# Understanding the Swelling Behavior of Individual Starch Granules by ParCS

by

Lanxin Mo

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the thesis entitled:

#### Understanding the Swelling Behavior of Individual Starch Granules by ParCS

submitted by <u>Lanxin Mo</u> in partial fulfilment of the requirements of the degree of <u>Master of Science</u>

in <u>Food Science</u>.

Dr. John M. Frostad, Faculty of Land and Food Systems, UBC Supervisor

Dr. Anubhav Pratap-Singh, Faculty of Land and Food System, UBC Supervisory Committee Member

Dr. Savvas Hatzikiriakos, Faculty of Chemical and Biological Engineering, UBC Supervisory Committee Member

Dr. Barbara Stefanska, Faculty of Land and Food System, UBC Additional Examiner

### Abstract

Starch is a widely used ingredient in food products, acting as a thickening, clouding, and gelling agent. Starch gelatinization is an important process that can influence the texture of food products, therefore, it has been studied extensively by many researchers. A Particle Cohort Study (ParCS) apparatus was used to observe the gelatinization process of individual starch granules from four types of legume starch: yellow pea, red bean, chickpea, and green lentil. This new method allows us to capture and understand the variability between individual granules during the swelling that occurs due to gelatinization. The size as a function of time was measured for a large number of individual granules in order to quantify the intra-sample variability for each type of starch, as this information is not available from the standard techniques for characterizing gelatinization: starch pasting and differential scanning calorimetry (DSC).

The swelling of individual starch granules under non-isothermal conditions was recorded and subjected to image analysis for quantifying their sizes. For each type of legume starch, around 180 granules were collected for data analysis. The cumulative size distribution measurements using image analysis were similar to that obtained from the laser diffraction method, except for red bean starch, which showed a smaller size by image analysis. We demonstrate that an empirical model, the Gompertz function, is highly effective at describing the size vs. time data. Using the Gompertz function, the data from image analysis are fitted to obtain and extract two new parameters related to gelatinization that we define for the first time in this manuscript: granule-swelling temperature and granule-swelling time scale. The accuracy of these new parameters is demonstrated by comparison with standard techniques. After proposing an alternative method for interpreting starch pasting data we show a very good correlation between all three techniques. The results indicate that these legume starches have a remarkably low variability in gelatinization properties. This new method of characterization is expected to enable optimization of starch gelatinization properties during large-scale processing of food products.

## Lay Summary

Understanding starch gelatinization properties is of much importance for optimizing the texture of food products containing starches. Gelatinization is marked by a large increase in the size of starch particles during heating as they absorb water. Gelatinization is traditionally studied through heating a mixture of starch and water while stirring (referred to as starch pasting) and/or measuring the temperature at which physical changes occur in the mixture under highly controlled heating conditions (Differential Scanning Calorimetry). In this thesis, we describe a new method to quantify and characterize the swelling behaviors of individual starch granules. This method can tell whether there is a significant variability in the gelatinization properties of one type of starch, and may be more convenient to use than traditional methods under some conditions. The results provide insights for optimization of processing food involving starch gelatization.

## Preface

The author, Lanxin Mo, under the guidance of Dr. John M. Frostad, carried out the experimental work, analyzed the data and wrote the thesis.

The work is original and has not been previously published. A separate manuscript containing this work has been submitted to a journal peer review and publication and is currently under review.

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# Dedication

To my beloved parents.

# Chapter 1

# Literature Review and Purpose of This Study

### 1.1 Literature review

#### 1.1.1 An introduction to starch

Starch is one of the major carbohydrate sources in human diets and has been widely used in many food and non-food products. Starches isolated from different sources have different molecular structures which lead to various functional properties [6]. Starch is produced during photosynthesis and stored as a major source of energy in plants [7]. Although starch is widely distributed in nature, only limited types of plants have been used extensively for commercial starches [8]. Starch is composed of two glucose polymers: amylose and amylopectin, which make up 20-30% and 70-80% respectively of most native starches [9–11]. Starch occurs naturally as discrete

particles, called granules. The size of granules can range from 1  $\mu$ m to more than 100  $\mu$ m, and the granule shapes include almost perfect spheres, ellipsoids, or irregular shapes [12, 13].

