



PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE  
ESCUELA DE INGENIERIA

**ON LINE IN SITU EXAMINATION:  
UNDERSTANDING MICROSTRUCTURAL  
CHANGES OF STARCH DURING  
ATMOSPHERIC AND VACUUM  
GELATINIZATION**

**NICOLÁS OVALLE DE LA FUENTE**

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 A.**

Santiago de Chile, June, 2012

© 2012, Nicolás Ovalle



PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE  
ESCUELA DE INGENIERIA

# **ON LINE IN SITU EXAMINATION: UNDERSTANDING MICROSTRUCTURAL CHANGES OF STARCH DURING ATMOSPHERIC AND VACUUM GELATINIZATION**

**NICOLÁS OVALLE DE LA FUENTE**

Members of the Committee:

**PEDRO BOUCHON A.**

**EDUARDO AGOSIN T.**

**VILBETT BRIONES L.**

**MARCELO ARENAS S.**

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

Santiago de Chile, June, 2012

To my family

## ACKNOWLEDGMENTS

I would like to express my sincerest gratitude to all the people that have supported and encouraged me during this time; specially my friends.

I would like to thank specially to Dr. Pedro Bouchon, my advisor, for all his support during the development of this research.

I would also like to express my gratitude to all the staff working at the Department of Chemical and Bioprocess Engineering, especially to Mrs. María Inés Valdebenito, for all her support and administrative help during this time.

My special gratitude to my partners and friends at the lab, Verónica Dueik, Mariel Farfán, Carolina Moreno, Salomé Mariotti, Loreto Muñoz, Pablo Cortés and Olajide Sobukola for their advice, support and friendship.

I would like to express my biggest gratitude to my family. Especially, I would like to thank them for their unconditional support through my whole life and during this time.

Finally I would like to thank the financial support from Fondecyt project number 1100851.

## GENERAL INDEX

Dedication .....	ii
Acknowledgements .....	iii
Table Index.....	vii
Figure Index .....	viii
Abstract .....	xi
Resumen .....	xi
1. Introduction .....	xii
1.1 Starch.....	2
1.1.1. Structure .....	2
1.1.2.Starch gelatinization .....	4
a) Differential Scanning Calorimetry (DSC) .....	5
b) Microscopy .....	6
c) Enzymatic procedures.....	8
1.2. Deep-fat frying .....	13
1.2.1. Atmospheric deep-fat frying.....	13
1.2.2. Vacuum deep-fat frying.....	16
1.3. Food process miniaturization: a vehicle to understand food dynamics.....	18
1.4. Image Analysis .....	20
2. Hypothesis .....	23
3. Objectives .....	24
4. Materials and Methods .....	25
4.1. Materials .....	25
4.2. Sample preparation .....	25

4.2.1. Isolated granules in excess of water .....	25
4.2.2. Isolated granules in solutions of pre-gelatinized corn starch .....	25
4.2.3. Starch granules in starch-gluten-water matrices.....	26
4.3. Experimental Setup.....	26
4.4. Experimental conditions .....	28
4.4.1. Atmospheric conditions.....	28
4.4.2. Vacuum conditions .....	28
4.5. Image acquisition and analysis .....	29
4.5.1. Atmospheric heating.....	29
4.5.2. Vacuum heating .....	37
4.6. Statistical Analysis .....	38
5. Results and Discussions .....	39
5.1. Gelatinization of isolated potato starch granules in excess of water.....	39
5.1.1. Atmospheric heating.....	39
5.1.2. Vacuum Heating .....	43
5.1.2.1. Water boiling point of 38 °C .....	44
5.1.2.2. Water boiling point of 69 °C .....	45
5.2. Gelatinization of isolated potato starch granules in pre-gelatinized corn starch solutions .....	49
5.2.1. Atmospheric heating.....	49
5.2.1.1. Diluted pre-gelatinized corn starch solution (3 % concentration) 49	
5.2.1.2. Diluted pre-gelatinized corn starch solution (5 % concentration) 51	
5.2.2. Vacuum Heating .....	56
5.2.2.1. Water boiling point of 38 °C .....	56
5.2.2.2. Water boiling point of 69 °C .....	57

5.3. Gelatinization of potato starch granules in a starch-gluten-water matrix.....	60
5.3.1. Atmospheric frying.....	60
5.3.2. Vacuum frying.....	67
5.3.2.1. Water boiling point of 38 °C .....	67
5.3.2.2. Water boiling point of 69 °C .....	68
6. Conclusions .....	71
References .....	74
Appendixes.....	82
Appendix A: Moisture content measurement of building blocks .....	83
Appendix B: Relationship between frame and temperature .....	84
B.1. Heating rate: 5 °C/min .....	84
B.2. Heating rate: 10 °C/min .....	85
B.3. Heating rate: 15 °C/min .....	86
Appendix C: Granule size distribution .....	87
Appendix D: Detail of on-set, ending and range of gelatinization temperatures heated at 5, 10 and 15 °C/min in excess of water .....	88
D.1. Heating rate: 5 °C/min.....	88
D.2. Heating rate: 10 °C/min.....	90
D.3. Heating rate: 15 °C/min.....	92

## TABLE INDEX

<b>Table 1.1:</b> Gelatinization temperature (°C) of different starches.....	10
<b>Table 4.1:</b> Experimental Design.....	29
<b>Table 5.1:</b> On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules heated in excess of water at 5, 10 and 15 °C/min .....	41
<b>Table 5.2:</b> On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules in a 3 % pre-gelatinized corn solution when heated at 5, 10 and 15 °C/min.....	50
<b>Table 5.3:</b> On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules heated in a 5 % pre-gelatinized corn solution at 5, 10 and 15 °C/min...52	
<b>Table 5.4:</b> Gelatinization temperature (°C) of isolated potato starch granules heated at 5, 10 and 15 °C/min in excess of water, 3 % and 5 % corn pre-gelatinized solutions.....	56
<b>Table 5.5:</b> On-set, ending and mean gelatinization temperatures (°C) of potato starch granules in a starch-gluten-water matrix (50 % water content) heated in oil at 5, 10 and 15 °C/min.....	61
<b>Table 5.6:</b> On-set, ending and mean gelatinization temperature (°C) of potato starch granules in a starch-gluten-water matrix (40% water) heated in oil at 5, 10 and 15 °C/min.....	62
<b>Table 5.7:</b> On-set, ending and gelatinization range of potato starch granules heated at 5, 10 and 15 °C/min in excess of water and in a starch-gluten-water matrix (40 % water content).....	65



## FIGURE INDEX

<b>Figure 1.1:</b> Chemical representation of an amylose molecule .....	3
<b>Figure 1.2:</b> Chemical representation of an amylopectin molecule.....	4
<b>Figure 1.3:</b> Representative DSC profile for potato starch granules heated at 10 °C/min. 6	
<b>Figure 1.4:</b> Representation of the polarizing mechanism. ....	7
<b>Figure 1.5:</b> State diagram of wheat starch. ....	13
<b>Figure 1.6:</b> Schematic representation of microstructural properties of some food building blocks at different temperatures.....	14
<b>Figure 1.7:</b> Representation of an image as a matrix array. ....	21
<b>Figure 4.1:</b> Experimental Setup. ....	27
<b>Figure 4.2:</b> Close up of the heating unit.....	27
<b>Figure 4.3:</b> Sequence of the main steps needed to obtain quantitative information. ....	31
<b>Figure 4.4:</b> Image of isolated potato starch granules acquired with a digital camera....	32
<b>Figure 4.5:</b> Resultant image after equalizing its light and contrast.....	33
<b>Figure 4.6:</b> Equalized image after applying a median filter (3x3).....	33
<b>Figure 4.7:</b> Filtered image after applying a sharpening filter (3x3).....	34
<b>Figure 4.8:</b> Histogram of the image of Figure 4.7. ....	35
<b>Figure 4.9:</b> Binarized image.....	35
<b>Figure 4.10:</b> Determination of the on-set gelatinization temperature of isolated potato starch granules heated in excess of water .....	36
<b>Figure 4.11:</b> Determination of the ending gelatinization temperature of isolated potato starch granules heated in excess of water. ....	37
<b>Figure 5.1:</b> Images of the gelatinization process of isolated potato starch granules heated in excess of water at 10 °C/min. ....	40

<b>Figure 5.2:</b> Gelatinization degree of isolated potato starch granules heated in excess of water at 5, 10 and 15 °C/min.....	43
<b>Figure 5.3:</b> Image gallery of a vacuum-heated (water boiling point of 38 °C) potato starch granule at 10 °C/min.....	45
<b>Figure 5.4:</b> Isolated potato starch granules in excess of water vacuum-heated at 15 °C/min and 300 mbar (water boiling point of 69 °C) . ....	46
<b>Figure 5.5:</b> Percentage of granules that either completed or started the gelatinization process (%G), and did not gelatinized (%NG) when vacuum-heating at 15 °C/min and 300 mbar (water boiling point of 69 °C up to 70, 80 and 90 °C.. ....	48
<b>Figure 5.6:</b> Degree of gelatinization of isolated potato starch granules heated in a 3 % pre-gelatinized corn solution when heated at 5, 10 and 15 °C/min. ....	50
<b>Figure 5.7:</b> Images of the gelatinization process of isolated potato starch granules in a solution of 5% pre-gelatinized corn starch at 15 °C/min. ....	51
<b>Figure 5.8:</b> Degree of gelatinization of isolated potato starch granules in a 5 % solution of pre-gelatinized corn starch when heated at 5, 10 and 15 °C/min. ....	53
<b>Figure 5.9:</b> Degree of gelatinization of isolated potato starch granules heated at (a) 5°C/min (b) 10°C/min and (c) 15°C/min in excess of water, 3 and 5 % corn pre-gelatinized starch solutions. ....	55
<b>Figure 5.10:</b> Vacuum-heated isolated potato starch granules in a pre-gelatinized corn solution at 3 % at a working pressure of 300 mbar (boiling point of water of 69 °C).....	58
<b>Figure 5.11:</b> Percentage of granules that are gelatinizing (%G) and that have not even start the gelatinization process (%NG) in vacuum conditions (300 mbar, water boiling point of 69 °C), at 80, 90 and 100 °C.....	59
<b>Figure 5.12:</b> Images of the gelatinization process of isolated potato starch granules heated in a starch-gluten-water matrix (40% water content). ....	63
<b>Figure 5.13:</b> Degree of gelatinization of isolated potato starch granules in a starch-gluten-water matrix (water content of 40%) heated at 5, 10 and 15 °C/min. ....	64

<b>Figure 5.14:</b> Degree of gelatinization of isolated potato starch granules heated in excess of water and in oil in a starch-gluten-water matrix (water content of 40%) at (a) 5°C/min (b) 10°C/min and (c) 15°C/min.....	66
<b>Figure 5.15:</b> Gallery of images of a starch-gluten-water matrix vacuum-fried in oil at 65 mbar.....	68
<b>Figure 5.16:</b> Structure of a starch-gluten-water matrix (40% water content) vacuum-heated in oil with a water boiling point of 69 °C.....	70

## ABSTRACT

Scientific knowledge generation is a key challenge in the manufacture of formulated products, to be able to satisfy emerging consumer trends towards healthier foods. In accordance, the main objective of this research was to understand the effect of water availability and processing conditions (temperature and vacuum level) in the microstructural changes that occur in potato starch granules heated in water and oil *in situ* and in real time to improve scientific knowledge in the new growing field of formulation engineering. Samples of isolated potato starch granules were heated in excess water and in two solutions of maize pre-gelatinized starch (3 and 5% w.b.). Then, matrices of starch-gluten-water with moisture contents of 40 and 50% were formulated and heated in oil. The microstructural changes undergone by starch granules were followed in a temperature and vacuum controlled stage under the lens of a polarized light microscope. Three heating rates (5, 10 and 15 °C/min) were used for atmospheric conditions. In addition, two vacuum conditions, leading to two different water boiling points (38 and 69 °C), were used in vacuum experiments. Heating rate increased resulted in a higher on-set gelatinization temperature which was determined as the loss of birefringence of the granules. Also, as the amount of water present in the system decreased, a higher on-set gelatinization temperature was observed. This could happen due to the competition for available water between the un-gelatinized starch and the other solutes (pre-gelatinized corn starch or gluten). For high vacuum experiments (water boiling point of 38 °C) no significant changes in the granules were observed, since there was not enough water in the system when the granules reached the gelatinization temperature. For lower vacuum experiments (water boiling point of 69 °C) some gelatinized and un-gelatinized granules were observed. This could be due to water evaporation as gelatinization occurred. Overall, this microstructural approach could help explaining why vacuum-fried starchy formulated products absorb more oil than atmospheric fried ones.

**Keywords:** hot-stage microscopy, starch gelatinization, atmospheric frying, vacuum frying, formulated products, microscopy, image analysis.

## RESUMEN

La generación de conocimiento científico en la fabricación de productos formulados es uno de los grandes desafíos de la industria alimentaria para satisfacer las tendencias de los consumidores por alimentos más sanos. El objetivo de este trabajo es entender el efecto que tiene la limitación de temperatura y agua, así como el efecto de la temperatura de ebullición del agua ( $T_{eb}$ ) (como consecuencia del procesamiento a vacío) en tiempo real en los cambios que sufre el almidón de papa, miniaturizando el proceso de fritura bajo el lente de un microscopio. Para esto, se calentó muestras de almidón aislado de papa en exceso de agua y en dos soluciones con almidón pre-gelatinizado de maíz (3 y 5 %). Luego, se formuló dos matrices de almidón-gluten-agua con contenidos de humedad de 40 y 50 % las que fueron calentadas en aceite. Los cambios microestructurales fueron seguidos en un microscopio de luz utilizando luz polarizada, controlando el proceso mediante el uso de una platina con control de temperatura y presión. Se utilizó 3 tasas de calentamiento (5, 10 y 15 °C/min) para los estudios a presión atmosférica y dos condiciones de vacío, definiendo dos puntos de ebullición, uno por bajo ( $T_{eb} = 38$  °C) y otro por sobre la temperatura de gelatinización ( $T_{eb} = 69$  °C). Se comprobó que a medida que aumenta la tasa de calentamiento y disminuye la cantidad de agua se obtiene una mayor temperatura de gelatinización. Esto se puede deber a una mayor competencia por el agua disponible entre el almidón no gelatinizado y los otros solutos (almidón pre-gelatinizado o gluten). En los experimentos con alto vacío ( $T_{eb} = 38$  °C) no se observó cambios significativos en la estructura del almidón. Esto puede atribuirse a que no hay suficiente agua cuando se alcanza la temperatura de gelatinización. Por otro lado, en los experimentos con un menor vacío ( $T_{eb} = 69$  °C) se observó la presencia de gránulos gelatinizados y no gelatinizados. Esto puede explicarse pues a medida que los gránulos gelatinizan parte del agua se evapora, por lo que no hay suficiente agua para poder completar la gelatinización. Este enfoque microestructural podría explicar el porqué productos amiláceos formulados absorben más aceite cuando son fritos a vacío en comparación con aquellos fritos en condiciones atmosféricas.

## 1. INTRODUCTION

In the last decades, the food industry has been challenged with new tasks. Consumers are more demanding. They desire to eat healthier and safer foods, but with adequate sensory attributes since they are not willing to sacrifice organoleptic properties (Garayo & Moreira, 2002). This has led researches and producers to study novel trends, processes and ingredients to develop new structures that may fulfill consumers' demands. Specifically, the snack industry is looking for new options to produce low-fat snacks.

A possible path to create new structures is through product formulation. To be able to design these new foods it is of paramount importance to understand the properties of the different ingredients (*e.g.* starch, gluten) and their possible interaction. However, in foods, most of the critical processes and transformations, such as transport properties, textural or rheological behavior occur in a scale range which is below 70  $\mu\text{m}$  (Wayne, 2008) and cannot be seen with the naked eye (Aguilera, 2005). Also, the main structural ingredients, also known as food building blocks, occur in this length scale. For this reason, it is critical to use appropriate tools that help understanding the main changes occurring during processing. Different microscopy techniques have allowed researchers to understand the microstructure of several food materials. Related to this, Aguilera (2005) describes food design (or food structure engineering) as the generation of food microstructure based on fundamental scientific knowledge.

As described above, the food industry is looking to fulfill consumer trends for low-fat and healthier products. A widely used operation in the snack industry to develop ready to eat products is deep-fat frying. In this process the main changes and properties, such as oil absorption and microstructure development, occur in the micrometric length scale. Thus, studying the microstructural changes in formulated products at the micro-scale could be an adequate technique to understand the main changes associated to the process. Through product formulation, properties such as lower oil absorption may be

achieved. Advantages of using specific food building blocks to create controlled formulated matrices include their reproducibility, uniformity and lack of defects.

It would be desirable to design a low-fat fried product with just enough fat to impart the desired quality attributes of deep-fried food (Gazmuri & Bouchon, 2009). To be able to design these new snacks it is important to understand the properties of starch, since starchy food represents an important product category. This includes the understanding of factors that may affect its gelatinization temperature as well as its microstructural changes during processing and storage, to comprehend the relationship between macro- (finished-product) and micro-properties.

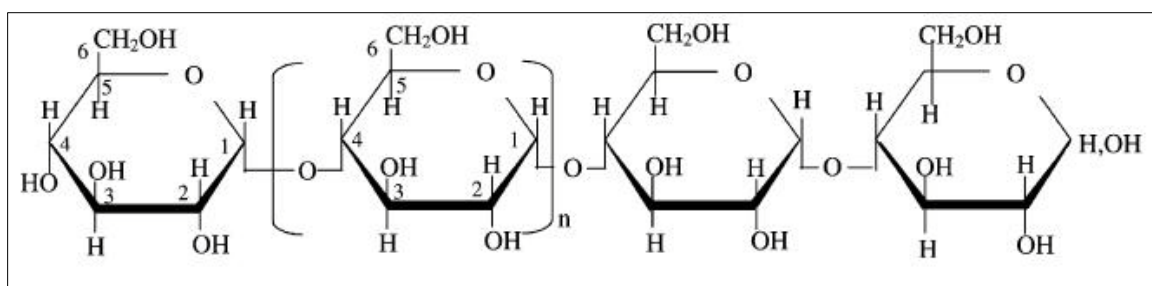
## **1.1 Starch**

Starch is the most common carbohydrate polymer in food. It is present in nature in diverse kinds of plant tissues, such as tubers (*e.g.* potato and cassava), roots, peas, cereals (*e.g.* wheat, corn and rice), among others (Eliasson & Gudmundsson, 1996; Flint, 1994). Their physical and thermal properties (morphology, gelatinization temperature, swelling, among others) vary depending on the botanical nature of starch.

### **1.1.1. Structure**

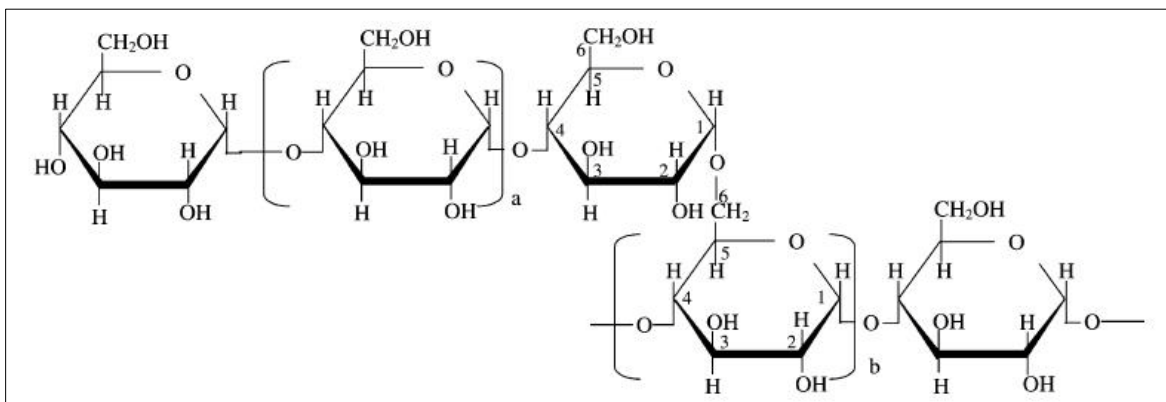
According to the source of the starch, it presents a particular shape, size and granule size distribution. The size of the granules is generally measured as their average diameter. However, it can be also measured as the average length between the major and minor axes, by means of the Feret diameter (*i.e.* diameter of a circumference that has the same area of the object), among others (Lindeboom, et al., 2004). For instance, potato starch granules have a long oval shape (Buleón, et al., 1998), a unimodal granule distribution (Tester, et al., 2004) with a granule mean size of approximately 30  $\mu\text{m}$  (Karlsson & Eliasson, 2003). Meanwhile, wheat starch has a spherical shape, a bimodal distribution

with two types of starch granules: large ones, which have a mean diameter ranging between 15 – 35  $\mu\text{m}$  and small ones, which have a granule size between 2 – 10  $\mu\text{m}$  (Tester, et al., 2004). The main uses of starch in the food industry are as a thickener, a gelling and a water retention agent, among others (Singh, et al., 2007). Starch is formed by  $\alpha$ -D-glucose, giving rise to the two main polymers that form starch: amylose and amylopectin, whose chemical representations are shown in Figures 1.1 and 1.2. Amylose is a linear polymer that consists in a 99 % of  $\alpha$  1 $\rightarrow$ 4 bonds (Buleón, et al., 1998) with very little branches due to  $\alpha$  1 $\rightarrow$ 6 bonds. Amylopectin is larger than amylose and consists of  $\alpha$  1 $\rightarrow$ 4 bonds with frequent branches due to  $\alpha$  1 $\rightarrow$ 6 bonds (Bertoft, 2004). Amylose and amylopectin are bound by hydrogen bonding and create a semi-crystalline structure (Eliasson & Gudmundsson, 1996; Singh, et al., 2003). The amorphous zones, which are disordered regions, are associated with the amylose molecules (Svihus, et al., 2005), while the crystalline zones, that are ordered regions, are represented by the amylopectin molecules (Singh, et al., 2003). The degree of cristallinity varies according to the botanical source of the starch. The amylopectin molecules form double helices and the amylose can be found dispersed between the crystalline regions. These regions (the amorphous and the crystalline) have a radial orientation, resulting from a high degree of molecular orientation, and grow in concentric rings, starting at the hilum, which may be in a central or in an eccentric position (Flint, 1994), ending at the surface of the granule (Aguilera & Stanley, 1999).



**Figure 1.1:** Chemical representation of an amylose molecule (Tester, et al., 2004).





**Figure 1.2:** Chemical representation of an amylopectin molecule (Tester, et al., 2004).

### 1.1.2. Starch gelatinization

Starch granules are insoluble in water at room temperature. In the presence of adequate temperature and water, the crystalline structure is disrupted, allowing the amorphous regions to become more accessible to absorb water and swell. This phenomenon is known as starch gelatinization. The swelling and gelatinization of starch granules can occur since the water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of the amylose and amylopectin molecules (Singh, et al., 2003). The gelatinization starts at the hilum of the granules and occurs first in the amorphous regions, since the hydrogen bonding is weakened earlier. Afterwards it occurs in the crystalline regions. The granule goes from a semi-crystalline structure to a disordered one.

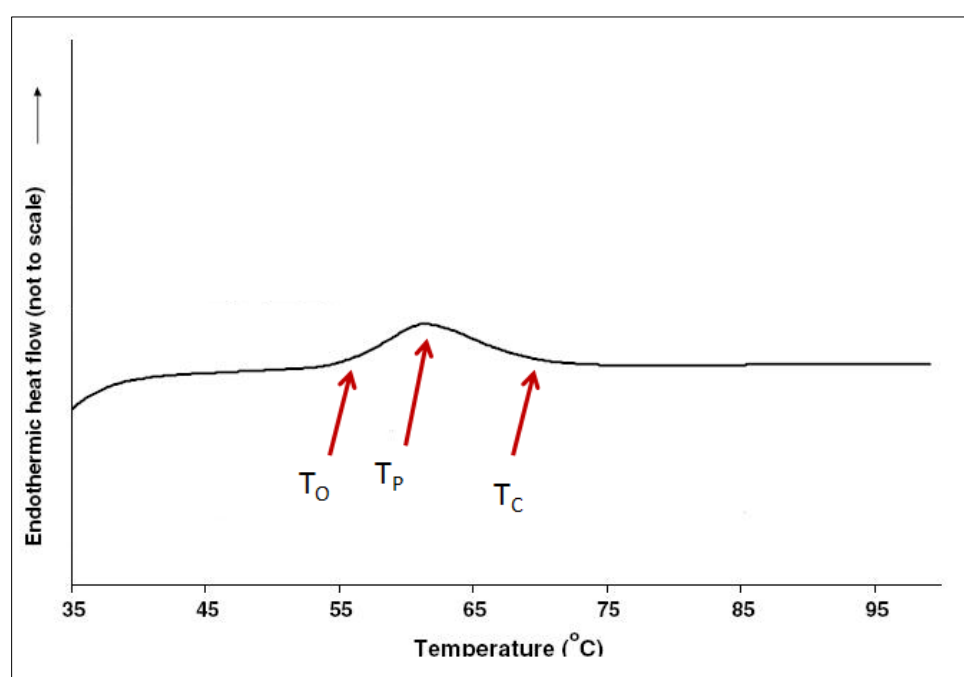
Starch gelatinization is not a phenomenon that occurs instantly. It occurs in a range of temperature and many irreversible changes may occur simultaneously (Fukuoka, et al., 2002; Ratnayake & Jackson, 2007). Thus, the gelatinization temperature is defined as a range of temperature, where the changes suffered by starch take place. This range of temperature is specific for the different starches, according to their botanical source.

Some of these changes are swelling, melting, loss of cristallinity (that can be observed as loss of birefringence under the lens of a polarized microscope), increase of viscosity of the suspension and leaching of amylose from the granules when the starch granules are fully hydrated. The absorption of water by the granules is often measured as the degree of their swelling (Eliasson & Gudmundsson, 1996). In frying starch gelatinization is very important, since it holds water and gives volume expansion (Chen, et al., 2001).

