials

Aqueous solutions of sucrose (Sigma–Aldrich, Dorset, UK) with concentrations of 5, 10, 15 and 20 wt.% were prepared with distilled water in triplicate for assays with two replicates.

3.2.2. Freezing procedure

Sucrose solutions (12 mL) contained in plastic tubes (internal diameter of 15 mm) were frozen in a static freezer at –20 °C for 12 h. As shown in Fig. 3.1, the external surface of

the plastic tubes was covered with thermal insulation made of foamed polystyrene so that heat transfer during freezing mainly occurred unidirectional (axially from top to bottom).

During freezing, the temperature in the sample was measured using needle-type thermocouples (Ellab A/S, Rodovre, Denmark; type T; copper-constantan; range –50 °C/135 °C; response time 0.8 s; accuracy < 0.2 %) located at the geometric center of the three test samples. Thermocouples were connected to a data acquisition system (model CTF84-S8; Ellab A/S, Copenhagen, Denmark) and temperatures were continuously registered. The freezing rate (mm min^{-1}) was calculated as the thickness divided by the freezing time (assuming that freezing occurs from one side) (Ramaswamy and Marcotte, 2006).

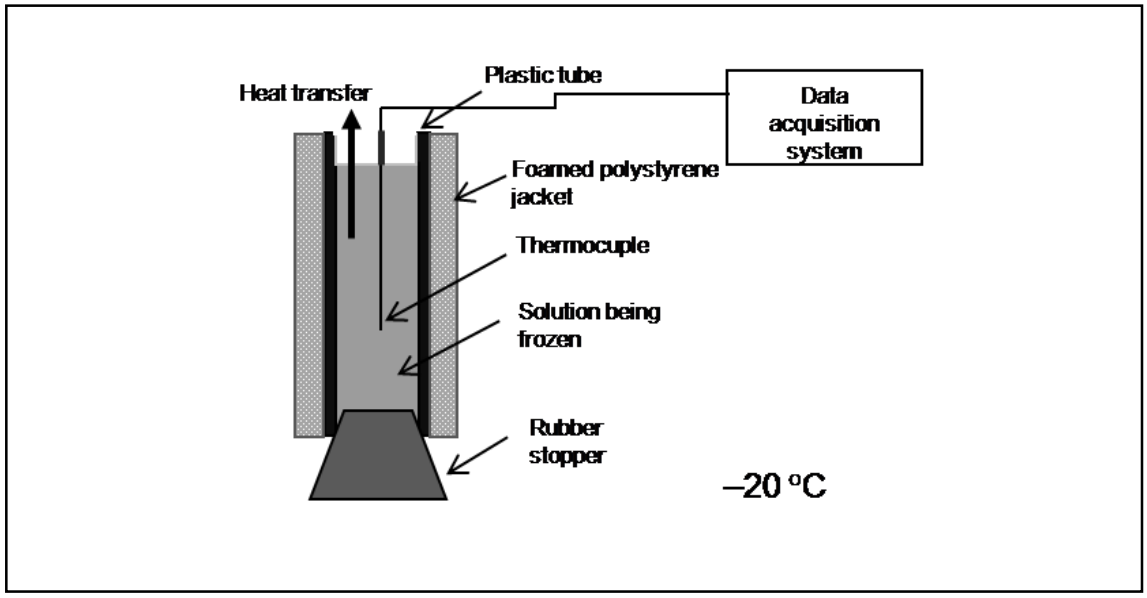

Figure 3.1. Freezing conditions of samples. The samples were frozen in a static freezer at –20 °C for 12 h where the heat transfer mainly occurred unidirectionally from top to bottom.

3.2.3. Vacuum suction procedures

The frozen samples were removed from the freezer (covered with a thermal insulation made of foamed polystyrene) and rapidly transferred to a suction stage as illustrated in

Fig. 3.2. The suction was generated by connecting a vacuum pump (model DOAP104-BN; GaST Mfg. Co., Benton Harbor, MI, USA) to the bottom of the frozen sample at ambient temperature and under vacuum (80 kPa), vacuum that remained constant throughout the test. Vacuum was controlled visually with a vacuum manometer of the pump and an external manometer. Initial and final weights of the ice fraction were registered. Process time (20 min in all cases) was registered until vacuum suction was terminated and the system returned to atmospheric pressure. The concentrated solution was collected and the remaining frozen fraction thawed so that the sugar concentration was determined in both fractions. The concentrations of the Cf and Cs fractions (sucrose in the molten frozen phase and solution, respectively) obtained after the assays were analyzed at ambient temperature (approximately 22 °C) with an ATAGO refractometer (model PAL-1; Tokyo, Japan) with a precision of ±0.1 Brix. All measurements were made in triplicate, and the average values were reported.

3.2.4. Control samples

Control samples were exposed to the same experimental conditions but without the vacuum pump. Process time was 60 min in all cases.

3.2.5. Calculations

3.2.5.1. Percentage of concentrate

Percentage of concentrate represents the evolution in time of the solution removal process. This percentage was calculated for each assay with the initial and final weights of the frozen fraction using the following equation Eq. (3.1):

$$\text{Concentrate}\,(\%) = \frac{\mathrm{W_i^0} - \mathrm{W_i^f}}{\mathrm{W_i^0}} 100 \qquad (3.1)$$

where W_i^0 and W_i^f are the initial and final weights of the frozen fraction, respectively.

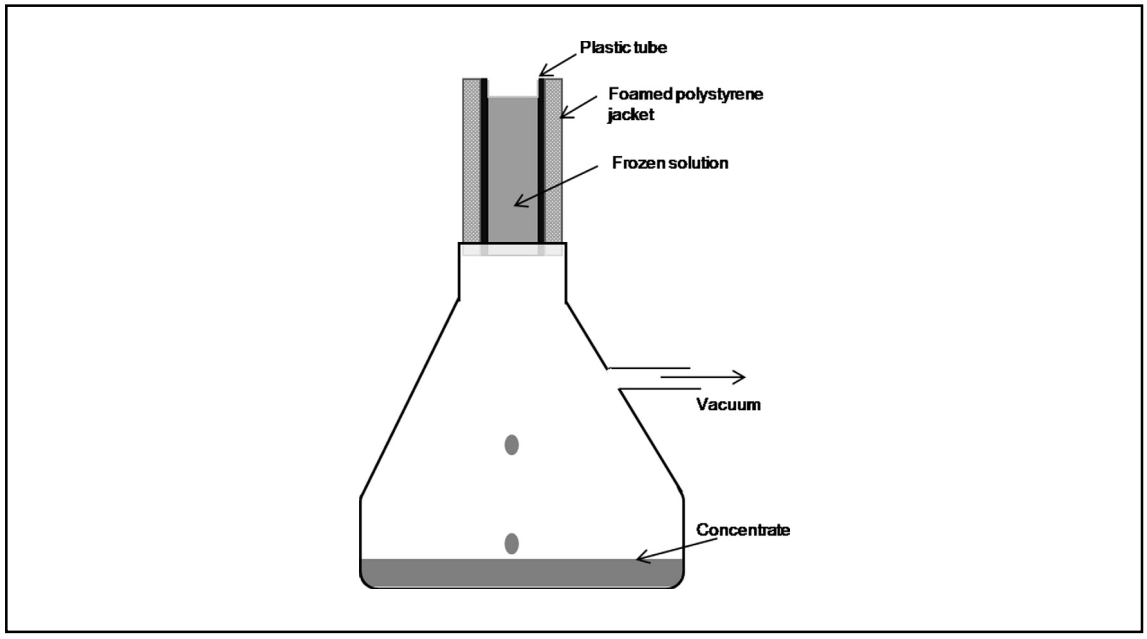

Figure 3.2. Vacuum suction procedure. The concentrated solution was collected until the system returned to atmospheric pressure leaving behind a spent frozen fraction.

3.2.5.2. Efficiency of concentration

The efficiency of each concentration run was defined as the increase in the concentration of the solution relative to the quantity of sugar remaining in the frozen fraction. In theory, the lower the sugar content remaining in the frozen fraction, the more concentrated the solution will be.

The following equation was used to calculate the efficiency Eq. (3.2):

$$\text{Efficiency}(\%) = \frac{C_s - C_f}{C_s} 100 \qquad (3.2)$$

where C_s and C_f are the concentrations of sucrose (Brix) in the concentrated solution and frozen fraction, respectively.

3.2.5.3. Validation of results

To validate the obtained experimental results, a mass balance of each test was made and compared to a theoretical value as follows Eq. (3.3):

$$W_p = \frac{C_s - C_0}{C_s - C_f} \tag{3.3}$$

where C_0 is the initial concentration of sucrose; and W_p is the predicted value of ice mass ratio W (kg ice/kg initial). Additionally, the quality of the fit between experimental (W_e) and predicted (W_p) values for *N* experimental points was evaluated by the root mean square (RMS) as follows Eq. (3.4):

$$RMS(\%) = 100\sqrt{\frac{\sum\left[\left(W_e - W_p\right)/W_e\right]^2}{N}} \tag{3.4}$$

3.2.5.4. Statistical analysis

Statistical analysis of data was performed through analysis of variance (ANOVA) using Statgraphics Centurion XVI Software (Statgraphics, 2009). Differences among mean values were established by the least significant difference (LSD) at 5%.

3.3 Results and discussion

3.3.1. Morphology of a frozen sample

When ice crystals begin to grow in a solution, solutes are rejected from the ice phase and accumulate at the solid/liquid interphase (Zaritzky, 2006). An important parameter of this solute redistribution is the crystal growth rate (given by the freezing rate in practical terms), which was approximately 0.5 mm min^{-1} in the present study. The morphology of a frozen sample is shown in Fig. 3.3. The concentrated phase was contained in veins between the ice crystals that formed an interconnected hydraulic system from where the

concentrated solution was removed under the application of an external force, such as by vacuum or gravity.