The majority of starch consumed by humans occurs naturally in cereals. Starch is the main component of foods such as pasta, bread, and noodles [8]. As a major source of calories, starch is inexpensive to produce [7]. Starch can also be used as a clouding agent, thickener, and gelling agent [14]. During thermal processing of food products, starch granules swell by absorbing water thereby changing the rheological properties of foods [15, 16]. By modifying the chemical structures, starch can be used in various ways [17–19].

#### **1.1.2** Starch structure and composition

Amylose and amylopectin make up for approximately 98-99% of the dry weight of starch. The ratio of these two polysaccharides varies according to the botanical source of starch. Amylopectin is the main component in most starches, and some waxy starches have almost 100% amylopectin [20, 21]. Potentiometric titration and colorimetric test are widely used to determine the ratio of amylose and amylopectin [3].

Amylose and amylopectin have different properties and structures (Figure 1.1). Amylose is a relatively long and linear molecule of  $(1-4)-\alpha$ -D-glucopyranosyl and slightly branched by  $(1-6)-\alpha$  linkages [22, 23]. Amylose has a right-handled helical shape with a molecular weight ranging from  $1 \times 10^5$  to  $1 \times 10^6$  [1]. Amylose has a degree of polymerization of 100 to 10,000 monermer units [8]. High-amylose corn starch and amylomaize starch, two common commercial starches, have amylose contents of 50% - 60% and 70% - 80% respectively [24, 25].

Amylopectin is one of the biggest molecules in nature and the major component of starch. Its structure is relatively more complex than amylose as shown in Figure 1.2 (a). Amylopectin is highly branched and formed through chains of (1-4)- $\alpha$ -Dglucopyranosyl with some (1-6) bonds at the branched points [23, 8]. A C chain is an amylopectin consisting of one chain. The basic organization of amylopectin includes a C chain which carries numerous B chains to which A chains are attached. A chain is unbranched and connected to another chain via 1-6 linkage. B chain is branched and connected to C chain via 1-6 linkage [8]. Amylopectin has a molecular weight of 200 to  $1 \times 10^9$  [22, 26]. Additionally, the interaction between amylopectin/amylose and lipid or protein plays a role in the functional properties of starch, which is of interest to the food application.

Starch granules have a semicrystalline structure and the organization of the amorphous and crystalline regions is shown in Figure 1.2 (b). The crystalline region consists primarily of amylopectin and the amorphous region contains amylose mainly but also some amylopectin [27, 8]. Starch with higher percentage of amylopectin has higher crystallinity, for example, waxy starch [21]. X-ray analysis is used to determined the extent of crystallinity and crystallite structure of starch [3]. Three types of patterns are shown in Figure 1.3. Usually, the A-type pattern can be detected in cereal starches and the B-type in tuber starch [23, 2]. C is a mixture of A and B type diagram and has been shown in bean starch, the complex of amylose and fatty acid exhibit a V pattern [23, 27].



Figure 1.1: Structure of (a) amylose and (b) amylopectin. [1]



Figure 1.2: (a) Essential structure of amylopectin. (b) Organization of amorphous and crystalline regions of starch granule [2]



Figure 1.3: X-ray diffraction patterns of V-, A-, B-, and C-type starches [3]

#### 1.1.3 Starch gelatinization and pasting

Starch granules are insoluble but hydrate in water at room temperature. In the presence of adequate temperature and water, the crystalline structure of granule is disrupted, and the amorphous regions becomes more accessible to absorb water and swell. This process is known as starch gelatinization [27, 8]. Understanding starch gelatinization during food processing is essential for food development and design since it has an effect on food texture and nutritional value.