Starch gelatinization has been widely studied, due to its great use in the food and non-food industry. The most common methods to determine starch gelatinization are described below:

**a) Differential Scanning Calorimetry (DSC):** This is the most used technique to measure starch gelatinization and other thermal properties of starch (Biliaderis, et al., 1986). The objective of this technique is to measure the change in the heat flow rate of a specific sample respect to a reference while they are submitted to a controlled temperature condition (Höhne, et al., 2003). In this analysis the samples are heated at a constant rate (*e.g.* 10 °C/min) and when a desired temperature is achieved, this temperature may remain constant. According to Donovan (1979), with this technique the first order transitions (melting) and second order transitions (glass transition) may be detected and provides information of paramount importance related to the order and disorder of the starch structure. When performing this technique, information about the on-set temperature, which is associated to a second order transition (from a crystalline structure to a disordered one), the peak temperature, and the conclusion temperature, which is achieved when all the granules have completed the gelatinization process and the enthalpy of gelatinization, can be obtained. The on-set temperature is achieved when the first difference in the heat flow is detected. The peak temperature is achieved when all the granules from the bulk initiated the gelatinization process (Bogracheva, et al., 2006) Figure 1.3 represents a typical profile curve obtained by DSC for the gelatinization of potato starch granules

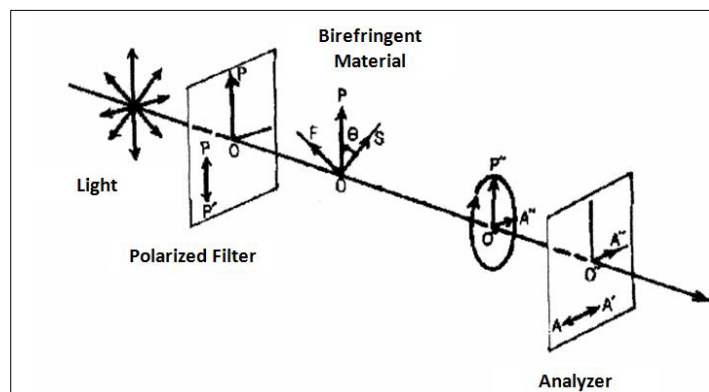
indicating the on-set temperature (the first granules that begin the process), the peak temperature (all the granules started the process) and the conclusion temperature (all the granules ended the gelatinization process). From this image it is possible to observe that the heat flow is positive, since the granules need to absorb heat to gelatinize.



**Figure 1.3:** Representative DSC profile for potato starch granules heated at 10 °C/min.  $T_O$ ,  $T_P$  and  $T_C$  represent the on-set, peak and conclusion gelatinization temperature respectively (adapted from Ratnayake and Jackson, 2007).

**b) Microscopy:** Many studies about starch gelatinization have been performed using polarized light microscopy (Bouchon & Aguilera, 2001; Brunnschweiler, et al., 2005; Parada, 2010; Varatharajan, et al., 2010; Zarguili, et al., 2006). Polarized light microscopy consists in forcing the light to travel in a

unidirectional way (by placing a polarized filter after the beam of light). The second filter (also called analyzer) is placed perpendicularly to the light plane formed, so the light cannot pass through it and extinguishes. If crystalline or semi-crystalline structures are studied using this technique, they can be observed, since they are able to rotate the incident beam of light. Due to incident light rotation, a parallel light component reaches the analyzer, which makes these objects visible. Figure 1.4 shows a schematic representation of the polarized light theory. When studying starch gelatinization under the lens of a polarized light microscope, the un-gelatinized starches show birefringence, since they are semi-crystalline structures. This property is represented by a Maltese cross (Aguilera & Stanley, 1999; Eliasson & Gudmundsson, 1996; Flint, 1994). When the on-set gelatinization temperature is achieved, the birefringence starts to disappear, indicating that the ordered structure is being disrupted. Due to structure disorder, the light starts to extinguish, since the light no longer has a parallel component to the analyzer.



**Figure 1.4:** Representation of the polarizing mechanism. The light is forced to come out forming a unidirectional light plane (P in the figure). Above the objective, an analyzer filter is placed, in a perpendicular position. The light cannot pass through and it extinguishes (adapted from Bouchon, 2010).

This process occurs in a range of temperature which is specific for each granule and can be associated with the granule size (Eliasson & Gudmundsson, 1996). The studies under polarized light have allowed determining the gelatinization temperature of the starch when submitted to different heat treatments and conditions (Aguilera, et al., 2001; Bouchon & Aguilera, 2001). This technique also allows performing a dynamic study of the changes that suffer starch when heated in different water and temperature conditions. In this sense, some properties like loss of birefringence and swelling can be determined using image analysis and extract quantitative information related to the morphologic properties of the starches. However, the literature does not give much information about examination of real time *in situ* gelatinization.

The changes that occur in starch gelatinization have also been studied using scanning electron microscopy (SEM). With this technique, some information about the growing pattern of the granules has been achieved, as well as some qualitative information about the structure of the granules before and after the gelatinization process (Bouchon & Pyle, 2004; Eliasson & Gudmundsson, 1996; Sobukola, et al., 2012).

**c) Enzymatic procedures:** Starch granules in their native form cannot be degraded by amylases. However, when the crystalline structure is disrupted, *e.g.* when heating the granules, amylases can degrade the chains into different maltose units. Using this concept, the gelatinization temperature of different starches and their degree of gelatinization can be determined. Sullivan and Johnson (1964) determined the gelatinization temperature of diverse starches (*e.g.* wheat, maize and potato) using this method and compared their results with those obtained using polarized microscopy. Solutions of 1 % of native starch were suspended in distilled water and heated up to 90 °C at 1.5 °C/min, and samples were extracted every 5 °C (from 55 to 90 °C). Once the samples were at room temperature, the solutions were treated with  $\beta$ -amylase for three hours. When the time was

completed, the amount of maltose was measured. They noticed that the amount of maltose reached a constant value after three hours and they determined the degree of gelatinization of the starches as the grams of maltose obtained per grams of starch treated. Results were consistent with the ones obtained using polarized light microscopy.

Starch gelatinization is a phenomenon that depends on different parameters, such as water availability, botanical source, chemical and/or physical treatments, among others. In accordance, the main factors that influence starch gelatinization are:

**Water content:** The amount of water and its availability is critical in starch gelatinization. If the amount of water is not adequate, the phenomenon cannot occur. To probe this hypothesis, Aguilera et al. (2001) heated isolated potato starch granules in oil under the lens of a polarized microscopy up to 180 °C without obtaining a significant change. Wang et al. (2010) studied the effect of vacuum freeze drying and vacuum microwave drying on potato slices. They did not observe any significant difference in the granules structure after the process. They attributed this behavior to the absence of water in the system. In the presence of solutes (such as sugar or salt), which are able to interact with water, the competition for water between the starch and the solutes retards the gelatinization process (Eliasson, 1983). In order to understand the effect of other components on starch gelatinization some authors (Bouchon & Aguilera, 2001; Karlsson & Eliasson, 2003) heated isolated potato starch granules in excess water and compared the results with the obtained heating potato tissue. They observed that an increase in the gelatinization temperature took place when the granules were in potato tissue. They proposed that this phenomenon was due to the competition for the available water between starch and the other compounds of potato cells. Also, water availability reduction makes starch gelatinization be retarded and may also result in a broader gelatinization temperature range (Gazmuri & Bouchon, 2009; Liu, et al., 2006; Ratnayake & Jackson, 2007; Svihus, et al., 2005). Fukuoka et al.

(2002) studied the effect of moisture content reduction on the gelatinization temperature of wheat starch by DSC. They concluded that, when decreasing the moisture content, a higher gelatinization temperature was reported. According to this, the degree of gelatinization of a starchy product will depend on temperature and water availability in the system (Primo-Martin, et al., 2007).

**Botanical source:** Starch gelatinization varies depending on the botanical source of the starch, since its morphological structure and its physicochemical properties differ from one to another (Mishra & Rai, 2006; Singh, et al., 2003). In Table 1.1 the range of gelatinization temperature of different kinds of starches, measured by DSC, is shown in order to observe the differences in the gelatinization temperature between different botanical sources. Some of the differences that may influence an increase or decrease in the gelatinization temperature of different starches are the ratio amylose/amylopectin, the presence of other components in different starches, such as phosphate groups in potato starches and lipids in maize starches (Singh et al., 2003), which vary the physicochemical properties such as the swelling power and solubility of the starches from different cultivars, significantly.

**Table 1.1:** Gelatinization temperature (°C) of different starches (adapted from Singh et al., 2003).

Botanical Source	Gelatinization Temperature (°C)
Potato	59 – 68
Maize	70 – 78
Rice	63 – 84
Wheat	55 – 67

**Chemical and physical modification:** In order to tailor starch for specific food applications and give them novel characteristics, some starches are modified (Tester, et al., 2004; Varatharajan, et al., 2010). This is relevant for the food industry, since some desired results may be obtained in order to fulfill different consumer needs, such as lower glycemic response due to the presence of un-gelatinized starch. Changes can be associated to chemical and physical transformations, such as pre-gelatinization, acetylation, cross linking, heat/moisture treatment, among others (Zavareze & Dias, 2011).

Pre-gelatinized starch has been used to produce soluble starch in cold water (Adedokun & Itiola, 2010). This physically modified starch has been used in order to avoid the use of heat in some processes. In accordance, this kind of starch is mainly used in foods as a thickener and adhesive (Anastasiades, et al., 2002).

The acetylation consists in the substitution of the hydroxyl groups for acetyl groups. This substitution results in a decrease in the gelatinization temperature, due to a weakening in the bonding of the molecules. This causes an early rupture of the amylopectin double helices. Also, an increase in solubility and in viscosity when heated has been reported (Singh, et al., 2004).

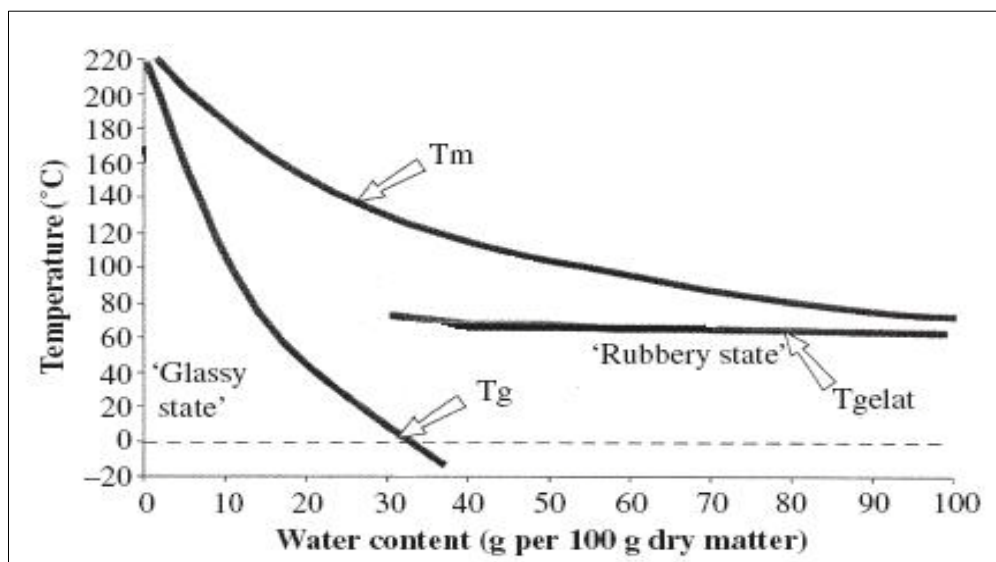
Cross linking refers to the bonding of two hydroxyl groups from the starch molecule. This gives rise to a stronger molecule (Luo, et al., 2009). When cross linking starches, a higher gelatinization temperature is observed, since the mobility of the amorphous zones of the starch is reduced due to the bridging of amylose molecules (Liu, et al., 1999).

The heat/moisture treatment induces heating the starch at a temperature above its gelatinization temperature (above 100 °C) with an un-sufficient amount of water (below 30 %) (Hoover & Manuel, 1996; Jiranuntakul, et al., 2011; Singh, et al., 2003). This physical treatment precludes gelatinization, increasing gelatinization temperature of starch, broadening the temperature range. Donovan et al. (1979)



proposed that this may be due to the creation of new crystalline zones or due to the re-crystallization of small crystalline regions. According to this, additional heat should be necessary so the gelatinization process could occur (Jiranuntakul, et al., 2011).

Gelatinization does not mean equilibrium. Starch molecules can re-crystallize after gelatinization, reordering the zones that were disordered due to gelatinization. This process is called starch retrogradation (Eliasson & Gudmundsson, 1996). As gelatinization, retrogradation is not an instant process; during aging, starch molecules can reassociate into crystalline segments. This process depends on the botanical source of the starch, the amylose/amylopectin ratio, the molecular weight and linearity of the starch molecules, the time and temperature of cooling, the moisture content, and the concentration of starch (Aguilera & Stanley, 1999). Special attention must be paid during the storage of starchy products, since starch retrogradation leads to water loss and hardening of the structures. Figure 1.5 shows a state diagram of wheat starch. This diagram allows to observe that when the amount of water is increased, the gelatinization ( $T_{gel}$ ), glass transition ( $T_g$ ) and melting ( $T_m$ ) temperatures decrease. However, when reaching a specific water content, the gelatinization temperature does not decrease anymore, since it can be considered as a starch granules suspension in excess of water



**Figure 1.5:** State diagram of wheat starch.  $T_m$  represents the melting temperature of the granules.  $T_{gelat}$  represents the gelatinization temperature and  $T_g$  the glass transition temperature of wheat starch (adapted from Cuq et al.,(2003)).

## 1.2. Deep-fat frying

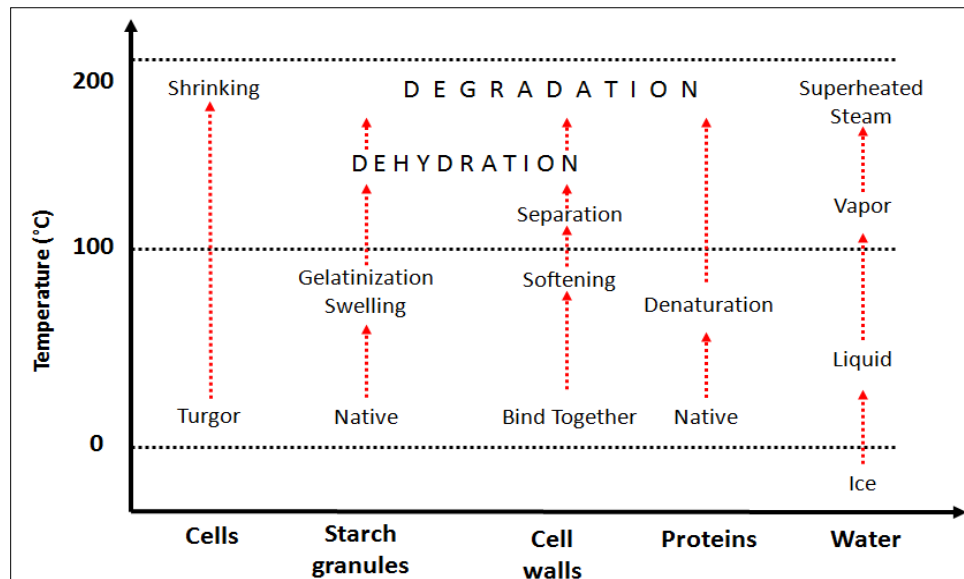
Deep-fat frying is a highly used technique for cooking food, which involves immersing a product in edible oil heated at a high temperature (generally above 150 °C). Fried products are very demanded by consumers, due to their crispy texture, flavor and sensation of satiety (Miranda & Aguilera, 2006).

### 1.2.1. Atmospheric deep-fat frying

From a food engineering perspective, frying is a complex unit operation that includes simultaneous heat and mass fluxes. This results in significant microstructural changes (Singh, 1995). The main changes that a starchy product may suffer, due to high

temperature processing, can be associated to fast dehydration, starch gelatinization, softening of cell walls, crust formation, among others (Bouchon & Aguilera, 2001; Llorca, et al., 2007). Figure 1.6 shows a schematic diagram of the main properties of some food building blocks at different temperatures in order to understand what are the main changes associated to the frying process.

The textural properties of a fried product are completely related to changes in its microstructure. For instance, firmness in potato chips is attributed to starch swelling and gelatinization with some other changes related to potato cell walls (Dueik & Bouchon, 2011a).



**Figure 1.6:** Schematic representation of microstructural properties of some food building blocks at different temperatures (adapted from Miranda and Aguilera, 2006).

An important issue related to frying is the oil absorbed by the fried product. This process is not yet fully understood, but it is believed that the escape of water during frying would

act as a barrier to oil absorption. According to Bouchon et al. (2001) most of the oil penetrates after the frying process and locates on the surface of the product, since, when the product is removed from the oil bath and starts to cool down, a competition between drainage and porous oil suction within the crust would take place (Bouchon, et al., 2003). On the other hand, Rajkumar et al. (2003), when studying structure formation of tortilla chips during frying, found that there was an inverse relationship between the degree of gelatinization and oil content, since the gelatinized starch would act as a barrier for the incoming oil towards the product. Overall, there is wide evidence that relates the microstructure of the crust with oil absorption (Bouchon & Aguilera, 2001; Pinthus, et al., 1995).

Gazmuri and Bouchon (2009) investigated the effect of gluten in the oil absorption of formulated products based on wheat gluten, wheat starch and water. Products with 8 and 12 % gluten and with different amounts of water (38 and 44 %) were studied. They determined the lowest oil content in samples containing 38 % of water and the higher gluten content. This agrees with the theory proposed by Bouchon and Pyle (2004), which states that a stronger and elastic network may be a good barrier against oil absorption. Working with dough that had a 44 % of water, no significant differences were observed when increasing the amount of gluten in the formulation. This was related to a disruption of the structure due to the higher amount of water escaping from the product as vapor bubbles. Even more, when frying pre-dried formulations using hot air, the samples absorbed half of the oil than the un-dried samples. This is associated to the formation of a barrier, due to starch gelatinization, before immersing the product in the hot oil. Moreno and Bouchon (2008) attributed this phenomenon to surface permeability of the product in fried potatoes. Both studies concluded that the initial water content did not have any significant effect in the oil absorbed by the product. In accordance, it would be of great interest to understand the combined effect of a stronger gluten network with different levels of starch gelatinization in the oil absorption of formulated products.

Moreno and Bouchon (2008) also studied the effect of freeze drying potato slices before frying and observed that these pre-dried samples absorbed 15.4 % more oil than the untreated ones. In the same line, Rajkumar et al. (2003) studied the effect of freeze drying tortilla chips before frying, which also resulted in higher oil uptake. They observed that after freeze drying no starch gelatinization occurred and a higher amount of small pores was created at the surface of the product due to water evaporation before immersing the products in the oil. After the frying process, most of the absorbed oil was located in these small pores.

Due to high temperature processing some toxic compounds may be generated, such as acrylamide (Granda, et al., 2004; Pedreschi, et al., 2004), and some beneficial compounds, like some antioxidants (*e.g.* ascorbic acid), may be deteriorated (Rojas-González, et al., 2006).

### **1.2.2. Vacuum deep-fat frying**

Vacuum frying is a novel technique that allows the removal of moisture in a low temperature and oxygen environment due to water boiling point depression (Garayo & Moreira, 2002; Shyu & Hwang, 2001). As Dueik and Bouchon (2011a) proposed, the temperature processing should not be too low, since the desired microstructural changes may be inhibited. Due to the characteristics of the process, in some food matrices an oil reduction has been achieved. However, the mechanism of oil uptake during vacuum frying is less understood than in atmospheric frying. In order to be able to compare the oil absorbed by the matrices fried under atmospheric and vacuum conditions, Mariscal and Bouchon (2008) coined the concept of equivalent thermal driving force, which defines the difference between the oil temperature and the water boiling point at a specific working pressure. For instance, if an equivalent thermal driving force of 60 °C was defined, in atmospheric conditions, the oil temperature should be 160 °C, but if the boiling point of water was reduced to 38 °C (*i.e.* the pressure was reduced from 1,000 to 65 mbar), the frying oil should be at 98 °C. When studying vacuum frying of apple

slices, they observed that atmospheric apple slices absorbed 21 % more oil than the vacuum fried ones. Similarly, Dueik and Bouchon (2011b) reported that vacuum fried potato and carrot chips absorbed 50 % less oil than the atmospheric fried ones. Vacuum frying may be an option for the production of healthier vegetable and fruit based snacks, since other techniques, such as extrusion, drying or baking may not be as successful as they may not impart the desired attributes that consumers want (Andrés-Bello, et al., 2011). However, when vacuum frying formulated matrices made from starch, gluten and water, a higher increase in oil absorption may occur (Kawas & Moreira, 2001; Sobukola, et al., 2012). This has been attributed to a decrease in the degree of gelatinization of the granules, which may not allow structure formation and therefore the creation of a barrier that inhibits oil absorption. Sobukola et al. (2012) studied the oil absorption of formulated products at different vacuum levels. When increasing the water boiling point from 38 to 71 °C, at a constant thermal driving force of 70 °C, a decrease in oil content of about 60 % was achieved. This result showed that, when there is enough temperature and water, gelatinization and structure formation may occur.

Since the temperature processing is substantially lowered, the toxic compounds generated during atmospheric frying may be diminished, and beneficial compounds may be preserved. During vacuum frying Dueik et al. (2010) studied and compared the amount of  $\alpha$ -carotene and  $\beta$ -carotene present in carrots after atmospheric and vacuum frying. They observed that a higher preservation of these components was achieved in vacuum fried samples, increasing from 36 to 90 % for  $\alpha$ -carotene and from 32 to 86 % for  $\beta$ -carotene. Also, other properties that are desired by consumers, such as color or texture, may be improved (Da Silva & Moreira, 2008; Dueik & Bouchon, 2011b). It would be of interest to study the effect of pre-treatments (*e.g.* conventional drying) in these products, which may promote gelatinization before vacuum frying, to better understand the relationship between structure formation and oil uptake. This may be a means to reduce oil uptake in vacuum fried formulated products.

### 1.3. Food process miniaturization: a vehicle to understand food dynamics

Process miniaturization is a term coined by Aguilera and Lillford (1996) which refers to the following-up of a tiny process in a particular stage (*e.g.* hot-stage) *in situ* and in real time under the lens of a microscope. Studying microstructural changes in real time in a hot-stage does not have its birth with process miniaturization. In fact, Watson (1964) studied the gelatinization temperature of starches under the lens of a microscope using a hot-stage. However, over the years, the technique has been improved and more accurate results have been obtained. Some advantages of process miniaturization are that it is a non-invasive technique, since the sample preparation does not require much processing, like in scanning electron microscopy, where the samples have to be dehydrated and then coated with a conducting metal before placing them under the lens of the microscope (Aguilera & Bouchon, 2008). For this reason, artifacts are minimized (Aguilera & Germain, 2007). Another advantage is that the operation conditions (*e.g.* temperature, pressure, relative humidity, among others) may be fully controlled, thus the microstructural changes can be associated to specific conditions. This technique becomes of particular interest in food processing, since it makes possible to study changes that may occur during the processing and storage of some food matrices in real time (Aguilera & Stanley, 1999). Special attention needs to be paid when studying different processes, since miniaturization is a representation of a real process, and it is not the real industrial unit operation itself. Generally, hot-stage experiments have been made under the lens of a light microscope; however, due to the development of new microscopy techniques, these experiences are plausible to be carried out under confocal laser scanning (Baier-Schenk, et al., 2005) and environmental scanning electron microscopes (James & Smith, 2009).

This technique has been used to study diverse processes. Bouchon and Aguilera (2001) studied atmospheric frying. Using a hot-stage, they studied the behavior of potato cells and starch when heating the tissue up to 180 °C at a controlled rate. A main conclusion of this study was that the granules tended to gelatinize at a higher temperature when they

were inside the cells than when they were isolated in excess water. Also, they studied the shrinkage of the cells and determined that even at high temperatures the cell walls were not disrupted, since oil was along the cell and not inside them. Kaushik and Ross (2007) studied the encapsulation of limonene during freeze drying. By controlling the process temperature and pressure, the authors studied different ingredients to microencapsulate limonene molecules. They concluded that a solution of arabic gum, sucrose and gelatin (1:1:1) was the best combination for freeze drying microencapsulated limonene molecules, since the matrix did not collapse during the process. Baier-Schenk et al. (2005) studied the microstructural changes that a wheat dough suffered due to the presence of ice crystal when freezing. To achieve this, they stained the samples with rhodamine and froze the dough up to -15 °C for 4 hours. Afterwards, they heated the sample to room temperature and they concluded that no irreversible changes were produced by ice crystals in wheat dough when freezing. The changes were followed under the lens of a confocal laser scanning microscope. Arellano et al. (2004) studied lactose crystallization kinetics *in situ* and in real time using polarized microscopy. To achieve the aim of their research, three different temperatures (10, 20 and 30 °C) and three different levels of initial relative super-saturation (1.95, 2.34 and 3.15) were investigated. They concluded that the study of this phenomenon could be an important tool to understand the effects of crystallization of different sugars in food processing. Saragoni et al. (2007) studied powder caking of coffee particles. By changing the relative humidity and temperature of the system, they studied the tendency of the particles to agglomerate and were able to understand them in the light of glass transition temperature (using DSC) and water activity (obtained with the isotherm curves) determination. James and Smith (2009) studied fat crystal growth in chocolates. In their research they used an environmental scanning electron microscope and determined *in situ* growth of fat and sugar crystals in chocolate (72 % cocoa) after freezing the sample to -5 °C. They concluded that if the sample was well tempered only fat crystals appeared on the surface of the chocolate, while if the sample was not well tempered, some sugar crystals were also able to emerge to the surface of the sample. Changes in corn syrup



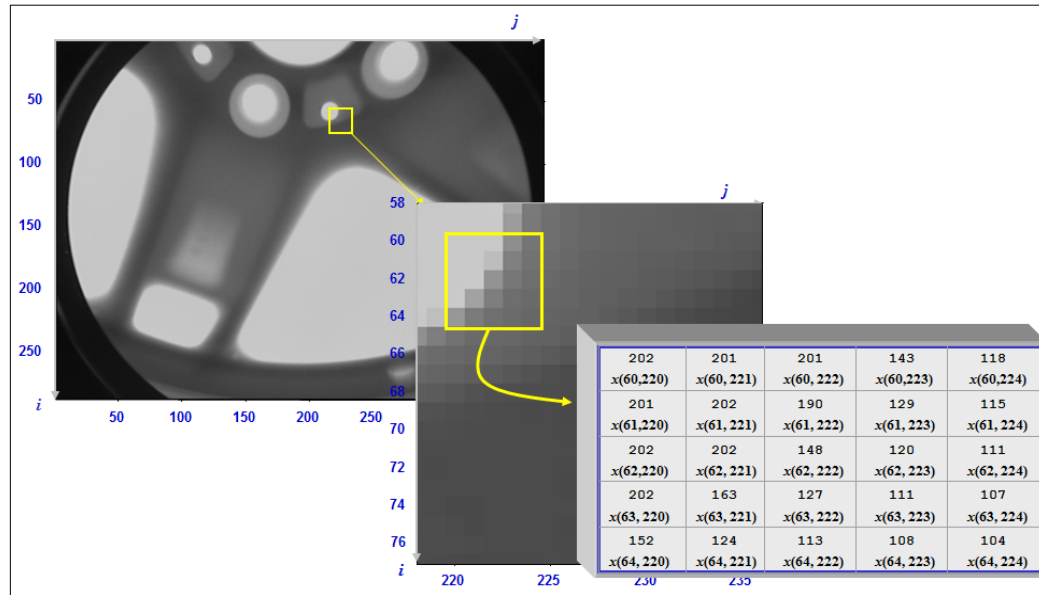
foam microstructure when submitted to pressure changes were studied by Heuer et al. (2007). To fulfill their mission, they changed the system pressure from 11 to 6 bar, from 6 to 1 bar and from 11 to 1 bar using a confocal laser scanning microscope. The objective was to determine the particle size distribution of the bubbles and the kinetics of the coalescence of the particles in real time. Due to an irregular coalescence pattern, no kinetic of the process could be determined.