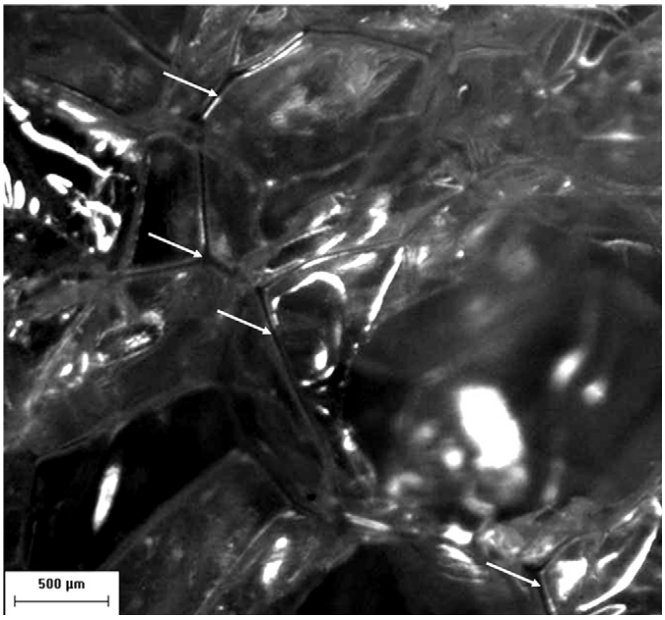

Figure 3.3. Photomicrograph of a thin section of a 15% wt sucrose sample frozen at –20 °C for 12 h. The frozen concentrated phase is observed between the ice crystals as indicated by the arrows. The bar indicates 500 µm. The temperature of sample was controlled in a Linkam® microscopy stage kept at –13 °C.

3.3.2. Ice mass ratio and mass balance

The ice mass ratio (W) decreased with the initial concentration of sucrose, and a significant drop in the ice mass ratio occurred with vacuum treatment (Fig. 3.4). A good agreement was observed between experimental (W_e) and predicted (W_p) ice mass ratios for each test. The obtained RMS values for vacuum and atmospheric treatments were 4.9% and 1.5%, respectively. The higher value observed in RSM samples with vacuum is attributed to differences between the experimental and predictive values (but within the acceptable range), especially at C_0 = 20% (see Fig. 3.4). These values of RSM were

much lower than 25%, which is what Lewicki (2000) considered as an acceptable fit, and these values were close to the value (5.2%) reported by Sánchez et al. (2010).

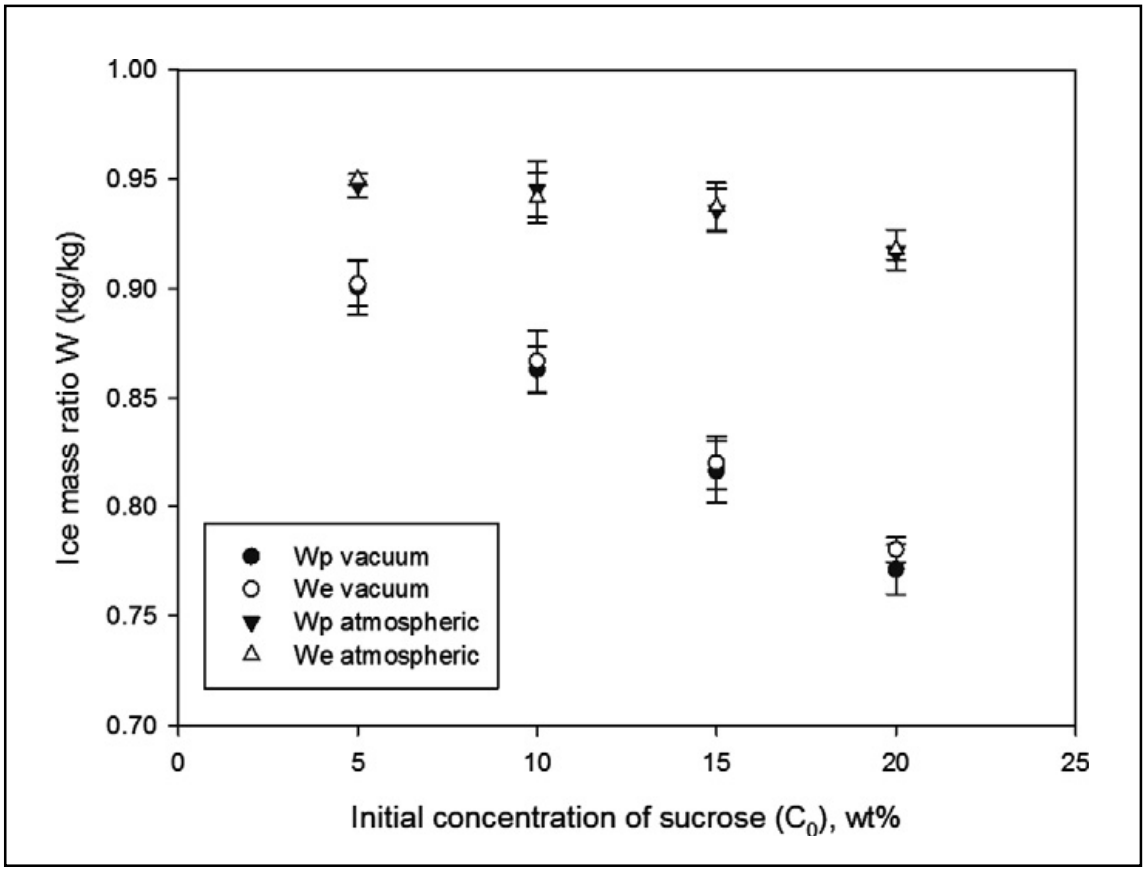

Figure 3.4. Experimental (W_e) and predicted (W_p) ice mass ratios as a function of initial concentration of sucrose (C_0).

3.3.3. Evaluation of vacuum-assisted freeze concentration

Table 3.1 shows the recovered solute, percentage of concentrate, viscosity of concentrate and C_s/C_0 ratio after vacuum and atmospheric treatments where C_0 is the concentration of the starting sucrose solution. For all values of C_0, the recovered solute was significantly larger ($p > 0.05$) with vacuum treatments than with atmospheric treatments. Approximately 0.5 kg of sucrose per 1 kg of initial sucrose was recovered for vacuum treatments as compared to values ranging from 0.16 to 0.3 kg/kg for the atmospheric

treatments. This value (0.5 kg/kg) can probably be closer to the conditions of maximum solute recovery effectiveness of using vacuum, given the low percentages of concentrate (approximately 9–22%). For the same value of C_0, the percentage of concentrate for vacuum treatments was significantly larger ($p > 0.05$) than atmospheric treatments with a direct proportional relationship between percentages of concentrate and the initial concentration of the solution (effect was less in atmospheric treatments). For similar solutions, the sucrose concentration in the concentrate was similar (C_s/C_0 ratios were not different between treatments for the same value of C_0), which is a direct consequence of the freezing process being the same for vacuum and atmospheric treatments.

Table 3.1. Recovered solute, percentage of concentrate, viscosity of concentrate and C_s/C_0 ratio*

Treatments	Recovered solute (kg sucrose/kg initial sucrose)	Percentage of concentrate (%)	Viscosity of concentrate** (mPa s)	C_s/C_0
Vacuum 5%	0.56 ± 0.06^{c}	8.79 ± 1.01^{c}	5.87	5.71 ± 0.65^{e}
Vacuum 10%	0.53 ± 0.04^{c}	13.33 ± 1.39^{d}	14.69	3.99 ± 0.39^{d}
Vacuum 15%	0.50 ± 0.05^{c}	17.99 ± 1.91^{e}	19.97	2.80 ± 0.11^{bc}
Vacuum 20%	0.50 ± 0.02^{c}	21.96 ± 0.78^{f}	28.76	2.27 ± 0.05^{ab}
Atm. 5%	0.30 ± 0.01^{b}	5.01 ± 0.24^{a}	6.43	6.03 ± 0.02^{e}
Atm.10%	0.20 ± 0.07^{a}	5.85 ± 2.17^{ab}	9.94	3.43 ± 0.10^{cd}
Atm.15%	0.16 ± 0.06^{a}	6.27 ± 0.15^{ab}	13.92	2.60 ± 0.03^{ab}
Atm.20%	0.16 ± 0.01^{a}	8.23 ± 0.09^{bc}	14.47	1.98 ± 0.01^{a}

*Significant differences at 5.0% between homogeneous groups in each variable according to a least significant difference (LSD) test are identified by different letters.
**Viscosity values calculated at 0°C from mean of concentration (°Brix) in the concentrate (Telis et al., 2007). Atm.: atmospheric treatments.

Fig. 3.5 shows the concentration of the frozen fraction (C_f) after the assays. More satisfactory results from the freeze concentration process imply lower values of C_f (i.e., lower values of ice impurity of sucrose retained in the ice matrix). A general tendency for impurity to increase with greater initial concentration of sucrose, a tendency similar to that reported by Raventós et al. (2007) in sucrose cryoconcentration assays. On the other hand, for similar solutions (same C_0), the C_f of vacuum treatments was

significantly less ($p > 0.05$) than the C_f of atmospheric treatments, which indicated a greater ice purity in the spent frozen matrix.

