PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

UNDERSTANDING THE EFFECT OF FREEZING ON STARCH MICROSTRUCTURAL CHANGES DURING HEATING AT HIGH RATES

MARÍA TERESA MOLINA MAYDL

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

Advisor:
PEDRO BOUCHON AGUIRRE

Santiago de Chile (December, 2014)

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To my parents, siblings, friends, and
to Rafael.

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RESUMEN

La congelación es un método tradicional de preservación de alimentos, pero la congelación previa al calentamiento podría afectar el desarrollo de una microestructura deseada durante la cocción de alimentos amiláceos. De hecho, la gelatinización de almidón, una transformación crítica durante el calentamiento que juega un rol fundamental en la formación de estructura y está asociada a atributos de calidad, podría verse afectada. Debido a su importancia, no hay estudios a escala microscópica sobre el efecto de la congelación en la gelatinización de almidón nativo durante el calentamiento.

La hipótesis de esta investigación es que a través de la miniaturización de procesos es posible entender el efecto de la congelación y el efecto de la accesibilidad de agua en la gelatinización de almidón, con el fin de mejorar nuestro conocimiento sobre el efecto de estos factores claves en los cambios microestructurales de almidón. Se espera observar y determinar un retraso en la gelatinización de almidón debido a la congelación. El objetivo principal fue estudiar el efecto de la congelación, junto con el efecto de la accesibilidad de agua, en los cambios microestructurales de almidón durante el calentamiento, a través de la miniaturización de procesos usando video-microscopía con luz polarizada y calorimetría diferencial de barrido.

Para llevar a cabo lo anterior, muestras de almidón nativo en agua, gel de carragenina o solución de sacarosa fueron, o bien directamente calentadas a 15°C/min, o congeladas durante 48 horas, descongeladas y luego calentadas a la misma tasa. Los cambios microestructurales fueron seguidos con una platina térmica adaptada a un microscopio con luz polarizada, y los cambios energéticos fueron monitoreados con calorimetría diferencial de barrido. Además, la morfología de los gránulos fue analizada usando microscopía electrónica de barrido y microscopía de fuerza atómica.

Los resultados indican que la congelación reduce el grado de gelatinización, lo que fue atribuido a un incremento en la cristalinidad del gránulo. Además, una restricción en la accesibilidad de agua produjo una reducción en el grado de gelatinización (DG(%) agua-almidón-sacarosa < DG(%) agua-almidón-carragenina < DG(%) agua-almidón) y un aumento en la entalpía de gelatinización (ΔH agua-almidón-sacarosa > ΔH agua-almidón-carragenina > ΔH agua-almidón). Esto fue atribuido a la presencia de grupos hidroxilos en las moléculas de carragenina y sacarosa.

El enfoque microestructural del proceso de gelatinización nos permitió entender lo que ocurre con gránulos de almidón cuando fueron sometidos a un proceso previo de congelación en sistemas con diferente disponibilidad de agua. Esto contribuye a tener una mejor compresión sobre el efecto de congelación-calentamiento en la macro-escala, es decir, en alimentos amiláceos sometidos a procesos de calentamiento que ocurren a altas tasas de transferencia de calor.

Palabras claves: Gelatinización de almidón, congelación-calentamiento, almidón nativo, miniaturización de procesos, análisis de imágenes.

ABSTRACT

Freezing is a traditional method for food preservation, but freezing prior to heating may modify the development of a desired microstructure during the cooking process of starchy products. In fact, starch gelatinization, a critical transformation during heating, which plays a key role in structure formation and associated quality attributes could be affected. Despite its importance, there are no studies that focus on the effect of freezing on native starch gelatinization during subsequent heating, at a microscopy scale.

Accordingly, the hypothesis of this research states that through process miniaturization it is possible to understand the effect of freezing as well as the effect of water accessibility in starch gelatinization to improve our knowledge about the effect of these key factors in starch microstructural changes. Specifically, it is expected to observe and determine a delay in starch gelatinization degree due to freezing and also due to solutes concentration. Accordingly, the main objective of this thesis was to study the effect of freezing together with the effect of water accessibility in starch microstructural changes during heating, through process miniaturization using *in situ* hot-stage polarized-light video-microscopy and differential scanning calorimetry.

To do so, samples of native starch in water, carrageenan gel, or sucrose solution were either heated at 15°C/min, or frozen for 48 h, defrosted, and then heated at 15°C/min. The microstructural changes were followed with hot-stage polarized light microscopy, and energy changes were monitored with differential scanning calorimetry. Moreover, surface morphology of granules was analyzed using scanning electron microscopy. Results showed that freezing reduces the gelatinization degree. This was attributed to an increase of crystallinity. Furthermore, restriction of water produced a reduction of the gelatinization degree (DG(%) sucrose < DG(%) carrageenan < DG(%) water), and an increase of the enthalpy of gelatinization (ΔH sucrose > ΔH carrageenan > ΔH water). This was attributed to the presence of hydroxyl groups in sucrose and carrageenan molecules.

Overall, the microstructural approach on the gelatinization process allows us to understand what happens to starch granules when subjected to freezing before heating in systems with different water availability. This contributes to a better understanding of the effect of freezing-heating on the gelatinization process at a macro scale, that is, on starchy products subjected to heating processes at a high rate of heat transfer.

Keywords: Starch gelatinization, freezing-heating cycle, native starch, process miniaturization, image analysis.

1. INTRODUCTION

1.1 Motivation

In Chile, consumption of frozen processed food has increased considerably in the last years. According to Euromonitor International, in 2001 Chilean citizens spent US$ 70.6 million in this type of food, and in 2013 this figure rose up to US$ 143 million. Most consumed products, in descending order, are vegetables, red meat, chicken, turkey, and ready-to-eat foods (El Mercurio Online, 2012).

Starchy products such as bread, *empanada* and pizza are often frozen either with or without a previous cooking step. Afterwards, they are usually subjected to processes that take place at high heat-transfer rates, such as baking or frying, in which the food undergoes important microstructural changes that confer them the desired organoleptic properties (Bouchon & Aguilera, 2001; Bouchon et al., 2001). In starchy products, starch granules undergo a process known as gelatinization, where its microstructure suffers irreversible changes. But, given that gelatinized starch molecules are in an unstable state (*i.e.*, they are not in thermodynamic equilibrium), when the food is frozen, they may retrograde (Gudmundsson, 1994). Throughout this process the starch molecules are reorganized, approaching to equilibrium. Retrogradation, however, may affect the quality of the frozen food (Gudmundsson, 1994). In accordance, the development of an adequate microstructure during the cooking process of a starchy product at high heating rate could be affected by previous storage at freezing temperatures (*e.g.*, between -18 and -25°C).

1.2 Structure of Starch

Starch is one of the most common carbohydrates present in foods. It is used as a thickening, gelling, and structure-forming agent. In the food industry, about 57% of starch is used for the production of syrup, sauces, and ready to eat food (Singh et al, 2007; Bertolini, 2010). It is extracted from different botanical sources, such as seeds (corn, wheat and rice starch), roots (tapioca starch), and tubers (potato and cassava starch) (Ratnayake & Jackson, 2009). Starch is a semicrystalline polysaccharide mainly formed by two kinds of polymers: amylose and amylopectin. Amylose is a linear polymer of D-glucopyranosyl units linked by α (1→4) linkages, whereas amylopectin is a ramified polymer of D-glucopyranosyl units linked by α (1→4) bonds, ramified though α (1→6) linkages (Shannon & Garwood, 2009). Figure 1 shows the chemical structure of amylose and amylopectin with their respective linkage zones.

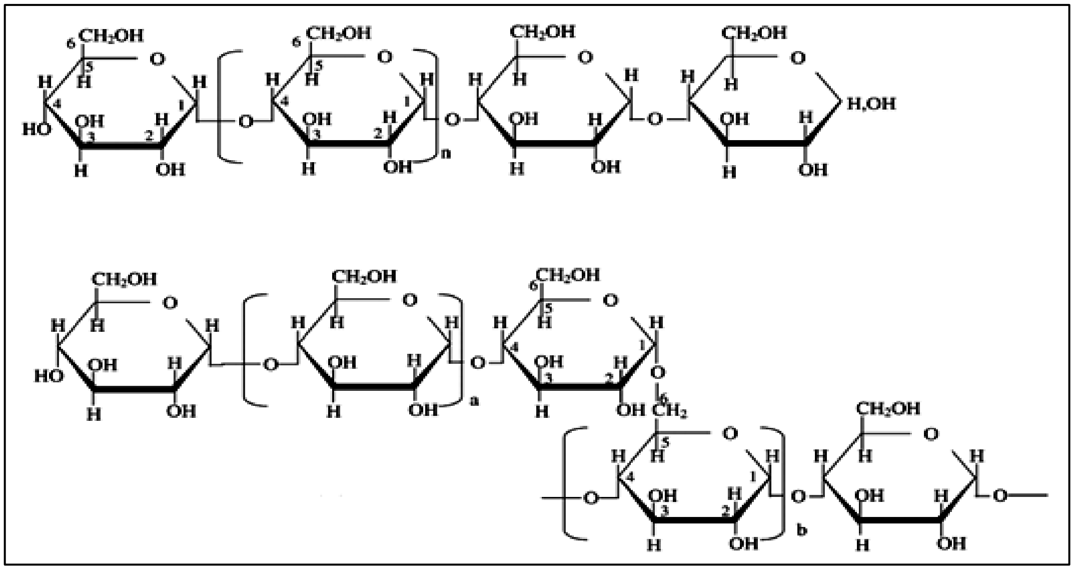

Figure 1. Chemical structure of amylose (above) and amylopectin (below) (from Tester et al., 2004).

These polymers are organized in concentric layers inside the starch granule, thus forming a semi-crystalline structure. Crystalline zones are mainly formed by amylopectin, and the amorphous zones are formed by amylose (Aguilera, 2012). Figure 2 shows a schematic diagram of the semi-crystalline structure within the starch granule.

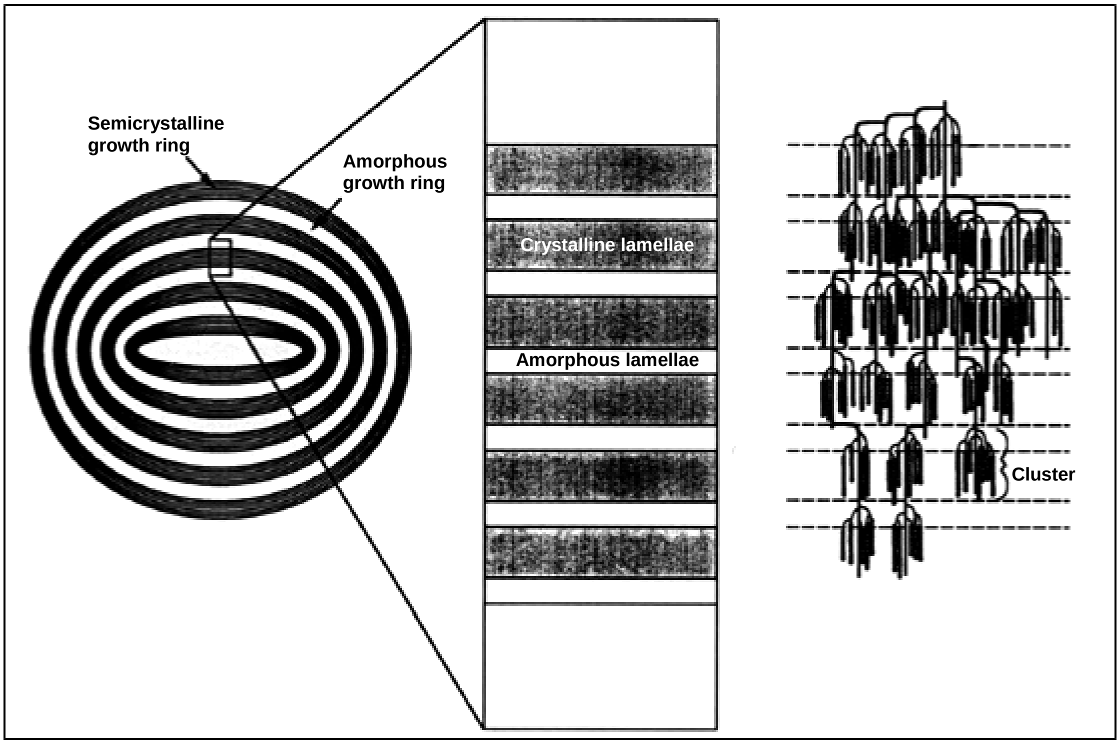

Figure 2. Representative diagram of the inner structure of a starch granule, showing the concentric layers formed by the amorphous and the crystalline zones (left), the inner structure of a layer (center), and a cluster of amylopectin in the semi-crystalline structure (right) (Adapted from Jenkins & Donald, 1995).