Transferring real experiments under the lens of a microscope to study in real time, on line and *in situ* the microstructural changes of some food matrices is of great interest not only for scientific purposes, but also for academic and pedagogic motivations, since as it is well known: an image is worth a thousand words.

#### **1.4. Image Analysis**

Digital image acquisition coupled to microscopy is a very powerful technique since it allows immediate recording and storage. But, the most important value of images relies on the numerical information and interpretation that we can extract from them (Aguilera & Germain, 2007).

A gray-scale digital image consists in a bi-dimensional numerical array. Each position of this array is called pixel (from picture element). Each pixel may have different intensity values: from 0 to 255, that is, from black to white color. These intensity values may be plotted as the number of pixels with a certain intensity value, which is referred as the histogram (Russ, 2005). Figure 1.7 shows a representation of an image as a matrix array, indicating the position  $x(a,b)$  and the value of each pixel within the matrix.



**Figure 1.7:** Representation of an image as a matrix array. From the picture it can be observed that each pixel has a specific position and intensity value (Bouchon, 2010).

Image processing helps enhancing details that are not visible as well as results communication, among other things. However, a great reason to process an image is to obtain quantitative information, which can be used to interpret results and understand the specific phenomena under study (Russ, 2005, Aguilera & Germain, 2007). González & Woods (2008) and Russ (2005) propose some methodologies that can be used for image processing:

- a) Image pre-processing: the aim of pre-processing an image is to improve the quality of the information contained in the image, in order to obtain better results in future steps (González & Woods, 2008). Some pre-processing treatments include:
  - Contrast enhancement: when acquiring a digital image the histogram does not always cover the full range of intensity values. This is associated to image low contrast. To enhance the lack of contrast, the intensity values of

the images are stretched to cover the full range of intensity (Russ, 2005, Aguilera & Germain, 2007).

- Noise removal: generally, noise refers to information contained in the image that is not part of the image itself, but was introduced in the image by the imaging system (González & Woods, 2008). Due to the artifacts introduced by noise removal (*e.g.* blurring), enhancement of details is generally performed.
- b) Thresholding: this step is critical in image processing. Thresholding an image consists in isolating the objects of interest from the background (Russ, 2005). The aim of thresholding is to obtain a binary image (*i.e.* a black and white image) that will be further segmented.
- c) Segmentation: the aim of image segmentation is to obtain an image with its main structural objects and be able to distinguish one from the other (González & Woods, 2008).
- d) Analysis: this final step consists in extracting quantitative information that will be interpreted according to specific needs (González & Woods, 2008).

## **2. HYPOTHESIS**

The comprehension of main transformations that occur during microstructure development in formulated products is of paramount importance for the food industry, since it helps to understand the functionality of the structural elements in their native state and during processing. Specifically, understanding the main processes involved in the development of fried snacks formulated products is of great importance, since its consumption has increased over the last decades and there is great concern in eating healthier products, which have lower oil content than the traditional ones.

Accordingly, the hypothesis of this thesis is that through process miniaturization it is possible to understand the effect of water and temperature limitation in the microstructural changes undergone by starch, in real time, when heating under excess or limited water conditions or oil, under atmospheric or vacuum conditions, to improve scientific knowledge in the new growing field of formulation engineering.

### 3. OBJECTIVES

The main objective of this research is to understand the effect of water availability and processing conditions (temperature and vacuum level) in the microstructural changes that occur in potato starch granules heated in water and oil *in situ* and in real time.

To achieve this objective, the specific aims of this thesis are:

- To understand the effect of temperature in the microstructural changes of isolated potato starch granules heated in excess water in real time and *in situ* at different vacuum levels (*i.e.* different water boiling points).
- To understand the effect of water availability and temperature in the microstructural changes of isolated potato starch granules heated in pre-gelatinized corn starch solutions in real time and *in situ* at different vacuum levels (*i.e.* different water boiling points).
- To understand the effect of water availability and temperature in the microstructural changes of potato starch embedded in a starch-gluten-water matrix heated in oil in real time and *in situ* at different vacuum levels (*i.e.* different water boiling points).

## **4. MATERIALS AND METHODS**

### **4.1. Materials**

Potato starch granules and pre-gelatinized corn starch were bought from Blumos S.A. (Santiago, Chile), while wheat gluten was acquired from Asitec S.A. (Santiago, Chile).

### **4.2. Sample preparation**

Different systems were prepared to understand the effect of water availability in potato starch behavior: 1) in excess of water, 2) in a lower water availability environment (3 and 5 % pre-gelatinized corn starch solutions) and 3) in a starch-gluten matrix (with either 40 or 50 % water content).

#### **4.2.1. Isolated granules in excess of water**

To study microstructural changes of isolated potato starch granules in excess of water droplets of water were added to the sample holder (microcapsule to be mounted in the microscope). Afterwards, potato starch granules were scattered over the sample holder.

#### **4.2.2. Isolated granules in solutions of pre-gelatinized corn starch**

First, the water content of the pre-gelatinized corn starch was determined drying in a forced air oven (LDO-080F, Labtech, Korea) at 105 °C for 24 h (to constant mass). Then, 3 and 5 % pre-gelatinized corn starch solutions were prepared by carefully determining the amount of water and pre-gelatinized starch to be added. Afterwards, as in excess of water conditions, droplets of the solution were added to the sample holder and a few potato starch granules were scattered over the solution.

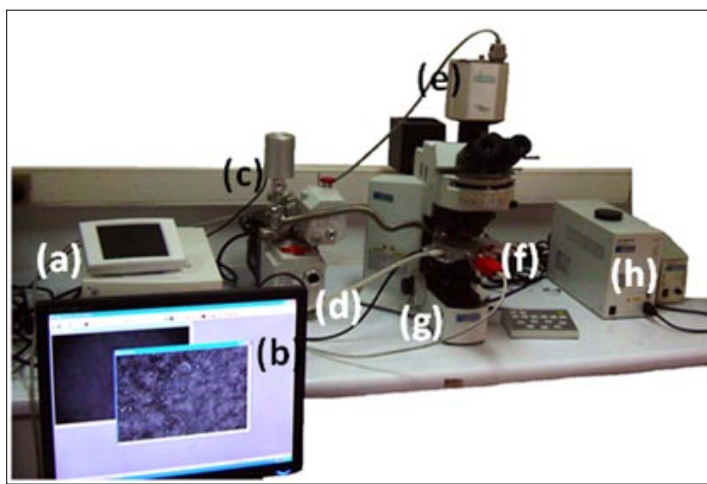
### **4.2.3. Starch granules in starch-gluten-water matrices**

Matrices of potato starch, wheat gluten and water were prepared in order to understand the role of gluten and water in the gelatinization process of potato starch. The matrices were prepared according to the methodology proposed by Moreno et al. (2010). The exact water content of the ingredients was determined experimentally by drying in a forced air oven (LDO-080F, Labtech, Korea) at 105 °C for 24 h (to constant mass), cooled in a desiccator and then weighted to obtain the dry weight. The amount of each ingredient was determined in order to observe and distinguish the granules from each other in the dough. Two different dough formulations were studied: with 50 and 40 % moisture content. This last water content was chosen, since products to be fried, generally have this amount of water (Moreno, et al., 2010). Both, starch and gluten remained constant. Once the matrices were prepared, a very thin sample of the dough was placed over the sample holder. Afterwards, ten droplets of sunflower oil (Natura, Córdoba, Argentina) were added to ensure that the sample was fully covered. Details about how the dry weight of the food building blocks was obtained are shown in Appendix A.

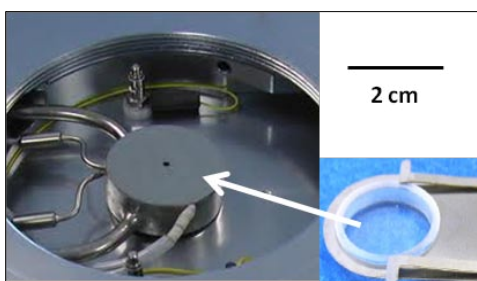
### **4.3. Experimental Setup**

The sample holder was placed on a Linkam hot-vacuum-stage (model THMS350V, Linkam Scientific Instruments, Surrey, UK) adapted to an Olympus BX61 light microscope (Olympus Optical Corporation, Tokyo, Japan). The microscope had a polarized filter and an analyzer, which allowed observing birefringent structures. The magnification used in this research was 10x. The temperature was controlled with a PE95 / T95 System controller which allows programming heating ramps from 0.1 to 30 °C/min. The temperature that can be achieved ranges from -196 to 350 °C. For vacuum experiments a Pfeiffer Duo 2.5 (Asslar, Germany) vacuum pump was attached to the hot-vacuum-stage. The vacuum pump had a Linkam MV196 motorized vacuum control

system which allowed controlling the pressure in the system from 0.001 to 300 mbar. Figure 4.1 shows a picture of the experimental setup with its main components. Figure 4.2 shows a close up of the hot-vacuum-stage (component f in Figure 4.1). The sample holder is placed over the heating unit, as shown in the figure.



**Figure 4.1:** Experimental setup. (a) Temperature controller; (b) PC/image analysis software; (c) vacuum controller; (d) vacuum pump; (e) digital camera; (f) hot-vacuum-stage; (g) light microscope; (h) source power.



**Figure 4.2:** Close up of the heating unit. The image from the right represents the sample holder, which is placed over the heating unit.



#### **4.4. Experimental conditions**

In order to characterize microstructural changes of potato starch granules and study the effect of temperature and water limitation, atmospheric and vacuum conditions were studied. In both conditions, once the sample was prepared, the sample holder was introduced into the hot-vacuum-stage, as shown in Figure 4.2.

##### **4.4.1. Atmospheric conditions**

In atmospheric experiments, three different heating rates were used: 5, 10 and 15 °C/min. These rates were chosen in order to approach to a real process where fast heating occurs. In experiments with excess of water, samples were heated up to 100 °C to ensure that all the granules ended the gelatinization process. Thereafter, the samples were allowed to cool to room temperature (25 °C approximately). The granules suspended in the pre-gelatinized corn starch solutions were heated up to 120 °C approximately to ensure that all the granules completed the gelatinization process. Afterwards, the temperature was also lowered to room temperature. The granules embedded in the matrix were heated in oil until they reached a frying temperature of 180 °C, as in frying, and then the temperature was lowered to room temperature.

##### **4.4.2. Vacuum conditions**

Two different vacuum levels were defined in order to understand the effect of water boiling point on potato-starch microstructural changes during heating. A vacuum pressure of 65 mbar, which correspond to a water boiling point of 38 °C, was used. This corresponds to a temperature that is lower to the one required to induce starch gelatinization, which ranges between 59 and 68 °C (Singh, et al., 2003). In addition, a vacuum pressure of 300 mbar was used, which corresponds to a water boiling point of

69 °C<sup>1</sup>, thus, above the gelatinization temperature. Once the system reached the required vacuum level, the heating started. A heating rate of 15 °C/min was used, since, under vacuum conditions, the motorized valve was not able to control low vacuum pressures (over 100 mbar) when using a lower heating rate heating (*e.g.* 5 and 10 °C/min). The system was heated up to 108 and 139 °C for water boiling points of 38 and 69 °C, respectively. Table 4.1 shows a summary of the main conditions studied during the research.

**Table 4.1:** Experimental Design ( $T_{bp}$  stands for water boiling point).

		Heating Rates (°C/min)				
		Atmospheric conditions			Vacuum conditions	
		$T_{bp} = 100\text{ °C}$			$T_{bp} = 38\text{ °C}$	$T_{bp} = 69\text{ °C}$
Heated in water	Excess water					
	3% Solution	5	10	15	15	15
	5% Solution					
Heated in oil	Starch-gluten-water matrix					

## 4.5. Image acquisition and analysis

### 4.5.1. Atmospheric heating

Figure 4.3 shows a schematic diagram of the steps that were needed to extract the relevant information from the images. First, pictures were captured using a Cool Snap Pro Color 289 digital camera (Photometrics Roper Division, Inc., Tucson, AZ, USA)

<sup>1</sup> The boiling point of water for this vacuum condition was  $69 \pm 4\text{ °C}$ , since the vacuum level oscillated between  $300 \pm 50\text{ mbar}$ .

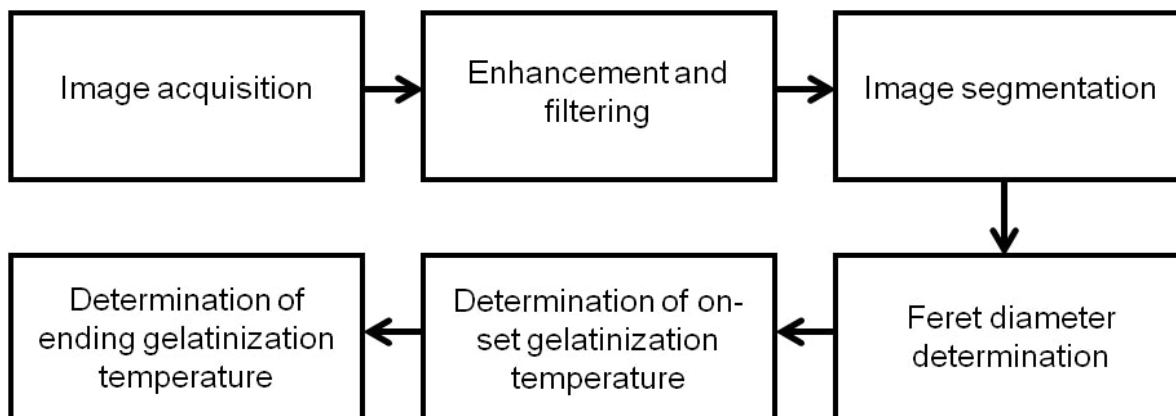
every 2 s. It is important to note that for each heating rate it was possible to clearly identify the corresponding temperature, as shown in Appendix B). Images were then processed and analyzed using Image ProPlus 4.5 (Media Cybernetics, Silver Spring, USA). Accordingly, they were processed by enhancing the contrast, noise was removed by applying a median filter and borders were enhanced using a sharpening filter. Afterwards, the granules were identified and their Feret diameter was determined in order to classify the granules according to their size. In Appendix C the granule size distribution of potato starch (according to their Feret Diameter is shown). For each granule the following parameters were obtained:

- **Feret diameter:** diameter of a circumference that has the same area than the object under study. It can be calculated according to equation (1).

$$D_f = \sqrt{\frac{4 \cdot Area}{\pi}} \quad (1)$$

- **On-set gelatinization temperature:** this temperature is achieved when the starch granule starts to lose its birefringence (the Maltese cross starts to disappear).
- **Ending gelatinization temperature:** this temperature is reached when the studied granule stops swelling and no more significant microstructural changes are observed.
- **Degree of gelatinization:** this concept refers to the percentage of granules that have completed the gelatinization process.
- **Gelatinization range:** this concept refers to the difference between the ending and on-set gelatinization temperatures (*i.e.* it refers to the range of temperature in which gelatinization process takes place). For example, if the on-set and ending

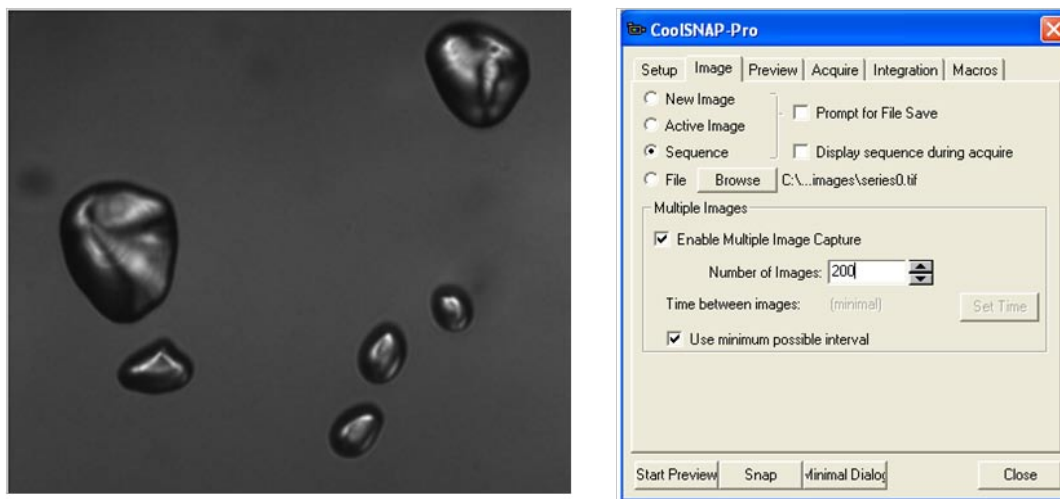
gelatinization temperatures are 65 and 72 °C respectively, the gelatinization range would be 7 °C. This parameter is studied in order to determine if the gelatinization process happens in a broader range of temperature.



**Figure 4.3:** Sequence of the main steps needed to obtain quantitative information.

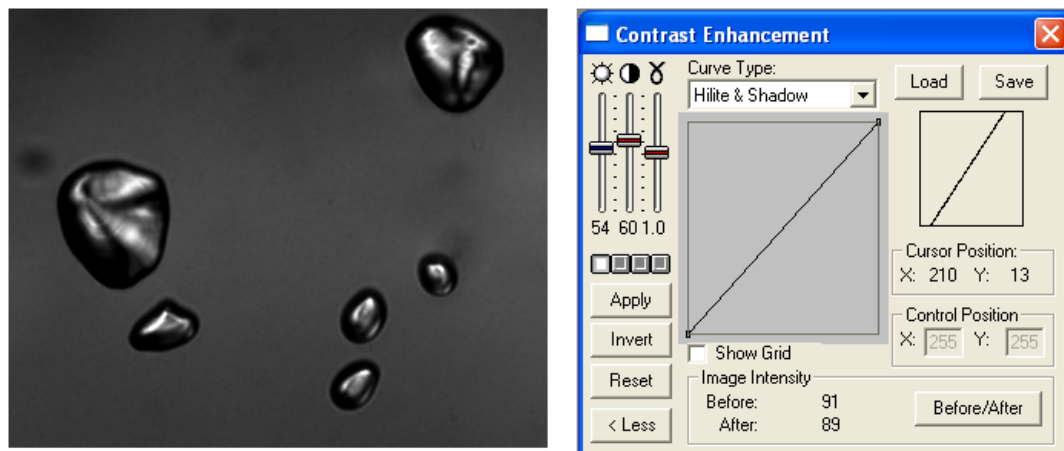
In order to get a better understanding of the proposed methodology, each box of Figure 4.3 will be explained with image support.

**Image acquisition:** Once the samples were under the lens of the microscope, images were acquired by carefully defining the time of exposure and the time interval between frames, among other parameters. Figure 4.4 shows an image of isolated potato starch granules in excess of water, as seen in the computer when acquiring the image. The dialog box, which shows the different parameters to be defined, is also shown.



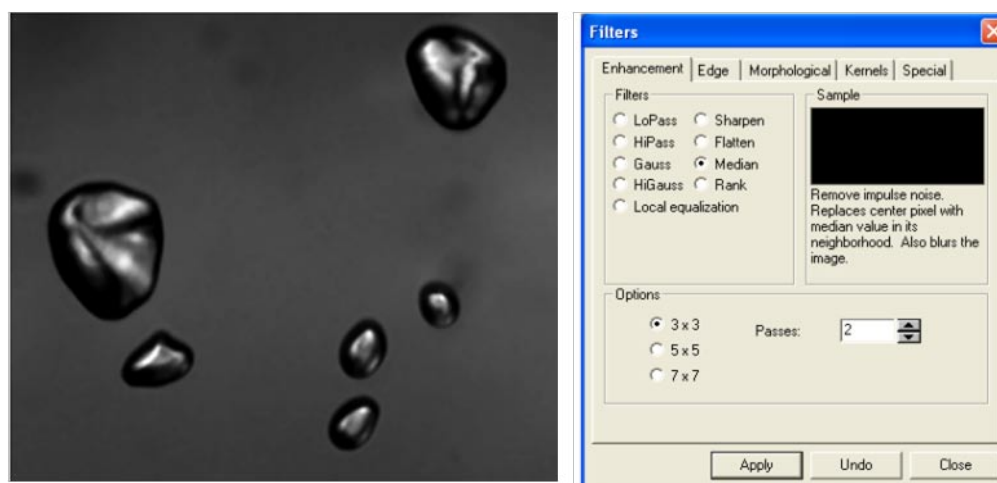
**Figure 4.4:** Image of isolated potato starch granules acquired with a digital camera. The dialog box shows the different parameters to be defined

**Enhancement and filtering:** To equalize the light, the contrast enhancement tool of Image ProPlus software was used. Figure 4.5 shows the different options that may be used in order to obtain the desired result. In this research, brightness and contrast parameters were adjusted.



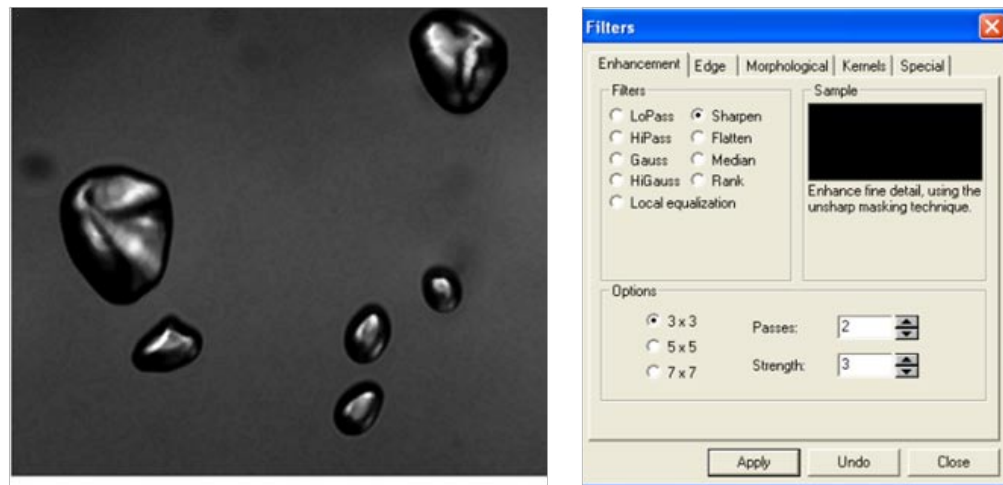
**Figure 4.5:** Resultant image after light and contrast equalization. The contrast enhancement toolbox is also shown.

Afterwards, the noise of the image was reduced using a median filter, as shown in Figure 4.6.



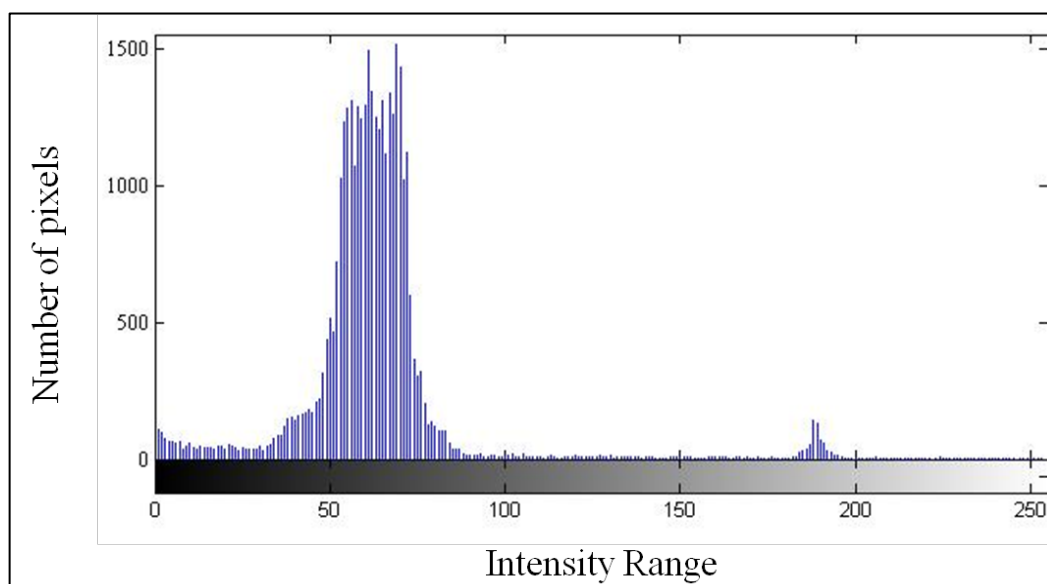
**Figure 4.6:** Equalized image after applying a median filter (3x3). The toolbox corresponding to the median filter is shown at the right side of the image.

Afterwards, a sharpening filter was used in order to enhance image details, as shown in Figure 4.7.

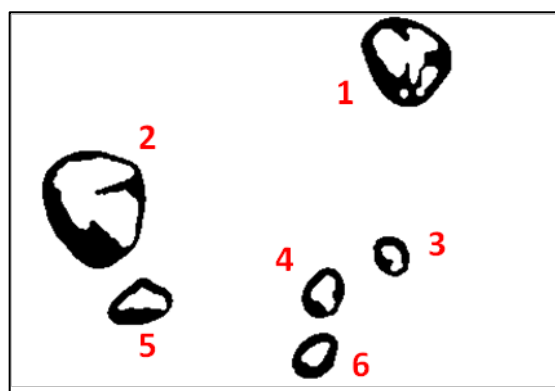


**Figure 4.7:** Filtered image after applying a sharpening filter (3x3). The corresponding toolbox is shown at the right side of the image.

**Image binarization and segmentation:** After processing, images were segmented. To do so, the threshold option of the Image ProPlus software was used. This option displays the image histogram and the threshold is defined manually, in order to obtain a binarized (black and white) image. Figure 4.8 shows the displayed histogram of Figure 4.7. If necessary, an additional median filter was applied to remove possible noise. Figure 4.9 shows the binarized image.



**Figure 4.8:** Histogram of the image shown in Figure 4.7.



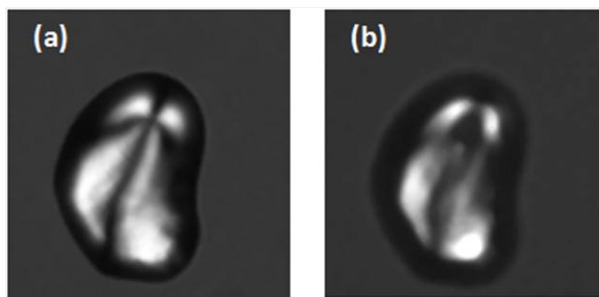
**Figure 4.9:** Binarized image.

**Feret diameter:** In order to measure the Feret diameter of the granules, the image was first calibrated using a micrometric ruler, which allows relating the number of pixels to a real length. Then, granules were identified using the count/size option (red numbers in



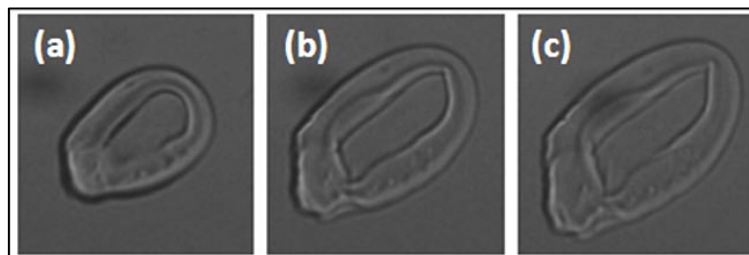
Figure 4.9). Finally, the area of the granules was obtained and the Feret diameter was determined using Equation (1).