The behavior of starch is primarily determined by the phase volume of swollen granules as well as their deformability [28]. As the granules keep swelling, the contents of granules leach into the water resulting in the increased viscosity. The structure change during gelatinization can be detected by differential scanning calorimetry (DSC), which is used to determine the crystalline melting point of starch. The endothermic heat flow is recorded as the starch sample is heated at a constant rate [29]. Pasting is the behavior following gelatinization. Swelling of granules is related to rheological properties and pasting behavior, often measured by a Rapid Visco Analyser (RVA), though can be done with any rheometer [30, 8]. RVA measures the apparent viscosity change when starch solution is heated up. The temperature at which the system shows a sudden viscosity increase is called the pasting temperature [31].

As the major components of starch granules, amylose and amylopectin play important roles in the gelatinization process. Many results have shown that amylopectin has an obvious effect on the starch gelatinization process, as large amounts of amylopectin were likely to result in high gelatinization temperature and restrict starch swelling [32–34]. The chain length as well as the amount of external and internal chains of amylopectin can both influence the gelatinization process of starch [11, 35]. It is also of great interest to understand how the interaction between starch and other components influence starch gelatinization when starch is present in a complex food matrix, such as bread, cake, and noodles. Ingredients like sugar and salts have significant influences on starch gelatinization, both of them can shift the gelatinization temperature to a higher value and increase the peak visicosity because they have limited the water availability to starch granules [36–38]. Mechanisms of starch gelatinization during heating of wheat flour have shown that proteins and non-starch polysaccharide were found to hinder starch gelatinization in grain flour by limiting water availability for starch gelatinization [39, 40].

To understand the mechanics of gelatinization, efforts have been made to model the size evolution of starch granules when swelling. For example, first order and second order kinetic models [41, 42] and some empirical models [16, 43] have been used to fit the size evolution kinetics. Previous studies usually heated the starch solution under the isothermal condition, however, most processes of food products are under non-isothermal condition. Using the average size obtained from laser diffraction method, the size distribution of wheat starch showed a right shift as the cooking temperature increased under the isothermal condition [16]. The change of average starch granule size indicated a rapid swelling when gelatinization temperature was reached, followed by a slower swelling before reaching equilibrium [43]. The starch granules were cooked under isothermal conditions and their size was measured using laser diffraction. However, the granules may continue swelling even when the heating stopped, which could bring some errors to the results. [41]. Therefore, non-isothermal experiment and continuous study of starch gelatinization are needed.

# 1.1.4 Starch granule swelling studied by hot-stage microscopy and image analysis

Starch gelatinization can not only be studied by analyzing thermal and rheological properties but can also be studied by observation under optical microscopy, which usually serves as a complementary result [20, 44, 45]. Hot-stage microscopy has the ability to observe *in situ* behavior of stach granule swelling and disapperance of crystallinity [46]. Starch granules exhibit an optical birefringence pattern when observed under polarized light. When a light is passed through a granule, the light is split into two rays when it passes through certain types of crystalline material, as amylose and amylopectin are radially oriented to different directions. This phenomenon is called birefringence [8]. Figure 1.4 shows potato starch granules viewed under polarized light. The polarization cross indicates the molecular order of starch granules. Image analysis has been used to estimate individual starch granule swelling under hydro-thermal treatment recorded under normal light microscope [47, 48], and polarized light images have been used to calculate the overall granule swelling change [45, 49]. There is also a study working on optimizing the tool to quantify starch granule swelling in a continuous experiment [50].

## 1.2 Purpose of this study

For food products involving starch gelatinization, overcooked or undercooked starch can both bring undesirable flavors. Starch is usually studied as a bulk, however, the variability between granules from the same type of starch has not been fully studied, especially for those with a monomodal size distribution. In some studies, the



Figure 1.4: Potato starch granules viewed under polarized light [3]

starch granules have been cooked under isothermal conditions and their size is measured by laser diffraction [16, 41]. The result from laser diffraction can only provide a distribution for the bulk population and cannot show the variability of individual granules. Therefore, a single-granule, *in-situ* monitoring method is preferable for accurate measurements. Some empirical and theoretical models have been proposed for the starch swelling process, and most of them used average granule size for fitting [43, 51, 16]. However, there is not currently a single method that has been widely adopted.