Under a polarized light microscope (see section 1.5), the starch granule shows a characteristic white cross, referred as “Maltese Cross”, as a result of starch birefringence, which results from the crystalline molecular organization of amylopectin. Figure 3 shows native corn and native potato starch viewed under a polarized light microscope, which also depicts the differences in size of the different granules.

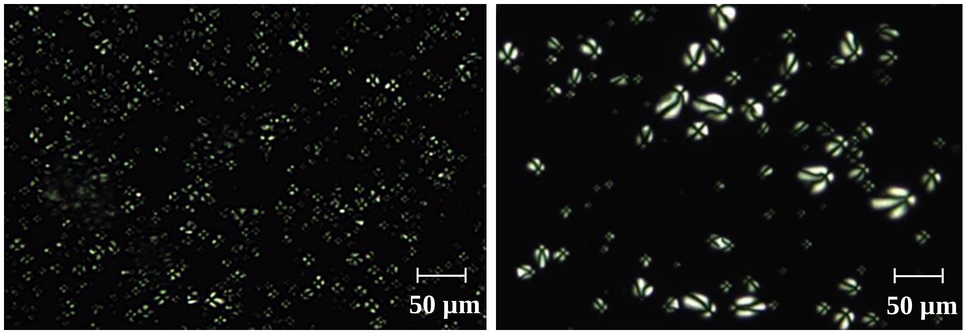

Figure 3. Birefringence (Maltese Cross) of corn (left) and potato (right) starch granules under polarized light microscopy.

In fact, both the form and the size of the granules depend on the botanical source. Some are very small (*e.g.*, rice starch granules), others are very big (*e.g.*, potato and banana starch granules), whereas wheat starch has a bimodal distribution, as reported in Table 1.

Table 1. Size (diameter) of starch granules of different botanic sources (Eliasson & Gudmundsson, 2006).

Starch botanical source	***Mean diameter (µm)***
Dent corn	10.3 – 11.5
Potato	37.9 – 50
Rice	5 – 7
Wheat	6.1 – 6.3, 18.2 – 19.3
Cassava	16.8
Tapioca	17.7

1.3 Starch Gelatinization

When starch granules are immersed in an aqueous medium they remain suspended, since they are not soluble at room temperature. However, when they are heated at a certain temperature, the granule begins to suffer irreversible transformations in its structure, which unleash the gelatinization process. Simultaneous changes take place in the granule during this process: (i) it loses its molecular order; (ii) it loses its crystallinity and birefringence; (iii) it swells as a result of water absorption; and finally, (iv) the amylose leaches into the aqueous medium, increasing viscosity (Colonna & Buleon, 2010; Biliaderis, 2009). Figure 4 shows the gelatinization process of a potato native granule.

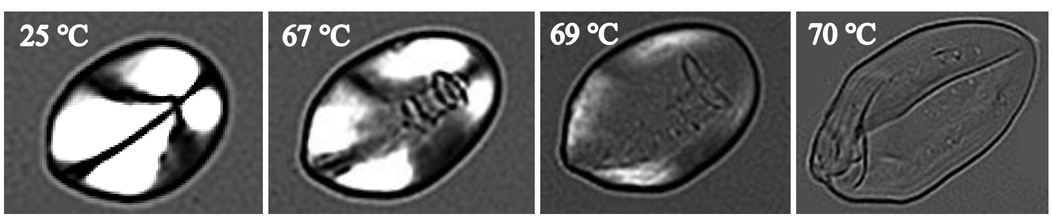

Figure 4. Gelatinization process of a native starch granule obtained through hot-stage polarized-light microscopy.

The gelatinization process occurs at a certain temperature interval and has specific energy requirements, which also depend on the botanical source. Table 2 shows the gelatinization temperature range of different starches. Moreover, it is important to note that this process depends on water availability and temperature; more specifically, the process can only take place if the medium is 60-80% w/w of water at a temperature above 50ºC. When water availability is insufficient, that is, lower than ≈60% w/w, the gelatinization process is not completed (gelatinization degree < 100%). In that case, a considerable increase in temperature may be needed to achieve it (Baks et al., 2007).

Table 2. Gelatinization temperature range of starch granules from different botanical sources (Biliaderis, 2009; Zaidul et al., 2008; Bogracheva et al., 2006).

Starch	***Temperature range (°C)***
Corn	62 – 72
Potato	56 – 75
Rice	68 – 78
Wheat	58 – 64
Tapioca	59 – 69

1.4 Most common methodologies used to asses starch thermal and physical properties

Differential scanning calorimetry (DSC) is the most used technique to measure the temperature range of starch gelatinization and associated energy requirements (Gonera & Cornillon, 2002; Liu & Lelievre, 1991; Burt & Russell, 1983). By means of this technique the heat capacity of any sample can be measured as a function of temperature, enabling the determination of phase transitions. The result of these measurements is an endothermic or an exothermic curve with some characteristic peaks, which reflect the induced transitions (Kaletunç, 2009). In the case of starch, the energy changes are endothermic and the resulting curve allows determining the onset (T_o), the peak (T_p), and the end (T_e) temperature of the gelatinization process. Figure 5 shows a representative curve of wheat starch gelatinization obtained by DSC. The presence of two endotherms becomes visible: the first one corresponds to the gelatinization process, whereas the second one corresponds to the melting of amylose-lipid complexes.

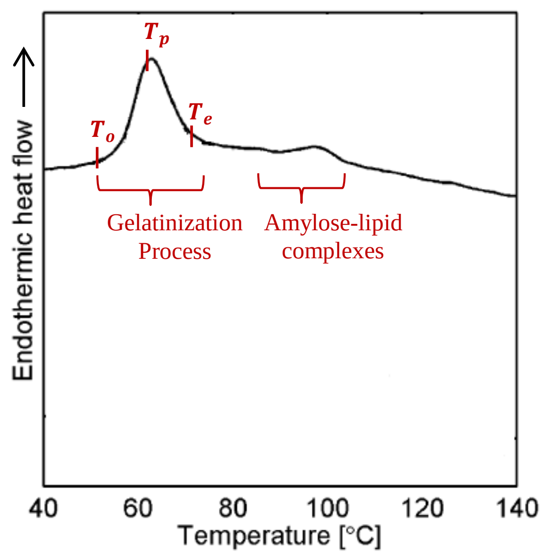

Figure 5. DSC thermogram of the gelatinization process of wheat starch (11% w/w) in excess of water (Adapted from Baks et al., 2007).

Another technique that may be used to assess starch gelatinization is hot-stage polarized-light video-microscopy. By miniaturizing a process, structural changes can be followed under the lens of a microscope, *in situ* and in real time under heating or cooling controlled conditions (Aguilera & Lillford, 1996). This technique makes it possible to observe the microstructural changes of native starch when it is subjected to heating, such as the loss of birefringence and the swelling of the granules. Birefringence may be observed under a polarized light microscope by means of two filters: the polarizer and the analyzer. The first one is located between the light source and the condenser, whereas the second one is placed above the objective. The polarization mechanism works as follows: the light coming from the light source propagates in all directions, but when it passes through the polarizer filter, it gets aligned along one single direction. This direction plane is referred as the polarization axe. When both, the polarizer and the analyzer, are positioned at right angles to each other, they are said to be crossed, with no light passing through the system and a dark viewfield present in the eyepieces. However, if the polarized light reaches a birefringent sample, as native starch (due to its crystalline zones), the light will be rotated into a perpendicular array, which will be able to get through the analyzer filter, making the object visible. Non-birefringent samples, as gelatinized starch, cannot rotate light, thus cannot be identified (Finzi & D Dunlap, 2001). Figure 6 shows a schematic diagram of the polarization mechanism.

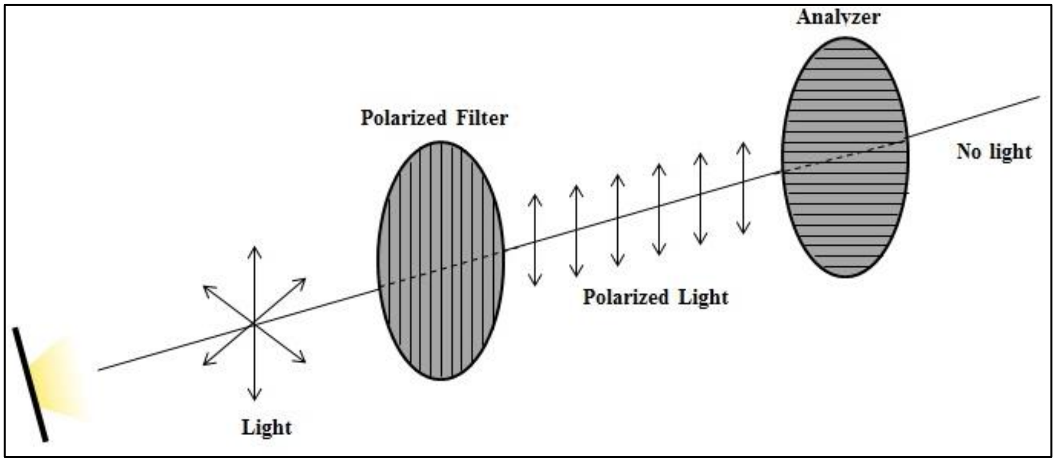

Figure 6. Representation of the polarizing mechanism (Sudhakaran, S., n.d.).

Using this approach, Bouchon & Aguilera (2001) studied the swelling of starch granules inside potato cells during oil immersion. Similarly, Ovalle et al. (2013) examined the microstructural changes of potato starch inside a starchy matrix when subjected to a frying process under atmospheric or vacuum conditions. The advantages of this technique are several, namely: first, it is non-invasive, since the manipulation of the sample is negligible and the sample requires no previous treatment; second, it allows the observation of a process in real time; third, the process conditions can be controlled; and fourth, some parameters of the process can be quantified, such as the loss of birefringence and Feret Diameter (Quian Li et al., 2014; Burt & Russell, 1983; Ghiasi et al., 1982).

In addition, scanning electron microscopy (SEM) has been used to characterize the morphological characteristics of native or gelatinized starch. Ratnayake (2007) studied the morphology of regular and high-amylose corn starches when it was isothermally heated. Bouchon & Pyle (2004) studied the morphology of potato chips after frying at 170°C, and were able to distinguish gelatinized from native potato starch in the matrix after the process. Tester et al. (1994) analyzed the surface damage of native wheat starch after it was milled in a ball miller for 1 up to 8 h. They were able to identify several clumps of small granules after 1 h of milling and hollow shells of large granules after 8 h of milling.

Likewise, X-ray diffraction (XRD) has been used to quantify the crystallinity of native starch as well as starch recrystallization after gelatinization. Zhou et al. (2008) analyzed the recrystallization of gelatinized wheat flour in the absence or presence of polysaccharides, and concluded that in the presence of polysaccharides, the degree of recrystallization decreased when the concentration was increased. Liu et al. (1991) studied the changes in the crystallinity of wheat, rice, and corn starch when heated in a diluted or in a concentrated aqueous suspension.

1.5 Effect of solute on starch gelatinization

The presence of some solutes may delay the gelatinization process. It has been reported that the presence of sugars in solutions containing starch, such as sucrose, glucose, and fructose, may delay the beginning of the gelatinization process, which thus may start at a higher temperature (Mason, 2009; Sopade et al., 2004; Perry & Donald, 2002; Kohyama & Nishinari, 1991). Furthermore, their presence may delay the retrogradation process of starch when it is stored at a low temperature, the delay being more effective as the sugar concentration increases (Baker & Rayas-Duarte, 1998; Kohyama & Nishinari, 1991). On the other hand, it has been shown that the presence of hydrocolloids in starch gels, such as alginate, guar gum, xanthan gum, and kappa-carrageenan, can generate an increase of the onset (T_o) and the end (T_e) temperatures of the gelatinization process, although in many cases this is insignificant (BeMiller, 2011; Rojas et al., 1999). Moreover, the addition of hydrocolloids in a starch gel may produce a decrease of starch retrogradation when subjected to freezing (Brennan et al., 2004; Lee et al., 2002).

1.6 Freezing of starchy products

Freezing is a traditional method for food preservation. This is mainly due to the positive effect of temperature reduction and water immobilization in the form of ice on microbiologic growth reduction and decrease of detrimental chemical reactions (Zaritzky, 2011). Accordingly, many ready to eat products containing high levels of starch are frozen. It has been reported that the storage of these products at a low temperature may cause syneresis and retrogradation, which may affect the organoleptic properties of the products (Feschi et al., 2014; Szymónska et al., 2003; Lee et al., 2002). Also, some texture properties, such as hardness, elasticity, and cohesiveness, may be affected when these products are subjected to freezing-heating cycles, increasing their hardness and decreasing their elasticity and cohesiveness (Wang et al., 2013; Teng et al., 2011).