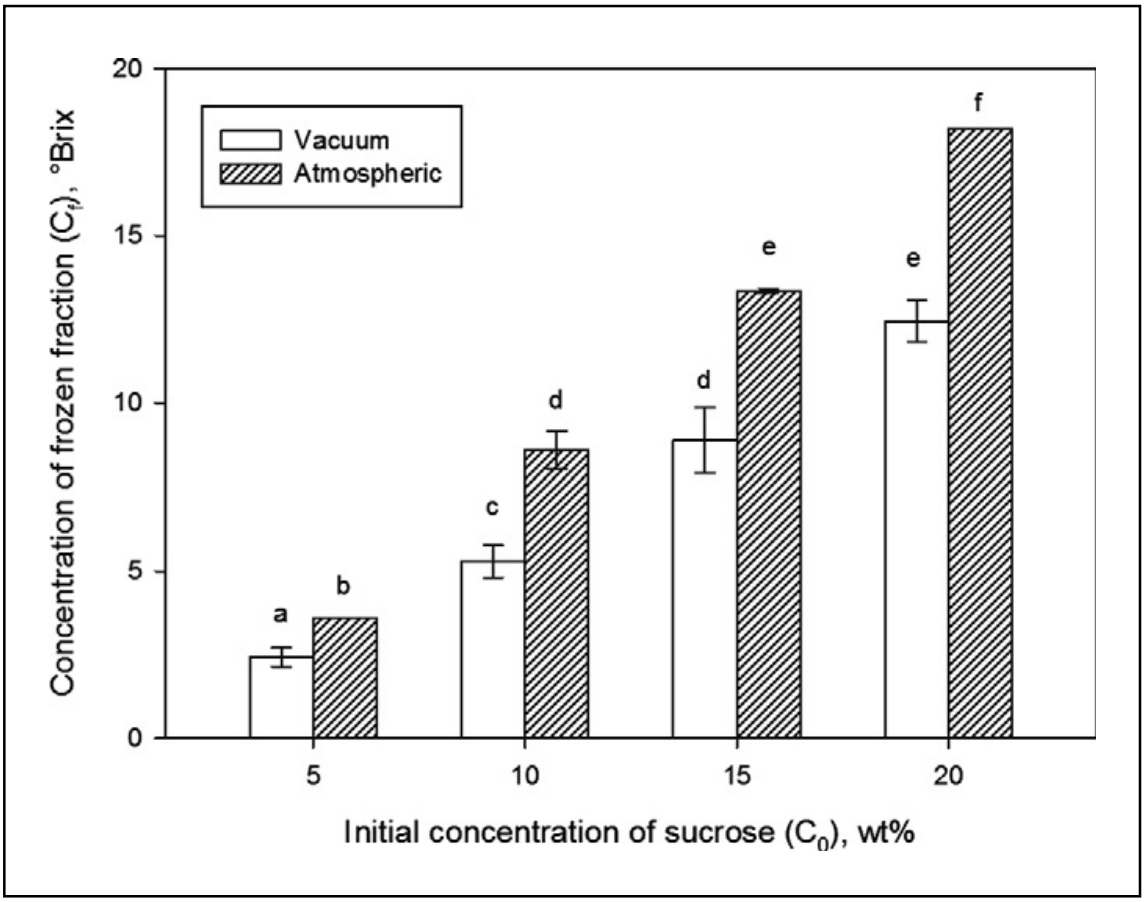

Figure 3.5. Concentration of frozen fraction as a function of initial concentration of sucrose (C_0). Concentration of the frozen fraction indicates the ice impurity. White bars represent treatments under vacuum (80 kPa) for 20 min. Gray bars represent the control treatments at atmospheric pressure after 60 min. Letters represent a least significant difference (LSD) test.

The efficiency was greater with vacuum conditions than with atmospheric conditions. When C_0 was equal to 10%, the differences were statistically significant ($p > 0.05$) (Fig. 3.6). On the other hand, the use of vacuum in the worst condition (C_0 = 20%) had an efficiency greater than 70%, while the efficiency with atmospheric conditions did not exceed 55%, although the viscosity of the concentrate in this condition is almost twice

(approximately 28 versus 14 mPa s, see Table 3.1), thus demonstrating the advantage of using vacuum as an assisting technique for freeze concentration. Both treatments experienced a decrease in efficiency with increasing C_0, which was reflected by a decline of approximately 6.1% and 11.4% in efficiency for every 5% increment in C_0 for vacuum and atmospheric treatments, respectively (Fig. 3.6). This effect was expected because an increase in C_s implies a higher viscosity of the solutions (see Table 3.1) and the concentration of the recovered solution generally depends on the viscosity of the concentrate in all freeze concentration processes (Welti-Chanes et al., 2004).

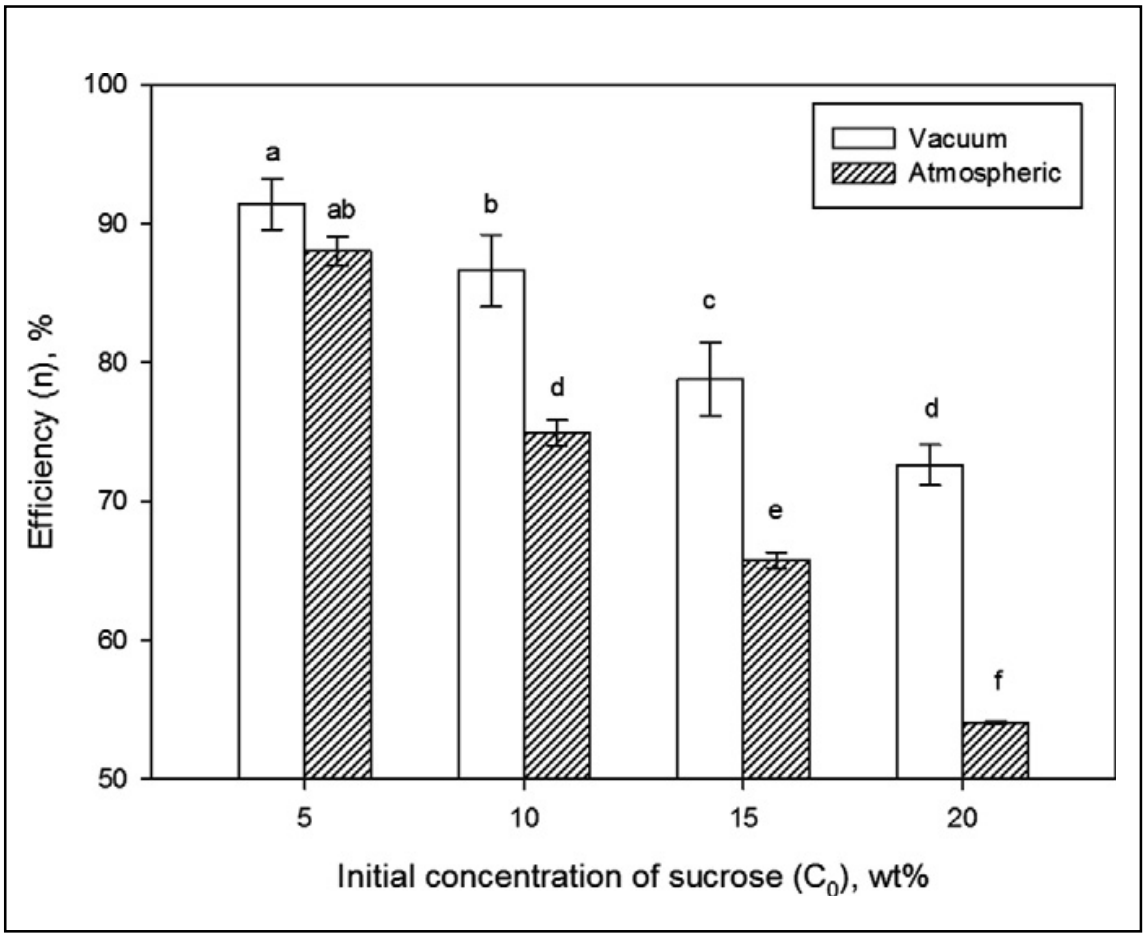

Figure 3.6. Efficiency (η) as a function of initial concentration of sucrose (C_0). White bars represent treatments under vacuum (80 kPa) for 20 min. Gray bars represent the control treatments at atmospheric pressure after 60 min. Letters represent a least significant difference (LSD) test.

Others have attributed the decrease in efficiency with increasing initial concentration to the presence of bound water (Aider and de Halleux, 2008a). In cryoconcentration assays of apricot and cherry juices, Aider and de Halleux, (2008b) argued that the amount of bound water in a solution becomes higher as the concentration increases and that this type of water does not readily freeze because it binds to sugars. Thus, considering that the bound water can barely freeze, it is expected that the amount of such water increases by increasing the initial concentration of the solution (Prawitwong et al., 2007).

The high separation efficiency of solutes with vacuum-assisted freeze concentration is a consequence of using an external driving force (vacuum) that improves the natural separation of gravitational thawing. This situation is similar to complete block cryoconcentration where the entire liquid food is frozen and then thawed followed by separation of the concentrated fraction from the ice fraction by gravitational means. Under these conditions, the ice block acts as a porous solid through which the concentrated fraction percolates, and control of the thawing process becomes critical to achieve an efficiency near 90% (Aider and de Halleux, 2008b, 2009; Aider et al., 2008).

On the other hand, Nakagawa et al., (2009, 2010) suggested that concentration behavior during thawing is not governed by the inter-phase equilibrium as in the case of conventional freeze concentration but would be controlled by the kinetics of mass transfer during the phase transition. Additionally, Nakagawa et al., (2010) reported that a device (rod heater) accelerate mass transfer rate in this frozen domain and the solutes (from commercial apple juice) could be recovered at high yield when the wall temperature was set higher.

In a previous study using the vacuum-assisted freeze concentration technique, Hsieh (2008) desalinized sea water using a low initial concentration of solute (approximately 3.5% salt) to achieve drinkable water (approximately 0.05% salt). However, this study did not show numeric results of efficiency or other numeric parameters of performance.