In this project, the swelling process of individual granules from legume starches with monomodal size distribution, including red bean, yellow pea, green lentil, and chickpea, was studied using ParCS (Particle Cohort Study) and image analysis. It would be useful to test some mathematical models on individual starch granules and compare swelling behavior of granules with pasting and thermal properties of bulk starch.

## **1.3** Research Hypothesis and Objectives

The first objective of this study is to observe and record the swelling process of red bean, yellow pea, green lentil, and chickpea starches using ParCS (Particle Cohort Study). It is hypothesised that by observing a large number of individual granules that we will find a statistically significant correlation between granule size and gelatinization temperature for four legume starches. The second objective is to examine some mathematical models for starch granule size evolution during swelling process. It is hypothesized that one model will be quantitatively superior at predicting the results. The third objective is to explore the correlation between the swelling behavior of starch granules and the pasting as well as thermal properties of starch. We hypothesize that there is a correlation between the temperature at which starch granules begin to swell and the temperature from pasting and DSC methods.

# Chapter 2

# Characterization of Swelling Behavior of Individual Starch Granules

## 2.1 Introduction

Starch is one of the major carbohydrate sources in the human diet. It has also been widely used as a thickener or binding agent in the pharmaceutical, food, and biomedical industries. Gelatinization is key to starch's ability to affect the texture of food, and the structural changes in starch during heating have been the focus of many studies [52–55, 4]. By controlling the heating process, it is possible to modify the texture and viscosity of food products [56, 57].

Within plants, the starch is located mostly in starch granules that have a semicrystalline structure composed of linear amylose and branched amylopectin of varying compositions depending on the type of plant [12, 58]. When heated in water, starch granules will lose their semi-crystalline structure and swell up to several times the initial size in a (mostly) irreversible process (i.e., starch gelatinization) [6]. Due to the increase of volume induced by swelling as well as the dissolution of starch molecules, the viscosity of the fluid increases [5]. The gelatinization process and the resultant viscosity both depend on the heating conditions [59, 51].

The size of starch granules can range from 1  $\mu$ m (e.g., rice and quinoa starch) to more than 100  $\mu$ m (e.g., potato and canna starch) [60]. It has been found that the functional characteristics of each starch can depend on morphology, composition, and structure [61]. For example, wheat starch has a large, flat "A-type" and a small, spherical "B-type" that can be separated and used for different applications [62]. Similarly, potato starch granules have a biomodal size distribution for which the gelatinization temperature appears to be correlated to granule size [63]. However, the variability between granules from the same type of starch has not been fully studied, especially for those with a monomodal size distribution.

Legumes are considered a healthy food; rich in proteins, carbohydrates, fiber, and minerals, and are an example of a starch source with a monomodal granule size distribution [10]. Legume starch has also been reported as a high-grade raw material in the food industry [64]. However, the characteristic properties of legume starches are less studied than those of tuber and grain starches. In this work, we examine four starches, one from each of the four main categories of legumes (specifically pulses) grown in Canada: dry beans, dry peas, chickpeas, and lentils.

Starch pasting and differential scanning calorimetry (DSC) have been widely used to characterize the rheological and phase transition properties of starches by many researchers [65–68]. The former provides information about the rheological changes associated with gelatinization (and is used heavily by the food industry), while the latter is primarily used for characterizing the thermodynamic properties of starch. In addition, starch gelatinization can also be observed under optical microscopy, which usually serves as a complementary method to pasting or DSC [16, 41, 69, 43]. For example, it was shown that the rheological properties of tapioca and rice starch granules depend on the size of the granules [70, 71]. In this work, we use all three methods to characterize the starch granules.

Recently, it was shown that there is a dependence between the size of individual starch granules and the pasting temperature for two types of starch: tapioca and rice starch [70, 71]. This was shown by using a sieve to obtain different fractions of the starch granules with different average sizes and then measuring the pasting properties of each fraction. A drawback of this method is that the results only provide a measure of the population average and cannot show the variability of individual granules. Therefore, a single-granule, in-situ monitoring method is preferable for proving the existence of a size dependence [49, 4, 46]. Here we hypothesize that by observing a large number of individual granules that we will find a statistically significant correlation between granule size and gelatinization temperature for the four legume starches.