**Determination of on-set gelatinization temperature:** The images were filed as a sequence. In order to determine the on-set gelatinization temperature, the behavior of each granule was followed through the sequence. The on-set gelatinization temperature was determined when the granule just lost its birefringence. Figure 4.10 shows two frames of a sequence, before reaching the on-set gelatinization temperature (notice that the granule has a birefringent structure with a well defined Maltese cross), and when this temperature was achieved. This sequence shows the moment when the birefringence disappears.



**Figure 4.10:** Determination of the on-set gelatinization temperature of isolated potato starch granules heated in excess of water. Image (a) represents the situation before reaching the on-set gelatinization temperature and (b) when reaching the on-set gelatinization temperature.

**Determination of ending gelatinization temperature:** Accordingly, the ending gelatinization temperature was determined when the granule stop swelling, as shown in Figure 4.11.



**Figure 4.11:** Determination of the ending gelatinization temperature of isolated potato starch granules heated in excess of water. Images (a) and (b) before reaching the ending gelatinization temperature; (c) when reaching the ending gelatinization temperature

#### 4.5.2. Vacuum heating

For granules heated under vacuum conditions (water boiling point of 38 °C) the same methodology of atmospheric conditions was used.

For granules heated when the water boiling point was 69 °C, images were acquired every two seconds. In order to understand the effect of vacuum and to compare the degree of gelatinization with atmospherically-heated potato starch, three different scenarios were studied. For starch suspended in excess of water, vacuum-heating up to 70, 80 and 90 °C was performed, while for 3% solutions of corn starch, vacuum heating was performed up to 80, 90 and 100 °C, because significant changes were observed at these temperatures. No significant changes were observed either below or above these temperatures. In starch-gluten-water matrices pictures were acquired during the whole heating process (from room temperature up to 138 °C. However, images acquired at room temperature and after the process was completed will be shown, since they are representative of the process. Image processing was carried out as described in section 4.5.1.

#### **4.6. Statistical Analysis**

Statistical analysis was done using Statgraphics Centurion XV for Windows software, version 15.2.06 (Manugistic Inc., Rockville, MD, USA). Results were compared using an ANOVA (t-test) with 95 % confidence level. When comparing the mean values, if the variances were not statistically equal, non-parametric tests were performed in order to compare the mean values. In these cases, a multiple range contrast and Kruskal-Wallis contrast with 95 % confidence level were performed.

## 5. RESULTS AND DISCUSSION

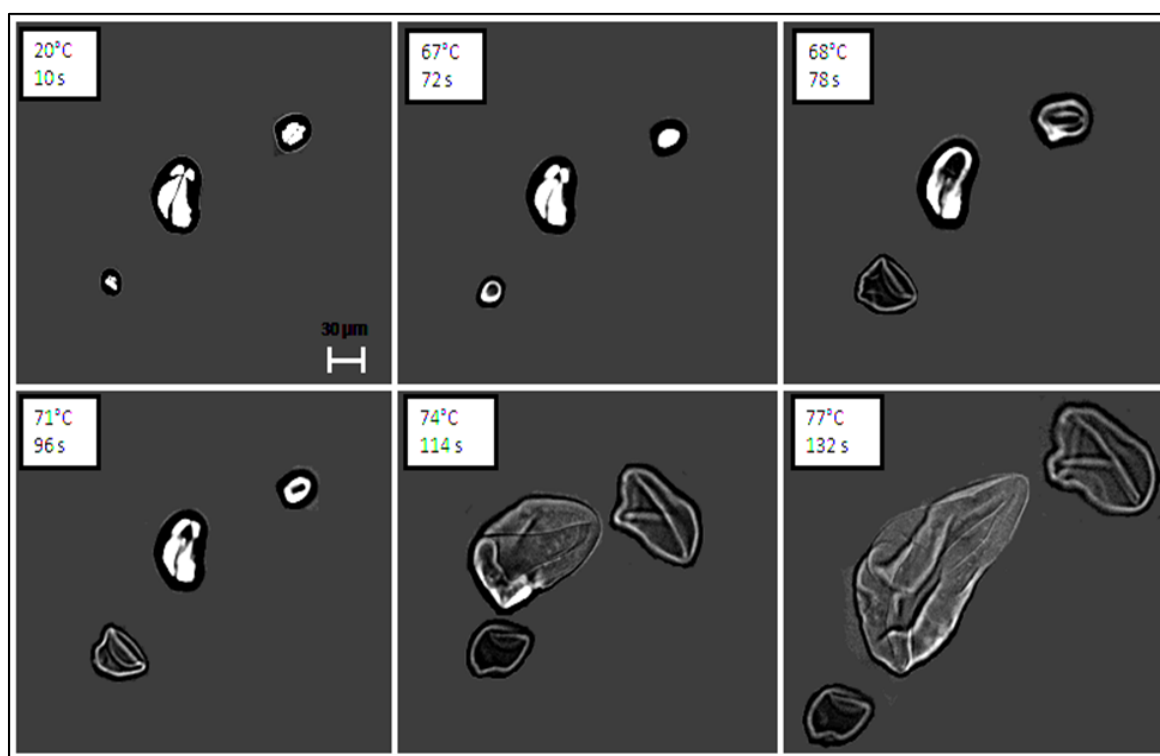
### 5.1. Gelatinization of isolated potato starch granules in excess of water

Isolated potato starch granules suspended in excess of water were heated under atmospheric and vacuum conditions. This was performed to get a better understanding of the effect of water and temperature in starch gelatinization, as well as the effect of water boiling-point.

#### 5.1.1. Atmospheric heating

Isolated potato starch granules were heated in excess of water at three different heating rates, 5, 10 and 15 °C/min, which were defined according to those used in DSC studies (Biliaderis et al., 1986; Fernandez-Martin et al., 2008; Karlsson & Eliasson, 2003; Parada & Aguilera, 2009; Primo-Martin et al., 2007; Ratnayake & Jackson, 2007), which approach to a real process where fast heating occurs. This is certainly a proxy, since rates in food processing may be much higher at some stages in the process. For instance, during frying, the heating rate is not constant during the whole process, and different stages may be differentiated: 1) *Initial Heating*, which lasts a few seconds and corresponds to the period of time whilst the surface temperature reaches the boiling point of the liquid; heat transfer is by natural convection and no vaporisation of water occurs, 2) *Surface Boiling*, which is characterised by the sudden loss of water, the beginning of the crust formation and a boiling convection regime due to high turbulence, associated with nucleate boiling 3) *Falling rate*, which is the longest, in which the internal moisture leaves the food, the core temperature rises to the boiling point, the crust layer increases in thickness, and finally the vapour transfer at the surface decreases and 4) Bubble End-Point (Farkas, 1994). During the second period, at a high heating rate occurs, which may reach 200 °C/min. Afterwards, the heating rate may decrease considerably to 30 °C/min and lower values (Dueik & Bouchon, 2011c).

Figure 5.1 shows an image gallery of isolated potato starch during heating at 10 °C/min. Images show that at room temperature (25 °C) starch is insoluble in water and shows the characteristic Maltese cross of un-gelatinized starch (Flint, 1994). When higher temperatures are reached, changes in the starch microstructure can be observed, such as loss of birefringence and swelling of the granules. It is possible to observe that not all the granules start the gelatinization process at the same temperature. This happens since the energy that the granules need to absorb is specific for each granule (Svihus, et al., 2005). In this research, the on-set gelatinization temperature of the granules is defined as the moment when the loss of birefringence occurs.



**Figure 5.1:** Images of the gelatinization process of isolated potato starch granules heated at 10 °C/min in excess of water.

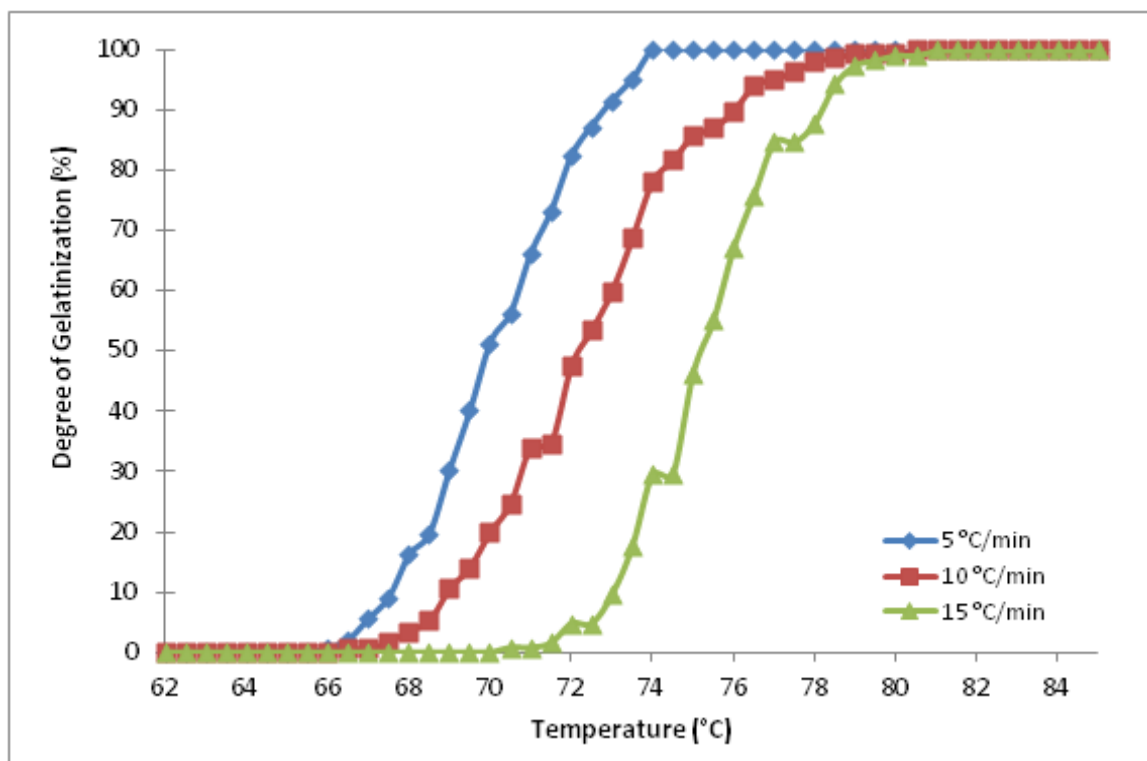
Table 5.1 shows the amount of granules studied for each heating rate, including the minimum, maximum and average on-set and ending gelatinization temperature for each one of the treatments studied. When increasing the heating rate from 5 to 10 °C/min and from 10 to 15 °C/min a higher on-set gelatinization temperature was observed (67.0, 69.3 and 70.9 °C respectively). Details about these results are shown in Appendix D.1. Considering the whole set of data, there were significant differences ( $p < 0.05$ ) between the on-set gelatinization temperature of granules heated at 5 and 10 °C/min and between those heated at 10 and 15 °C/min. This may be due to the great importance of the thermal history in the gelatinization process (Fukuoka, et al., 2002; Lund & Lorenz, 1984; Marchant & Blanshard, 1978). Although, an important variability in the gelatinization temperature was found within each heating rate, no significant differences were found for granules of different sizes within each rate ( $p > 0.05$ ). However, some authors proposed for starches from other botanical sources, such as wheat starch, that larger granules are weaker than the smaller ones, and gelatinize first (Chiotelli & Le Meste, 2002; Llorca, et al., 2007; Svihus, et al., 2005).

**Table 5.1:** On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules heated in excess of water heated at 5, 10 and 15 °C/min.

Heating Rate	5 °C/min		10 °C/min		15 °C/min	
N	142		170		125	
	On-set	Ending	On-set	Ending	On-set	Ending
Mean (°C)	67 ± 0.1	70.1 ± 0.2	69.3 ± 0.2	72.4 ± 0.2	70.9 ± 0.2	75.3 ± 0.2
Minimum (°C)	63.2	65.6	62.7	66.3	64	70.3
Maximum (°C)	72.5	74.0	78.3	80.3	76.4	80.6

Also, for each heating rate, the degree of gelatinization was studied. Results are shown in Figure 5.2. It can be seen that when the heating rate is increased, gelatinization is retarded, as earlier discussed. For example, at 70 °C (see Figure 5.2) none of the granules heated at 15 °C/min has completed the gelatinization process. On the other hand, 51 and 20 % of the granules completed gelatinization at 70 °C when heating them at 5 and 10 °C/min, respectively. However, if the granules are heated up to 70 °C and the temperature is maintained for 30 s, all granules are able to end the gelatinization process. This may be happening, since starch gelatinization is a time and temperature related phenomenon (Lund & Lorenz, 1984; Marchant & Blanshard, 1978). It is possible to observe that the gelatinization happens in a broader range of temperature when increasing the heating rate from 5 to 15 °C/min, being  $3.2 \pm 0.1$  °C for 5 °C/min and  $4.4 \pm 0.1$  °C for 15 °C/min ( $p < 0.05$ ). No significant differences were found for the granules heated at 5 and 10 °C/min.

For all the heating rates a complete degree of gelatinization was achieved. This is consistent with results reported by Parada and Aguilera (2009), who studied the degree of gelatinization of starch by DSC heating the samples at 10 °C/min. These authors proposed that samples heated above 65 °C for several minutes, always reached a total degree of gelatinization.



**Figure 5.2:** Gelatinization degree of isolated potato starch granules heated in excess of water heated at 5, 10 and 15 °C/min.

### 5.1.2. Vacuum Heating

Two different vacuum levels were studied. First, a vacuum pressure of 65 mbar, which corresponds to a water boiling point of 38 °C, was used, which is lower than the gelatinization temperature of isolated potato starch granules. Afterwards, a vacuum pressure of 300 mbar (boiling point of water of 69) was used<sup>2</sup>. This range in the boiling point of water includes the gelatinization temperature of isolated potato starch granules, according to experimental results. In order to compare the results obtained during vacuum-heating and atmospheric heating, the granules were heated up to 100 °C, since

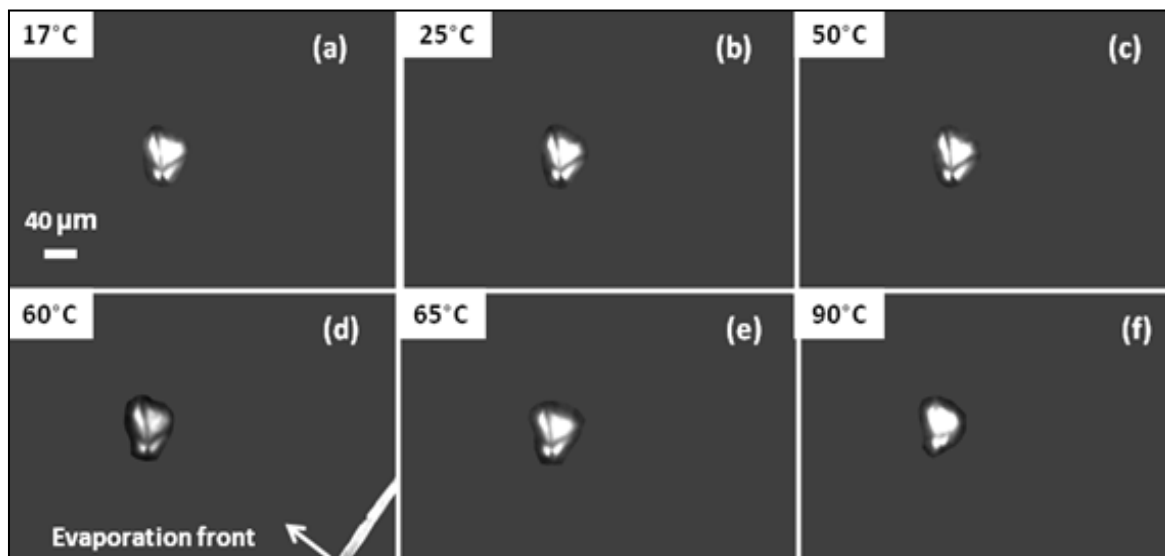
<sup>2</sup> The boiling point of water for this vacuum condition was  $69 \pm 4$  °C, since the vacuum level oscillated between  $300 \pm 50$  mbar.



at that temperature, under atmospheric conditions, a total degree of gelatinization is achieved.

#### **5.1.2.1. Water boiling point of 38 °C**

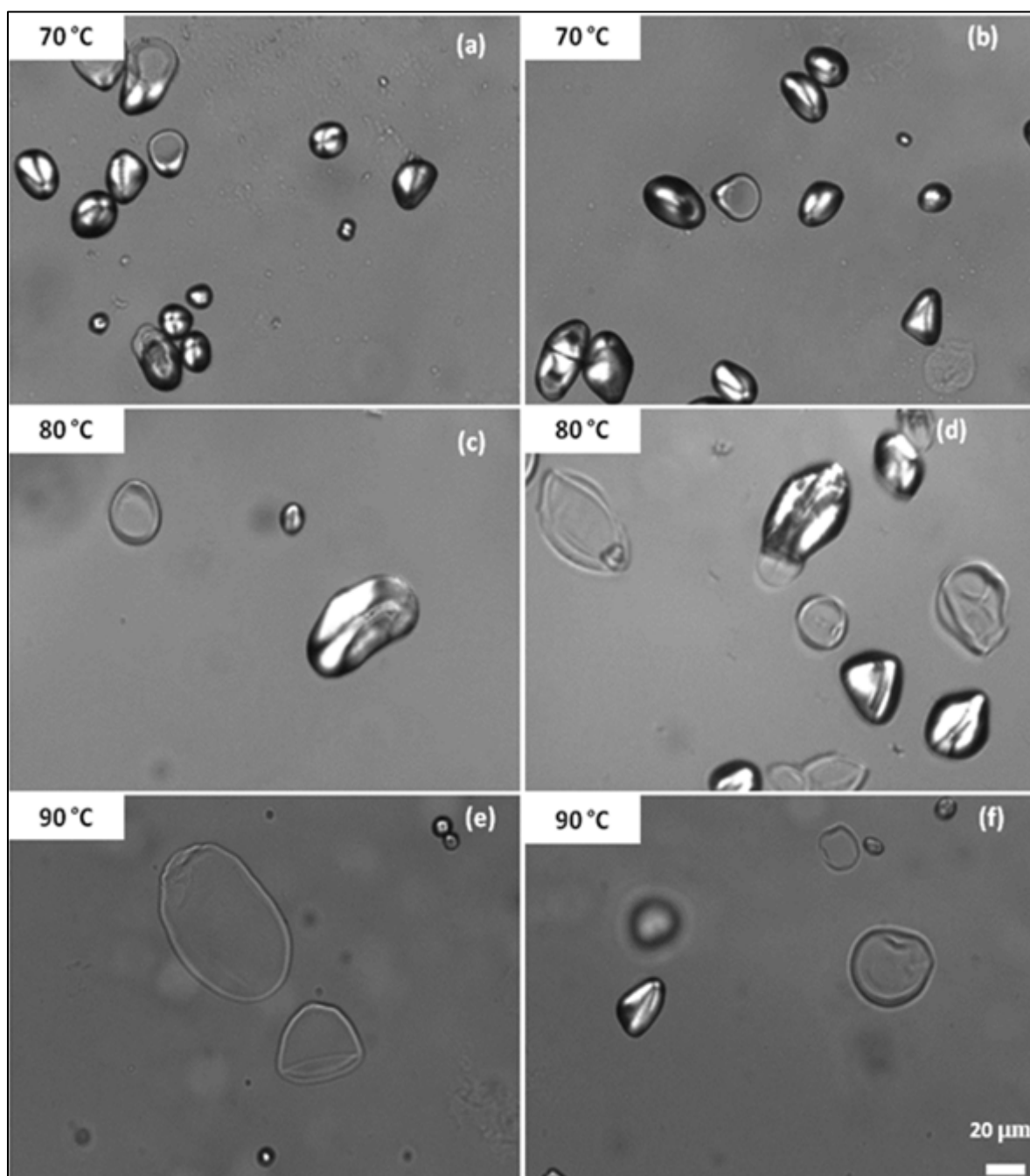
When vacuum-heating the granules working at a vacuum pressure of 65 mbar, no starch gelatinization was observed. This may be happening, since at 38 °C (corresponding water boiling point), the granules have not absorbed the required energy to gelatinize. On the other hand, when the gelatinization temperature was reached, there was not enough water to make the granules swell and gelatinize (the gelatinization temperature ranged between 64 to 76.4 °C, when heating at 15 °C/min in excess of water). This does not mean though that the water was totally evaporated at 38 °C, since the system was not in equilibrium and the gelatinization process is a kinetically dependent process (Bogracheva, et al., 2006). In Figure 5.3 a sequence of images is shown in order to illustrate the process. From image (d) it can be observed that the evaporation front is moving towards the granule, showing that water evaporation is not instant and that there may be presence of water even at temperatures above the boiling point of water (non-equilibrium conditions). From the other images it is possible to observe that no gelatinization takes place when heating at this working pressure. Other authors (Kawas & Moreira, 2001; Rajkumar, et al., 2003) have studied the effect of vacuum on starch gelatinization when frying tortilla chips (water boiling point of 12 °C approximately). They concluded that when vacuum frying the tortillas, no starch gelatinization was observed.



**Figure 5.3:** Image gallery of a vacuum-heated (water boiling point of 38 °C) potato starch granule at 10 °C/min. No starch gelatinization takes place at this working pressure. In picture (d) it is possible to observe the evaporation front coming towards the granule. In picture (f) there is no water left in the system.

#### 5.1.2.2. Water boiling point of 69 °C

Vacuum-heating with a water boiling point of 69 °C showed a certain degree of gelatinization. Vacuum-heating up to 70, 80 and 90 °C was performed. In Figure 5.4 the images represent the changes in the granules microstructure when they were heated at the mentioned temperatures. It can be observed that a small amount of granules did gelatinize when the granules were heated up to 70 °C (images a and b), whereas when heating up to 80 °C (images c and d) a larger amount of granules started and completed the process. When the granules were heated up to 90°C (images e and f) it can be observed that almost all the granules did gelatinize or at least started the gelatinization process.

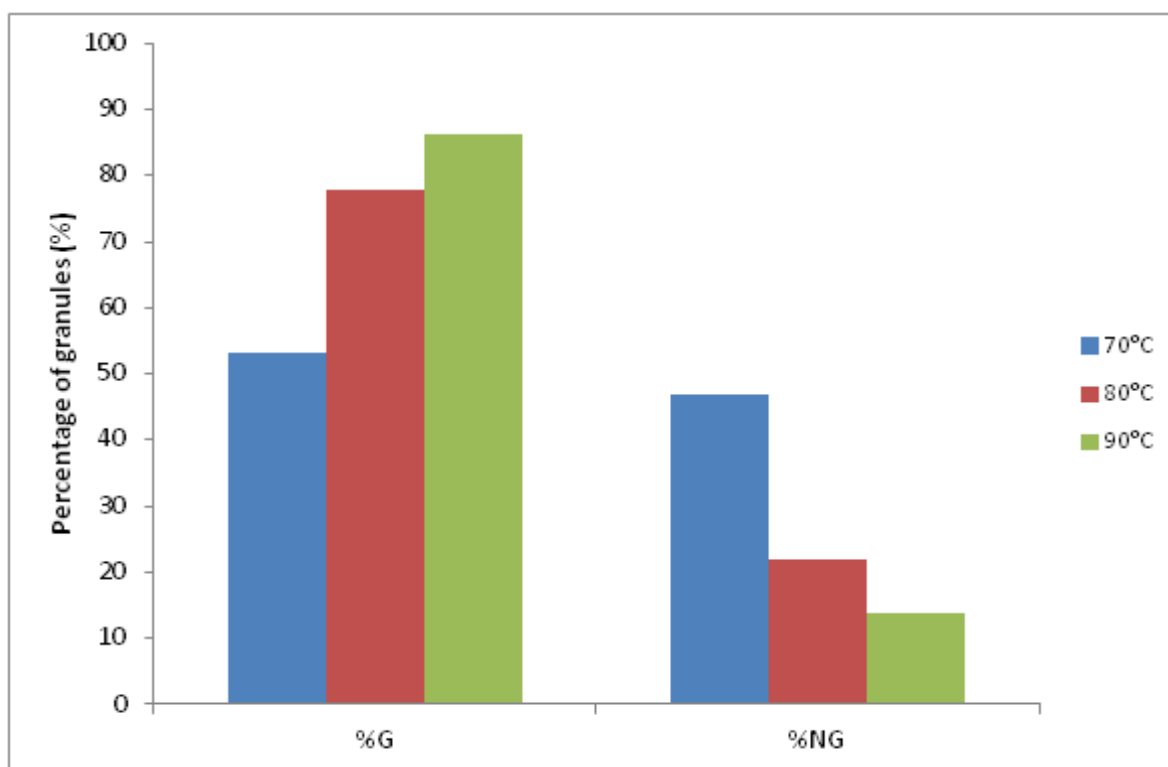


**Figure 5.4:** Isolated potato starch granules in excess of water vacuum-heated at 15 °C/min and 300 mbar (water boiling point of 69 °C) up to 70 (images a and b), 80 (images c and d) and 90 °C (images e and f).

Figure 5.5 shows the percentage of granules that completed the gelatinization process or are being gelatinized (%G) and those that did not even start the process (%NG), at different temperatures. When heating up to 70 °C (n = 137), 53 % of the starch started or completed the gelatinization process, whereas 47 % of the granules did not show any sign of change in their structure. When increasing the temperature to 80 °C (n = 127), the percentage of gelatinizing starch rose to 78 % and the amount of starch that did not even start the process to 22%; these figures are significantly different ( $p < 0.05$ ) from those obtained at 70 °C. Moreover, when the temperature was increased up to 90 °C (n = 123), 86 % of the granules started the process and 14 % of the granules did not even start the process. The raise of gelatinized granules when increasing the temperature may be explained due to the dynamics of the process, since not all the water had evaporated at 90°C (non-equilibrium conditions), thus, there was enough liquid water to induce gelatinization. In accordance, some of the granules that were not able to start gelatinization at 80 °C were now able to start the process. Due to water boiling point depression, the amount of water present in the system at 70, 80 and 90 °C is lower than under atmospheric conditions. However, when the system reaches the water boiling point, it does not mean that the equilibrium is achieved instantly and that all the water available for starch gelatinization has been evaporated. In fact, as explained above, the amount of gelatinized granules increases with temperature until there is no water left to increase the number of gelatinized granules.

The results obtained from the comparison between vacuum and atmospheric conditions suggest that the difference in the boiling point of water and therefore the presence of liquid water have an influence in the degree of gelatinization of starch. In fact, under atmospheric conditions, at 80 and 90 °C all of the studied granules started the gelatinization process, versus 78 and 86 %, respectively, under vacuum conditions. Blaszcak et al. (2005) when studying the effect of high pressures in the gelatinization of starch observed that at high pressures the gelatinization of starch could be reduced from 65 to 57 °C approximately. These authors proposed that high pressures would influence the behavior of the gelatinization of potato starch granules, since the crystalline structure

may be disrupted before the hydration of the amorphous zones of the granules. This could result in the destruction of the granular structure and in an earlier hydration of the granules. Accordingly, lower temperatures might be necessary to start swelling and gelatinization. Results obtained from this research suggest that the decrease in the amount of gelatinized granules should be happening due to a decrease in the available water that allows the process to occur.