From a practical point of view, the process efficiency (greater than 70%) and shorter process time (one-third of the process time for atmospheric treatments) demonstrated that vacuum treatment is an effective assisting technique to enhance the separation

efficiency. However, the efficiency of vacuum treatments was lower than the efficiency of block cryoconcentration assays (approximately 90%), which was attributed to lower percentages of concentrate in the vacuum assays (fluctuating from 9% to 22%) (Table 3.1) than in the block cryoconcentration assays (50%) (Aider and de Halleux, 2009) or thawing-concentration from commercial apple juice for 16 h (Nakagawa et al., 2010).

The present investigation with sucrose solutions is not entirely applicable to other basic sugars such as fructose and glucose, so tests should be performed under such conditions to ensure their applicability. However, Raventós et al. (2007) suggest that they would not have many differences when using these sugars, since these authors found no major differences when using sucrose, fructose and glucose in cryoconcentration assays, which they attribute to the small differences in their physical properties.

Theoretically, freeze concentration must be more effective than evaporation for given water removal (latent heat of fusion: 0.33 kJ/g-water; latent heat of evaporation: 2.26 kJ/g-water). However, the capital cost investment of freeze concentrators is greater than the cost of evaporators. High operating costs are mainly due to the juice loss that accompanies the formation of ice crystals and the difficulty of removing ice crystals without losing food solids, and additionally the degree of concentration achieved is lower than by evaporation (Ramaswamy and Marcotte, 2006). Therefore, the priority of vacuum assisted freeze concentration over the evaporation is explained for the quality of concentrate products: freeze concentration has been known to be the best among the methods of concentration giving the highest retention in flavors and thermally fragile compounds (Deshpande et al., 1982).

On the other hand, the next work would be to employ this concentration method to investigate the effect in solutions with more volume and using food samples as fruit juices.

3.4 Conclusions

As compared to atmospheric treatments, vacuum treatments can improve the efficiency of freeze concentration of sucrose solutions. In addition, vacuum treatment showed an

evident advantage in process time (which was one-third of the process time of atmospheric treatments), and it had high values of recovered solute (approximately 0.5 kg of sucrose obtained per 1 kg of initial sucrose). The performance of vacuum-assisted freeze concentration was attributed to ice matrix acting as a porous solid matrix of filtration improved by vacuum suction.

4. CENTRIFUGAL FREEZE CONCENTRATION

4.1. Introduction

Freeze concentration is a method for recovering a food solute from a solution based on the separation of pure ice crystals from a freeze-concentrated aqueous phase. As compared to evaporation and membrane technology, freeze concentration has some significant potential advantages for producing a concentrate with high quality because the process occurs at low temperatures where no vapor/liquid interface exists resulting in minimal loss of volatiles. Thus, the flavor and quality of freeze-concentrated products are exceptionally high, especially relative to their counterparts produced by evaporation. These benefits make freeze concentration particularly suitable for the concentration of some products, such as fruit juices, coffee and tea extracts, and aroma extracts (Morison and Hartel, 2007).

Recently, the industrial future of freeze concentration has been associated more with developments in the configuration of one-step systems (block freeze concentration or progressive freeze concentration) than conventional freeze concentration systems (suspension crystallization), because of the simpler separation step (Petzold and Aguilera, 2009; Sánchez et al., 2009; Sánchez et al., 2010; Miyawaki et al., 2012). An additional advantage of these one-step systems is their simplicity in terms of both the construction and operation of the equipment (Sánchez et al., 2009). In block freeze concentration, the whole liquid food is frozen (ice block), then thawed and the concentrated fraction is separated from the ice fraction by gravitational means. Under these conditions the control of thawing process becomes critical to achieve an efficiency of near 90% (Aider & de Halleux, 2008b, 2009; Aider et al., 2008). Progressive freeze concentration is based on a similar concept because a large ice mass is formed and grown on the cooling surface so that the separation from the mother solution is relatively easy (Liu et al., 1997).

Assisted techniques that improve the efficiency of processing in one-step configurations of freeze concentration are important in achieving commercial viability. Examples of the

assisted technique are the use of ultrasound to control ice nucleation in progressive freeze concentration (Matsuda and Kawasaki, 1997; Matsuda et al., 1999; Kawasaki et al., 2004) and the use of ice nucleation agents in progressive freeze concentration to avoid impurities in the ice phase to suppress the initial supercooling (Liu et al., 1997). Other alternatives are the use of external forces such as vacuum or centrifugation. In this way, vacuum (suction by a pump) has been proposed by Hsieh (2008) to obtain drinkable water from sea water to separate salt, thereby converting the ice of sea water into fresh water. Recently, Petzold et al. (2013) applying a vacuum (80 kPa) improved the efficiency over atmospheric conditions in freeze concentration of sucrose solutions. Centrifugation has been proposed by Bonilla-Zavaleta et al. (2006) in frozen pineapple juice to separate ice from unfrozen concentrated juice, while Luo et al (2010) obtained ice crystals of high purity during the freezing concentration of brackish water, and recently Virgen-Ortíz et al. (2012) proposed a simple and effective cryoconcentration method that elutes the concentrated protein solution from the frozen ice matrix via centrifugation.

Centrifugation is a type of separation where the force of gravity is largely replaced by a higher driving force, through the application of centrifugal force (Toledo, 2007). Thus, an alternative for separating the concentrated solution from the ice fraction is the use of centrifugation. The process takes advantage of the hydraulic system existing in the frozen matrix formed by veins (or channels) between the ice crystals occluding the concentrated solution. This matrix in a frozen system is responsible for differences in the concentration of impurities in ancient polar ice, where solutes migrated through the microchannels between the ice crystals under the pressure of upper ice layers (Rempel et al., 2001).

The aim of this part of the thesis was to analyze the use of centrifugation as a driving force to remove the concentrated solution from the ice matrix in a one-step freeze concentration of sucrose solutions.

4.2. Materials and methods

4.2.1 Materials

Aqueous solutions of sucrose (Sigma-Aldrich, Dorset, UK) with concentrations 5, 10, 15 and 20 wt.% were prepared with distilled water for assays.

4.2.2 Experimental procedure

4.2.2.1 General experimental procedure

A general experimental procedure is schematized in Fig. 4.1. Sucrose solutions were frozen separately by radial and unidirectional freezing, and then the samples were transferred to a refrigerated centrifuge to force the separation of solutes from the frozen solutions.

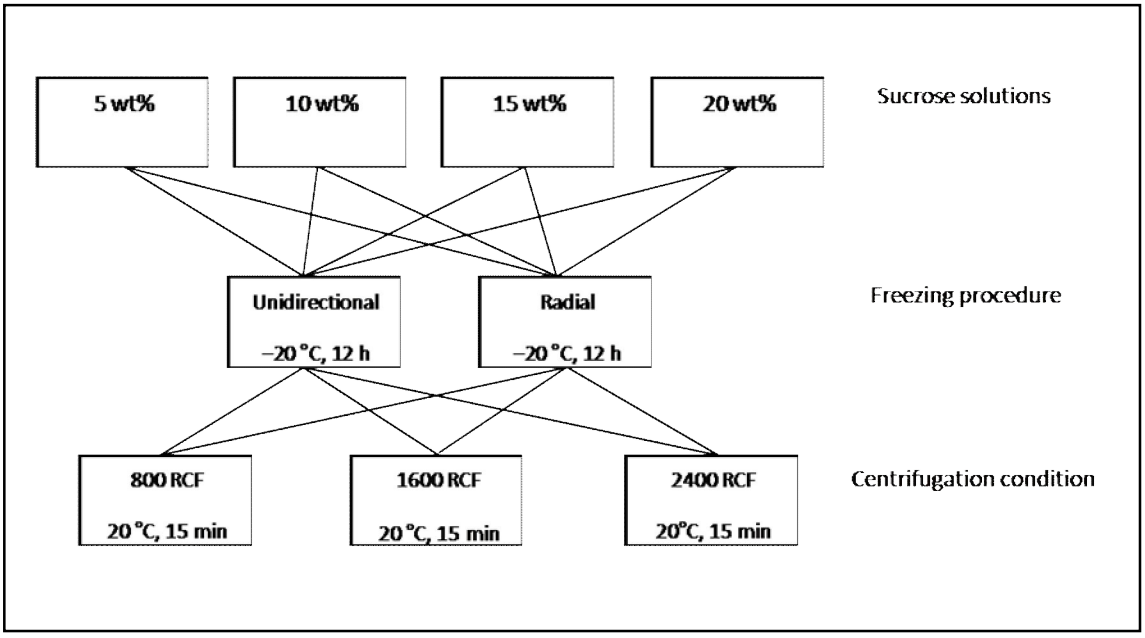

Figure 4.1. General experimental procedure. Sucrose samples were frozen in unidirectional and radial orientations, and then transferred to a refrigerated centrifuge to force the separation of solutes from the frozen solutions.

4.2.2.2 Freezing and centrifugation

Sucrose solutions (12 mL) contained in plastic centrifugal tubes (internal diameter D = 15 mm) were frozen in a static freezer at –20 °C for 12 h. As shown in Figure 4.2, the external surface of the plastic tubes was covered with a thermal insulation made of foamed polystyrene (8 mm thickness, thermal conductivity K = 0.035 W m^{-1} K^{-1}) so that heat transfer during freezing mainly occurred either unidirectionally (axially from top to bottom) (Fig. 4.2a) or radially from walls to the center (Fig. 4.2b). During freezing, the temperature in the sample was measured using needle-type copper-constantan thermocouples (Ellab A/S, Rodovre, Denmark; range –50 °C/135 °C; response time 0.8 s; accuracy < 0.2 %) located at the geometric center of each of three test samples. Thermocouples were connected to a data acquisition system model CTF84-S8 (Ellab A/S, Copenhagen, Denmark) and registered continuously. The freezing rate (°C min^{-1}) was calculated as the rate of temperature decrease from the freezing temperature to the temperature 10 °C below the freezing point (Ramaswamy and Marcotte, 2006).