To aid in the analysis of our data in this work, we examine a few possible mathematical models for the starch granule swelling process. We further hypothesize that of the many models proposed by other researchers, that one of them will be quantitatively superior at predicting the results. The models that have been proposed include first-order and second-order kinetic models [70, 41], first-principles models [51, 16], and a range of empirical models [41, 47, 48]. Each of these models can be said to have their own strengths and weaknesses and there is not currently a single method that has been widely adopted.

In summary, our objective in this work was to measure the size evolution of individual starch granules from four legume starches using a Parcticle Cohort Study (ParCS) technique [4]. To test our hypotheses about the size dependence of gelatinization temperature we first needed to develop a clear and reproducible method for quantifying the gelatinization temperature of individual starch granules and then determine if these measurements are correlated to bulk measurements. We therefore further hypothesized that a correlation will exist between the temperature at which starch granules begin to swell and at least one of two standard techniques: pasting and DSC.

By studying a large number of granules, we were able to establish the intra-sample variability in gelatinization temperature, which cannot be determined from standard techniques. Our results will provide future researchers with reliable data about a full population of starch granules for use in statistical calculations. Finally, because of the low profit margins that are common in the food industry, these results and techniques are expected to be valuable for optimizing food production processes involving starch granules.

## 2.2 Materials and Methods

#### 2.2.1 Starch isolation

Starches from red bean (Vigna angularis), green lentil (Lens culinaris), yellow pea (Pisum sativum), and chikpea (Cicer arietinum) (supplied by President's Choice Canada) were isolated following a previously published method with some minor modifications [72]. Approximately 100 g of dried seeds were steeped in 200 mL of 0.5 wt% sodium bisulfite solution for 20 h at 4 °C and drained, then ground in a blender for 5 min with 500 mL of distilled water. The resulting slurry was filtered through a 125  $\mu$ m sieve (120 mesh size). The filtered suspension was then transferred to centrifuge tubes and centrifuged at 1500 × g for 15 min. The supernatant was discarded and the starch was resuspended in distilled water for centrifuging again under the same conditions. The upper, non-white layer of the final centrifuged pellet was scraped off and discarded. Finally, the starch was then dried at 40 °C for 24 h and gently ground using a mortar and pestle in order to pulverize the pellet and separate individual granules from clumps.

#### 2.2.2 Particle size analysis

The composition of these legume starches is well-studied in previous works and the relative proportion of amylose to amylopectin is expected to be greatest relevance to this study [10, 73, 74]. However, our hypotheses relate to differences between individual granules and there are no known methods for characterizing the amylose content of single granules. Therefore, here we report the amylose content of each type of legume starch as measured in the literature (see Table 2.1) and focus our efforts on measuring the size of the granules.

Granule size distributions of each starch type were determined by using two methods. First, laser diffraction was done on 0.5 g of starch suspended in 10 mL deionized water using a Microtrac Series 5000 Sync, Particle Size Analyzer (ATS Scientific Inc, Burlington, ON, Canada). The refractive index of the starch was taken to be 1.54 and each measurement was repeated three times.

Second, image analysis was carried out using a custom script written in Python 3.7 on Windows. The boundaries of individual starch granules were manually outlined and the projected area (A) was computed. The equivalent spherical diameter (d) of individual granules and volume ( $V = \frac{\pi}{6}d^3$ ) was then calculated according to the following formula:

$$d = 2\sqrt{\frac{A}{\pi}}.$$
(2.1)

For each type of starch, around 180 granules were analyzed at approximately 30 to 40 time points.

In addition to size distribution, the morphology of the starch granules was observed using scanning electron microscopy (SEM). Starches were prepared on SEM stubs using conductive carbon tape. The samples were sputter coated with a 10 nm film of iridium to minimize charging before imaging. The conductive coatings were deposited using a Leica EM MED020 coating system. SEM imaging was performed using a Helios FEI Helios NanoLab 650 FIB-SEM (Thermo Fisher Scientific, Waltham, MA, USA) with a 1 kV accelerating voltage.