Furthermore, some studies have shown that the surface area, the volume, and the diameter of the surface pores of native granules may increase considerably after the first freezing-heating cycle (Szymónska & Wodnicka, 2005; Szymónska et al., 2003; Szymónska et al., 2000). Despite its importance, there are no scientific studies that focus on the effect of freezing on native starch gelatinization during subsequent heating.

2. HYPOTHESIS

A proper understanding of microstructural changes during food processing and storage is of critical importance to control product performance and ensure quality, by minimizing product variation from batch to batch. In the case of starchy food, starch gelatinization during heating plays a critical role in structure formation and associated quality attributes. Accordingly, the hypothesis of this research states that through process miniaturization it is possible to understand the effect of freezing as well as the effect of water accessibility (*i.e.*, the amount of available water in the medium enabling the gelatinization of starchy molecules) in starch gelatinization to improve our knowledge about the effect of these key factors in starch microstructural changes. Specifically, it is expected to observe and quantify a delay in starch gelatinization degree due to freezing and also due to presence of solutes.

3. OBJECTIVES

Accordingly, the main objective of this thesis was to study the effect of freezing together with the effect of water accessibility in starch microstructural changes during heating, through process miniaturization, using *in situ* hot-stage polarized-light video-microscopy, and differential scanning calorimetry. To achieve this objective, the specific aims of this thesis are:

(i) To understand the effect of freezing prior to heating, in the microstructural changes of native potato starch either dispersed in distilled water, in a kappa-carrageenan gel, or in a sucrose solution, when heated under controlled conditions.

(ii) To understand the effect of water accessibility in the microstructural changes of native potato starch either dispersed in distilled water, in a kappa-carrageenan gel, or in a sucrose solution, when directly heated or when heated after freezing.

(iii) To understand the effect of water accessibility in the enthalpy of gelatinization of native potato starch either dispersed in distilled water, in a kappa-carrageenan gel, or in a sucrose solution, when directly heated or when heated after freezing.

4. MATERIALS AND METHODS

4.1 Materials

Native potato starch with 20% amylose was obtained from Blumos S.A. (Santiago, Chile), kappa-carrageenan powder was provided by Gelymar S.A. (Santiago, Chile) and sucrose analytic degree was obtained from Winkler Ltda. (Santiago, Chile).

4.2 Sample preparation

Three systems were prepared, in order to understand the effect of water accessibility in starch gelatinization, as well as the effect of freezing prior to heating. A hydrocolloid was selected because, first, it is used in starchy gels, such as sauces and pastry filling; second, it stabilizes the structure of gels when they are frozen; and finally, it reduces the syneresis and retrogradation rate of starchy molecules. Kappa-carrageenan was specifically selected because it is a gelling agent and it is stable to temperature fluctuations. On the other hand, we used sucrose because it is commonly used in starchy products such as donut, crescent, cake, and extruded cereals. Moreover, it gives smoothness, color and flavor to sweet food, and delays retrogradation rate of starchy molecules.

4.2.1 Starch in water

Native starch granules were added to a crucible (microcapsule to be mounted in the microscope) together with 300 µL of distilled water.

4.2.2 Starch in carrageenan gel

First, 1% w/w of kappa-carrageenan powder was dispersed in distilled water. Then the dispersion was heated in a heating magnetic stirrer up to 80°C and was maintained at this temperature for 1 h with constant stirring. Finally, the temperature was reduced to 35°C and 300 µL of gel were extracted. The sample was added to a crucible together with native potato starch granules.

4.2.3 Starch in sucrose solution

A solution was prepared by diluting 30% w/w of powder sucrose in distilled water with constant stirring at room temperature. 400 µL were then extracted and added to a crucible together with native potato starch granules.

4.3 Video-microscopy: hot-stage light microscopy

4.3.1 Experimental set-up

Starch microstructural changes were followed in a Linkam hot-stage (model THMS350V, Linkam Scientific Instruments, United Kingdom) adapted to an Olympus light microscope (model BX61, Olympus Optical Corporation, Japan), as shown in Figure 7. A PE95/T95 temperature controller allowed a precise control of the desired heating rate.

A polarized filter and an analyzer were incorporated to the microscope, which allowed the observation of birefringent native starch granules. Changes were recorded on line using a CoolSnap Pro Color 289 digital camera (Photometrics Roper Division, Inc., USA) connected to the microscope and viewed with a 10x magnifying lens.

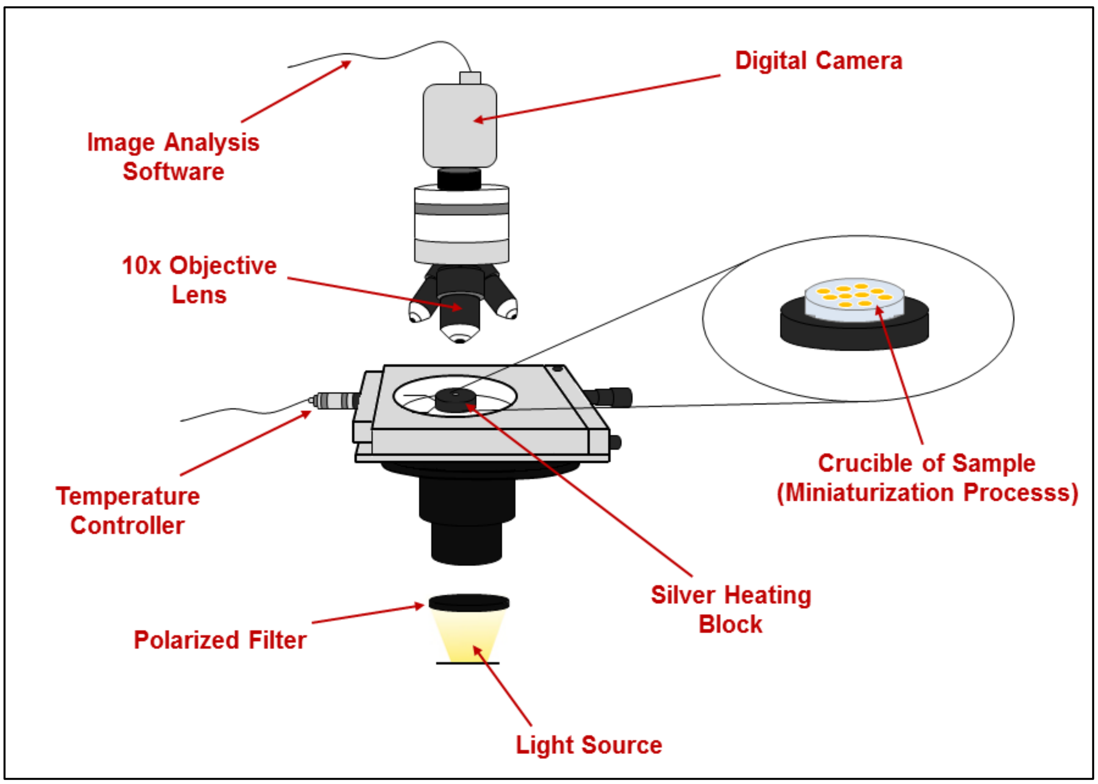

Figure 7. Hot-stage polarized-light video-microscopy experimental set-up used to characterize starch microstructural changes *in situ* and in real time.

4.3.2 Heating and freezing-heating of the samples

Once the samples were prepared they were either heated directly (H samples) in the hot-stage at 15°C/min, or they were frozen (FH samples) for 48 h in a conventional freezer General Electric (model TBZ 16 NA, General Electric Company, USA). Frozen samples were later defrosted at room temperature and heated under the same conditions.

4.3.3 Image acquisition and processing

Images were processed and analyzed using Image ProPlus 4.5 software (Media Cybernetics, USA), where each frame was linked to a specific temperature. First, color-scale images were changed from RGB to 8 bits grey scale. Thereafter, the noise was reduced using a high pass Gaussian filter (7x7 *HiGauss,* 1 pass and strength of 10) and features were enhanced using a sharpening filter (5x5 *Sharpen*, 1 pass and strength of 10). Finally, images were segmented using a grey intensity threshold of 110 for water-starch and water-starch-sucrose systems, and 160 for the water-starch-carrageenan system in order to get quantitative information. Binarized images were then analyzed to get specific parameters such as the Feret Diameter and the birefringent area of the granules, among others. Figure 8 shows an original image as well as some processed and segmented images to illustrate the procedure.

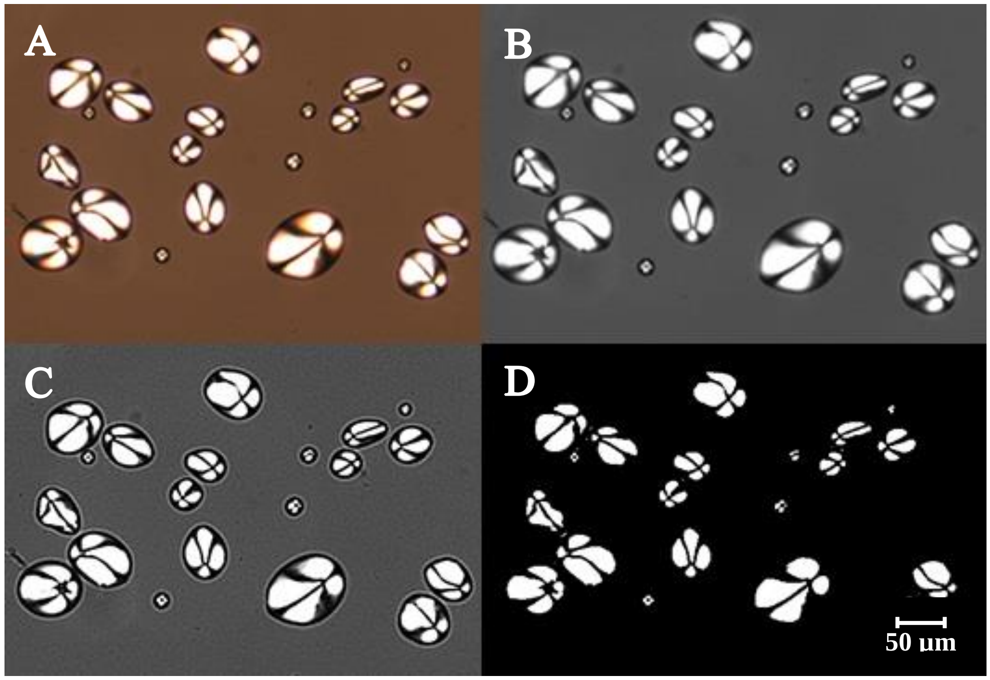

Figure 8. Image processing sequence, showing: A) an original image, B) a grey scale image, C) a processed image, and D) a segmented image.

4.3.4 Quantification of the degree of gelatinization

The degree of gelatinization (DG %) was quantified by measuring the gradual loss of birefringence. The gelatinization process, which defined the onset temperature (T_o), was considered to begin when at least one granule had lost 1.5% of its birefringent area. In turn, the end temperature (T_e) was defined when all the granules had lost 100% of their birefringence (Li et al., 2013). In accordance, for each set of images, DG was quantified according to Equations 1 and 2:

$$DG_{ij}(\%) = \left(\frac{A_{i0} - A_{ij}}{A_{i0}}\right) \cdot\ 100\ \%, \qquad where\ DG_{ij}(\%) \geq 1.5\% \qquad (1)$$

$$DG_j(\%) = \frac{\sum_{i=1}^{N} DG_{ij}(\%)}{N} \qquad (2)$$

where:

0 and j are the first and the j^{th} frames of the sequence.

i is the i^{th} granule of the sequence.

N is the total number of granules of the sequence.

A_{i0} is the birefringent area of granule i in the first frame of the sequence.

A_{ij} is the birefringent area of granule i in the j^{th} frame of the sequence.

In order to replicate the results, four groups of starch granules were followed in each system (water, carrageenan, and sucrose) under each experimental condition (directly heated and frozen-heated), as shown in Table 3.

Table 3. Number of granules studied in each system under each experimental condition.

	Starch - water		Starch – water - carrageenan		Starch – water - sucrose	
	Heated	**Frozen-Heated**	**Heated**	**Frozen-Heated**	**Heated**	**Frozen-Heated**
Group 1	40	76	50	41	65	57
Group 2	66	54	61	50	62	49
Group 3	62	48	68	77	59	27
Group 4	33	57	43	48	53	46
Total granules	201	235	222	206	239	179

In order to have a representative parameter of granules population in each system, the Sauter Diameter (μm) was calculated according to Equation 3 (Richardson & Harker, 2002):

$$\overline{D}_{sauter} = \frac{\sum_{i=1}^{n} N_i \cdot D_i^3}{\sum_{i=1}^{n} N_i \cdot D_i^2} \qquad (3)$$

where:

i is a size interval of Feret Diameter.

n is the total number of intervals of Feret Diameter.