The frozen samples were removed from the freezer and rapidly transferred to a refrigerated centrifuge (centrifuge Hettich model D-7853, Tuttlingen, Germany) operated at 20 °C for 15 min to force the separation of solutes from the frozen samples by different centrifugation speeds, expressed as a relative centrifuge force (RCF), see Fig. 4.1.

After centrifugation, the concentrated solution was collected and the remaining frozen core was thawed so that the sugar concentration was determined in both fractions. The concentration of fractions C_f and C_s (sucrose in the molten frozen phase and solution, respectively) obtained after assays was analyzed at ambient temperature (approx. 22 ºC) with an ATAGO refractometer (model PAL-1, Tokyo, Japan) with a precision of ± 0.1 °Brix. All measurements were made in triplicate and average values are reported.

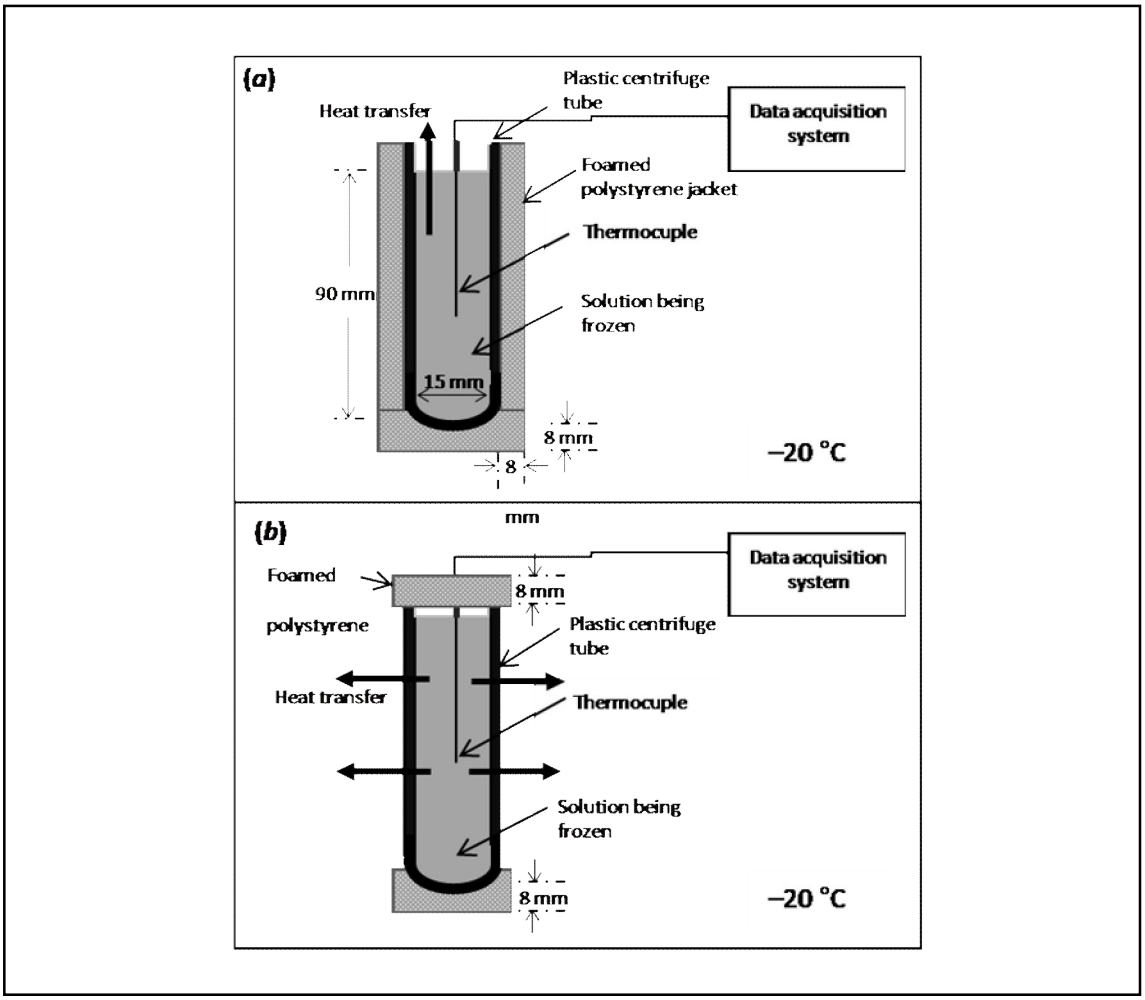

Figure 4.2. Freezing conditions of samples. The samples were frozen in a static freezer at –20 °C for 12 h, where the heat transfer mainly occurs unidirectionally from top to bottom (*a*), and mainly radially from walls to the center (*b*).

4.2.3 Calculations

4.2.3.1 Percentage of concentrate

Percentage of concentrate represents the evolution in time of the removal of solution from the frozen phase. This percentage was calculated for each assay with the initial and final weights of the frozen fraction using the following equation:

$$PC\,(\%) = \frac{W_i^0 - W_i^f}{W_i^0} 100 \tag{4.1}$$

Where W_i^0 and W_i^f are the initial and final weight of the frozen fraction, respectively.

4.2.3.2 Efficiency of concentration

The efficiency of each concentration run was defined as the increase in the concentration of the solution relative to the quantity of sugar remaining in the frozen fraction. In theory, the lower the sugar content remaining in the frozen fraction, the more concentrated the solution will be.

The following equation was used to calculate the efficiency:

$$\eta\ (\%) = \frac{C_s - C_f}{C_s} 100 \tag{4.2}$$

where C_s and C_f are the concentrations of sucrose (°Brix) in the concentrated solution and frozen fraction, respectively.

4.2.3.3 Recovered solute

The recovered solute represents a ratio of the sucrose concentration in the concentrated solution to that in the initial solution, C_s/C_0, converted into solute yield as follows:

$$Y = \frac{C_s}{C_0} \frac{m_s}{m_0} \tag{4.3}$$

where Y has units (kg sucrose/kg initial sucrose), C_0 is the concentration of sucrose (°Brix) in the initial solution, m_s and m_0 are mass (kg) in the concentrated and initial solution, respectively.

4.2.4 Experimental design

A multilevel factorial 4 × 3 design (one factor at four levels and one factor at three levels) was performed with two blockings, namely: radial and unidirectional freezing procedures. The independent variables (factors) were the initial concentration of sucrose (C_0) and centrifuge speed (CS), while the responses analyzed (dependent variables) were the efficiency of concentration (η), percentage of concentrate (PC) and recovered solute (Y). The factors and levels of the experimental design are shown in Table 4.1.

These dependent variables ($\hat{y}$) were expressed individually as a function of the independent variables resulting in a response function:

$$\hat{y} = b_0 + b_1 \times C_0 + b_2 \times CS + b_{12} \times C_0 \times CS + b_{11} \times C_0^{\,2} + b_{22} \times CS^2 \qquad (4.4)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (linear coefficients), b_{12} (interactive coefficient), b_{11} and b_{22} (quadratic coefficients), and C_0 and CS were the independent variables.

Table 4.1. Factors and levels of the experimental design[a]

Factors	**Levels**			
Initial concentration of sucrose (C_0), wt.%	5	10	15	20
Centrifuge speed (CS), RCF	800	1600		2400

[a] Was performed with two blocking: radial and unidirectional freezing.

4.2.5 Statistical analysis

The analysis of variance (ANOVA) was carried out using Statgraphics Centurion XVI Software (Statgraphics, Virgina, USA, 2009) at a probability (p) of 0.05. Additionally, this software gave the value of coefficients in the polynomial functions, the residual analysis of polynomial functions, and graphics of the surface responses. The

experimental and predicted values were plotted in order to visualize the results of the model, and the lineal coefficient of correlation (R^2 value) was registered.

4.3. Results and discussion

4.3.1 Evolution of sucrose concentration in ice-melted over thawing time

A preliminary experiment was conducted, a solution sample C_0 = 10 wt.% was frozen unidirectionally (from top to bottom, see Fig. 4.2a) in the same conditions of Section 4.2.1 and melted at ambient temperature (approximately 22 °C) and the molten ice fraction (concentrate) was analyzed over thawing time (Fig. 4.3). The concentrated solution was collected at each thawing time and the sugar concentration was determined. The concentration of sucrose in the ice-melted solution produced at short times (approximately 31 wt.%) was much higher than the initial concentration (C_0 = 10 wt.%) and decreased with time, reaching approximately 13 wt.% after almost 2 h and approximately 65% of PC. A similar tendency was reported by Luo et al. (2010) in thawing assays of frozen brackish water (where the solutes were the total dissolved solid in water) and recently by Miyawaki et al. (2012) in progressive freeze concentration by partial melting of sucrose solutions. This behavior indicated that the solute (sucrose) in the frozen sample was discharged not only with melting, but also with a solute diffusion from cryo-concentrated phase; and also only in larger cylinders is possible a radial stratification of the frozen concentrate, causing the ice to become more concentrated toward the surface (Bakal and Hayakawa, 1973). In this way, Nakagawa et al. (2009, 2010) suggested that ice-melted concentration in a freeze-thawing process is not governed by the inter-phase equilibrium as in the case of conventional freeze concentration but by the kinetics of mass transfer during the phase transition. In addition, this preliminary experiment showed similar results to the natural phenomena of the ice thawing process in a frozen river, where the initial fraction of melting solutions were concentrated in impurities while the last fractions of the melting solutions had a much lower concentration (Leung and Carmichael, 1984).