**Figure 5.5:** Percentage of granules that either completed or started the gelatinization process (%G) and did not gelatinized (%NG) when vacuum-heating at 15°C/min and 300 mbar (water boiling point of 69 °C) up to 70, 80 and 90 °C.

## **5.2. Gelatinization of isolated potato starch granules in pre-gelatinized corn starch solutions**

In order to understand the effect of water limitation in the gelatinization process, 2 solutions were prepared as explained before. Changes in the gelatinization temperature and the degree of gelatinization at each temperature were reported and compared between the granules heated in excess of water.

### **5.2.1. Atmospheric heating**

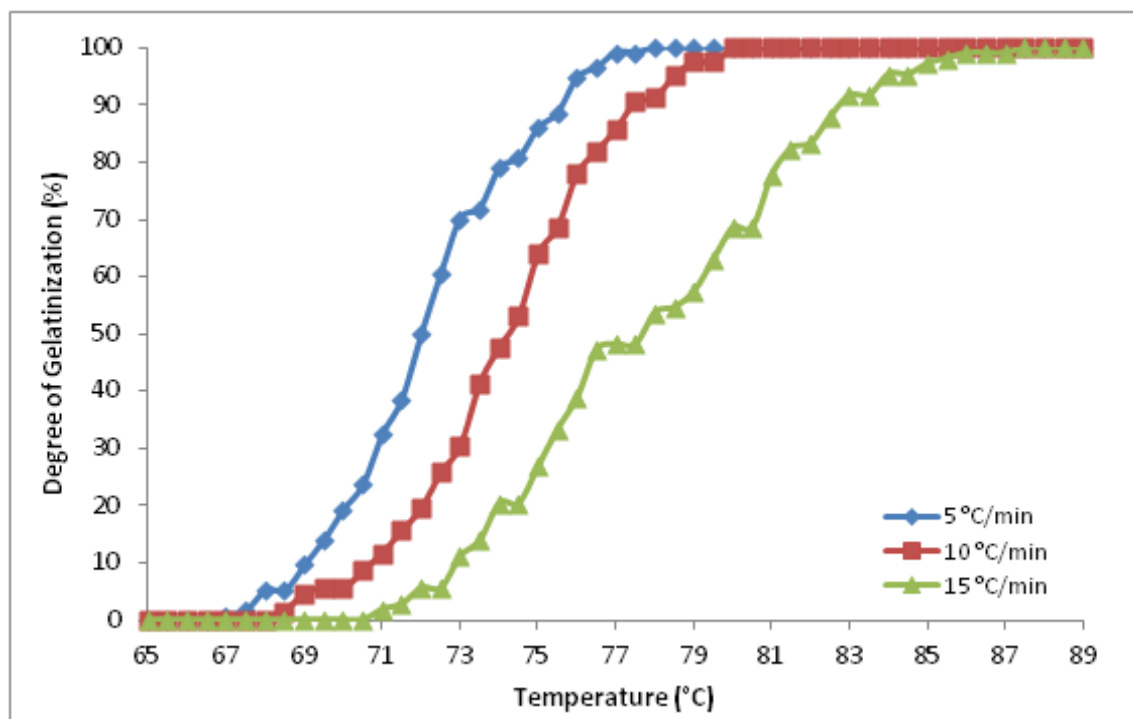
Similar conditions than the studied in the samples heated in excess water (5, 10 and 15 °C/min) were used to compare the gelatinization temperature of isolated potato starch granules suspended in pre-gelatinized corn starch solutions.

#### **5.2.1.1. Diluted pre-gelatinized corn starch solution (3 % concentration)**

The on-set, ending and mean gelatinization temperature for isolated starch granules suspended in the 3 % solution for the different heating rates is shown in Table 5.2. As in the excess of water experiments, when increasing the heating rate of the treatment, a higher gelatinization temperature was observed being 69.1, 71.3 and 72.2 °C when heating at 5, 10 and 15 °C/min ( $p < 0.05$ ), respectively. In Figure 5.6 the degree of gelatinization of the granules is presented. As in excess of water conditions, when the heating rate was increased, starch gelatinization is also retarded ( $p < 0.05$ ) and reaches 100 % gelatinization at a higher temperature (from 78 °C at 5 °C/min to 88 °C at 15 °C/min). Also, the gelatinization range is significantly higher when the heating rate is increased from 5 to 15 °C/min, being  $3.0 \pm 0.2$  °C for the granules heated at 5 °C/min and  $5.6 \pm 0.1$  °C for the granules heated at 15 °C/min ( $p < 0.05$ ).

**Table 5.2:** On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules in a 3 % pre-gelatinized corn solution when heated at 5, 10 and 15 °C/min.

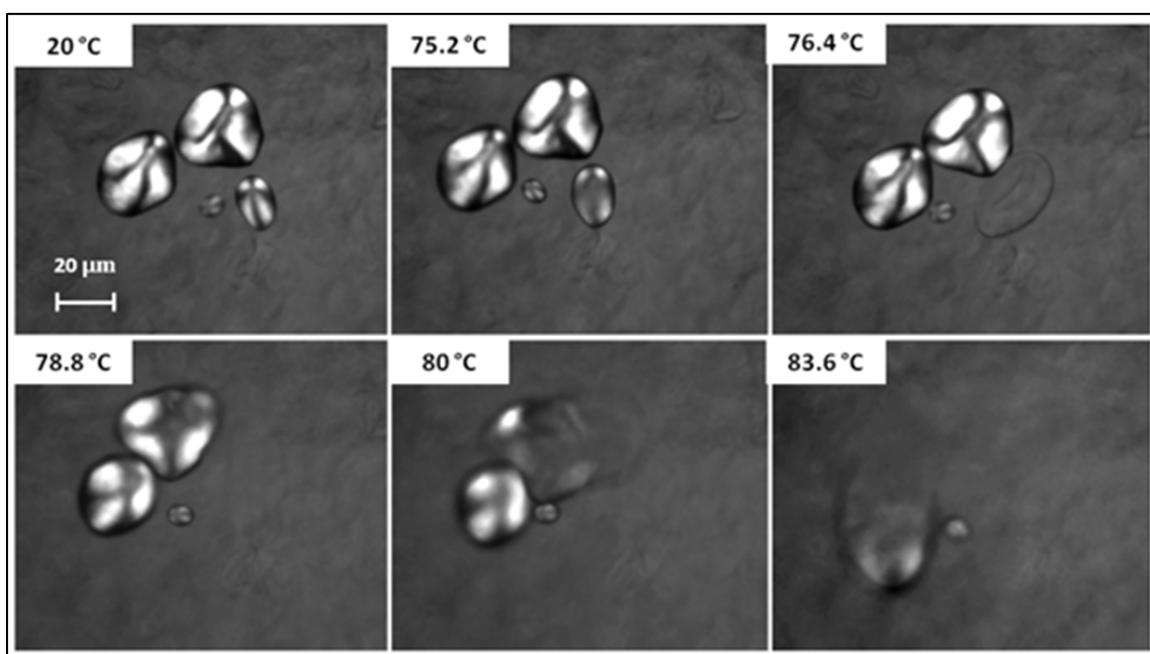
Heating Rate	5 °C/min		10 °C/min		15 °C/min	
N	114		128		125	
	On-set	Ending	On-set	Ending	On-set	Ending
Mean (°C)	69.1 ± 0.1	72.2 ± 0.2	71.3 ± 0.2	74.2 ± 0.2	72.2 ± 0.2	77.9 ± 0.2
Minimum (°C)	63.6	66.8	64.8	68.2	65.4	70.8
Maximum (°C)	74.2	77.8	77.4	80.0	80.0	87.2



**Figure 5.6:** Degree of gelatinization of isolated potato starch granules in a 3 % pre-gelatinized corn solution when heated at 5, 10 and 15 °C/min.

### 5.2.1.2. Diluted pre-gelatinized corn starch solution (5 % concentration)

In Figure 5.7 a gallery of images of the gelatinization process of isolated potato starch granules suspended in a 5 % pre-gelatinized corn solution is presented. As in the previous experiments, the granules show their characteristic Maltese cross at room temperature, which is lost as the temperature is increased (loss of birefringence) and swelling occurs. Not all the granules gelatinize at the same temperature, and it is possible to observe that, as the solution is made of pre-gelatinized starch, once the un-gelatinized granules gelatinize, they cannot be distinguished from the background.



**Figure 5.7:** Images of the gelatinization process of isolated potato starch granules in a solution of 5% pre-gelatinized corn starch heated at 15 °C/min.

In Table 5.3 and Figure 5.8 the gelatinization temperature of isolated potato starch granules suspended in a 5 % solution of pre-gelatinized corn starch and the degree of



gelatinization of the starch respectively are shown when heated at 5, 10 and 15 °C/min. As in the previous scenarios (excess of water and 3 % solution of pre-gelatinized corn starch), when the heating rate is increased from 5 to 15 °C/min, a higher gelatinization temperature of the granules is observed, being 70.9, 73.5 and 76.7 °C when heating at 5, 10 and 15 °C/min, respectively ( $p < 0.05$ ).

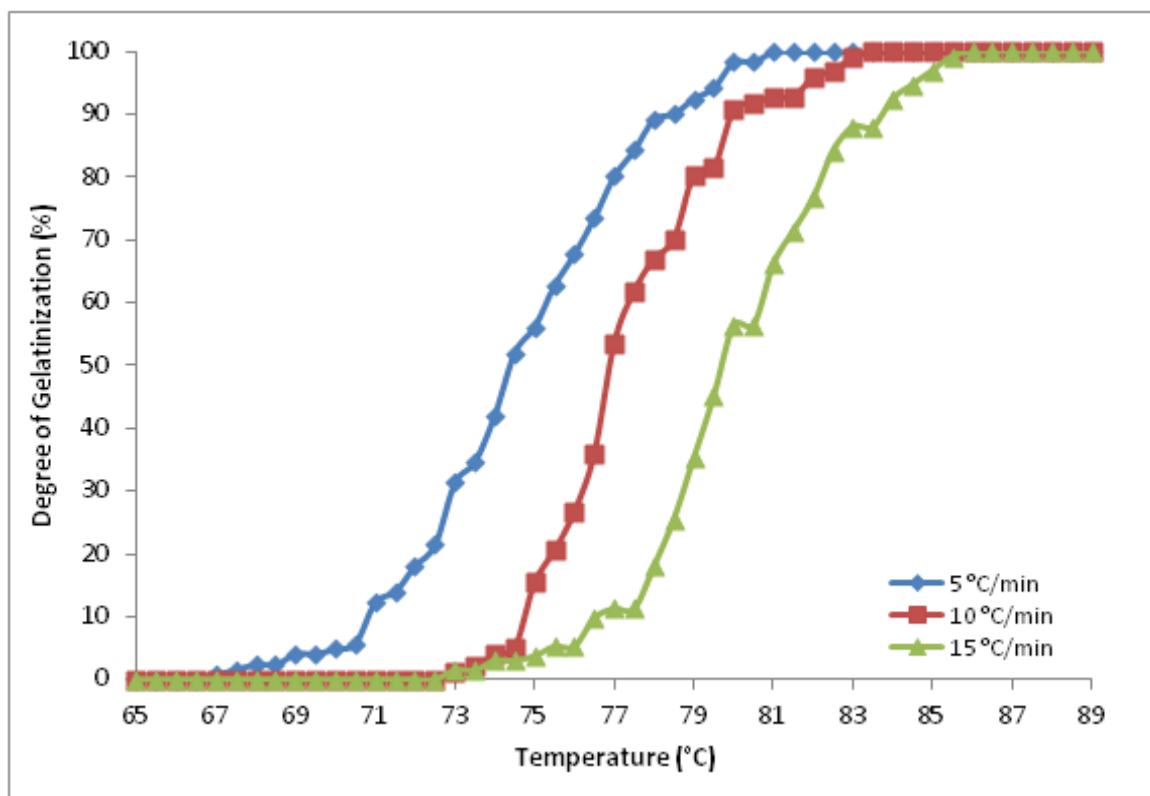
**Table 5.3:** On-set, ending and mean gelatinization temperatures (°C) of isolated potato starch granules heated in a 5 % pre-gelatinized corn solution at 5, 10 and 15 °C/min.

Heating Rate	5 °C/min		10 °C/min		15 °C/min	
N	121		102		133	
	On-set	Ending	On-set	Ending	On-set	Ending
Mean (°C)	70.9 ± 0.2	74.6 ± 0.3	73.5 ± 0.2	77.4 ± 0.2	76.7 ± 0.2	80 ± 0.2
Minimum (°C)	64.2	66.6	67.7	73	68.6	72.7
Maximum (°C)	77.0	81.0	78.7	83.3	82.4	86

As in excess of water and in the 3 % pre-gelatinized corn starch solution, when increasing the heating rate from 5 to 15 °C/min, the granules reached 100% gelatinization at a higher temperature (from 81 °C at 5 °C/min to 86 °C at 15 °C/min). Also, when studying the gelatinization range, it is possible to observe that this occurs in a significantly wider range of temperature when increasing the heating rate from 5 to 15 °C/min, being  $3.6 \pm 0.2$  °C for the granules heated at 5 °C/min and  $5.4 \pm 0.1$  °C for the granules heated at 15 °C/min ( $p < 0.05$ ).

In order to compare the effect of the heating rate and water availability of the system, the results of the gelatinization process of isolated potato starch granules are presented in Figure 5.9; where figure (a) compares the experiments at 5 °C/min, figure (b) at 10

°C/min and figure (c) at 15 °C/min. In each of the figures, excess of water, 3 % and 5 % solutions are compared.

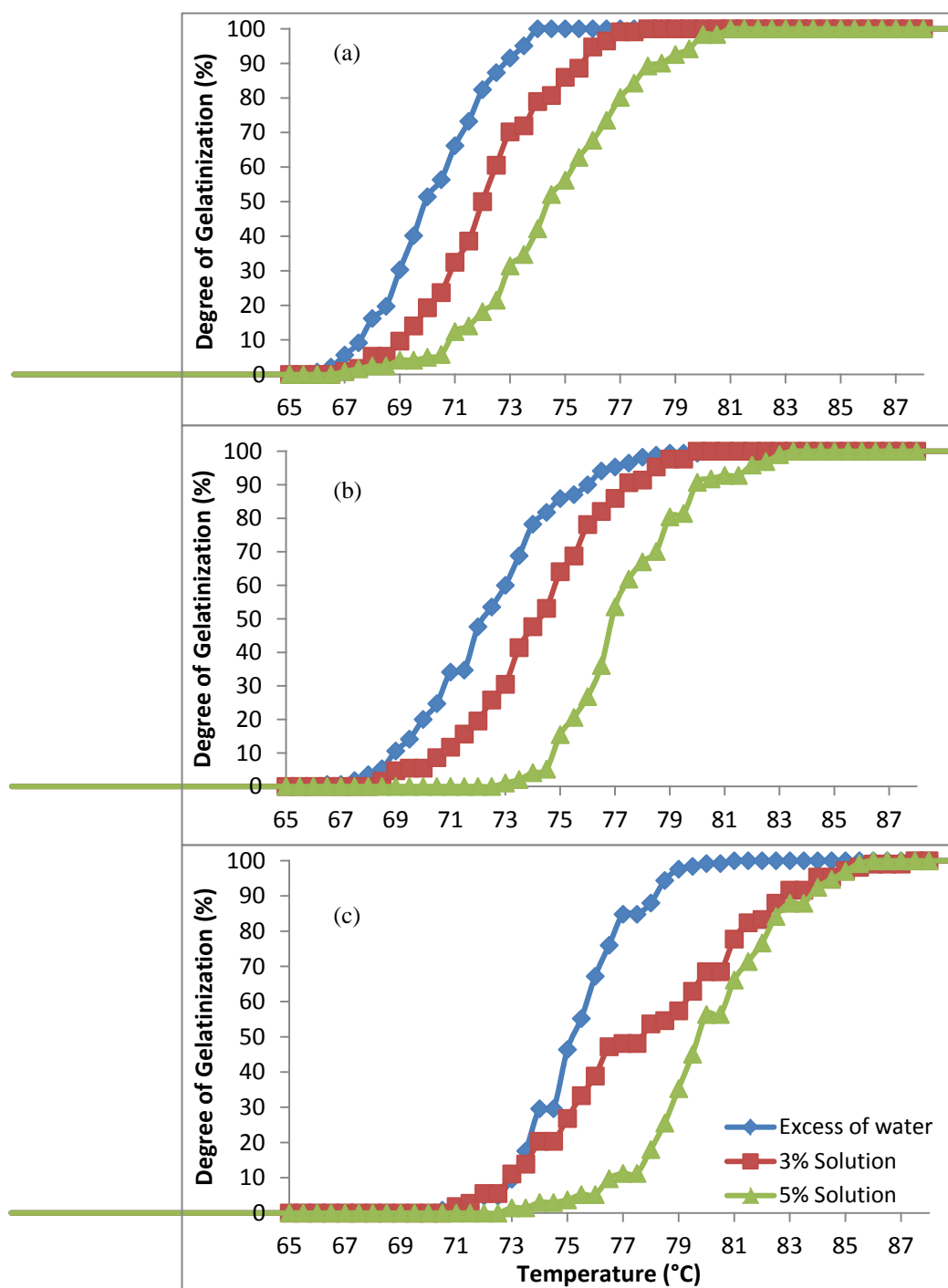


**Figure 5.8:** Degree of gelatinization of isolated potato starch granules in a 5% solution of pre-gelatinized corn starch when heated at 5, 10 and 15 °C/min.

When heating the starch granules in the different solutions, the gelatinization process is retarded when increasing the amount of solute. This may be happening due to a competition for the available water of the system between the un-gelatinized starch and the pre-gelatinized starch during heating (Bouchon & Aguilera, 2001; Fukuoka, et al., 2002; Primo-Martin, et al., 2007; Zhang, et al., 2011). According to this, when the solution is more concentrated (5 %) more heat is needed to complete gelatinization,

since there may be heat transfer differences that retard the gelatinization process due to the presence of solute and less water is available to fulfill the energy requirements of starch gelatinization (Blaszczak, et al., 2005; Karlsson & Eliasson, 2003; Svihus, et al., 2005). It can be seen that the gelatinization process occurs in a broader range of temperature when increasing the heating rate from 5 to 15 °C/min and also when increasing the solute concentration (excess of water v/s 3 and 5 % solute concentration). When heating at 5 °C/min the gelatinization range increases significantly from  $3.2 \pm 0.1$  °C to  $3.6 \pm 0.2$  °C when heated in excess water and in the 5 % solution respectively ( $p < 0.05$ ). For the granules heated at 10 °C/min the gelatinization range increases from  $3.1 \pm 0.1$  °C to  $3.9 \pm 0.2$  °C when heated in excess water and in the 5 % solution. When heating the granules at 15 °C/min, the gelatinization range increases from  $4.4 \pm 0.2$  °C to  $5.4 \pm 0.2$  °C when increasing the amount of solute from excess water to the 5 % solution. In 3 % solution concentrations, no significant changes were observed in the gelatinization range.

In Table 5.4 it is possible to observe that when increasing the heating rate and solute concentration, a higher gelatinization temperature can be observed for isolated potato starch granules. The lowest gelatinization temperature is obtained when heating the granules in excess of water at 5 °C/min ( $67 \pm 0.1$  °C) and increases when increasing the heating rate; the highest is reached when heating at 15 °C/min the solution with 5 % pre-gelatinized corn starch ( $76.7 \pm 0.2$  °C). Also, the degree of gelatinization is also retarded when increasing the heating rate and the solute concentration. When heating the samples in excess of water at 5 °C/min total gelatinization is achieved at 74 °C, whereas, when the starch is heated at 15 °C/min in the 5 % solution, 100 % gelatinization is only reached at 87 °C. In this sense, it is possible to say that starch gelatinization is a dynamic process, which is governed by moisture content (since when there is less available water, the granules tend to present a higher gelatinization temperature) and depends on the heating rate that is being used, increasing the gelatinization temperature, when increasing the heating rate (Sobukola, et al., 2012).



**Figure 5.9:** Degree of gelatinization of isolated potato starch granules heated at (a) 5°C/min, (b) 10°C/min and (c) 15°C/min in excess of water, 3 and 5 % corn pre-gelatinized starch solutions.

**Table 5.4:** Gelatinization temperature (°C) of isolated potato starch granules heated at 5, 10 and 15°C/min in excess of water, 3 and 5 % corn pre-gelatinized starch solutions

	5 °C/min	10 °C/min	15 °C/min
Excess Water	67.0 ± 0.1	69.3 ± 0.2	70.9 ± 0.2
3 % Solution	69.1 ± 0.2	71.3 ± 0.2	72.2 ± 0.4
5 % Solution	70.9 ± 0.2	73.5 ± 0.2	76.7 ± 0.2

### 5.2.2. Vacuum Heating

In the solutions of pre-gelatinized corn starch (3 and 5 %) vacuum heating was also performed at two different working pressures (water boiling point of 38 and 69 °C), as in the excess of water conditions.

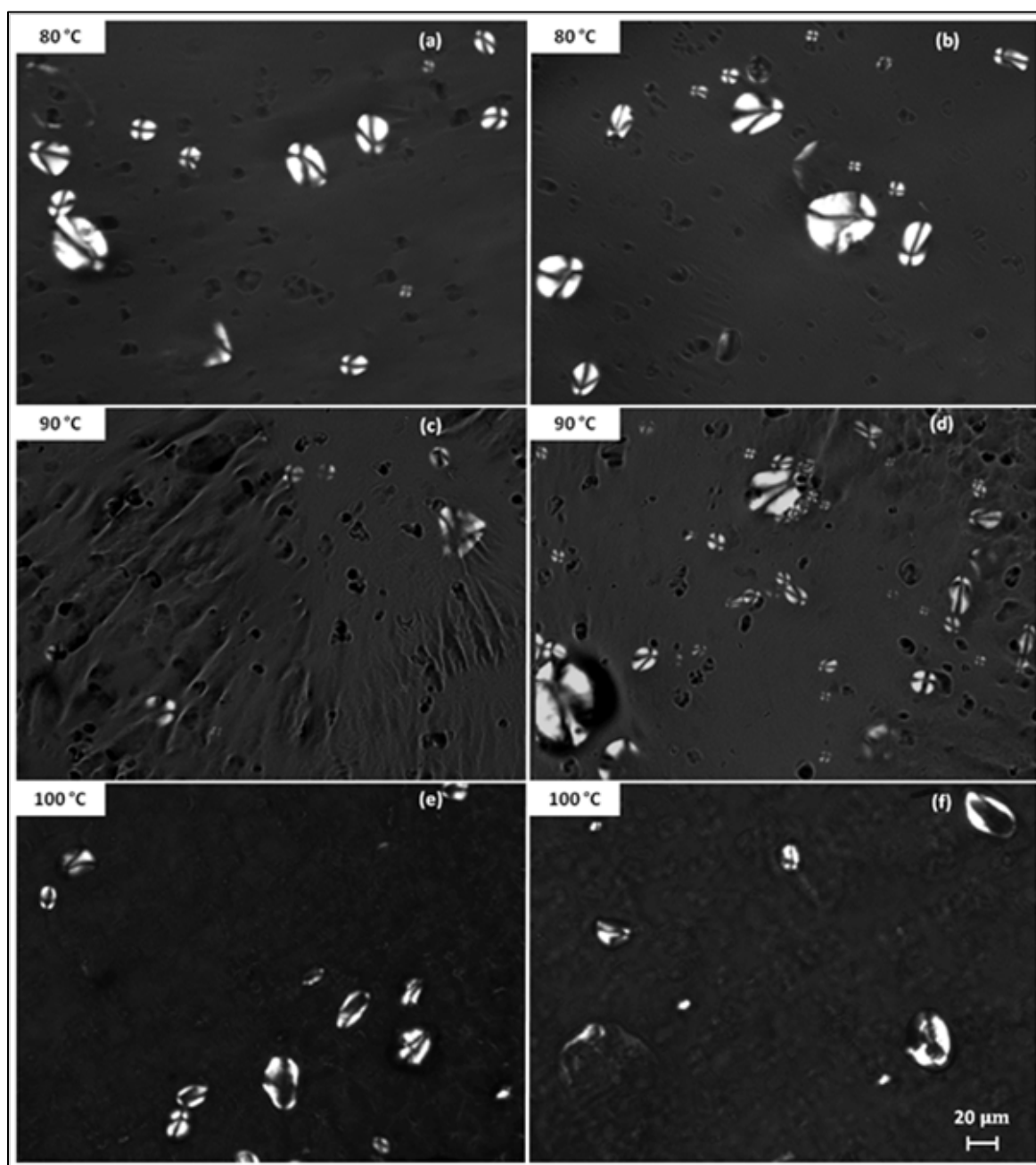
#### 5.2.2.1. Water boiling point of 38 °C

For a water boiling point of 38 °C, as when vacuum-heating the granules in excess water, no starch gelatinization was observed. As discussed above (see section 5.1.2.1) this may be happening due to the absence of water when reaching the gelatinization temperature of the granules. On the other hand, when there is still water available to gelatinize the granules, the on-set gelatinization temperature has not been achieved. However, it is important to emphasize that the system does not reach the equilibrium at 38 °C, since the water boiling point of water, in this case, may be also increased, due to the presence of other components (pre-gelatinized corn solution).