N_i is the total number of granules in the i^{th} interval.

D_i is the mean diameter in the i^{th} interval.

4.4 Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) analysis was done in triplicate using a Mettler Toledo Star System (model 821e, Mettler-Toledo International Inc., USA). First, 10 mg of native potato starch were added to an aluminum crucible of 160 μL. Then, 90 mg of distilled water, kappa-carrageenan gel, or sucrose solution were added to the crucible in order to obtain a starch:medium ratio of 1:9 (Baks et al., 2007). An empty pan was used as a reference. The crucible was hermetically sealed and the samples were equilibrated for 18 h at room temperature. The samples were then immediately heated or frozen in a conventional freezer for 48 h. A 10°C/min heating rate was used, since a higher heating rate (15°C/min) the endothermic peak was less pronounced. The temperature intervals were 25-90°C for the samples with water and carrageenan gel, and 25-95°C for those with a sucrose solution.

4.5 Scanning electron microscopy

In order to study the morphology and possible surface damages in starch granules after freezing, surface micro-images were obtained using a Scanning Electron Microscope (SEM, model LEO 1420VP, LEO Electron Microscopy Ltd, England). Unfrozen native starch granules were directly attached to aluminum stubs with double-sided adhesive tape. On the other, frozen starch granules immersed in water were centrifuged at 5000 rpm (1677 g) for 20 min. The supernatant was then separated from the sediment, which was attached to aluminum stubs with double-sided adhesive tape. Subsequently, frozen and unfrozen samples were coated with approximately 20 nm of gold using a sputter coater vacuum evaporator (model PS 10E, Varian Inc. Vacuum Division, USA). Finally, the samples were examined at an accelerating potential of 25 kV, using magnifications ranging from 500× to 5000×.

4.6 Atomic force microscopy

The atomic force microscopy (AFM) analysis was carried out in order to visualize and quantify surface roughness. Unfrozen and frozen starch granules were fixed on an AFM sample holder with double-sided adhesive tape to immobilize the samples (Krok et al., 2000). Surface areas ranging from 1×1 to 3×3 μm^2 were measured using an atomic force microscope (NanoWizard 3, JPK Instruments, Germany) using a PPP-NCHAuD AFM probe. Four granules per sample were analyzed. Surface roughness was quantified by means of the root mean square roughness (R_q), which is one of the most important physical parameters used to describe surface roughness (ASME 1995) and is considered to be more sensitive to peaks and valleys than the average roughness (Kumar & Rao, 2012).

4.7 Statistical analysis

Reported results correspond to the arithmetic mean ± standard error. The statistical analysis was carried out using Statgraphics Centurion XV software (Manugistic Inc., USA) for Windows. Results were compared using ANOVA table with a confidence level of 95%.

5. RESULTS AND DISCUSSIONS

5.1 Starch granules population analysis

Figure 9 shows the size distribution of the granules that were examined in the different systems, with and without previous freezing. Overall, results showed that the size distribution followed a similar trend in all systems. Specifically, it can be noted that the density of the population was higher when the Feret Diameter (μm) was between [10-20) and [20-30), and that the population density was further reduced in the following intervals. The Sauter Diameter (μm) was calculated with Equation (3) in order to have a representative parameter for each population (Richardson & Harker, 2002). The Sauter Diameter of the granules that were directly heated or were frozen previous to heating were 42.34 ± 7.88 (H) and 47.80 ± 1.04 (FH) in the starch-water system, 47.11 ± 3.05 (H) and 42.94 ± 4.84 (FH) in the starch-water-carrageenan system and 44.13 ± 2.78 (H) and 41.91 ± 9.26 (FH) in the starch-water-sucrose system, respectively. That is, values were extremely similar in all systems and no significant differences were found ($p < 0.05$). Bouchon & Aguilera (2001) proved that bigger starch granules (60.0 μm) may gelatinize faster than smaller ones (12.6 μm). Accordingly, it was possible to ensure from the beginning that results would not be biased due to this factor.

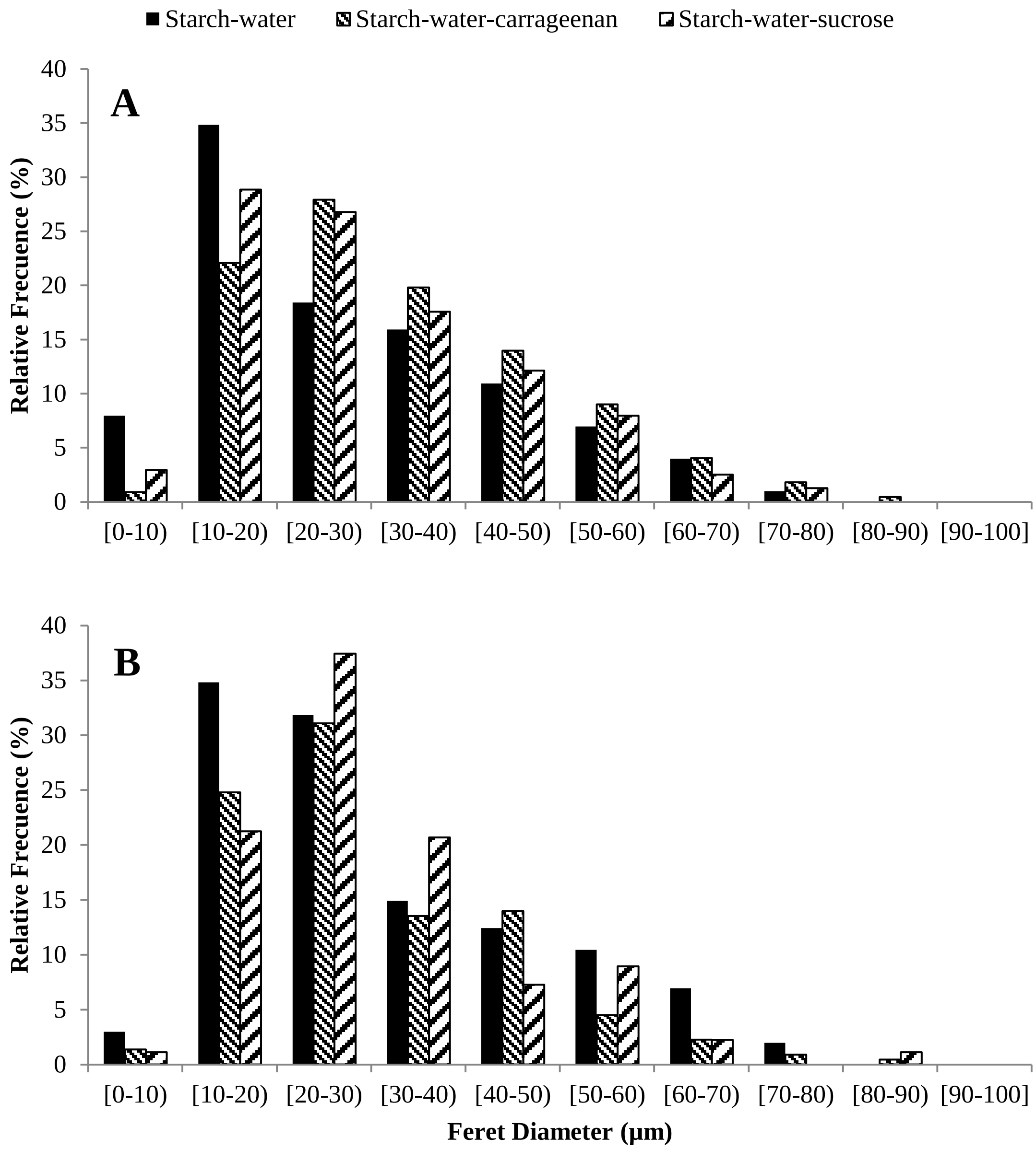

Figure 9. Size distribution of the population of granules native starch, which were either: A) directly heated or B) heated after freezing.

5.2 Effect of freezing on starch gelatinization

Figure 10 shows an image gallery of the gelatinization process of native potato starch in carrageenan gel heated at 15°C/min, to illustrate a standard process. The degree of gelatinization was quantified by means of image analysis using Equations (1) and (2). As expected, when the temperature increased the birefringence of the starch granules diminished and the degree of gelatinization increased.

Figure 11 shows the curves of the degree of gelatinization at different temperatures, obtained from image analysis. Overall, in all systems, the degree of gelatinization decreased when the samples were subjected to previous freezing. For instance, at 70°C, the degree of gelatinization of the samples directly heated or frozen previous to heating were 83.25 ± 8.00% (H) and 54.94 ± 11.00% (FH) for the starch-water system, 41.42 ± 12.20% (H) and 13.30 ± 3.60% (FH) for the starch-water-carrageenan system and 5.69 ± 3.70% (H) and 3.48 ± 2.9% (FH) for the starch-water-sucrose system. Furthermore, unfrozen and frozen samples had gelatinization degrees significantly different ($p<0.05$) in each system over a wide temperature interval. Differences were determined over the following temperature intervals: 62-74°C (13°C) in the starch-water system, 68-75°C (8°C) in the starch-water-carrageenan system and 79-84°C (6°C) in the starch-water-sucrose system.

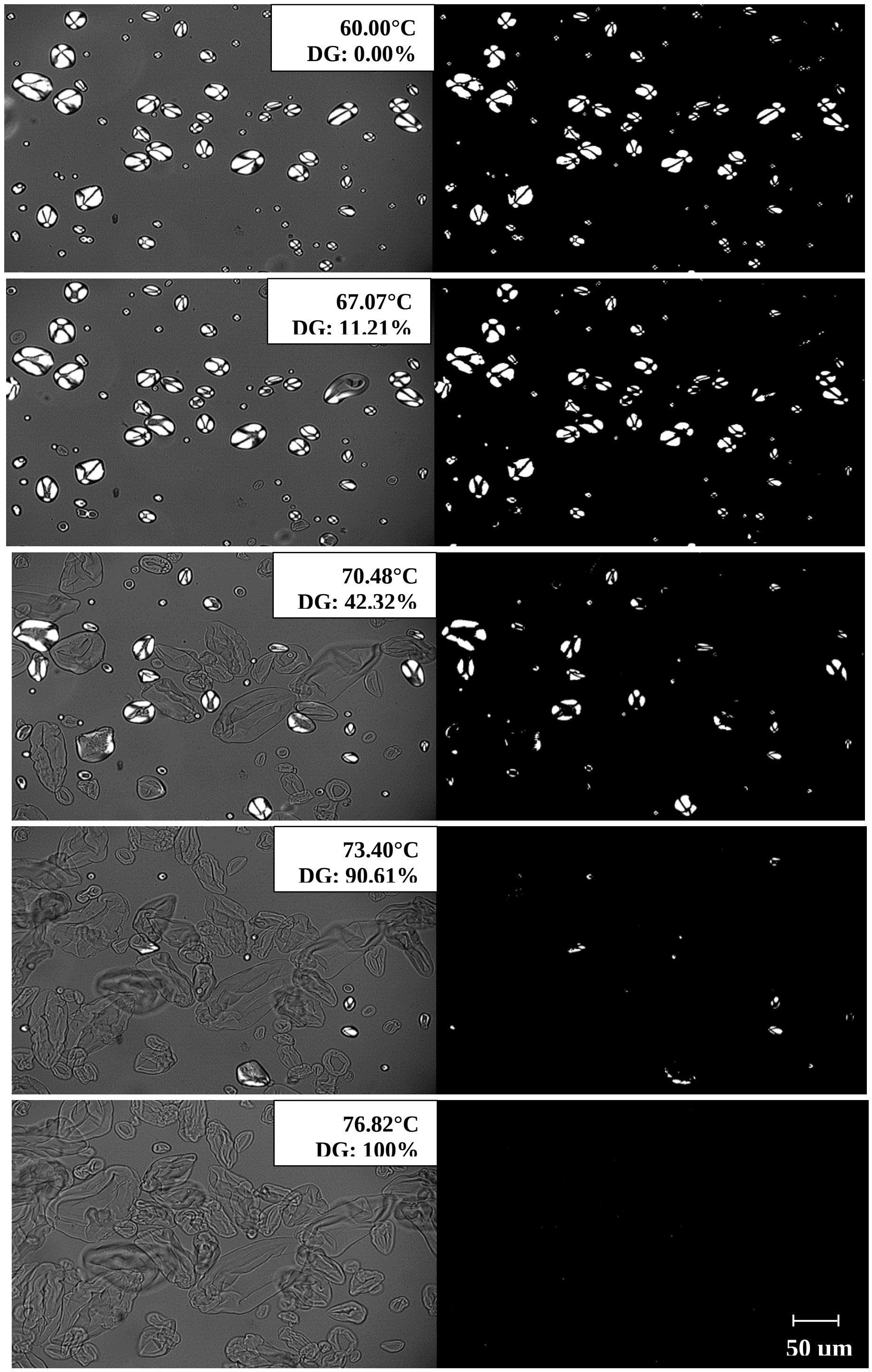

Figure 10. Images and degree of gelatinization (DG) of a starch–carrageenan system directly heated at 15°C/min, using polarized-light hot-stage video microscopy (left column) and segmented images (right column).