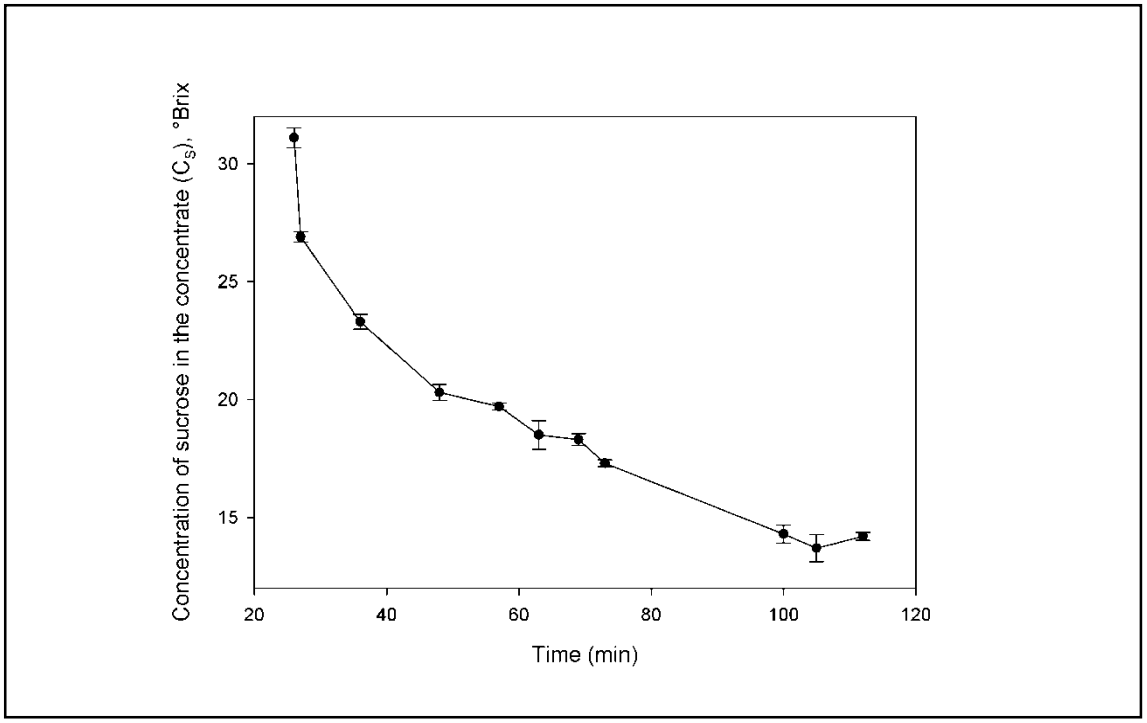

Figure 4.3. Evolution of sucrose concentration in ice-melted (concentrate) over time at ambient temperature (approx. 22 ºC). A solution sample C_0 = 10 wt.% was unidirectional frozen and melted.

4.3.2 Evaluation of centrifugal freeze concentration

Table 4.2 shows the fitted polynomial models for centrifugal freeze concentration. The R^2 values of these relations were in all but one case (0.89) higher than 0.91, thus ensuring satisfactory fitness of the models to the experimental data (Fig. 4.4). In addition, only linear and quadratic coefficients appeared in the polynomial models, indicative of no significant interactive effects of factors ($C_0 \times CS$) for the resulting responses.

Fig. 4.5a and b shows the surface plot of the estimated efficiency (η) as a function of factors C_0 and CS for radial and unidirectional freezing, respectively. For radial freezing, the surface plot shows a predominance of the linear effect of C_0 (with a decrease in

efficiency with increasing C_0), independent of *CS* values. For unidirectional freezing, the surface plot shows a similar decrease in η with *Co*, but the linear effect of *CS* became

Table 4.2. Fitted polynomial models for centrifugal freeze concentration

Freezing treatment	Fitted polynomial models*	R^2
Radial	$\eta = 74.9122 - 1.20916 \times C_0$	0.89
	$PC = -17.028 + 0.667578 \times Co + 0.0645984 \times CS - 0.0000173948 \times CS^2$	0.93
	$Y = 0.0358073 + 0.000775196 \times CS$ □ 2.10894E-7 × CS^2	0.97
Unidirectional	$\eta = 56.7678 - 1.83402 \times C_0 + 0.0258096 \times CS - 0.00000654319 \times CS^2$	0.97
	$PC = -28.6325 + 1.1247 \times C_0 + 0.0752925 \times CS - 0.000020805 \times CS^2$	0.93
	$Y =$ □0.0476812 + 0.000859547×CS □ 2.32695E-7× CS^2	0.92

***** η = efficiency of concentration; *PC* = percentage of concentrate; *Y* = recovered solute

curved (see table 4.2). This non-linear effect of *CS* for unidirectional freezing means an increase in efficiency when *CS* changes from 800 to 1600 RCF followed by a slight decrease when *CS* reaches the value of 2400 RCF. The effect of C_0 on the efficiency was reflected by a decline of approximately 6.1% and 9.2% in efficiency for every 5% increment in *Co* for radial and unidirectional freezing treatments, respectively. This effect was expected because an increase in *Co* implies a higher concentration of sucrose in the concentrated solution (*C*s) with higher viscosity, and the concentration of the recovered solution generally depends on the viscosity of the concentrate in all freeze concentration processes (Welti-Chanes et al., 2004). On the other hand, the use of radial freezing in the worst condition (C_0 = 20%) had an efficiency greater than 50%, while the efficiency with unidirectional freezing ranged from 36.6% (C_0 = 20%, *CS* = 800 RCF) to 44.6% (C_0 = 20%, *CS* = 1600 RCF).

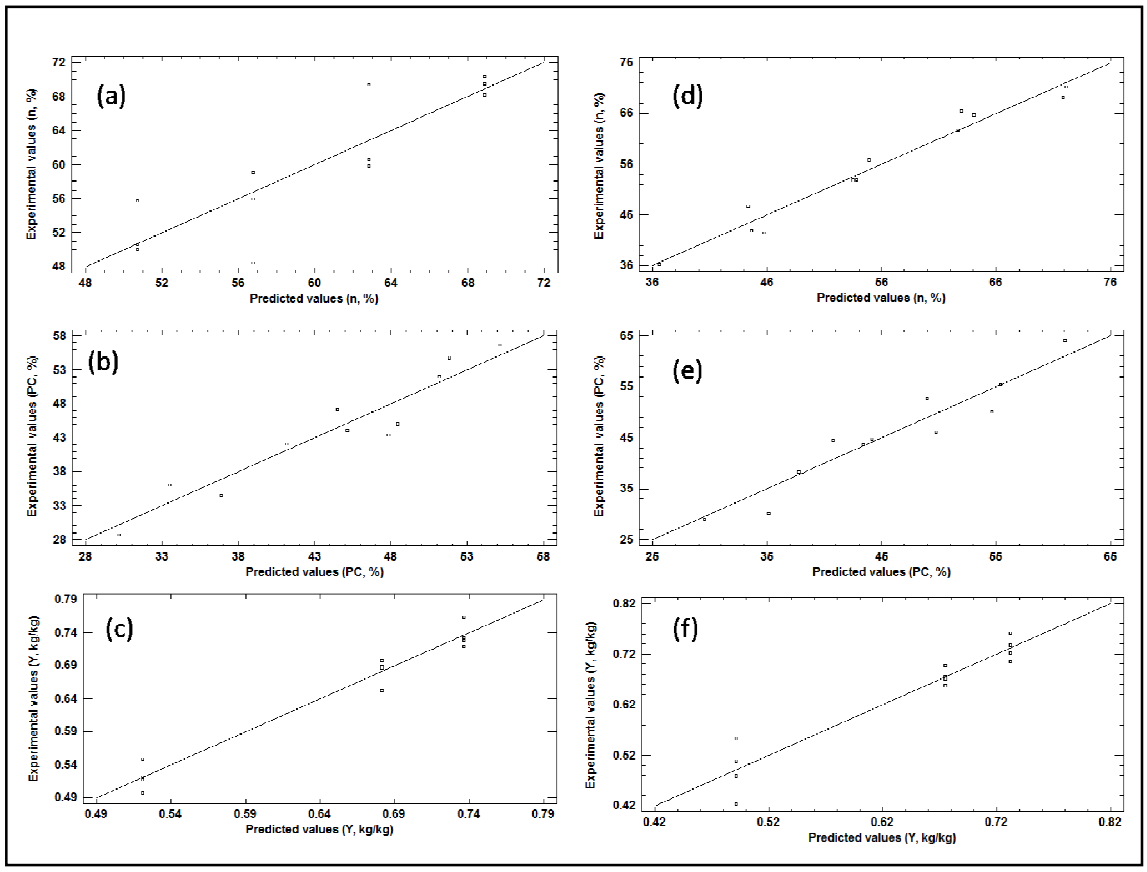

Figure 4.4. Experimental and predicted plot of responses. a-c: radial freezing, d-f: unidirectional freezing; a) and d): efficiency (η); d) and e): percentage of concentrate (PC); c) and f): recovered solute (Y).

The efficiency at lower solute concentration (C_0 = 5%) in the present study is comparable to the results obtained by Luo et al. (2010) in crushing ice and centrifugation of brackish water, where centrifuging efficiency was above 73% in the range of 4300 and 560 ppm of total dissolved solids although a completely different mixture of solutes (ions of the water) were used. In conclusion, the best conditions for maximal efficiency (radial freezing) were C_0 = 5% independent of CS (η = 68.9%), and for unidirectional freezing were C_0 = 5% and CS = 1600 RCF (η = 73.1%).