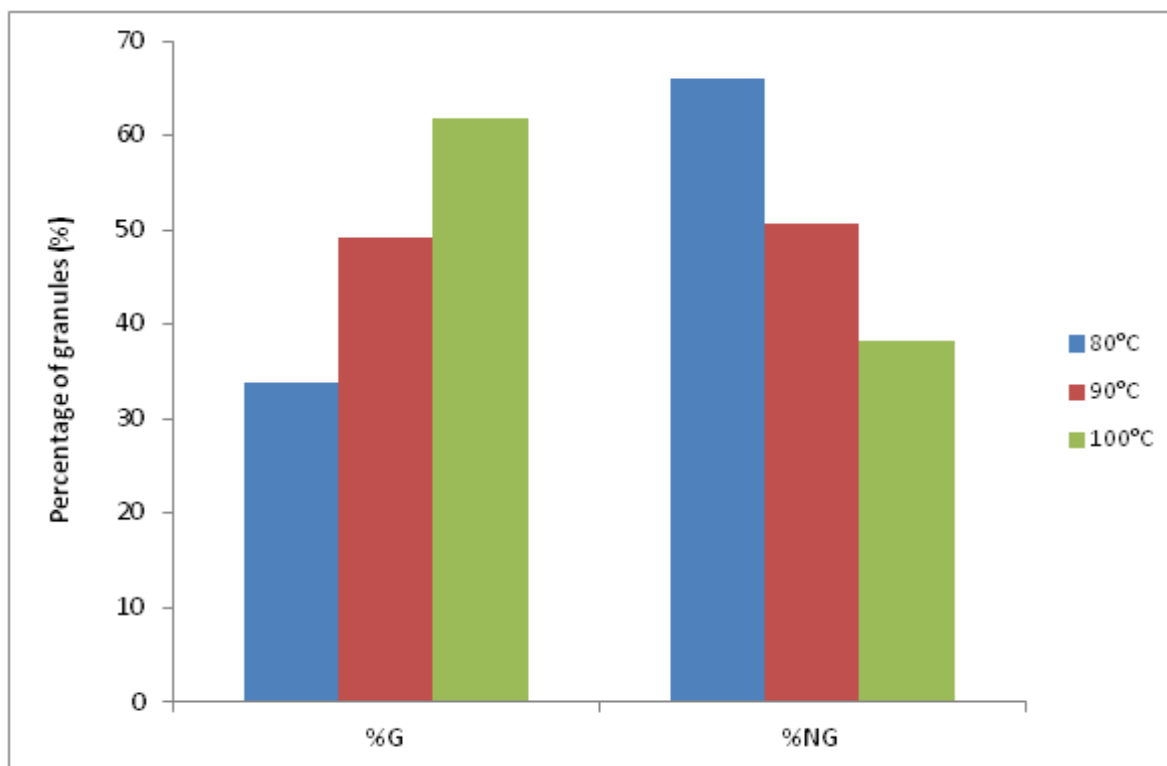
#### 5.2.2.2. Water boiling point of 69 °C

When vacuum-heating the granules suspended in the 3 % solution of pre-gelatinized corn starch using a water boiling point of 69 °C three different conditions were studied: vacuum heating until 80, 90 and 100 °C. When heating up to temperatures below 80 °C no starch gelatinization was observed. When vacuum-heating up to 80 °C a certain degree of gelatinization was observed. At 90 °C a larger amount of granules completed the gelatinization process and at 100 °C the solution was completely dehydrated with more gelatinized granules. No significant changes in the granules were found when vacuum-heating them above 100 °C at the experimental conditions described before.

Figure 5.10 shows a gallery of images of the process. Pictures a and b represent the heating process up to 80 °C, where it is possible to observe that a few granules have lost their native birefringent structure. In fact, at 80 °C ( $n = 127$ ), 34 % of the granules have initiated the gelatinization process and in 66 % of the granules studied, no significant changes in their microstructure were observed. Whereas when the temperature was increased up to 90 °C (images c and d) a larger amount of granules had gelatinized, or at least, reached the on-set gelatinization temperature. At the mentioned temperature, 49 % of the granules initiated the gelatinization process ( $n = 142$ ), while the un-gelatinized granules decreased to 51 %, showing differences between the samples heated up to 80 °C. When vacuum-heating up to 100°C (images e and f) it may be observed that a higher amount of granules started the gelatinization process. In fact, the gelatinized granules increased to 62 % ( $n = 123$ ). This may be explained, since when raising the temperature of the system, more water has been evaporated, and less water is available for the granules to start and complete the gelatinization process. The raise in the amount of gelatinized granules may be explained since the water does not instantly evaporate at the mentioned water boiling point, and due to the presence of solute, the water boiling point may be retarded. It is also possible to observe that the solution becomes more dehydrated when increasing the temperature.



**Figure 5.10:** Vacuum-heated isolated potato starch granules in a pre-gelatinized corn solution at 3% at a working pressure of 300 mbar (boiling point of water of 69 °C).



**Figure 5.11:** Percentage of granules that are gelatinizing (%G) and that have not even start the gelatinization process (%NG) in vacuum conditions (300 mbar, water boiling point of 69 °C), at 80, 90 and 100 °C.

When comparing the results obtained under vacuum and atmospheric conditions, it is possible to observe that under atmospheric conditions all the granules at least started the gelatinization process at 80, 90 and 100 °C, while under vacuum conditions, 34 % of the granules started the process at 80 °C, 49 % at 90 °C and 62 % at 100 °C, reaffirming the theory that, as the water boiling point is reduced from 100 to 69 °C, the available water to gelatinize the granules is decreased and may explain the difference in the amount of granules that started the process between atmospheric and vacuum conditions.

For isolated potato starch granules suspended in the 5% pre-gelatinized corn solutions, no microstructural changes were observed when vacuum heating at a water boiling point



of 38 °C and a boiling point of water of 69 °C. This may be happening since at the temperatures studied all the water present in the system had evaporated before the granules could start the gelatinization process.

### **5.3. Gelatinization of potato starch granules in a starch-gluten-water matrix**

Heating isolated potato starch granules in water and in the pre-gelatinized corn solutions was performed as a first approach to the frying process. In order to understand the effect of other ingredients and a formulated matrix in the gelatinization temperature of potato starch granules 2 matrices were designed. The amount of starch and gluten remained constant and was chosen to be able to observe and distinguish the granules one from each other when studying the process. The amount of water was modified, decreasing from 50 to 40 %, in order to understand the effect of moisture content in the gelatinization temperature of the granules embedded in the matrix.

#### **5.3.1. Atmospheric frying**

The objective of this study was to understand the effect of the matrix in the gelatinization process. To do so, the matrices were heated at 5, 10 and 15 °C/min in oil, in order to miniaturize the frying process.

Table 5.5 shows the on-set and ending gelatinization temperature of the granules heated in the matrix. When heating the matrix with an amount of water corresponding to a 50 %, a higher mean on-set gelatinization temperature than the one obtained when heating them in excess of water, was observed; however, the difference was not significant ( $p > 0.05$ ). This may be explained since it is considered that a 50 % of water content is closest to be excess water (Liu, et al., 2006). In the 3 and 5 % pre-gelatinized corn solutions this situation does not occur, since in pre-gelatinized starch solutions water is strongly bound at room temperature because hydrogen bonding of the starch granules

are previously disrupted and the hydrophilic tendency of the granules increases (Singh, et al., 2009); thus at room temperature they are able to swell more water. Therefore, when heated, water becomes more linked, since there are still some hydrogen bonds undisrupted, making the granules to absorb even more water. This shows an increase in the viscosity of solutions of pre-gelatinized starch, when compared to the viscosity of non pre-gelatinized starch granules (Karaoğlu, et al., 2001). On the other hand, at room temperature pre-gelatinized starch granules can hold more water than wheat starch, making that small amounts of pre-gelatinized starch absorb higher amounts of water (Traynham, et al., 2007)

**Table 5.5:** On-set, ending and mean gelatinization temperatures (°C) of potato starch granules in a starch-gluten-water matrix (50 % water content) heated in oil at 5, 10 and 15 °C/min.

Heating Rate	5 °C/min		10 °C/min		15 °C/min	
n	147		153		160	
	On-set	Ending	On-set	Ending	On-set	Ending
Mean (°C)	67.4 ± 0.2	73.3 ± 0.3	70.4 ± 0.2	79.8 ± 0.4	72.4 ± 0.4	81.1 ± 0.4
Minimum (°C)	63	65.8	64.2	71.6	64	70.8
Maximum (°C)	74.4	82.6	78	91.8	90.2	96.7

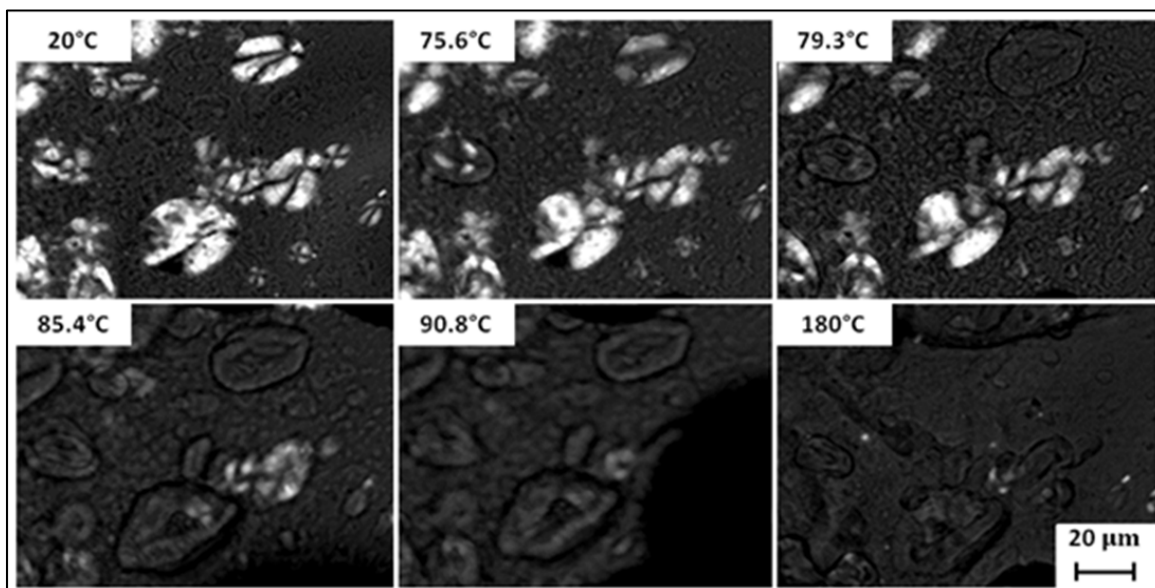
Table 5.6 shows the gelatinization temperature of potato starch granules when reducing the amount of water from 50 to 40 %, after heating at three rates. A higher gelatinization temperature was observed, when increasing from 69.1 to 73.6 and 74.9 °C when heating at 5, 10 and 15 °C/min respectively ( $p < 0.05$ ). Also, the whole set of data corresponding to the granules heated in excess of water, corn pre-gelatinized solutions (3 and 5 %) and the granules heated in oil in the matrix with a moisture content of 40 % were compared,

in order to study if there were significant differences between these conditions. The on-set gelatinization temperature was significantly increased when heating the matrix in oil compared with the granules heated in excess of water, as can be observed in Table 5.7 ( $p < 0.05$ ). This may be happening since other ingredients, such as gluten, may retard starch gelatinization, since it has a high capacity to retain water, reducing water availability (Eliasson, 1983; Fessas & Schiraldi, 2001; Parada & Aguilera, 2011).

**Table 5.6:** On-set, ending and mean gelatinization temperatures ( $^{\circ}\text{C}$ ) of potato starch granules in a starch-gluten-water matrix (40 % water content) heated in oil at 5, 10 and 15  $^{\circ}\text{C}/\text{min}$ .

Heating Rate	5 $^{\circ}\text{C}/\text{min}$		10 $^{\circ}\text{C}/\text{min}$		15 $^{\circ}\text{C}/\text{min}$	
n	149		144		153	
	On-set	Ending	On-set	Ending	On-set	Ending
Mean ( $^{\circ}\text{C}$ )	$69.1 \pm 0.2$	$76.6 \pm 0.3$	$73.6 \pm 0.3$	$84.6 \pm 0.4$	$74.9 \pm 0.3$	$86.4 \pm 0.4$
Minimum ( $^{\circ}\text{C}$ )	63.4	66.4	65.9	76.1	68	77.6
Maximum ( $^{\circ}\text{C}$ )	82.8	88.8	83.1	96.1	88.7	99.7

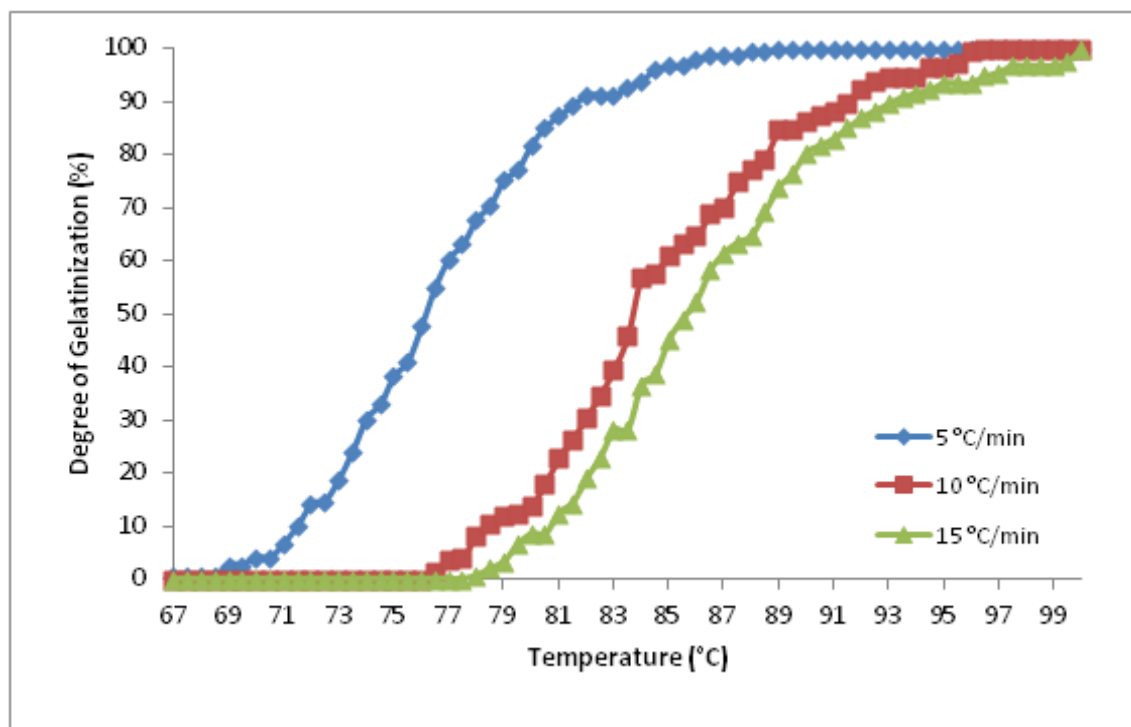
However, no significant differences in the on-set gelatinization temperature were found when comparing with granules heated in the 3 and 5 % pre-gelatinized corn starch solutions. This may be explained, since as the water limitation was increased, due to the added solute, retarding the on-set gelatinization for the granules may be similar to the designed matrix condition. Also, the gelatinization is retarded due to a decrease in water availability.



**Figure 5.12:** Images of the gelatinization process of isolated potato starch granules heated in a starch-gluten-water matrix (40% water content).

When increasing the heating rate, lower degree of gelatinization was reached at the same temperature (see Figure 5.13). For example, at 80 °C, the degree of gelatinization for 5, 10 and 15 °C/min was 82, 14 and 8 % respectively.

These results not only indicate that when increasing the heating rate the gelatinization is retarded, they also indicate that the process occur in a broader range of time (Svihus, et al., 2005). There are significant differences in the range of temperature in which the gelatinization range takes place between the heating at 5 and 15 °C/min ( $p < 0.05$ ). Although an increase in the degree of gelatinization was observed between heating at 10 and 15 °C/min, no significant differences were observed between these conditions ( $p > 0.05$ ).



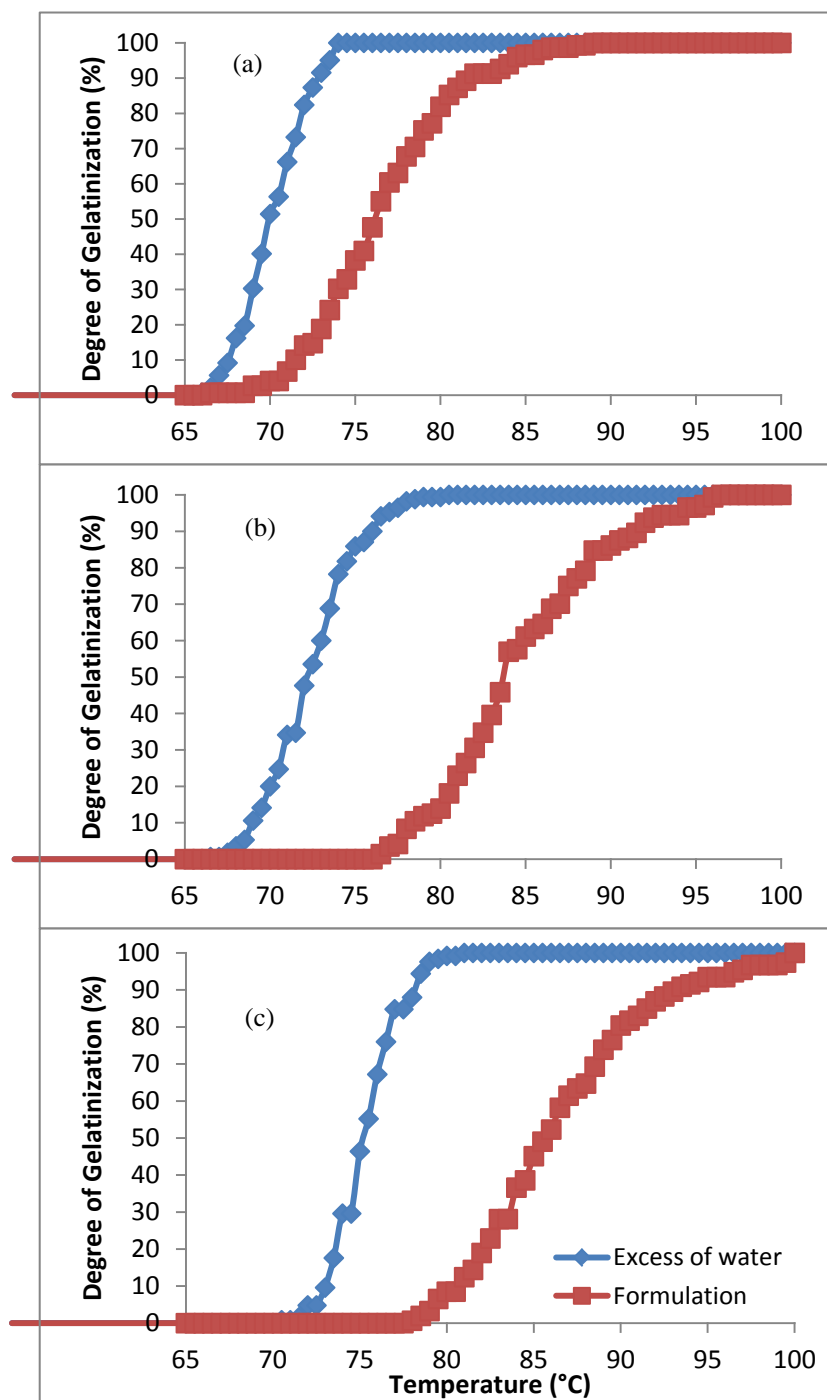
**Figure 5.13:** Degree of gelatinization of isolated potato starch granules in a starch-gluten-water matrix (water content of 40%) heated at 5, 10 and 15 °C/min.

As in the on-set gelatinization temperature, significant differences were found between the granules heated in excess of water and in the designed matrix in all heating rates conditions ( $p < 0.05$ ). However, for the granules heated in the 3 and 5 % solution, no significant differences were found. This may be explained, since the pre-gelatinized corn solutions were prepared as a model system, and the interaction between water and the pre-gelatinized starch differs from the obtained between the gluten and water; thus the behavior of the available water for starch gelatinization is different in each situation, as explained above. To illustrate this situation only the degree of gelatinization of the granules heated in excess water and in the starch-gluten-water matrix is shown in Figure 5.14. When studying the rate of gelatinization of the granules, it can be observed that this is significantly increased in the granules embedded in the starch-gluten-water matrix

than those heated in excess water, as reflected in the slopes in Figure 5.14. Table 5.7 shows a comparison between the on-set, ending and gelatinization range of the described granules. It is also possible to observe some changes in the gluten network due to high temperature processing, such as dehydration and some expansion which may be produced by the escaping of water vapor (Gazmuri & Bouchon, 2009).

**Table 5.7:** On-set, ending and gelatinization range of potato starch granules heated at 5, 10 and 15 °C/min in excess of water and in a starch-gluten-water matrix (40 % water content).

Heating Rate	5 °C/min		10 °C/min		15 °C/min	
Condition	Ex water	Dough	Ex water	Dough	Ex water	Dough
On-set (°C)	67.0 ± 0.2	69.1 ± 0.2	69.3 ± 0.2	73.6 ± 0.3	70.9 ± 0.2	74.9 ± 0.3
Ending (°C)	70.1 ± 0.2	76.6 ± 0.2	72.4 ± 0.2	84.6 ± 0.4	75.3 ± 0.2	86.4 ± 0.4
Gelatinization range (°C)	3.2 ± 0.1	7.4 ± 0.2	3.3 ± 0.1	10.9 ± 0.2	4.4 ± 0.1	11.5 ± 0.2



**Figure 5.14:** Degree of gelatinization of isolated potato starch granules heated in excess of water and in oil in a starch-gluten-water matrix (water content of 40%) at (a) 5°C/min (b) 10°C/min and (c) 15°C/min.

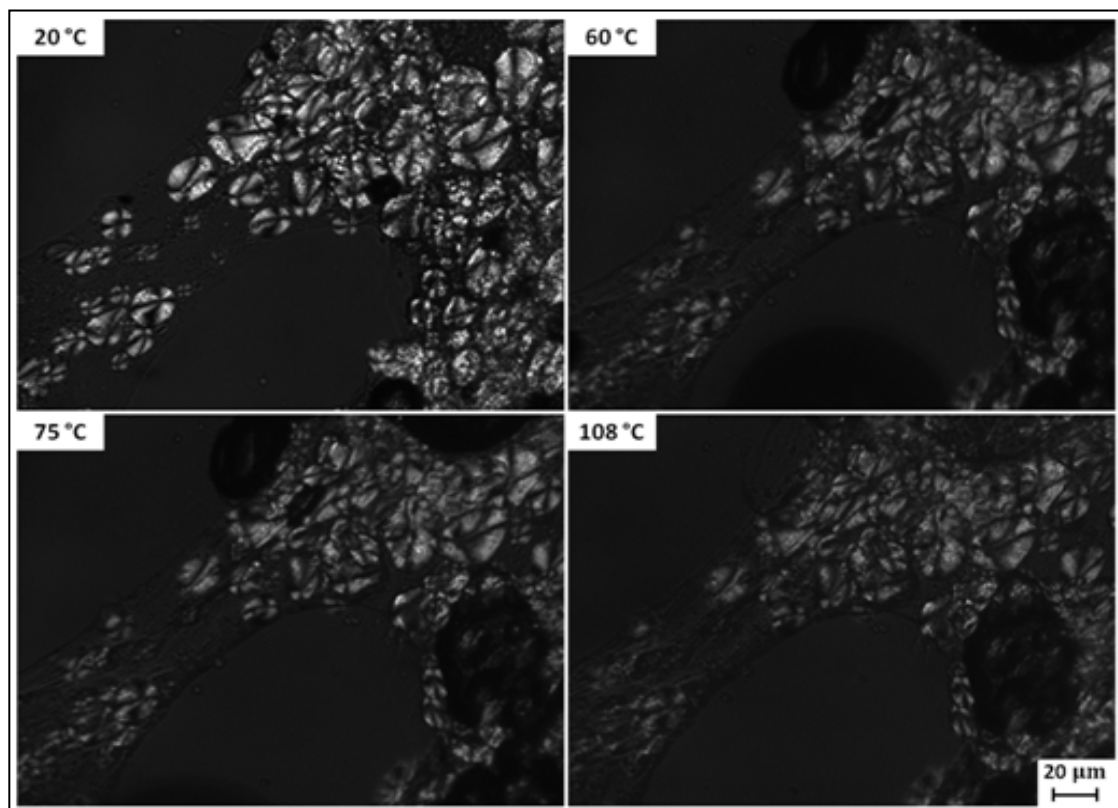
### **5.3.2. Vacuum frying**

The starch-gluten-water matrices were heated using the 2 vacuum levels.

#### **5.3.2.1. Water boiling point of 38 °C**

The samples were heated up to 108 °C at 15 °C/min. When studying this condition, no starch gelatinization was observed as in excess of water and in the pre-gelatinized corn solutions. Figure 5.15 shows a gallery of images that represent the process. No images of what is happening between 30 and 50 °C are shown, since in this range of temperatures a vigorous escape of water vapor bubbles is taking place, due to water evaporation. As temperature increases, the water boiling is reduced, and when the matrix reaches the gelatinization temperature (approximately 70 °C), no more available water is left to start the gelatinization process (Rajkumar, et al., 2003; Wang, et al., 2010). In the images it is possible to observe some black bubbles, which correspond to vapor bubbles that are emerging from the product to the environment. As in previous experiments under vacuum (excess water and pre-gelatinized corn solutions), it does not mean that the water present in the dough evaporates instantly, it is a dynamic process; however, when reaching temperatures near 70 °C, the water that started the evaporation process at nearly 38 °C (since it is not a system in equilibrium and the presence of solute may retard the evaporation process) is not available in its liquid state to gelatinize the starch granules embedded in the matrix. On the other hand, other microstructural changes may be observed in the matrix such as an apparent expansion and dehydration of the gluten network. This can be seen in the images, since the granules that are embedded in the gluten network start to change their shape, from a circular to a more oval shape.



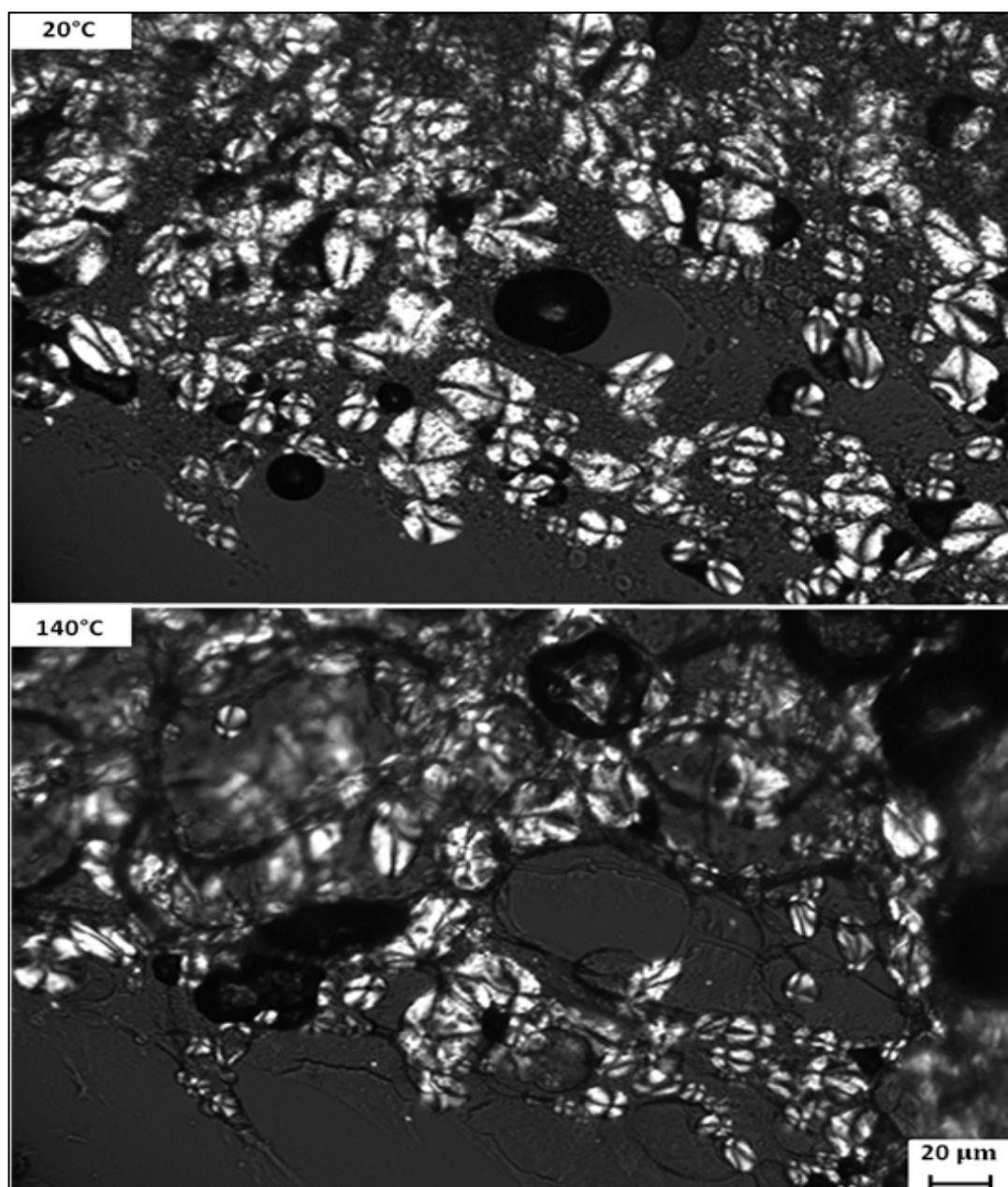


**Figure 5.15:** Gallery of images of a starch-gluten-water matrix vacuum-fried in oil at 65 mbar. Black bubbles represent water vapor escaping from the product. Some expansion and dehydration of the gluten network may be observed when increasing the temperature.