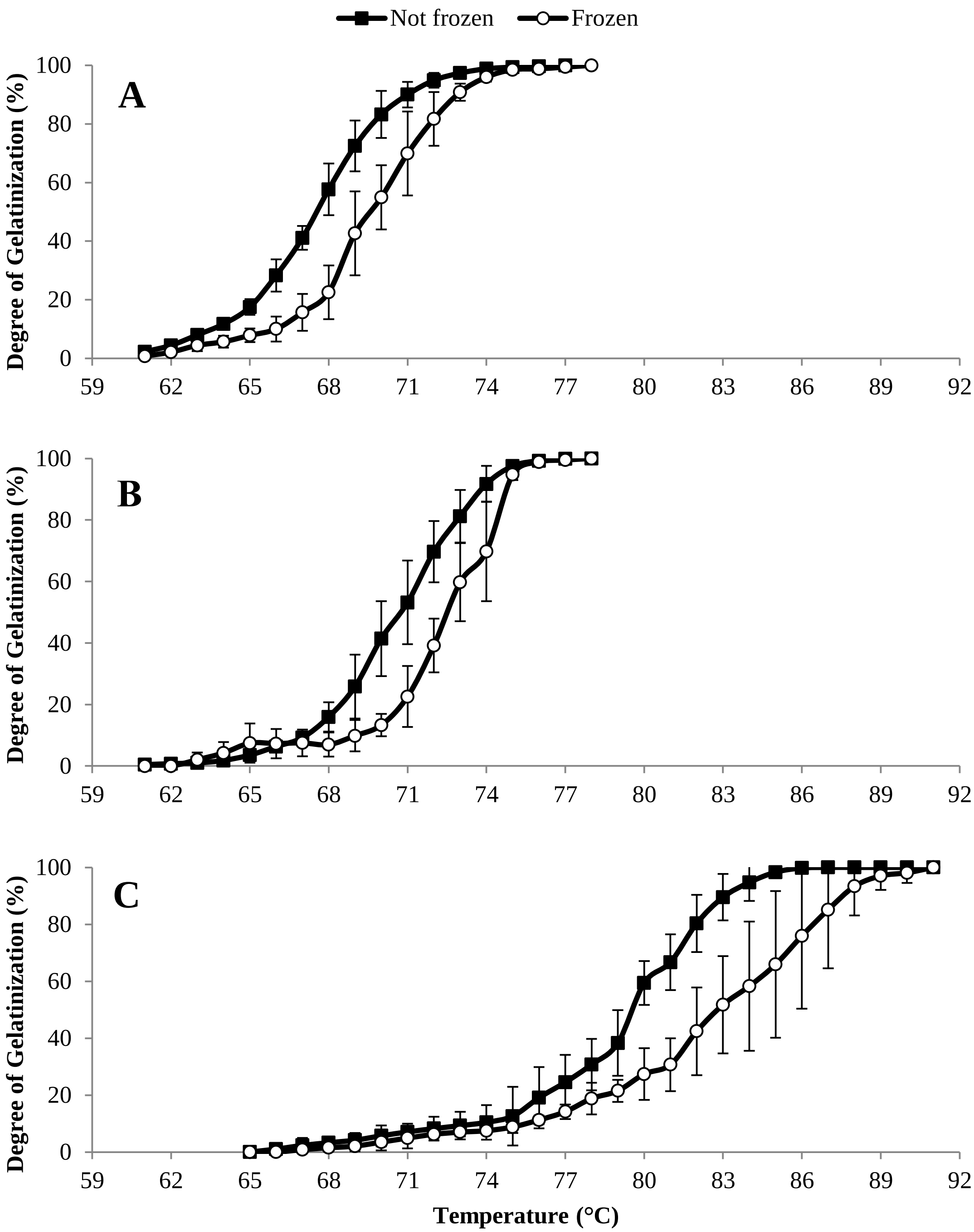

Figure 11. Degree of gelatinization of: A) Starch-water, B) Starch-water-carrageenan and C) Starch-sucrose systems of directly heated and pre-frozen heated samples.

In order to get further insight about the effect freezing over starch gelatinization, scanning electron microscopy and force atomic microscopy were used. Figure 12 shows some representative SEM microphotographs as well as some AFM topographical images of the surface of non-frozen and frozen native starch granules. It is possible to observe that the unfrozen native starch granule had a smooth surface, whereas the frozen one had a rougher surface and with some protuberances. These observations were in agreement with surface roughness quantification, which showed that the root mean square roughness (R_q) of unfrozen samples (3.74 ± 0.48 nm) was significantly lower than that of frozen granules (5.62 ± 0.86 nm). Similar observations were found by Szymónska & Wodnicka (2005), Szymónska & Krok (2003), and Szymónska et al. (2000) when studying the physical changes of native potato starch when subjected to freezing-heating cycles. They explained that these external changes could make the granule more accessible to water molecules, inducing hydration and water redistribution within the granule, and leading to an increase in crystallinity. Accordingly, the retarded gelatinization degree of frozen samples could also be related to an increased crystallinity, as a consequence of water redistribution. Certainly, further experiments should be carried out to confirm this hypothesis.

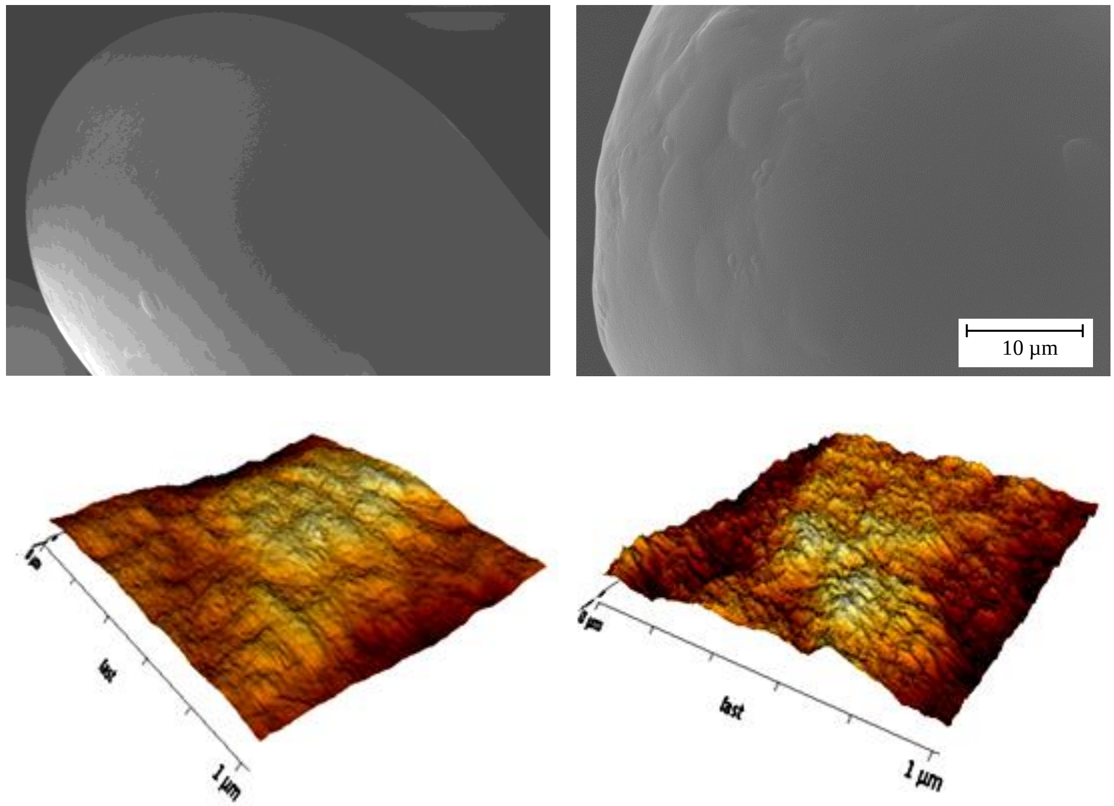

Figure 12. Scanning electron microphotograph (above) and atomic force microscopy images (below) of the surface of a native potato starch granule without freezing (left) and after freezing when immersed in water (right).

The transition temperatures determined by microscopy (T_o and T_e) and by DSC (T_o, T_p y T_e) for each system and experimental condition are summarized in Table 4. Overall, it is possible to observe that freezing did not alter the temperature range over which gelatinization occurred. Significant differences were only found in the starch-water-sucrose system through video-microscopy. Moreover, when T_o and T_e are compared in both tables for each system, it can be observed that $T_o(DSC) <$

$T_o(Microscopy)$ and $T_e(DSC) < T_e(Microscopy)$. The temperature range over which gelatinization occurred in the water-starch system fallen within the temperature that has been previously reported for native potato starch in excess of water (56-75^{o}C) through DSC (Zaidul et al., 2008; Bogracheva et al., 2006; Liu et al., 2002). On the other hand, the differences between both techniques may be due to the different heating rates that were used in each procedure. In fact, Ovalle et al. (2013) proved that an increase in the heating rate generates a positive displacement (towards higher temperatures) of the temperature range over which the gelatinization process takes place. Accordingly, the higher temperature range obtained through microscopy is consistent with these results.

5.3 Effect of water accessibility on starch gelatinization

Figure 13 shows the degree of gelatinization at different temperatures of the different systems, directly heated or heated after freezing, to assess the effect of water accessibility on starch gelatinization. First, it can be observed that the gelatinization degree was higher in the starch-water system than in the starch-carrageenan systems, and that both were consistently higher than the one of the starch-water-sucrose system in both conditions (directly heated or heated after freezing). For instance, at 72°C the gelatinization degrees of the starch-water system were 94.87 ± 2.50% (H) and 81.71 ± 9% (FH), that of the starch-water-carrageenan system were 69.68 ± 10% (H) and 39.18 ± 8.70% (FH), and that of

Table 4. Gelatinization temperatures obtained from differential scanning calorimetry and polarized-light hot-stage video microscopy.

	Starch - water		Starch – water - carrageenan		Starch – water - sucrose	
	Heated	**Frozen-Heated**	**Heated**	**Frozen-Heated**	**Heated**	**Frozen-Heated**
Video Microscopy						
T_o (°C)	60.74 ± 0.02[a]	60.86 ± 0.25[a]	61.47 ± 1.08[ab]	63.06 ± 0.90[b]	66.13 ± 0.35[c]	66.98 ± 0.48[d]
T_e (°C)	74.45 ± 1.44[a]	76.49 ± 1.71[a]	77.07 ± 0.31[b]	76.90 ± 0.56[ab]	85.72 ± 0.85[c]	89.00 ± 1.83[d]
DSC						
T_o (°C)	58.91 ± 0.01[a]	59.57 ± 0.44[a]	59.68 ± 0.51[a]	59.35 ± 0.15[a]	68.19 ± 0.22[b]	68.18 ± 0.11[b]
T_p (°C)	64.33 ± 0.31[a]	65.07 ± 0.43[a]	64.23 ± 0.23[ab]	63.78 ± 0.19[b]	73.23 ± 0.23[c]	73.27 ± 0.08[c]
T_e (°C)	71.73 ± 0.27[a]	72.17 ± 0.60[a]	71.21 ± 0.74[a]	71.56 ± 0.33[a]	79.60 ± 0.21[b]	79.36 ± 0.13[b]
ΔH (J/g)	0.69 ± 0.01[a]	0.63 ± 0.02[b]	1.04 ± 0.04[c]	1.04 ± 0.03[c]	1.36 ± 0.11[d]	1.16 ± 0.05[e]

Values with different letters in the same row are significantly different ($p < 0.05$).

the starch-water-sucrose system were 8.22 ± 4.20% (H) and 6.23 ± 2.10% (FH) for directly heated and frozen-heated samples, respectively.