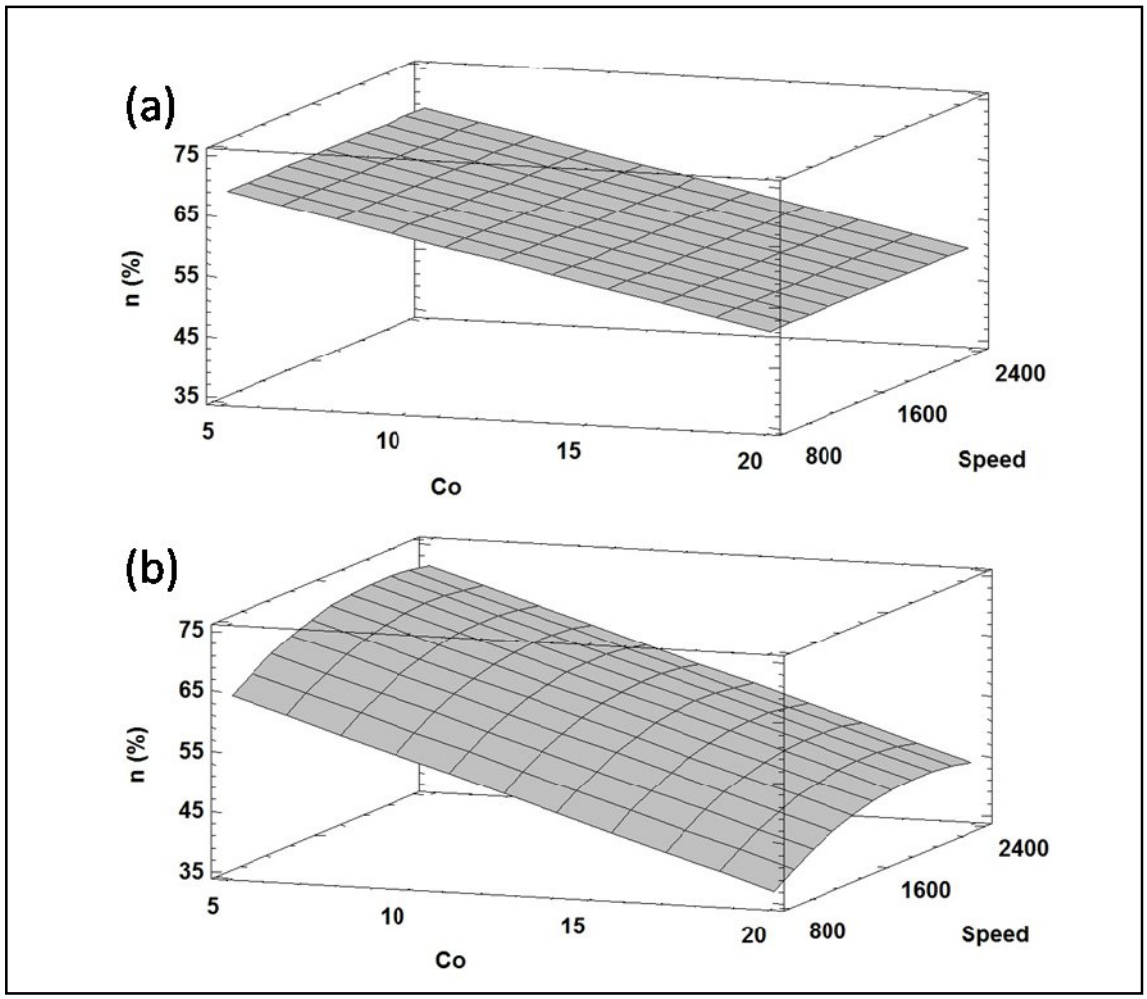

Figure 4.5. Surface plot of the estimated efficiency (η) as a function of factors C_0 (initial concentration of sucrose) and *CS* (centrifugal speed) for radial (*a*) and unidirectional freezing (*b*).

Fig. 4.6a and b shows the surface plot of the estimated percentage of concentrate (*PC*). A direct proportional relationship between *PC* and the initial concentration of the solution (linear effect of C_0 for radial and unidirectional freezing), and a linear and a curvature effect of *CS* can be observed. C_0-effect is more pronounced in unidirectional freezing than in radial freezing, which is reflected in the values of the C_0-linear coefficients (see polynomial models in table 4.2). A similar tendency of the effect of C_0 on *PC* was reported by Petzold et al. (2013) and Luo et al. (2010) in freeze concentration using vacuum (sucrose solutions) and centrifugation (brackish water), respectively. In

conclusion, the best conditions for maximal *PC* were C_0 = 20% and *CS* = 1600 RCF, reaching values of *PC* = 56.3% and *PC* = 61.9% for radial and unidirectional freezing, respectively.

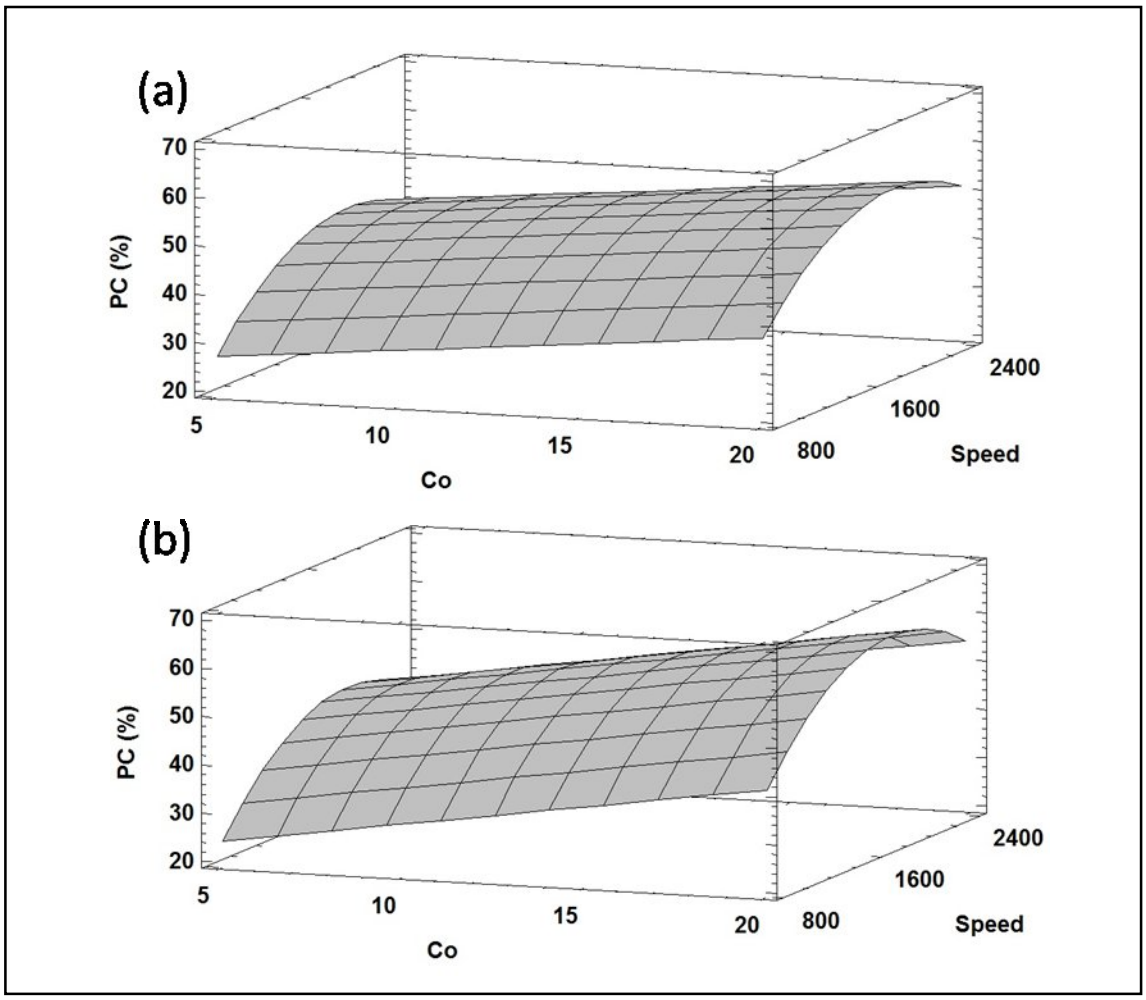

Figure 4.6**.** Surface plot of the estimated Percentage of Concentrate (*PC*) as a function of factors C_0 (initial concentration of sucrose) and *CS* (centrifugal speed) for radial (*a*) and unidirectional freezing (*b*).

Fig. 4.7a and b shows the surface plot of the estimated recovered solute (*Y*). A similar behavior is observed for radial and unidirectional freezing with a linear and a curvature effect of *CS* (see Table 4.2), independent of C_0 values. The values of *Y* increased from 0.5 (*CS* =800 RCF) to 0.73 (*CS* = 1600 RCF), then diminished to 0.6 (*CS* = 2400 RCF).

A similar of trend of the *CS* effect on recovered solute was reported by Virgen-Ortíz et al. (2012) in concentrate protein solutions, where an increase in the centrifugation speed allowed a significant increase in the amount of whey protein recovered. In conclusion, the best conditions for maximal *Y* were *CS* = 1600 RCF, reaching values of approximately 0.73 kg of sucrose obtained per 1 kg of initial sucrose.