#### 5.3.2.2. Water boiling point of 69 °C

In order to study if potato starch granules embedded in a starch-gluten-water matrix with a water content of 40 % gelatinized in a vacuum system with a water boiling point of 69 °C, the matrices were heated up to 140 °C at 15 °C/min. In Figure 5.16 a comparison between the gluten-starch-water matrix at 20 °C and at 140 °C is shown. No images of the whole process could be acquired, since the vigorous vapor escape, due to water evaporation, takes place when the gelatinization of the granules is occurring. Comparing

these images, it is possible to observe that some of the granules did gelatinize and that some of them did not do it. This may be explained since, as in the cases of excess water and the pre-gelatinized corn solution (3 %) the water available for the gelatinization of the granules is lower due to water evaporation. As the system does not reach the equilibrium, it is not possible to ensure that there is no water left at temperatures above 70 °C. Moreover, water boiling point may be retarded, due to the presence of solutes. However, since the amount of water used to perform the miniaturization is reduced, the available water in the system tends to highly decrease at the mentioned temperatures. The diffuse zones in the picture of the bottom of Figure 5.18 represent the gelatinized and partially gelatinized granules. Also, as when vacuum-frying with a water boiling point of 38 °C, it is possible to observe changes in the gluten network as in atmospheric vacuum-frying, such as dehydration and some expansion (Sobukola, et al., 2012). The dehydration of the gluten network may be identified since, in the picture that represents the matrix at 20 °C, tiny dots may be seen present in the gluten network; whereas in the picture that represents the 140 °C situation, these dots have been eliminated, or are present in a much lower amount.



**Figure 5.16:** Structure of a starch-gluten-water matrix (40% water content) vacuum-heated in oil with a water boiling point of 69 °C. The upper picture shows the structure before heating and the bottom picture after vacuum-heating.

## 6. CONCLUSIONS

Process miniaturization allowed the following up of microstructural changes undergone by isolated potato starch granules when heated in excess of water and in two solutions of pre-gelatinized corn starch under atmospheric and vacuum conditions in a polarized light microscope. It also allowed following up the microstructural changes of potato starch granules heated in oil (fried) in a starch-gluten-water matrix under the same conditions.

When studying the effect of the heating rate in starch gelatinization, it was observed that increasing the heating rate from 5 to 15 °C/min resulted in a higher on-set gelatinization temperature for the granules heated in excess of water, in the solutions of pre-gelatinized corn starch (3 and 5 %) and in the starch-gluten-water formulation. Also, the granules heated at higher heating rates completed the process in a significant broader range of temperature (higher gelatinization range) ( $p < 0.05$ ). This may be explained since, as the granules gelatinize due to energy absorption, and the process is a time and temperature dependent phenomenon, the history of the temperature becomes critical in the process.

On the other hand, when reducing water availability, either in the solution of pre-gelatinized corn starch or in the formulated dough, a significant increase in the on-set gelatinization temperature was observed ( $p < 0.05$ ). This may be happening, since starch gelatinization occurs when an adequate amount of water is available to hydrate the granules and interact with them to fulfill the gelatinization process. In pre-gelatinized corn solutions a competition for the available water takes place between the ungelatinized granules and the pre-gelatinized starch when heating. In the formulated product, the gluten network absorbs water to become hydrated, so the amount of water that becomes accessible for the granules to gelatinize decreases. Therefore, a significantly higher on-set gelatinization temperature is achieved and a lower degree of gelatinization for different temperatures may be observed when comparing it with the degree of gelatinization obtained when heating isolated granules in excess water ( $p < 0.05$ ). Also, when decreasing the amount of water in the system, a significantly higher

gelatinization range was observed, indicating that when decreasing the amount of water, the granules gelatinize in a broader range of temperature. This may be also explained due to a competition for the available water in the system between the un-gelatinized granules and the other solutes (pre-gelatinized corn or wheat gluten).

When vacuum-heating under pressure conditions that defined a water boiling point of 38 °C condition (lower than the gelatinization temperature according to the literature and experimental results) no starch gelatinization was observed in any of the studied conditions. This may be happening, due to rapid water evaporation before reaching the gelatinization temperature of the granules. Although the system might not reach thermodynamic equilibrium when achieving the gelatinization temperature of potato starch granules, there is not enough water to gelatinize the granules.

When the water boiling point was increased up to 69 °C, some of the granules were able to start the gelatinization process. In order to understand the temperature effect in vacuum-heated granules, images were taken at 70, 80 and 90 °C when vacuum-heating granules in excess of water and at 80, 90 and 100 °C when vacuum-heating the granules in the pre-gelatinized corn solutions. The degree of gelatinization reached by the granules depended on the final temperature of the treatment. This can be due to the fact that water evaporation is not an instant process (non-equilibrium conditions), therefore, enough liquid water to induce gelatinization may be present. This could be seen in most conditions (excess of water, 3 % solution and the formulated matrix). When samples were heated to higher temperatures using this vacuum level, a higher degree of gelatinization was achieved. In the case of the 5 % solution of pre-gelatinized corn starch, no starch gelatinization was observed. This may be due to insufficient available water to induce gelatinization, since the pre-gelatinized starch absorbs high amounts of water, limiting the available water to gelatinize the granules. When vacuum-frying the formulated matrix, a certain degree of gelatinization was observed, as in the granules heated in excess of water. This lower degree of gelatinization in the vacuum-fried matrices compared to the atmospheric fried ones, may explain the higher oil absorption

reported by other studies in formulated starchy products, due to deficient structure development (in this study, starch gelatinization).

It would be of great interest to study in real time and *in situ* the microstructural changes of the granules when more ingredients are considered in the formulation, such as different kinds of fiber (soluble and insoluble) or other solutes (*e.g.* salt or microencapsulated ingredients), since it would help to understand the behavior of diverse food building blocks in the frying process when they are isolated and the interaction between them when they are part of a formulation. Then, it would be interesting to try to relate the microstructural and macro-structural properties of the designed matrix. Also, staining the samples would be of great interest to try to elucidate the oil absorption phenomenon in these formulated products and therefore be able to determine, where the oil is located in this type of formulated products and understand the relationship between the different food building blocks and oil absorption, since the aim of formulating these products is to achieve lower oil contents in the final fried product. This microstructural approach may be a very useful tool in formulation engineering, since understanding the phenomena at the microstructural level gives valuable information of what is happening at the macro-structural level.

## REFERENCES

- Adedokun, M. O., & Itiola, O. A. (2010). Material properties and compaction characteristics of natural and pregelatinized forms of four starches. *Carbohydrate Polymers*, 79(4), 818-824.
- Aguilera, J., & Germain, J. (2007). Advances in image analysis for the study of food microstructure. *Understanding and controlling the microstructure of complex foods*. Abington Cambridge, England & Boca Raton, Fla.: Woodhead Publishing Limited and CRC Press LLC, 261-287.
- Aguilera, J. M. (2005). Why food microstructure? *Journal of Food Engineering*, 67(1-2), 3-11.
- Aguilera, J. M., & Bouchon, P. (2008). Scanning Electron and Transmission Electron Microscopies in Food Analysis. In: *Otles, S., Handbook of food analysis instruments, Boca raton: CRC Press. Pp. 495-511.*
- Aguilera, J. M., Cadoche, L., Lopez, C., & Gutierrez, G. (2001). Microstructural changes of potato cells and starch granules heated in oil. *Food Research International*, 34(10), 939-947.
- Aguilera, J. M., & Lilford (1996). Microstructure as applied to food engineering. In *P. Fito, E. Ortega-Rodríguez, G.V. Barbosa-Cánovas (Eds.). Food engineering 2000, Chapman and Hall, London (1996), 20-36*
- Aguilera, J. M., & Stanley, D. W. (1999). *Microstructural principles of food processing and engineering*: Springer.
- Anastasiades, A., Thanou, S., Loulis, D., Stapatoris, A., & Karapantsios, T. D. (2002). Rheological and physical characterization of pregelatinized maize starches. *Journal of Food Engineering*, 52(1), 57-66.
- Andrés-Bello, A., García-Segovia, P., & Martínez-Monzó, J. (2011). Vacuum frying: An alternative to obtain high-quality dried products. *Food Engineering Reviews*(3), 1-16.
- Arellano, M. P., Aguilera, J. M., & Bouchon, P. (2004). Development of a digital video-microscopy technique to study lactose crystallisation kinetics in situ. *Carbohydrate Research*, 339(16), 2721-2730.

- Baier-Schenk, A., Handschin, S., von Schonau, M., Bittermann, A. G., Bachi, T., & Conde-Petit, B. (2005). In situ observation of the freezing process in wheat dough by confocal laser scanning microscopy (CLSM): Formation of ice and changes in the gluten network. *Journal of Cereal Science*, 42(2), 255-260.
- Bertoft, E. (2004). Analysing starch structure. In A.C. Eliasson (Ed.), *Starch in Food: Structure, function and applications*, Woodhead Publishing Ltd. and CRC Press LLC, USA. .
- Biliaderis, C. G., Page, C. M., Maurice, T. J., & Juliano, B. O. (1986). Thermal characterization of rice starches: a polymeric approach to phase transitions of granular starch. *Journal of Agricultural and Food Chemistry*, 34(1), 6-14.
- Blaszcak, W., Valverde, S., & Fornal, J. (2005). Effect of high pressure on the structure of potato starch. *Carbohydrate Polymers*, 59(3), 377-383.
- Bogracheva, T. Y., Meares, C., & Hedley, C. L. (2006). The effect of heating on the thermodynamic characteristics of potato starch. *Carbohydrate Polymers*, 63(3), 323-330.
- Bouchon, P. (2010). Principios de Análisis de Imágenes. *Curso: Avances en Tecnología de Alimentos*, PUC.
- Bouchon, P., & Aguilera, J. A. (2001). Microstructural analysis of frying potatoes. *International Journal of Food Science and Technology*, 36(6), 669-676.
- Bouchon, P., Aguilera, J. M., & Pyle, D. L. (2003). Structure oil-absorption relationships during deep-fat frying. *Journal of Food Science*, 68(9), 2711-2716.
- Bouchon, P., Hollins, P., Pearson, M., Pyle, D. L., & Tobin, M. J. (2001). Oil distribution in fried potatoes monitored by infrared microspectroscopy. *Journal of Food Science*, 66(7), 918-923.
- Bouchon, P., & Pyle, D. L. (2004). Studying oil absorption in restructured potato chips. *Journal of Food Science*, 69(3), E115-E122.
- Brunnschweiler, J., Luethi, D., Handschin, S., Farah, Z., Escher, F., & Conde-Petit, B. (2005). Isolation, physicochemical characterization and application of yam (*Dioscorea* spp.) starch as thickening and gelling agent. *Starch/Stärke*, 57(34), 107-117.
- Buleón, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International journal of biological macromolecules*, 23(2), 85-112.



- Cuq, B., Abecassis, J., & Guilbert, S. (2003). State diagrams to help describe wheat bread processing. *International Journal of Food Science & Technology*, 38(7), 759-766.
- Chen, C. S., Chang, C. Y., & Hsieh, C. J. (2001). Improving the texture and color of fried products. . In J.B. Rossell (Ed.), *Frying: Improving Quality*, Woodhead Publishing Ltd. and CRC Press LLC, USA.
- Chiotelli, E., & Le Meste, M. (2002). Effect of small and large wheat starch granules on thermomechanical behavior of starch. *Cereal Chemistry*, 79(2), 286-293.
- Da Silva, P. F., & Moreira, R. G. (2008). Vacuum frying of high-quality fruit and vegetable-based snacks. *Lwt-Food Science and Technology*, 41(10), 1758-1767.
- Donovan, J. W. (1979). Phase transitions of the starch/water system. *Biopolymers*, 18(2), 263-275.
- Dueik, V., & Bouchon, P. (2011a). Development of Healthy Low-Fat Snacks: Understanding the Mechanisms of Quality Changes During Atmospheric and Vacuum Frying. *Food Reviews International*, 27(4), 408-432.
- Dueik, V., & Bouchon, P. (2011b). Vacuum Frying as a Route to Produce Novel Snacks with Desired Quality Attributes According to New Health Trends. *Journal of Food Science*, 76(2), E188-E195.
- Dueik, V., Robert, P., & Bouchon, P. (2010). Vacuum frying reduces oil uptake and improves the quality parameters of carrot crisps. *Food Chemistry*, 119(3), 1143-1149.
- Eliasson, A. C. (1983). Differential scanning calorimetry studies on wheat starch-gluten mixtures: I. Effect of gluten on the gelatinization of wheat starch. *Journal of Cereal Science*, 1(3), 199-205.
- Eliasson, A. C., & Gudmundsson, M. (1996). *Starch: physicochemical and functional aspects*. In: Eliasson, A.C. (ed.). *Carbohydrates in food*. New York. pp. 431-503.
- Farkas, B. E. (1994). *Modeling immersion frying as a moving boundary problem*. Ph.D. Thesis, University of California, Davis, USA.
- Fessas, D., & Schiraldi, A. (2001). Water properties in wheat flour dough - I: Classical thermogravimetry approach. *Food Chemistry*, 72(2), 237-244.

Flint, O. (1994). *Food microscopy: a manual of practical methods, using optical microscopy* (Vol. 30): Garland Science.

Fukuoka, M., Ohta, K., & Watanabe, H. (2002). Determination of the terminal extent of starch gelatinization in a limited water system by DSC. *Journal of Food Engineering*, 53(1), 39-42.

Garayo, J., & Moreira, R. (2002). Vacuum frying of potato chips. *Journal of Food Engineering*, 55(2), 181-191.

Gazmuri, A. M., & Bouchon, P. (2009). Analysis of wheat gluten and starch matrices during deep-fat frying. *Food Chemistry*, 115(3), 999-1005.

González, R. C., & Woods, R. E. (2008). *Digital Image Processing*. Upper Saddle River: Pearson Prentice Hall.

Granda, C., Moreira, R. G., & Tichy, S. E. (2004). Reduction of acrylamide formation in potato chips by low-temperature vacuum frying. *Journal of Food Science*, 69(8), E405-E411.

Heuer, A., Cox, A. R., Singleton, S., Barigou, M., & van Ginkel, M. (2007). Visualisation of foam microstructure when subject to pressure change. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 311(1-3), 112-123.

Höhne, G., Hemminger, W., & Flammersheim, H. J. (2003). *Differential scanning calorimetry*. Berlin: Springer Verlag.

Hoover, R., & Manuel, H. (1996). Effect of heat/moisture treatment on the structure and physicochemical properties of legume starches. *Food Research International*, 29(8), 731-750.

James, B. J., & Smith, B. G. (2009). Surface structure and composition of fresh and bloomed chocolate analysed using X-ray photoelectron spectroscopy, cryo-scanning electron microscopy and environmental scanning electron microscopy. *Lwt-Food Science and Technology*, 42(5), 929-937.

Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Pancha-arnon, S., & Uttapap, D. (2011). Microstructural and physicochemical properties of heat-moisture treated waxy and normal starches. *Journal of Food Engineering*, 104(2), 246-258.

- Karaoğlu, M. M., Kotancilar, H. G. r. z., & Çelik, İ. (2001). Effects of Utilization of Modified Starches on the Cake Quality. *Starch - Stärke*, 53(3-4), 162-169.
- Karlsson, M. E., & Eliasson, A. C. (2003). Gelatinization and retrogradation of potato (*Solanum tuberosum*) starch in situ as assessed by differential scanning calorimetry (DSC). *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 36(8), 735-741.
- Kaushik, V., & Roos, Y. H. (2007). Limonene encapsulation in freeze-drying of gum Arabic-sucrose-gelatin systems. *Lwt-Food Science and Technology*, 40(8), 1381-1391.
- Kawas, M. L., & Moreira, R. G. (2001). Effect of degree of starch gelatinization on quality attributes of fried tortilla chips. *Journal of Food Science*, 66(2), 300-306.
- Lindeboom, N., Chang, P. R., & Tyler, R. T. (2004). Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch - Stärke*, 56(3-4), 89-99.
- Liu, H., Ramsden, L., & Corke, H. (1999). Physical Properties of Cross-linked and Acetylated Normal and Waxy Rice Starch. *Starch - Stärke*, 51(7), 249-252.
- Liu, H., Yu, L., Xie, F., & Chen, L. (2006). Gelatinization of cornstarch with different amylose/amylopectin content. *Carbohydrate Polymers*, 65(3), 357-363.
- Lund, D., & Lorenz, K. J. (1984). Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. *C R C Critical Reviews in Food Science and Nutrition*, 20(4), 249-273.
- Luo, F., Huang, Q., Fu, X., Zhang, L., & Yu, S. (2009). Preparation and characterisation of crosslinked waxy potato starch. *Food Chemistry*, 115(2), 563-568.
- Llorca, E., Hernando, I., Perez-Munuera, I., Quiles, A., Larrea, V., & Lluch, M. A. (2007). The structure of starch granules in fried battered products. *Food Hydrocolloids*, 21(8), 1407-1412.
- Marchant, J. L., & Blanshard, J. M. V. (1978). Studies of the Dynamics of the Gelatinization of Starch Granules Employing a Small Angle Light Scattering System. *Starch - Stärke*, 30(8), 257-264.
- Miranda, M. L., & Aguilera, J. M. (2006). Structure and Texture Properties of Fried Potato Products. *Food Reviews International*, 22(2), 173-201.

- Mishra, S., & Rai, T. (2006). Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocolloids*, 20(5), 557-566.
- Moreno, M. C., & Bouchon, P. (2008). A different perspective to study the effect of freeze, air, and osmotic drying on oil absorption during potato frying. *Journal of Food Science*, 73(3), E122-E128.
- Moreno, M. C., Brown, C. A., & Bouchon, P. (2010). Effect of food surface roughness on oil uptake by deep-fat fried products. *Journal of Food Engineering*, 101(2), 179-186.
- Parada, J. (2010). *Effect of microstructure on the biophysical degradation of starch*. Ph.D. thesis. School of Engineering. Pontificia Universidad Católica de Chile, Chile.
- Parada, J., & Aguilera, J. M. (2009). In vitro Digestibility and Glycemic Response of Potato Starch is Related to Granule Size and Degree of Gelatinization. *Journal of Food Science*, 74(1), E34-E38.
- Parada, J., & Aguilera, J. M. (2011). Microstructure, mechanical properties, and starch digestibility of a cooked dough made with potato starch and wheat gluten. *Lwt-Food Science and Technology*, 44(8), 1739-1744.
- Pedreschi, F., Kaack, K., & Granby, K. (2004). Reduction of acrylamide formation in potato slices during frying. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 37(6), 679-685.
- Pinthus, E. J., Weinberg, P., & Saguy, I. S. (1995). Deep-fat fried potato product oil uptake as affected by crust physical properties. *Journal of Food Science*, 60(4), 770-772.
- Primo-Martin, C., van Nieuwenhuijzen, N. H., Hamer, R. J., & van Vliet, T. (2007). Crystallinity changes in wheat starch during the bread-making process: Starch crystallinity in the bread crust. *Journal of Cereal Science*, 45(2), 219-226.
- Rajkumar, V., Moreira, R., & Barrufet, M. (2003). Modeling the structural changes of tortilla chips during frying. *Journal of Food Engineering*, 60(2), 167-175.
- Ratnayake, W. S., & Jackson, D. S. (2007). A new insight into the gelatinization process of native starches. *Carbohydrate Polymers*, 67(4), 511-529.
- Rojas-González, J., Avallone, S., Brat, P., Trystram, G., & Bouchon, P. (2006). Effect of deep-fat frying on ascorbic acid, carotenoids and potassium contents of platatin cylinders. *International Journal of Food Sciences & Nutrition*, 57(1-2), 123-136.

- Saragoni, P., Aguilera, J. M., & Bouchon, P. (2007). Changes in particles of coffee powder and extensions to caking. *Food Chemistry*, 104(1), 122-126.
- Shyu, S. L., & Hwang, L. S. (2001). Effects of processing conditions on the quality of vacuum fried apple chips. *Food Research International*, 34(2-3), 133-142.
- Singh, G. D., Bawa, A. S., Riar, C. S., & Saxena, D. C. (2009). Influence of Heat-Moisture Treatment and Acid Modifications on Physicochemical, Rheological, Thermal and Morphological Characteristics of Indian Water Chestnut (*Trapa natans*) Starch and its Application in Biodegradable Films. *Starch - Stärke*, 61(9), 503-513.
- Singh, J., Kaur, L., & McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications - A review. *Food Hydrocolloids*, 21(1), 1-22.
- Singh, J., Kaur, L., & Singh, N. (2004). Effect of acetylation on some properties of corn and potato starches. *Starch/Stärke*, 56(12), 586-601.
- Singh, N., Singh, J., Kaur, L., Sodhi, N. S., & Gill, B. S. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, 81(2), 219-231.
- Singh, R. P. (1995). Heat and mass-transfer in foods during deep-fat frying. *Food Technology*, 49(4), 134-137.
- Sobukola, O. P., Dueik, V., & Bouchon, P. (2012). Understanding the effect of vacuum level during frying of restructured wheat starch and gluten based product. Accepted.
- Sullivan, J. W., & Johnson, J. A. (1964). Measurement of starch gelatinization by enzyme susceptibility. *Cereal Chemistry*, 41, 73-79.
- Svihus, B., Uhlen, A. K., & Harstad, O. M. (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology*, 122(3-4), 303-320.
- Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch composition, fine structure and architecture. *Journal of Cereal Science*, 39(2), 151-165.
- Traynham, T., Myers, D., Carriquiry, A., & Johnson, L. (2007). Evaluation of Water-Holding Capacity for Wheat-Soy Flour Blends. *Journal of the American Oil Chemists' Society*, 84(2), 151-155.

- Varatharajan, V., Hoover, R., Liu, Q., & Seetharaman, K. (2010). The impact of heat-moisture treatment on the molecular structure and physicochemical properties of normal and waxy potato starches. *Carbohydrate Polymers*, 81(2), 466-475.
- Wang, R., Zhang, M., & Mujumdar, A. S. (2010). Effects of vacuum and microwave freeze drying on microstructure and quality of potato slices. *Journal of Food Engineering*, 101(2), 131-139.
- Watson, S. A. (1964). Determination of starch gelatinization temperature. In R. L. Whistler (Ed.), *Methods in carbohydrate chemistry* (Vol. 4, pp. 240-242). New York: Academic Press.
- Wayne, R. O. (2008). *Light and video microscopy*: Academic Press.
- Zarguili, I., Maache-Rezzoug, Z., Loisel, C., & Doublier, J. L. (2006). Influence of DIC hydrothermal process conditions on the gelatinization properties of standard maize starch. *Journal of Food Engineering*, 77(3), 454-461.
- Zavareze, E. D., & Dias, A. R. G. (2011). Impact of heat-moisture treatment and annealing in starches A review. *Carbohydrate Polymers*, 83(2), 317-328.
- Zhang, M., Chen, H., & Zhang, Y. (2011). Physicochemical, thermal, and pasting properties of Chinese chestnut (*Castanea mollissima* Bl.) starches as affected by different drying methods. *Starch - Stärke*, 63(5), 260-267.