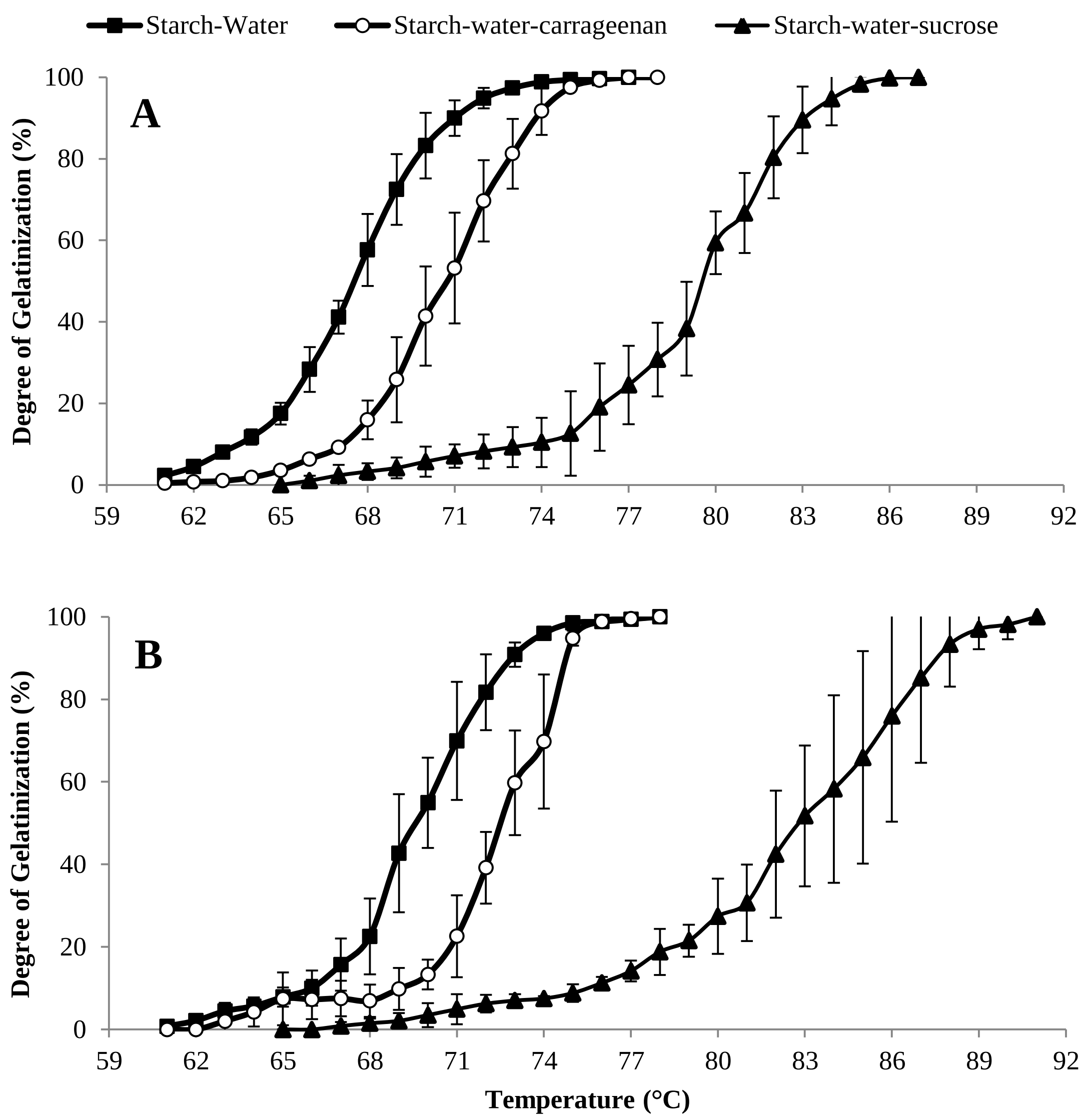

Figure 13. Effect of water accessibility on the degree of gelatinization of starch: A) directly heated and B) heated after freezing.

The addition of carrageenan delays the gelatinization process, and the latter is further delayed in the presence of sucrose. The same behavior is observed when the gelatinization enthalpies are analyzed (Table 4), where $\Delta H_{starch-water} < \Delta H_{starch-water-carrageenan} < \Delta H_{starch-water-sucrose}$, in the systems with and without previous freezing. This may be due to two phenomena, which are a consequence of the presence of hydroxyl groups in starch, carrageenan, and sucrose molecules:

1. The carrageenan and the sucrose molecules may get hydrated due to the presence of hydroxyl groups in their structures (Davis, 1995). Such hydration is expressed through the formation of new hydrogen bonds between these groups and the water molecules present in the medium. Considering the quantity of hydroxyl groups, the hydration of sucrose is higher than that of carrageenan, because the former has 9 hydroxyl groups in its structure, whereas the latter only has 3 per structural unit. This may explain why the starch-water-sucrose system requires more energy during heating compared to the starch-water-carrageenan system, which in turn requires more energy than the starch-water system. According to Viturawong et al. (2008) and Khanna & Tester (2006), a restriction in water mobility due to the presence of an hydrocolloid may induce an incomplete gelatinization of starch, which translates into a lower energy requirement, that is, $\Delta H_{without\ hydrocolloid} < \Delta H_{with\ hydrocolloid}$. Yet in this study the three systems analyzed were in

excess of water (over 70%), thus a complete gelatinization of starch was achieved, as shown in Figure 10. Accordingly, in this set of experiments, the lower gelatinization degree of carrageenan and sucrose containing systems may be better attributed to the higher hydration due to the presence of hydroxyl groups rather than to partial gelatinization.

2. The presence of hydroxyl groups makes the interaction between carrageenan and starch possible, as well as the one between sucrose and starch. This interaction reduces the mobility of starchy chains, which may result in a higher energy requirement for the system to break the interactions and thus for the gelatinization process to take place, as reported by Li et al. (2015), Matignon et al. (2014), Huc et al. (2004) and Chiotelli et al. (2000). Moreover, the higher energy required for the starch-water-sucrose system compared to the starch-water-carrageenan system may be due to the higher degree of functionalization of sucrose compared to carrageenan, which can make sucrose more susceptible to interact with the starch. Also, the presence of sulfate groups in the carrageenan molecules as well as in the starch chains may decrease their interaction.

With respect to the onset and end temperatures (Table 4) the starch-water and starch-water-carrageenan systems did not show clear differences in T_0 and T_e. This is consistent with the results reported by Viturawong et al. (2008), Tester &

Sommerville (2003), Gonera & Cornillon (2002), according to which the addition of hydrocolloids may cause minor or even negligible effects on T_o, T_p and T_e. However, this does not mean that the addition of carrageenan has no effect on starch gelatinization. As previously discussed, a clear effect in the gelatinization enthalpy was observed. Also, the temperature range over which the degree of gelatinization differs between frozen and unfrozen samples is lower ($8°C$ for starch-water-carrageenan) *versus* ($13°C$ for starch-water).

If T_o, T_p and T_e of the starch-water-sucrose are compared with those of the other two systems, a considerable increase in these temperatures can be seen, which is consistent with the reports from Ratnayake et al. (2009), Gonera & Cornillon (2002), and Perry & Donald (2002). This increment was observed in the results obtained by microscopy and by DSC, and is attributed to the high hydration capacity and functionalization of sucrose. In addition, the temperature range over which the degree of gelatinization differs between frozen and unfrozen samples is reduced ($6°C$). Overall, it can be said that the temperature range of such differences decreased when the accessibility to water was reduced ($\Delta T_{starch-water} > \Delta T_{starch-water-carrageenan} > \Delta T_{starch-water-sucrose}$).

6. CONCLUSIONS

Hot-stage polarized-light video-microscopy was an instrumental technique to miniaturize and characterize the gelatinization process of native starch granules when immersed either in water, in a carrageenan gel, or in a sucrose solution. Specifically, it was possible to follow in real time, and with minimal intrusion, the structural changes of native potato starch during when heating or during heating after prolonged freezing. On the other hand, differential scanning calorimetry allowed monitoring the enthalpy changes related to the gelatinization process, and scanning electron microscopy allowed comparing the surface morphology of native and previous frozen starch granules.

With respect to the effect of freezing, in all systems, it was possible to conclude that the degree of gelatinization decreased when the samples were subjected to previous freezing. This was attributed to a possible increase in the crystallinity of the starch granule due to hydration and water redistribution within the granule. These observations are certainly preliminary, and further studies should be carried out in order to dig further into this phenomenon. For instance, future studies could consider the use of X-ray diffractometry. Through this technique it should be possible to examine and quantify if there actually is an increase in crystallinity during freezing.

In addition, it was possible to conclude that there was a significant effect of water restriction on the degree of gelatinization as well as on the enthalpy of gelatinization. These results were consistent with previously reported results during heating, which were now extended to pre-frozen samples and different conditions. The ascending order regarding the degree of gelatinization was starch-water-sucrose < starch-water-carrageenan < starch-water, which was confirmed when analyzing the gelatinization enthalpies, in the systems with and without previous freezing. This behavior was attributed to the higher presence of hydroxyl groups in sucrose (9) compared to carrageenan (3), which increases their hydration capacity and functionalization degree. In addition, it was possible to conclude that the temperature range over which the degree of gelatinization differed between frozen and unfrozen samples was diminished when the accessibility to water was reduced.

Overall, these results contribute to get a better understanding of the effect of freezing and water accessibility/restriction during the heating of starchy products at high heating rates. This knowledge may help to optimize product formulation, frozen storage and processing conditions to control product performance and improve quality, and thus, may be a valuable contribution in the growing field of food product design. In order to get further knowledge about how aggressive the effect of freezing is, it would be of interest to study the effect of different freezing rates together with the effect of different freezing-heating cycles as well as the effect of thawing in the microstructural changes of native starch and subsequent changes during processing. Furthermore, it would be relevant to study the effect of freezing under the aforementioned conditions of a starchy matrix, at a pilot plant scale, to understand the relationship between micro- and macro-structural properties, together with their link to desired organoleptic properties. Further, starch gelatinization within a structured matrix could be also studied under the lenses of a microscope using hot-stage polarized-light video-microscopy, based on this research.

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APPENDIXES

APPENDIX A: FERET DIAMETER OF STARCH GRANULES POPULATION

Starch-water System – Directly heated			
M. 1	**M. 3**	**M. 5**	**M. 7**
23,80	10,62	61,49	30,89
22,55	26,04	36,63	26,13
25,52	9,04	34,75	25,56
14,11	17,63	43,43	16,47
8,38	10,21	70,04	37,51
32,47	11,86	19,62	41,96
10,83	11,92	26,61	43,52
22,10	13,80	40,13	19,12
15,05	13,93	20,34	29,53
53,00	28,67	15,91	47,89
12,18	12,14	55,52	60,32
7,02	10,38	54,42	11,82
38,37	35,28	45,53	47,73
5,68	38,96	52,34	43,40
13,75	42,35	17,93	43,55
16,90	47,94	56,00	50,88
12,23	12,84	61,15	45,48
25,56	29,19	38,89	14,22
21,07	38,04	25,40	10,90
21,97	10,21	16,71	20,80
21,87	53,34	34,58	44,32
9,76	15,30	67,92	43,88
23,62	14,77	62,01	24,26
39,44	27,50	13,16	40,27
41,10	13,52	30,42	33,79
13,90	35,28	29,65	35,07
9,68	16,48	30,19	22,66
11,47	21,92	18,91	39,79
10,01	12,16	50,08	25,94
16,49	9,85	27,62	31,49
17,53	24,06	17,12	33,60
20,48	9,99	9,80	7,28
14,53	17,11	12,26	16,36
25,00	12,32	17,05	
26,92	49,13	14,33	
39,18	10,26	65,86	
9,04	8,21	40,01	
13,52	32,24	16,54	
8,80	7,26	54,74	
13,98	10,76	12,40	
	29,63	16,16	
	14,85	59,44	
	32,02	23,76	
	45,16	37,64	
	34,93	49,39	
	22,97	30,15	
	39,66	14,68	
	14,08	28,97	
	43,60	14,13	
	16,00	12,55	
	30,68	18,21	
	47,65	20,10	
	9,16	12,91	
	10,19	66,29	
	38,04	36,34	
	77,02	27,55	
	12,71	19,67	
	12,58	24,67	
	56,43	39,28	
	12,40	14,74	
	50,70	52,99	
	54,85	39,34	
	13,10		
	60,58		
	11,02		
	9,40		