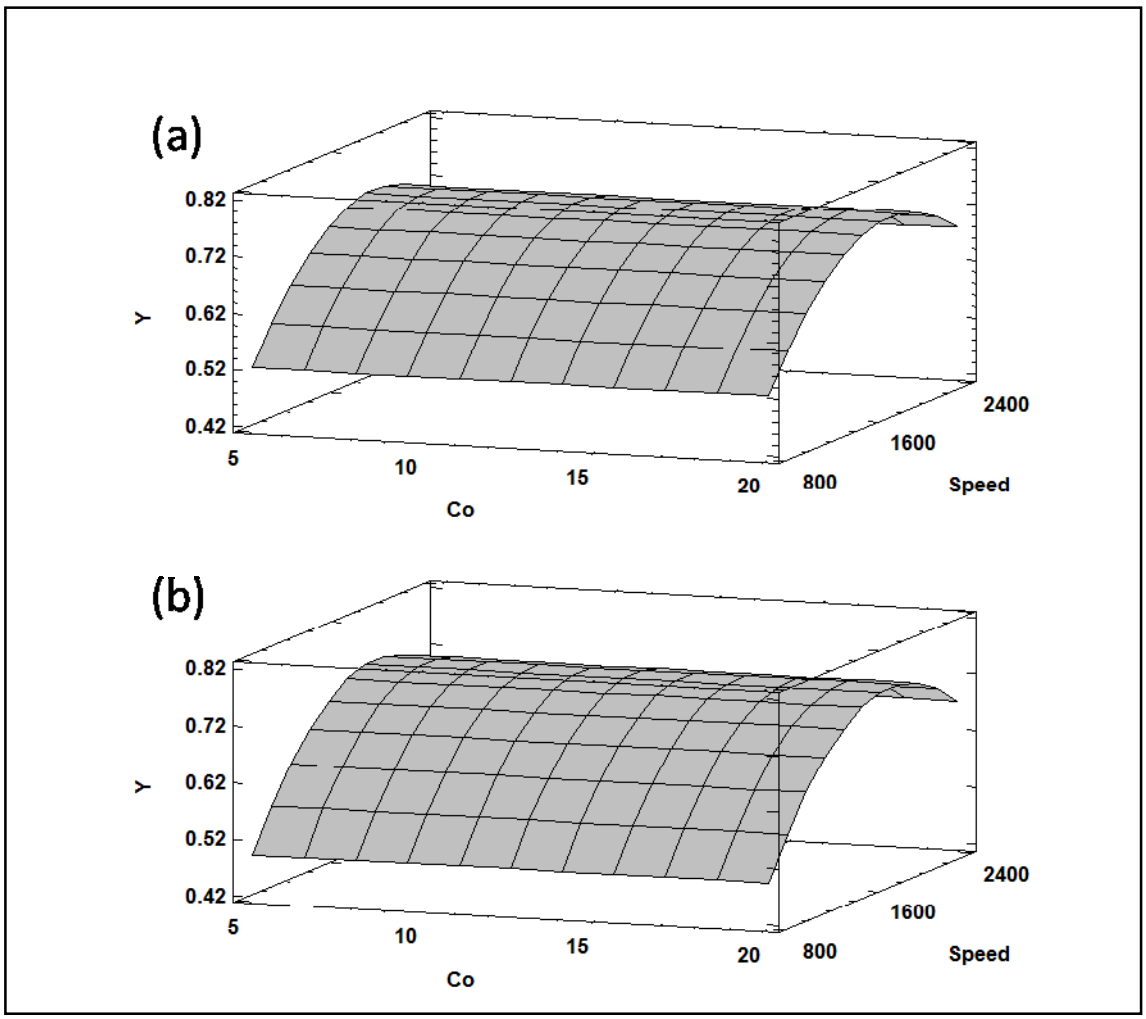

Figure 4.7. Surface plot of the solute recovered (*Y*) as a function of factors C_0 (initial concentration of sucrose) and *CS* (centrifugal speed) for radial (*a*) and unidirectional freezing (*b*).

The curvature with *CS* of response surfaces shown in Figs. 4.5b, 4.6a-b and 4.7a-b leads to optimal values of the response variables around *CS* = 2000 RCF, a condition not considered in the experimental design. So, it is recommended that a new experimental design should be performed with values close to 2000 RCF to confirm this conclusion.

On the other hand, the ice morphology influences the separation process in freeze concentration, for example in suspension crystallization large ice crystals are favorable to ease the separation of ice crystals from the concentrated mother solution (Petzold and Aguilera, 2009), therefore, it was expected that the method of freezing (radial or unidirectional) would affect the results of the freeze concentration process by centrifugation. However, no major differences between unidirectional and radial frozen samples were found (figures 4.5 to 4.7 show similar trends for both frozen methods in efficiency, percentage of concentrate and solute recovered). However, it has to be kept in mind that freezing rates were different: approximately 0.13 and 0.06 °C min^{-1} for radial and unidirectional freezing, respectively. The high separation efficiency of solutes with centrifugal freeze concentration is a consequence of using an external driving force (pressure difference induced by centrifugation) much higher than that operating in separation by gravitational thawing. Under centrifugation conditions the ice block acts as a porous solid through which the concentrated solution percolates through drainage channels between ice crystals in a similar fashion as reported for vacuum-assisted freeze concentration (Petzold et al., 2013) but under a higher driving pressure differential.

4.4. Conclusions

Centrifugation is an effective assisted technique to remove the concentrated solution from the ice matrix in a one-step freeze concentration of sucrose solutions. This technique has high values of solute recovered, reaching approximately 0.73 kg of sucrose obtained per 1 kg of initial sucrose at 1600 RCF of centrifugation speed, independent of initial concentration of sucrose and freezing method. The performance of centrifugal freeze concentration was attributed to ice matrix acting as a porous solid

through which the concentrated solution percolates through drainage channels of the ice improved by the centrifugal force.

5. GENERAL CONCLUSIONS AND FUTURE PROSPECTS

5.1 General conclusions

Ice morphology plays an important role in frozen and partly frozen systems and technological process involving freezing. So far, the study of the morphology of the frozen phase (size and shape of crystals, arrangement of ice crystals in the frozen structure, channels between groups of crystals, etc) has not been given an adequate attention in its relation to food processes, in particular, to freeze concentration. Thus, the main contribution of this thesis is the demonstration that the architecture or spatial arrangement of elements in the structure of the frozen phase plays an important role in the efficiency of the freeze concentration process.

Ice is a very interesting material, in that when an ice nucleus begins to grow in a solution solutes are rejected from the ice phase and accumulate at the solid–liquid interphase. This exclusion phenomenon is a major principle of the freeze concentration techniques. Assisted freezing techniques (such as use of ultrasound or ice nucleation agents) have proven to be important in providing a morphology of the frozen phase that improves the efficiency of freeze concentration. Other alternatives are the use of external driving forces such as vacuum or centrifugation in the recovery of concentrated solutions (subjects of this thesis), which are similar to the principle used by children to suck the sugar solution containing colorants and sugar from popsicles. The process takes advantage of the hydraulic system existing in the frozen matrix (ice phase) formed by veins (or channels) between the ice crystals occluding the concentrated solution.

The proposed assisted techniques presented in this thesis, vacuum and centrifugation, can improve the performance of freeze concentration in the case of sucrose solutions. As compared to control samples, vacuum or centrifugation treatments showed higher values of recovered solute. Thus, by applying vacuum (80 kPa), the efficiency of the freeze concentration was significantly improved over atmospheric conditions showing a higher

recovery of solute, approximately 0.5 kg of sucrose obtained per 1 kg of initial sucrose compared to recovery values ranging from 0.16 to 0.3 kg/kg for atmospheric treatments. On the other hand, by applying centrifugation, high yields of solute, reached approximately 0.73 kg of sucrose obtained per 1 kg of initial sucrose in solution at 1600 relative centrifuge force (RCF). In comparison, using a less centrifuge speed (800 RCF) achieved a solute recovery significantly minor (approximately 0.5 kg/kg).

The high separation efficiency of solutes with assisted techniques on freeze concentration is a consequence of using an external driving force that improves the natural separation of gravitational thawing and taking advantage of the microstructural features of the frozen phase. In a sense, this situation is similar to complete block cryoconcentration where the entire liquid food is frozen and then thawed followed by separation of the concentrated fraction from the ice fraction by gravitational means. Under these conditions, the ice block acts as a porous solid through which the concentrated fraction percolates.

Finally, we can conclude that the use vacuum or centrifugation in freeze concentration is an effective assisted technique to enhance the separation efficiency of solutes.

5.2 Future prospects

At the end and through the development of this thesis, some interesting possibilities for further studies were raised; mainly related to the formation of a morphology of the ice phase that in combination with adequate driving forces will favor the performance of technological processes, in this case, freeze concentration.

The most important step in the crystallization process for controlling the crystal size and crystal size distribution is nucleation. By controlling nucleation, a desired crystalline microstructure and ice morphology may be attained that will favour the performance of

specific technological processes. So, a challenge is to investigate into the control of nucleation techniques, such as the use of ice nucleation agents and antifreeze proteins, as well as into crystal growth mechanisms like ultrasound and high pressure freezing.

Undoubtedly ice morphology is complex and highly dependent on the sample (composition) and conditions in which the frozen phase is formed. In effect, there are still no systematic studies incorporating how the morphology of the frozen phase quantitatively affects the quality of frozen or partially frozen foods and the efficiency of some processes based on freezing techniques such as freeze-drying or freeze concentration.

Although freeze concentration is theoretically more energy efficient in removing water than evaporation, the capital costs of freeze concentrators are greater than those of evaporators. Therefore, it would be desirable to have a detailed cost study of assisted techniques for freeze concentration (vacuum or centrifugation), and the commercial potential of the postulated techniques. Additionally, an important advantage of freeze concentration is that the obtained concentrated product is of high quality (or at least different) and therefore, it may be interesting to determine whether the postulated techniques for freeze concentration improve the preservation of some compounds of interest whether micronutrients (as vitamins or compounds with antioxidant capacity) or some odorous volatile compounds. Since it is expected that a freeze concentrated extract will have different organoleptic properties that their evaporated counterparts, unique applications may be found in high-quality industrial products as well as in gastronomy.

On the other hand, an interesting aspect is to apply predictive equations that may explain the behavior of freeze concentration process assisted by centrifugation or vacuum, using liquid foods having Newtonian and / or non-Newtonian rheology (i.e., more complex systems).

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