## **APPENDIXES**

## APPENDIX A: MOISTURE CONTENT MEASUREMENT OF BUILDING BLOCKS

Potato Starch							
Samples	Petri Dish (g)	Petri dish + sample (g)	Petri dish + dry sample (g)	Starch + Water (g)	Dry Starch (g)	Water (g)	Moisture Content (Water/Starch+Water)
<b>1</b>	19,555	29,3852	28,2270	9,831	8,6725	1,1582	11,78%
<b>2</b>	13,957	24,7232	23,4324	10,766	9,4755	1,2908	11,99%
<b>3</b>	19,674	30,8870	29,5381	11,213	9,8642	1,3489	12,03%

<b>Average</b>	<b>11,93%</b>
Std. Dev	0,13%

Maize Pre-gelatinized Starch							
Samples	Petri Dish (g)	Petri dish + sample (g)	Petri dish + dry sample (g)	Starch + Water (g)	Dry Starch (g)	Water (g)	Moisture Content (Water/Starch+Water)
<b>1</b>	34,3910	40,004	39,7159	5,613	5,3249	0,2881	5,13%
<b>2</b>	29,8784	35,900	35,5929	6,0214	5,7145	0,3069	5,10%
<b>3</b>	30,5433	38,438	38,0274	7,8949	7,4841	0,4108	5,20%

<b>Average</b>	<b>5,14%</b>
Std. Dev	0,05%

Wheat Gluten							
Samples	Petri Dish (g)	Petri dish + sample (g)	Petri dish + dry sample (g)	Starch + Water (g)	Dry Starch (g)	Water (g)	Moisture Content (Water/Starch+Water)
<b>1</b>	30,6855	38,8687	38,3532	8,1832	7,6677	0,5155	6,30%
<b>2</b>	31,2542	38,4126	37,9245	7,1584	6,6703	0,4881	6,82%
<b>3</b>	31,104	37,6078	37,2077	6,5038	6,1037	0,4001	6,15%

<b>Average</b>	<b>6,42%</b>
Std. Dev	0,35%



## APPENDIX B: RELATIONSHIP BETWEEN FRAME AND TEMPERATURE

### B.1. Heating rate: 5 °C/min

Frame	Temperature (°C)
1	60
2	60,3
3	60,5
4	60,8
5	61
6	61,2
7	61,4
8	61,6
9	61,8
10	62
11	62,2
12	62,4
13	62,6
14	62,8
15	63
16	63,2
17	63,4
18	63,6
19	63,8
20	64
21	64,2
22	64,4
23	64,6
24	64,8
25	65
26	65,2
27	65,4
28	65,6
29	65,8
30	66

Frame	Temperature (°C)
31	66,2
32	66,4
33	66,6
34	66,8
35	67
36	67,2
37	67,4
38	67,6
39	67,8
40	68
41	68,2
42	68,4
43	68,6
44	68,8
45	69
46	69,2
47	69,4
48	69,6
49	69,8
50	70
51	70,2
52	70,4
53	70,6
54	70,8
55	71
56	71,2
57	71,4
58	71,6
59	71,8
60	72

Frame	Temperature (°C)
61	72,2
62	72,4
63	72,6
64	72,8
65	73
66	73,2
67	73,4
68	73,6
69	73,8
70	74
71	74,2
72	74,4
73	74,6
74	74,8
75	75
76	75,2
77	75,4
78	75,6
79	75,8
80	76
81	76,2
82	76,4
83	76,6
84	76,8
85	77
86	77,2
87	77,4
88	77,6
89	77,8
90	78

**B.2. Heating rate: 10 °C/min**

Frame	Temperature (°C)
1	60
2	60,4
3	61
4	61,5
5	62
6	62,3
7	62,7
8	63,2
9	63,8
10	64,1
11	64,6
12	65
13	65,4
14	65,9
15	66,4
16	66,9
17	67,4
18	67,8
19	68,3
20	68,8
21	69,3
22	69,8
23	70,2
24	70,7
25	71,2
26	71,6
27	72,1
28	72,5
29	73
30	73,4

Frame	Temperature (°C)
31	73,8
32	74,3
33	74,7
34	75,2
35	75,6
36	76,1
37	76,6
38	77
39	77,5
40	78
41	78,4
42	78,9
43	79,3
44	79,8
45	80,2
46	80,7
47	81,2
48	81,6
49	82,1
50	82,6
51	83,1
52	83,6
53	84
54	84,5
55	85
56	85,4
57	85,9
58	86,3
59	86,8
60	87,2

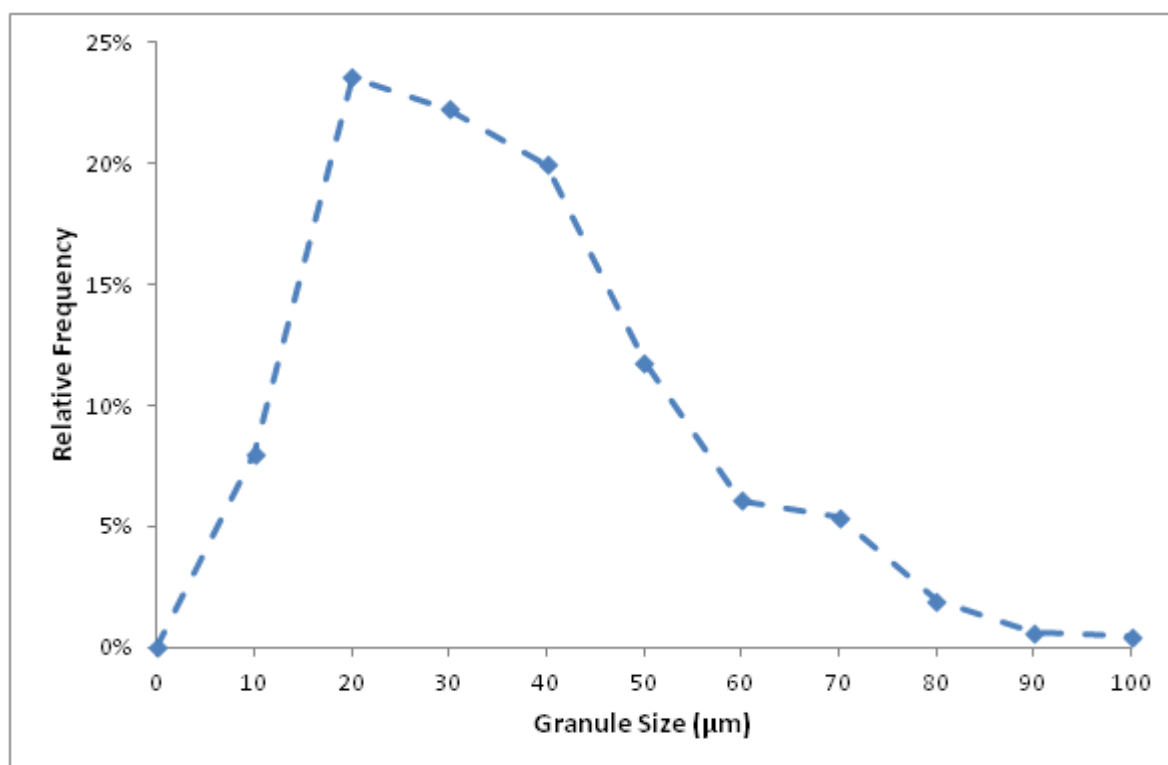
Frame	Temperature (°C)
61	87,7
62	88,1
63	88,6
64	89
65	89,5
66	90
67	90,4
68	90,9
69	91,3
70	91,8
71	92,3
72	92,8
73	93,4
74	93,9
75	94,2
76	94,8
77	95,2
78	95,6
79	96
80	96,6
81	97,2
82	97,7
83	98,3
84	98,8
85	99,2
86	99,7
87	100
88	100,5
89	101
90	101,4

**B.3. Heating rate: 15 °C/min**

Frame	Temperature (°C)
1	60
2	60,5
3	61
4	61,5
5	62
6	62,68
7	63,36
8	64,04
9	64,72
10	65,4
11	66,04
12	66,68
13	67,32
14	67,96
15	68,6
16	69,16
17	69,72
18	70,28
19	70,84
20	71,4
21	72,04
22	72,68
23	73,32
24	73,96
25	74,6
26	75,2
27	75,8
28	76,4
29	77
30	77,6

Frame	Temperature (°C)
31	78,2
32	78,8
33	79,4
34	80
35	80,6
36	81,2
37	81,8
38	82,4
39	83
40	83,6
41	84,2
42	84,7
43	85,2
44	85,7
45	86,2
46	86,7
47	87,2
48	87,7
49	88,2
50	88,7
51	89,2
52	89,7
53	90,2
54	90,7
55	91,2
56	91,7
57	92,2
58	92,7
59	93,2
60	93,7

Frame	Temperature (°C)
61	94,3
62	94,9
63	95,5
64	96,1
65	96,7
66	97,3
67	97,9
68	98,5
69	99,1
70	99,7
71	100,3
72	100,9
73	101,5
74	102,1
75	102,7
76	103,3
77	103,9
78	104,5
79	105,1
80	105,7

**APPENDIX C: GRANULE SIZE DISTRIBUTION**

**Figure C.1:** Granule size distribution of potato starch granules, measured as Feret Diameter.

**APPENDIX D: DETAIL OF ON-SET, ENDING AND RANGE OF  
GELATINIZATION TEMPERATURES OF GRANULES HEATED AT 5, 10  
AND 15 °C/MIN IN EXCESS WATER**

**D.1. Heating rate: 5 °C/min**

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)	Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
4	#1	44,34	71	73,2	2,2	2	#1	45,68	69,5	70	0,5
	#2	12,89	63,2	67	3,8		#2	22,37	67,5	69,4	1,9
	#3	26,33	65,8	72,6	6,8		#3	32,97	68	69	1
	#4	15,37	64,5	67	2,5		#4	41,61	67,5	70	2,5
	#5	52,15	67,2	71,6	4,4		#5	13,24	67,6	70,6	3
	#6	46,6	66,8	72	5,2		#6	39,09	71,5	72,2	0,7
	#7	61,99	68,2	73,2	5		#7	12,89	68	70,6	2,6
	#8	10,49	67,2	71	3,8		#8	43,52	67,8	70,6	2,8
	#9	11,42	65	71,2	6,2		#9	16,5	72,5	73	0,5
	#10	8,66	65,4	71,4	6		#10	16,82	65,5	68	2,5
	#11	16,94	65,2	68,8	3,6		#11	48,35	66,4	69,4	3
	#12	9,97	65,6	72	6,4		#12	21,48	68,4	71,2	2,8
	#13	10,25	67,4	72,4	5		#13	27,56	67,5	71,6	4,1
	#14	13,45	64	72	8		#14	20,16	68	69,8	1,8
	#15	16,17	70,6	73,6	3		#15	22,71	68,2	69,8	1,6
	#16	46,84	65,4	70,4	5		#16	31,41	68,6	74	5,4
	#17	58,09	66	70,2	4,2		#17	54,38	65	73,4	8,4
	#18	51,19	64,8	68,6	3,8		#18	42,83	68,7	74	5,3
	#19	13,02	65,5	71,2	5,7		#19	20,19	65,5	68,2	2,7
	#20	12,96	64	72,8	8,8		#20	24,27	68,7	73,2	4,5
	#21	12,34	64,8	71	6,2		#21	30,39	66	70,6	4,6
	#22	33,92	66,5	71,6	5,1		#22	23,71	67,5	69	1,5
	#23	39,02	68	69	1		#23	10,94	64,5	72,8	8,3
	#24	38,55	67,4	71,6	4,2		#24	21,65	69	74	5
	#25	31,47	67,8	74	6,2		#25	49,3	68	72,6	4,6
	#26	32,27	68	71,6	3,6		#26	37,09	66,2	72,4	6,2
	#27	19,69	66,5	70	3,5		#27	30,37	65,2	71,6	6,4
	#28	47,45	66,5	71,6	5,1		#28	44,45	66,6	72	5,4
	#29	47,82	66,2	68,8	2,6		#29	19,55	66	67,8	1,8
	#30	22,55	65,8	69	3,2		#30	19,26	72	73,8	1,8
	#31	55,78	65,4	71,2	5,8		#31	17,5	71,5	74	2,5
	#33	53,62	65,8	69,6	3,8		#32	50,05	68,5	71,2	2,7
	#34	21,25	67,6	69	1,4		#33	19,26	66,6	71,2	4,6
	#35	63,36	68,5	71	2,5		#34	13,72	65	65,6	0,6
	#36	65,77	65,2	70,6	5,4		#35	48,76	67,8	73,4	5,6
	#37	66,21	64,3	70,2	5,9		#36	17,18	66,6	69,4	2,8
	#38	49,48	68,6	72,2	3,6		#37	16,65	69,2	73	3,8
	#39	66,87	66,5	69,2	2,7		#38	20,51	67,2	69,8	2,6
	#40	67,12	67,8	69,6	1,8		#39	13,05	66,5	69,2	2,7
	#41	67,43	68,4	70,6	2,2		#40	17,76	65,8	70	4,2
	#42	67,54	66,7	71	4,3		#41	44,08	66,6	70,8	4,2

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
3	#1	13,25	66	66,8	0,8
	#2	18,9	65	67,2	2,2
	#3	15,08	66,2	67	0,8
	#4	17,47	65	66,2	1,2
	#5	16,47	65,2	67,6	2,4
	#6	20,87	66,6	69	2,4
	#7	16,71	66,2	67,6	1,4
	#8	8,83	65,2	67	1,8
	#9	8,51	65,4	67	1,6
	#10	25,49	67,2	68,8	1,6
	#11	14,72	64,5	67,4	2,9
	#12	12,54	65,8	67,6	1,8
	#13	20,69	66	68	2
	#14	15,83	65	68,4	3,4
	#15	13,78	66,8	68,8	2
	#16	14,99	69,6	70,8	1,2
	#17	17,37	67,4	69,2	1,8
	#18	16,05	71	72,2	1,2
	#19	19,78	66,4	68,6	2,2
	#20	19,13	70,5	71,8	1,3
	#21	24,14	67	69,8	2,8
	#22	25,31	66,4	68,6	2,2
	#23	23,9	65,5	69	3,5
	#24	20,42	68,2	70,6	2,4
	#25	12,09	68,2	69,6	1,4
	#26	11,51	67,8	69,6	1,8
	#27	20,82	64,8	67,8	3
	#28	68,33	67,5	71	3,5
	#29	68,46	66,4	70,8	4,4
	#30	69,65	66,8	71,4	4,6
	#31	70,13	67,2	69,6	2,4
	#32	70,24	65,6	69,2	3,6
	#33	70,32	64,4	69,8	5,4

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
1	#1	8,83	67,8	70,4	2,6
	#2	12,6	66,5	69,6	3,1
	#3	10,05	67	68,6	1,6
	#4	11,65	67,2	69,4	2,2
	#5	10,98	66,8	70,2	3,4
	#6	13,91	71	72	1
	#7	11,14	69,2	72,4	3,2
	#8	5,88	68,2	71	2,8
	#9	5,67	67,7	69,4	1,7
	#10	17	65	67,4	2,4
	#11	9,82	66,4	67,6	1,2
	#12	8,36	66,8	68,8	2
	#13	13,79	65,8	67,6	1,8
	#14	10,56	66	68	2
	#15	9,18	66,2	68,2	2
	#16	9,99	66	67,8	1,8
	#17	11,58	67,4	69	1,6
	#18	10,7	69,2	70,4	1,2
	#19	15,93	70	71,6	1,6
	#20	13,62	66,2	67,8	1,6
	#21	8,06	67,2	68,8	1,6
	#22	7,67	67,8	69,6	1,8
	#23	13,88	65	66,6	1,6
	#24	72,87	68	69	1
	#25	76,77	68,5	70,2	1,7
	#26	79,54	67,2	69,4	2,2
	#27	67,87	64,5	66,4	1,9

## D.2. Heating rate: 10 °C/min

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)	Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
Papa 1_1	#1	8,4	66,3	70,3	4,0	Papa 1_3	#1	9,0	64,0	69,3	5,3
	#2	8,8	64,7	67,3	2,6		#3	10,4	70,3	73,3	3,0
	#3	4,1	64,3	70,0	5,7		#4	10,1	70,3	73,3	3,0
	#4	37,3	69,0	72,3	3,3		#5	6,2	72,3	75,0	2,7
	#5	8,7	75,0	75,7	0,7		#6	7,3	71,0	72,7	1,7
	#6	63,4	69,4	72,0	2,6		#8	7,7	73,7	75,7	2,0
	#7	20,7	68,4	71,0	2,6		#9	24,7	70,0	73,7	3,7
	#8	39,5	73,7	76,3	2,6		#10	10,3	71,7	74,3	2,6
	#9	7,2	65,7	67,7	2,0		#11	6,2	63,0	66,3	3,3
	#10	57,0	70,4	72,3	1,9		#12	6,0	64,4	68,3	3,9
	#11	8,1	72,7	73,7	1,0		#13	47,7	67,4	70,7	3,3
	#12	6,6	63,7	67,3	3,6		#14	11,3	74,0	76,3	2,3
	#13	11,2	72,0	73,7	1,7		#15	20,4	71,4	74,3	2,9
	#14	10,1	64,0	68,3	4,3		#16	4,5	68,7	75,7	7,0
	#15	7,5	72,7	74,0	1,3		#17	6,1	64,7	68,7	4,0
	#16	7,8	71,0	72,7	1,7		#18	24,0	70,4	72,7	2,3
	#17	8,8	73,0	75,7	2,7		#19	9,4	72,0	73,7	1,7
Papa 1_2	#1	17,9	70,7	73,7	3,0		#20	24,1	70,4	72,7	2,3
	#2	10,2	70,0	73,3	3,3		#21	5,1	65,7	68,7	3,0
	#3	4,9	63,0	68,3	5,3		#22	8,7	64,0	69,0	5,0
	#4	6,1	70,3	73,7	3,4		#23	9,9	67,3	73,3	6,0
	#5	7,6	68,7	71,3	2,6		#24	7,2	77,3	79,0	1,7
	#6	6,2	67,3	72,0	4,7		#25	59,0	69,7	72,7	3,0
	#7	13,6	68,0	74,3	6,3		#26	7,5	69,0	70,7	1,7
	#8	41,0	67,7	71,0	3,3		#27	13,8	67,3	70,7	3,4
	#9	6,9	67,3	69,0	1,7		#28	6,6	78,3	80,3	2,0
	#10	9,6	71,7	76,3	4,6		#29	11,6	67,3	70,7	3,4
	#11	8,1	67,3	69,7	2,4		#30	42,9	65,0	69,3	4,3
	#12	7,0	67,3	69,3	2,0		#31	54,6	70,4	73,7	3,3
	#13	9,4	70,0	72,0	2,0		#32	50,2	68,7	72,3	3,6
	#14	3,7	67,3	69,0	1,7		#33	5,3	69,7	73,3	3,6
Papa 2_1	#1	19,2	67,4	69,7	2,3	Papa 3	#1	16,8	68,0	72,3	4,3
	#2	22,3	62,7	67,7	5,0		#3	41,3	67,7	73,3	5,6
	#3	13,6	62,7	67,7	5,0		#4	32,0	68,4	71,0	2,6
	#4	21,5	65,0	69,3	4,3		#5	11,4	68,7	72,0	3,3
	#5	22,0	69,4	72,7	3,3		#6	89,4	67,7	75,0	7,3
	#6	26,7	67,0	69,0	2,0		#7	32,4	69,7	73,7	4,0
	#7	57,8	67,4	70,3	2,9		#8	19,3	72,4	78,0	5,6
	#8	22,7	70,7	74,0	3,3		#9	23,9	68,7	71,7	3,0
	#9	57,9	67,4	70,3	2,9		#10	14,1	67,7	74,0	6,3
	#10	21,5	71,4	74,3	2,9		#11	24,9	71,7	75,3	3,6
	#11	43,5	65,0	71,0	6,0		#12	10,1	71,3	76,3	5,0
	#12	19,9	65,7	70,3	4,6		#13	14,6	69,7	73,7	4,0
	#13	22,4	67,7	70,3	2,6		#14	28,7	69,7	73,3	3,6
	#14	64,0	67,7	70,0	2,3		#15	36,0	70,7	74,3	3,6

Papa 3	#16	11,4	67,3	71,7	4,4
	#17	21,1	68,7	71,0	2,3
	#18	72,6	69,7	73,3	3,6
	#19	38,2	69,0	72,7	3,7
	#20	27,0	68,7	71,7	3,0
	#21	76,7	72,4	78,3	5,9
	#22	19,4	72,7	78,0	5,3
	#23	75,5	69,0	72,0	3,0
Papa 4	#1	28,5	72,4	78,0	5,6
	#2	16,2	68,7	71,7	3,0
	#3	42,9	68,4	72,0	3,6
	#4	36,6	72,4	76,7	4,3
	#5	16,8	68,0	71,0	3,0
	#6	26,2	72,0	74,7	2,7
	#7	48,4	72,4	76,7	4,3
	#8	29,9	69,0	72,0	3,0
	#9	16,2	72,4	76,3	3,9
	#10	78,4	70,7	74,0	3,3
	#11	31,2	70,0	72,3	2,3
	#12	18,0	69,7	71,7	2,0
	#13	20,6	72,4	74,7	2,3
	#14	21,2	67,7	69,3	1,6
	#15	26,0	71,7	74,3	2,6
	#16	12,5	72,0	74,0	2,0
	#17	18,9	69,7	71,7	2,0
	#18	49,5	68,7	70,3	1,6
	#19	39,4	69,4	71,7	2,3
	#20	26,6	71,4	73,7	2,3
	#21	38,6	68,7	71,7	3,0
	#22	18,0	68,7	72,3	3,6
	#23	14,9	70,7	73,3	2,6
	#24	68,6	68,7	71,7	3,0
	#25	25,4	67,4	69,0	1,6
	#26	41,2	68,7	70,7	2,0
	#27	14,0	69,3	72,0	2,7
	#28	40,7	73,0	76,3	3,3
	#29	25,8	70,7	74,7	4,0
	#30	38,5	73,4	77,3	3,9
	#31	15,1	68,0	70,7	2,7
	#32	33,3	68,7	70,3	1,6
	#33	55,7	69,4	71,7	2,3
	#34	41,3	68,0	70,0	2,0
	#35	34,1	72,4	76,3	3,9
	#36	46,9	71,4	74,7	3,3
	#37	40,1	70,7	74,7	4,0
Papa 4	#41	51,3	68,0	70,0	2,0
	#42	53,3	70,4	71,7	1,3
	#43	24,7	70,0	71,0	1,0
	#44	50,7	71,4	73,3	1,9
	#45	40,6	71,0	73,3	2,3
	#46	47,5	71,0	72,7	1,7
	#47	32,3	71,0	73,7	2,7
	#48	15,4	68,0	70,0	2,0
	#49	20,2	70,4	71,7	1,3
	#50	29,5	71,0	73,3	2,3
	#51	30,7	70,0	72,3	2,3
	#52	19,1	68,7	70,0	1,3
	#53	52,8	68,0	71,7	3,7
	#54	55,5	73,7	77,3	3,6
	#55	12,1	68,7	72,3	3,6
	#56	27,6	68,0	70,0	2,0
	#57	25,6	68,0	69,3	1,3
	#58	12,6	70,3	72,3	2,0
	#59	25,7	66,7	69,0	2,3
	#60	24,2	68,4	70,7	2,3
	#61	32,9	72,0	73,3	1,3
	#62	80,2	68,7	70,7	2,0
	#63	15,7	69,7	73,3	3,6
	#64	15,4	69,4	71,7	2,3
	#66	16,8	68,4	70,0	1,6
	#67	8,3	70,7	72,7	2,0
	#68	59,9	70,4	72,3	1,9
	#69	42,2	69,7	72,7	3,0
	#70	22,3	70,7	73,3	2,6
	#71	70,1	68,0	70,7	2,7
	#72	18,7	72,0	75,3	3,3
	#73	14,5	72,7	75,7	3,0
	#74	14,4	66,3	69,0	2,7
	#75	53,2	70,7	72,7	2,0
	#76	19,1	67,4	70,3	2,9



### D.3. Heating rate: 15 °C/min

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
15Cmin1	1	54,46	64	70,3	6,3
	2	54,79	69,2	74,6	5,4
	3	41,48	67,3	73,3	6
	4	47,34	66,7	72	5,3
	5	60,25	74	78,2	4,2
	6	64,29	68,6	73,3	4,7
	7	63,86	67,3	72	4,7
	8	61,32	69,7	75,2	5,5
	9	64,22	69,7	74,6	4,9
	10	76,33	68	74	6
	11	29,40	68,6	73,3	4,7
	12	34,99	71,4	75,8	4,4
	13	73,24	70,8	75,8	5
	14	14,85	70,3	74,6	4,3
	15	66,34	69,7	74	4,3
	16	25,92	69,7	73,3	3,6
	17	28,29	70,3	75,2	4,9
	18	32,13	66,7	72,7	6
	19	22,78	71,4	75,2	3,8
	20	14,31	70,3	74,6	4,3
	21	24,67	69,2	73,3	4,1
	22	48,17	71,4	75,8	4,4
	23	18,22	70,3	74,6	4,3
	24	29,29	70,3	74	3,7
	25	53,38	70,8	76,4	5,6
	26	55,95	70,3	73,3	3
	27	52,50	70,8	75,8	5
	28	85,26	70,3	74	3,7
	29	26,26	69,7	74,6	4,9
	30	24,76	70,3	74,6	4,3
	31	60,55	71,4	76,4	5
	32	29,04	70,8	74	3,2
	33	65,69	68,6	74	5,4
	34	55,10	72	77	5
	35	41,90	72,7	78,8	6,1
	36	58,03	69,2	73,3	4,1
	37	52,14	71,4	75,8	4,4
	38	23,90	73,3	77,6	4,3
	39	24,28	69,7	74	4,3
	40	43,82	69,7	74,6	4,9
	41	34,06	72	77	5

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
15Cmin2	1	60,61	70,8	76,4	5,6
	2	76,14	71,4	77	5,6
	3	25,56	70,8	74,6	3,8
	4	36,51	72	76,4	4,4
	5	31,38	68	72,7	4,7
	6	30,58	71,4	76,4	5
	7	12,30	69,2	72,7	3,5
	8	28,45	70,3	74	3,7
	9	40,37	69,7	74,6	4,9
	10	51,51	70,8	74	3,2
	11	16,42	70,3	73,3	3
	12	28,28	71,4	74,6	3,2
	13	53,24	68,6	73,3	4,7
	14	39,96	70,8	75,2	4,4
	15	38,88	68,8	72,7	3,9
	16	46,21	69,7	74,6	4,9
	17	32,25	71,4	75,8	4,4
	18	44,82	70,8	75,2	4,4
	19	17,94	73,3	75,8	2,5
	20	59,49	72	75,8	3,8
	21	10,74	72,7	76,4	3,7
	22	33,02	70,3	74	3,7
	23	36,81	68,6	74,6	6
	24	39,15	70,3	74,6	4,3
	25	63,72	70,8	75,2	4,4
	26	43,93	69,7	74,6	4,9
	27	17,26	74	77,6	3,6

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
15Cmin3	1	18,28	70,8	74	3,2
	2	30,76	72,7	75,8	3,1
	3	23,28	70,3	73,3	3
	4	22,83	70,8	74,6	3,8
	5	23,00	76,4	80	3,6
	6	49,79	72	76,4	4,4
	7	65,51	70,8	75,8	5
	8	45,85	71,4	77	5,6
	9	59,98	71,4	75,8	4,4
	10	78,08	69,2	74	4,8
	11	26,28	72,7	78,2	5,5
	12	18,59	72	76,4	4,4
	13	36,92	73,3	77,6	4,3
	14	44,27	70,8	75,2	4,4
	15	27,32	70,8	75,8	5
	16	24,67	74	78,2	4,2
	17	35,72	74,6	79,4	4,8
	18	24,66	71,4	75,2	3,8
	19	25,58	72	77	5
	20	33,09	66,7	71,4	4,7
	21	24,69	70,3	74,6	4,3
	22	20,49	72	77	5
	23	34,81	71,4	75,2	3,8
	24	23,11	70,8	74	3,2
	25	35,22	69,7	74,6	4,9
	26	55,69	74,6	78,8	4,2
	27	19,99	69,2	72,7	3,5
	28	26,05	69,7	74	4,3
	29	29,91	72	77	5
	30	29,16	69,7	74,6	4,9

Sample Name	Object	Feret Diameter (μm)	On-set Temperature (°C)	Ending Temperature (°C)	Gelatinization Rate (°C)
15Cmin3	31	27,77	72,7	77	4,3
	32	17,73	75,2	78,8	3,6
	33	22,81	72	75,2	3,2
	34	67,85	73,3	77	3,7
	35	28,32	70,8	74,6	3,8
	36	31,53	74	77	3
	37	31,71	70,8	77	6,2
	38	17,16	71,4	75,8	4,4
	39	33,48	75,2	78,8	3,6
	40	28,89	72,7	76,4	3,7
	41	21,44	72	76,4	4,4
	42	32,35	71,4	75,8	4,4
	43	14,77	68,6	72	3,4
	44	37,44	72	77,6	5,6
	45	26,43	69,2	72,7	3,5
	46	27,01	68	72	4
	47	36,84	72,7	78,2	5,5
	48	27,76	72,7	78,2	5,5
	49	28,39	71,4	76,4	5
	50	20,08	70,3	74,6	4,3
	51	28,65	72	75,8	3,8
	52	44,61	73,3	78,2	4,9
	53	32,96	69,7	74	4,3
	54	38,45	73,3	78,2	4,9
	55	34,27	72,7	78,2	5,5