Starch-water system – Heated after freezing			
M. 2	**M. 3**	**M. 4**	**M. 5**
18,39	14,92	22,95	25,07
42,37	27,60	24,71	19,70
18,76	32,67	26,22	13,60
13,05	24,85	58,90	28,46
34,23	27,73	25,08	17,49
15,29	71,79	15,35	13,30
25,19	26,17	59,77	16,65
48,51	3,51	16,05	64,88
37,84	58,78	35,98	12,02
79,17	21,48	46,34	12,64
67,97	31,37	16,01	14,18
28,73	32,81	23,82	36,16
47,00	67,20	22,42	26,40
9,77	23,85	9,87	35,41
59,63	14,15	24,85	46,11
30,51	20,97	55,14	12,29
13,96	53,69	26,05	73,04
14,43	15,00	44,72	59,84
27,59	31,39	47,51	39,64
21,32	25,27	23,83	43,66
27,68	44,03	69,81	54,10
23,84	32,88	55,08	42,90
48,33	21,03	18,91	30,65
61,74	39,55	40,97	19,03
19,69	25,14	16,28	31,48
46,73	43,86	23,60	17,06
20,12	66,67	24,13	14,86
23,04	10,27	10,63	15,61
29,20	49,33	28,22	35,57
14,86	22,69	29,88	45,05
25,92	58,59	54,44	52,68
29,48	38,34	41,29	28,78
32,93	26,93	19,48	42,27
9,51	25,79	25,99	33,48
12,56	27,69	14,32	22,42
14,45	29,77	14,34	56,07
31,07	18,11	41,40	15,46
13,77	54,59	25,75	21,79
11,12	15,85	13,41	18,85
13,66	28,88	17,91	21,27
19,34	22,51	20,95	23,79
12,93	19,62	14,20	20,50
12,52	37,02	60,34	61,60
43,80	56,98	22,76	16,87
43,65	11,47	68,45	12,82
35,91	33,37	24,71	23,10
11,17	52,10	52,19	15,31
40,01	28,94	53,34	48,87
17,06		68,33	39,36
33,85		18,15	39,94
44,59		22,68	13,89
62,65		35,31	18,72
43,97		15,26	19,10
9,26		15,43	34,12
52,11			30,55
9,92			69,77
66,81			
10,78			
13,31			
13,44			
13,12			
35,03			
23,75			
11,58			
15,08			
63,48			
13,79			
22,09			
52,74			
20,52			
73,52			
54,95			
17,88			
24,04			
20,85			
29,07			

Starch-water-carrageenan System – Directly heated			
M. 1	**M. 2**	**M. 3**	**M. 5**
69,72	25,58	57,25	23,37
45,81	29,62	32,24	46,06
32,79	51,20	10,11	31,94
35,87	30,64	64,24	16,28
15,28	34,48	42,39	25,49
16,37	32,47	43,47	18,61
57,63	48,98	50,76	54,21
33,90	53,93	79,90	24,55
16,51	30,05	35,09	20,63
13,80	21,42	40,09	32,82
12,24	13,21	21,54	26,16
78,02	32,65	42,62	25,93
26,70	22,87	32,33	21,45
47,45	26,46	21,28	81,00
27,09	34,69	17,32	37,53
61,70	25,63	32,07	41,18
48,72	31,58	38,20	45,40
40,95	19,24	14,10	47,27
61,04	16,72	47,73	35,82
31,90	40,21	18,96	28,38
53,39	53,64	25,32	54,78
17,84	44,01	31,59	24,10
27,48	14,13	37,56	23,27
39,45	12,05	22,13	37,04
42,42	78,49	33,98	50,16
11,11	16,84	30,62	45,71
52,16	22,78	22,28	29,39
66,07	13,63	27,36	20,05
51,19	30,65	35,95	18,25
11,50	21,28	26,51	37,84
10,53	18,19	19,27	24,00
10,81	39,20	16,93	18,67
25,62	16,81	38,68	16,20
61,52	17,77	57,36	42,25
14,36	22,48	30,65	37,57
49,28	11,24	21,69	31,15
17,21	58,17	47,35	16,56
21,83	40,03	35,22	18,53
21,40	31,92	46,14	34,73
33,56	21,47	51,15	21,79
21,35	31,07	26,36	16,01
27,74	29,34	22,47	19,89
7,76	32,47	28,55	23,78
14,69	53,46	44,61	
52,54	43,72	51,28	
44,96	40,94	29,21	
26,18	42,78	77,65	
12,78	22,72	30,59	
55,15	24,64	62,58	
43,74	13,54	23,26	
	52,36	17,45	
	17,83	29,42	
	33,89	14,97	
	9,62	29,33	
	22,08	32,29	
	65,82	10,59	
	29,71	47,60	
	15,91	22,14	
	13,43	27,73	
	25,45	39,46	
	23,25	27,93	
		21,63	
		69,97	
		11,82	
		48,79	
		13,56	
		26,36	
		22,95	

Starch-water-carrageenan System – Heated after freezing			
M. 1	**M. 2**	**M. 3**	**M. 5**
39,35	36,33	56,04	27,43
22,32	26,55	32,82	26,97
32,72	15,33	28,36	21,57
17,76	43,55	46,71	44,65
15,49	21,15	23,32	40,37
24,26	11,72	37,34	49,52
13,01	27,61	22,33	9,03
23,74	20,85	18,06	59,74
16,44	18,06	14,45	10,76
21,63	10,33	28,96	17,16
56,43	15,72	22,60	24,17
17,94	45,93	47,78	17,82
11,14	17,31	21,20	48,59
16,38	25,78	20,36	51,92
37,63	9,79	22,17	41,56
32,00	18,85	29,67	23,95
9,95	50,13	24,60	51,36
13,51	15,74	19,60	10,12
40,54	27,49	40,25	27,00
28,88	15,77	35,70	46,54
21,71	23,16	25,43	40,57
19,68	33,04	46,31	41,16
37,98	12,03	12,34	25,99
21,74	13,45	28,28	55,68
27,24	38,08	40,86	12,27
50,59	16,78	22,94	72,03
14,16	20,42	41,55	58,46
41,81	49,90	23,05	25,17
16,79	53,89	31,16	33,88
38,07	29,81	13,75	65,63
16,22	21,93	34,87	42,10
49,69	14,82	26,84	43,57
13,89	35,77	20,28	40,88
43,84	30,60	40,28	42,03
11,13	28,35	28,97	24,60
26,97	60,91	25,90	27,35
12,78	19,09	19,19	15,89
21,98	24,36	33,75	32,25
29,61	32,69	20,45	
39,79	20,27	41,97	
45,99	20,73	19,95	
	19,60	21,45	
	22,11	38,38	
	13,74	21,85	
	23,72	20,79	
	21,53	35,87	
	39,05	38,80	
	42,66	32,13	
	23,19	39,90	
	68,35	14,28	
		15,33	
		32,17	
		14,66	
		16,80	
		18,49	
		14,98	
		24,60	
		16,00	
		19,68	
		21,60	
		17,91	
		18,40	
		61,26	
		29,68	
		21,51	
		36,19	
		46,07	
		15,26	
		41,58	
		28,00	
		37,29	
		22,11	
		28,06	
		24,62	
		84,71	
		76,16	
		65,32	

Starch-water-sucrose System – Directly heated			
M. 1	**M. 2**	**M. 3**	**M.4**
20,91	8,04	23,83	22,63
29,18	14,24	19,29	23,23
18,00	15,95	15,82	22,86
47,18	11,49	14,74	16,53
15,02	15,12	23,86	36,21
37,12	13,75	31,50	23,34
11,67	18,84	19,37	57,24
11,31	61,88	31,81	48,73
52,36	35,17	27,21	29,55
25,61	49,63	15,03	22,71
20,83	12,88	32,49	39,64
17,17	66,80	20,17	54,75
29,20	10,72	30,95	26,90
13,98	20,55	13,79	39,61
11,74	20,84	21,23	13,72
11,95	10,38	69,06	19,09
60,46	19,12	24,01	31,27
56,95	10,16	32,78	28,61
47,29	18,26	19,38	33,20
48,09	22,53	23,05	20,62
15,48	29,07	30,93	20,53
46,06	27,19	9,42	38,99
39,39	42,25	33,02	24,75
41,69	23,78	40,38	22,55
15,53	47,39	14,26	54,20
8,80	27,74	52,99	37,04
15,93	21,95	36,12	16,86
10,23	73,35	52,33	52,17
20,58	13,61	22,95	28,40
43,41	52,58	16,73	42,50
55,19	49,34	18,60	21,56
26,71	11,91	19,28	28,18
43,49	46,95	32,88	77,65
21,92	19,16	24,22	20,08
15,71	56,91	34,93	25,77
71,47	55,19	20,43	38,34
16,19	20,49	16,92	19,13
11,04	19,46	19,83	66,38
16,97	21,89	41,03	38,68
9,62	9,79	21,23	28,82
28,83	38,16	43,07	32,88
10,22	13,30	46,91	34,16
9,85	35,35	51,64	41,09
30,44	10,04	45,77	40,61
9,97	47,12	50,87	45,39
52,26	10,38	42,07	32,69
26,46	19,99	29,86	29,25
51,54	56,60	18,77	20,51
56,58	35,15	36,02	36,83
14,27	32,05	25,88	36,85
23,09	24,16	36,48	21,30
40,20	49,67	35,09	30,00
62,66	14,31	33,40	27,57
45,70	11,08	30,09	
24,85	36,10	16,67	
38,84	14,40	27,11	
20,67	28,07	19,55	
10,03	44,83	19,58	
23,55	30,29	49,31	
17,64	26,81		
18,90	51,11		
34,28	13,10		
23,86			
12,03			
16,73			

Starch-water-sucrose System – Heated after freezing

M. 2	M. 4	M. 3	M. 5
45,53	53,32	26,48	49,11
14,57	39,56	25,12	56,26
21,60	13,19	38,08	29,53
9,60	14,69	33,58	26,81
39,04	21,86	21,87	15,26
36,10	19,85	25,65	18,71
25,71	28,05	20,88	37,40
45,16	29,80	25,74	39,77
15,72	20,07	28,23	35,95
20,32	20,31	43,69	36,60
29,06	22,89	27,80	44,75
30,88	33,62	19,99	29,75
14,61	62,50	27,74	43,61
51,05	23,93	19,87	32,72
43,12	31,02	25,77	22,05
25,13	16,82	22,72	57,73
12,95	23,71	18,29	31,05
23,07	56,86	19,82	31,77
13,36	66,36	20,89	31,04
29,10	61,51	40,25	29,87
50,69	18,25	24,15	37,30
27,27	24,70	18,68	33,41
36,13	17,92	22,85	36,83
30,48	25,37	21,54	36,64
36,73	52,78	17,25	54,50
22,98	28,86	30,01	20,45
28,78	22,56	19,60	36,16
12,49	31,58	23,93	
27,53	29,48	18,99	
38,96	36,25	23,39	
18,49	41,99	19,82	
20,44	35,40	35,34	
31,41	58,84	38,80	
51,20	21,79	30,04	
29,89	15,75	53,41	
27,84	5,87	26,74	
13,73	87,93	16,97	
54,93	47,33	14,38	
24,94	29,29	23,15	
17,58	59,11	49,39	
28,14	17,55	23,17	
53,10	57,24	26,18	
33,61	19,38	21,31	
17,29	16,64	26,31	
15,17	82,14	31,53	
22,79	26,95	21,11	
26,02	63,02		
14,51	21,22		
24,09	47,44		
13,93			
46,22			
59,95			
18,70			
34,77			
31,77			
18,33			
20,88			

APPENDIX B: DEGREE OF GELATINIZATION STARCH-WATER SYSTEM DIRECTLY HEATED

M. 1 (40 granules)	
Temperature (°C)	**DG (%) /100**
60,25	0,00
60,75	0,04
61,25	0,05
61,75	0,06
62,25	0,07
62,75	0,08
63,25	0,09
63,75	0,11
64,25	0,13
64,75	0,16
65,25	0,23
65,75	0,27
66,25	0,34
66,75	0,41
67,25	0,47
67,75	0,56
68,25	0,64
68,75	0,71
69,25	0,76
69,75	0,78
70,25	0,82
70,75	0,88
71,25	0,92
71,75	0,95
72,25	0,96
72,75	0,97
73,25	0,97
73,75	0,97
74,25	0,98
74,75	0,98
75,75	0,98
76,25	1,00

M. 3 (66 granules)	
Temperatura (°C)	**DG (%) / 100**
60,24	0,00
60,72	0,02
61,20	0,04
61,68	0,03
62,16	0,07
62,64	0,08
63,12	0,09
63,60	0,11
64,08	0,13
64,56	0,17
65,04	0,24
65,52	0,34
66,00	0,34
66,48	0,36
66,96	0,37
67,44	0,40
67,92	0,47
68,4	0,54
68,88	0,61
69,36	0,68
69,84	0,75
70,32	0,80
70,80	0,85
71,28	0,87
71,76	0,92
72,24	0,96
72,72	0,97
73,20	0,98
73,68	0,99
74,16	0,99
74,64	1,00
75,12	1,00
75,60	1,00

M. 5 (62 granules)	
Temperature (°C)	**DG (%) / 100**
60,24	0,00
60,72	0,02
61,20	0,03
61,68	0,05
62,16	0,06
62,64	0,07
63,12	0,08
63,60	0,10
64,08	0,12
64,56	0,15
65,04	0,19
65,52	0,21
66,00	0,27
66,48	0,33
66,96	0,40
67,44	0,53
67,92	0,60
68,40	0,69
68,88	0,78
69,36	0,85
69,84	0,89
70,32	0,93
70,80	0,95
71,28	0,96
71,76	0,98
72,24	0,99
72,72	0,99
73,20	0,99
73,68	0,99
74,16	0,99
74,64	0,99
75,12	1,00
75,60	1,00
75,84	1,00

M. 7 (33 granules)	
Temperature (°C)	**DG (%) / 100**
60,25	0,00
60,75	0,01
61,25	0,02
61,75	0,04
62,25	0,06
62,75	0,09
63,25	0,12
63,75	0,14
64,25	0,17
64,75	0,21
65,25	0,24
65,75	0,30
66,25	0,37
66,75	0,46
67,25	0,57
67,75	0,68
68,25	0,77
68,75	0,80
69,25	0,85
69,75	0,91
70,25	0,92
70,75	0,92
71,25	0,94
71,75	0,94
72,25	0,95
72,75	0,97
73,25	1,00
73,75	1,00
74,25	1,00

APPENDIX C: DEGREE OF GELATINIZATION STARCH-WATER SYSTEM HEATED AFTER FREEZING

M. 2 (76 granules)	
Temperature (°C)	**DG (%) / 100**
60,25	0,00
60,75	0,01
61,25	0,01
61,75	0,02
62,25	0,03
62,75	0,05
63,25	0,05
63,75	0,06
64,25	0,09
64,75	0,10
65,25	0,12
65,75	0,16
66,25	0,20
66,75	0,24
67,25	0,31
67,75	0,36
68,25	0,41
68,75	0,50
69,25	0,56
69,75	0,65
70,25	0,79
70,75	0,83
71,25	0,86
71,75	0,89
72,25	0,92
72,75	0,93
73,25	0,95
73,75	0,96
74,25	0,98
74,75	0,99
75,25	0,99
77,50	1,00

M. 4 (54 granules)	
Temperature (°C)	**DG (%) / 100**
60,24	0,00
60,73	0,01
61,22	0,02
61,70	0,03
62,19	0,03
62,67	0,03
63,16	0,04
63,65	0,05
64,13	0,05
64,62	0,05
65,10	0,06
65,59	0,06
66,08	0,08
66,56	0,10
67,05	0,12
67,53	0,14
68,02	0,21
68,50	0,29
68,99	0,36
69,48	0,44
69,96	0,56
70,45	0,63
70,93	0,69
71,42	0,75
71,91	0,79
72,39	0,85
72,88	0,90
73,36	0,93
73,85	0,96
74,34	0,97
74,82	0,97
75,31	0,97
75,79	0,98
76,28	0,98
76,77	1,00
77,01	1,00

M. 3 (48 granules)	
Temperature (°C)	**DG (%) / 100**
60,24	0,00
60,72	0,01
61,20	0,01
61,68	0,02
62,16	0,02
62,64	0,03
63,12	0,04
63,60	0,04
64,08	0,04
64,56	0,10
65,04	0,09
65,52	0,10
66,00	0,10
66,48	0,12
66,96	0,16
67,44	0,17
67,92	0,19
68,40	0,21
68,88	0,26
69,36	0,35
69,84	0,39
70,32	0,44
70,80	0,50
71,28	0,60
71,76	0,70
72,24	0,79
72,72	0,87
73,20	0,91
73,68	0,94
74,16	0,98
74,64	1,00
75,12	1,00
75,36	1,00

M. 5 (57 granules)	
Temperature (°C)	**DG (%) / 100**
60,74	0,00
61,23	0,02
61,71	0,02
62,20	0,02
62,68	0,07
63,17	0,08
63,65	0,08
64,14	0,07
64,62	0,07
65,11	0,08
65,59	0,08
66,08	0,10
66,57	0,13
67,05	0,17
67,54	0,21
68,02	0,38
68,51	0,53
68,99	0,58
69,48	0,56
69,96	0,60
70,45	0,68
70,93	0,78
71,42	0,85
71,90	0,89
72,39	0,91
72,87	0,93
73,36	0,95
73,84	0,97
74,33	0,98
74,81	0,98
75,30	0,98
77,00	0,99
78,00	1,00

APPENDIX D: DEGREE OF GELATINIZATION STARCH-WATER-CARRAGEENAN SYSTEM DIRECLTY HEATED

M. 1 (50 granules)		M. 2 (61 granules)	
Temperature (°C)	**DG (%) / 100**	**Temperature (°C)**	**DG (%) / 100**
60,24	0,000	60,24	0,000
60,73	0,012	60,73	0,005
61,22	0,014	61,22	0,036
61,71	0,012	61,70	0,011
62,19	0,017	62,19	0,015
62,68	0,017	62,68	0,012
63,17	0,024	63,16	0,019
63,66	0,020	63,65	0,022
64,14	0,027	64,13	0,026
64,63	0,037	64,62	0,035
65,12	0,045	65,11	0,054
65,61	0,065	65,59	0,063
66,09	0,077	66,08	0,080
66,58	0,095	66,57	0,104
67,07	0,112	67,05	0,137
67,55	0,140	67,54	0,175
68,04	0,172	68,03	0,212
68,53	0,207	68,51	0,247
69,02	0,290	69,00	0,283
69,50	0,424	69,48	0,330
69,99	0,432	69,97	0,388
70,48	0,423	70,46	0,463
70,97	0,497	70,94	0,536
71,45	0,614	71,43	0,613
71,94	0,713	71,92	0,676
72,43	0,806	72,40	0,733
72,92	0,856	72,89	0,797
73,40	0,906	73,38	0,904
73,89	0,938	73,86	0,954
74,38	0,960	74,35	0,971
74,87	0,970	74,84	0,982
75,35	0,979	75,32	0,993
75,84	0,994	75,81	0,996
76,33	0,994	76,29	0,999
76,82	1,000	76,78	1,000

M. 3 (68 granules)	
Temperature (°C)	**DG (%) / 100**
60,93	0,000
61,40	0,007
61,87	0,007
62,34	0,009
62,81	0,011
63,28	0,012
63,74	0,016
64,21	0,037
64,68	0,047
65,15	0,052
65,62	0,060
66,08	0,072
66,55	0,086
67,02	0,104
67,49	0,144
67,96	0,217
68,43	0,318
68,89	0,408
69,36	0,491
69,83	0,566
70,30	0,634
70,77	0,712
71,23	0,769
71,70	0,820
72,17	0,857
72,64	0,896
73,11	0,926
73,58	0,948
74,04	0,971
74,51	0,978
74,98	0,982
75,45	0,988
75,92	0,993
76,38	0,997
76,85	0,999
77,32	1,000

M. 5 (43 granules)	
Temperature (°C)	**DG (%) / 100**
62,54	0,000
63,02	0,005
63,50	0,012
63,97	0,014
64,45	0,021
64,93	0,024
65,41	0,039
65,89	0,065
66,36	0,075
66,84	0,084
67,32	0,093
67,80	0,106
68,28	0,133
68,76	0,171
69,23	0,230
69,71	0,271
70,19	0,319
70,67	0,384
71,15	0,480
71,62	0,578
72,10	0,633
72,58	0,700
73,06	0,758
73,54	0,830
74,02	0,896
74,49	0,933
74,97	0,967
75,45	0,975
75,93	0,988
76,41	0,990
76,89	0,999
77,36	1,000

APPENDIX E: DEGREE OF GELATINIZATION STARCH-WATER-CARRAGEENAN SYSTEM HEATED AFTER FREEZING

M. 1 (41 granules)	
Temperature (°C)	**DG (%) / 100**
60,86	0,00
62,59	0,04
64,32	0,14
66,05	0,13
67,77	0,12
69,50	0,12
70,36	0,17
71,23	0,27
72,09	0,46
72,95	0,68
73,82	0,92
74,68	0,95
75,54	0,98
76,41	0,99
77,27	1,00

M. 3 (77 granules)	
Temperature (°C)	**DG (%) / 100**
61,82	0,00
63,46	0,04
65,10	0,05
66,75	0,08
68,39	0,15
70,03	0,29
70,85	0,37
71,67	0,47
72,49	0,60
73,31	0,65
74,13	0,78
74,95	0,97
75,77	0,99
76,60	0,99
77,42	1,00

M. 2 (50 granules)	
Temperature (°C)	**DG (%) / 100**
60,70	0,00
62,09	0,04
63,48	0,09
64,87	0,07
66,27	0,05
67,66	0,03
69,05	0,09
70,45	0,18
71,14	0,25
71,84	0,42
73,23	0,53
74,62	0,92
75,32	1,00
76,02	1,00
76,71	1,00

M. 5 (48 granules)	
Temperature (°C)	**DG (%) / 100**
63,31	0,00
64,11	0,01
64,92	0,01
65,72	0,03
66,53	0,04
67,33	0,04
68,14	0,06
68,94	0,09
69,75	0,17
70,56	0,18
71,36	0,41
72,17	0,52
72,97	0,70
74,58	0,95
75,39	0,99
76,19	1,00

APPENDIX F: DEGREE OF GELATINIZATION STARCH-WATER-SUCROSE SYSTEM DIRECTLY HEATED

M. 1 (65 granules)	
Temperature (°C)	DG (%) / 100
65,00	0,00
65,79	0,02
66,58	0,03
67,37	0,04
68,16	0,06
68,94	0,07
69,73	0,07
71,31	0,07
72,10	0,09
72,89	0,10
73,68	0,09
76,04	0,19
76,83	0,23
77,62	0,33
78,41	0,46
79,20	0,63
79,99	0,69
80,78	0,76
81,56	0,84
82,35	0,89
83,14	0,95
83,93	0,97
84,72	1,00
85,51	1,00

M. 1 (62 granules)	
Temperature (°C)	DG (%) / 100
65,00	0,00
65,87	0,02
66,74	0,06
69,34	0,10
70,21	0,11
71,07	0,11
71,94	0,14
72,81	0,16
73,68	0,19
74,11	0,21
74,55	0,24
74,98	0,28
75,41	0,32
75,85	0,33
76,28	0,34
76,72	0,37
77,58	0,40
78,02	0,43
78,45	0,47
78,88	0,50
79,32	0,54
80,19	0,54
81,05	0,59
81,92	0,67
82,36	0,79
83,22	0,83
83,66	0,85
84,09	0,88
84,53	0,93
84,96	0,96
85,39	0,98
85,83	0,99
86,26	0,99
86,69	1,00

M. 3 (59 granules)	
Temperature (°C)	**DG (%)**
65,00	0,00
66,50	0,00
68,00	0,01
69,50	0,02
70,25	0,05
71,00	0,06
76,25	0,07
77,00	0,14
77,75	0,19
78,50	0,26
79,25	0,42
80,00	0,63
80,75	0,72
81,50	0,80
82,25	0,86
83,00	0,93
83,75	0,96
84,50	0,97
85,25	1,00
86,00	1,00

M. 4 (53 granules)	
Temperature (°C)	**DG (%)**
65,68	0,00
66,36	0,02
67,71	0,03
70,43	0,04
71,79	0,05
73,14	0,07
75,18	0,15
75,86	0,19
76,54	0,24
77,22	0,31
79,25	0,34
79,93	0,52
80,61	0,64
81,29	0,77
81,97	0,90
82,65	0,98
83,32	0,99
84,00	1,00
84,68	1,00

APPENDIX G: DEGREE OF GELATINIZATION STARCH-WATER-SUCROSE SYSTEM HEATED AFTER FREEZING

M. 2 (57 granules)	
Temperature (°C)	**DG (%) / 100**
65,00	0,00
71,38	0,04
72,65	0,05
73,93	0,07
75,20	0,11
76,48	0,15
77,75	0,21
78,39	0,25
79,03	0,32
79,66	0,38
81,58	0,43
82,85	0,48
83,49	0,54
84,13	0,68
84,76	0,79
85,40	0,86
86,04	0,94
86,68	0,97
87,31	0,99
87,95	1,00

M. 4 (49 granules)	
Temperature (°C)	**DG (%) / 100**
65,00	0,00
66,59	0,02
68,97	0,04
72,94	0,08
74,53	0,10
76,12	0,17
77,71	0,24
79,29	0,32
80,09	0,39
81,68	0,50
82,47	0,65
83,26	0,67
84,06	0,71
84,85	0,78
85,65	0,83
86,44	0,88
87,23	0,95
88,03	0,97
88,82	0,98
89,62	1,00
90,41	1,00

M. 3 (46 granules)	
Temperature (°C)	**DG (%) / 100**
65,50	0,00
66,83	0,01
70,14	0,07
70,80	0,08
71,46	0,08
74,78	0,11
78,75	0,18
80,08	0,21
82,73	0,29
85,38	0,38
86,71	0,55
87,37	0,78
88,03	0,84
88,69	0,90
89,36	0,93
90,02	0,98
90,68	1,00

M. 5 (27 granules)	
Temperature (°C)	**DG (%) / 100**
66,00	0,00
67,68	0,03
71,03	0,08
71,87	0,08
72,71	0,07
75,23	0,13
77,74	0,19
80,26	0,25
81,10	0,39
81,94	0,56
82,77	0,65
83,61	0,83
84,45	0,88
85,29	0,96
86,13	1,00
86,97	1,00