#### 2.2.3 Developing a new version of ParCS apparatus

The ParCS (Particle Cohort Study) apparatus has three versions. The first one was built by Do as shown in Figure 2.1[4]. The chamber was machined from a block of aluminum with the dimensions of 51 mm(length)  $\times$  25 mm (width)  $\times$  9 mm (height). It has an upper (X) and lower (Z) window for observation, and the upper one is removable and sealed by sandwiching a silicone rubber gasket. Sample solution is injected by a syringe from the inlet hole (Y) and we wait until all samples reach the bottom glass (Z). Temperature is controlled by a cartridge heater and a thermocouple with PID controlled.

Since the first one is used to observe cotyledon cells, which are much larger than starch granules, cells do not move when the solution is heated up. Starch granules move rapidly in heated chamber and we cannot track the same granules if using the first chamber. The second version was designed by a previous lab member as shown in Figure 2.2. This one is smaller (1 inch in length and width) to minimize the influence of heat flow and the coated glass was used.

The view of the second version is small (1/4 in diameter), which makes it hard for cleaning. Also, if the bottom glass is not flat when sealed, granules will all move to the one side of chamber, therefore, a third version was created. The third one is slightly bigger (2 inch in length and width) and the height of ribber/glass is considered, so that the bottom glass is flat. The third version was designed by me as shown in Figure 2.3.



Figure 2.1: Schematic layout and photograph of chamber (top view) [4].



Figure 2.2: Bottom view of the second version of ParCS.



Figure 2.3: Top and bottom view of the third version of ParCS.

#### 2.2.4 Heating treatment

A ParCS apparatus was used to monitor the swelling process of individual starch granules when heated with an increasing temperature. First,  $2.0 \pm 0.3$  mg starch sample was weighed and dispersed in 17 mL deionized water in a 20 mL vial. Immediately after stirring (to avoid settling of the granules), a small amount of the starch solution was transferred into the ParCS chamber using a syringe.

After loading the granules into the chamber, the temperature of the chamber was equilibrated to 50 °C. The temperature was maintained at 50 °C for 2 minutes, and then increased according to the following function with T in units of Celcius and t in units of seconds:

$$T = \begin{cases} 50 & t < 120 \ s \\ -1.92 \times 10^{-4} t^2 + 0.221t + 26 & 120 \le t \le 540 \ s \ , \end{cases}$$
(2.2)  
90  $t > 540 \ s$ 

This function was based on the temperature profile from PID controller as a linear function was not able to achieve. Once the temperature reached 89 to 90 °C, it was maintained at this temperature for 2 minutes.

#### 2.2.5 Microscope slide surface treatment

When heating starch granules in the ParCS apparatus, the natural convection currents that are generated can cause more buoyant granules to move, which is not desirable. To avoid this, the bottom glass was coated with APTES (3-aminopropyltriethoxysilane) to change the surface charge from negative to positive. The positive charge on the glass surface provides an electrostatic attraction with the starch granules that are negatively charged due to phosphate monoester groups [75].

The coating procedures followed a previous method with some minor modifications [76]. Glass microscope slides were cleaned with detergent, flushed in deionized water, and dried in a fume hood. Then the slides were immersed in 1% HCl/ethanol (v/v) for 30 min and then rinsed in running DI water. After drying, the glass was immersed in 5% APTES/toluene for 24 h and rinsed with toluene and acetone. The glass was then ready to use once it was fully dry.

#### 2.2.6 Starch pasting

The pasting properties of each starch were measured using a starch pasting cell in a rheometer (MCR 302 Anton Paar, Austria).

The method was based on Anton Paar's standard protocol with some minor modifications. A starch sample of 2.16 g was transferred into the cell along with 18 mL of distilled water. The suspension was then heated to 50 °C and stirred at 960 rpm for 10 s to thoroughly disperse the sample. The starch cell is equipped with a stirrer measuring the apparent viscosity of starch suspension as shown in Figure 2.4.

Subsequently, while stirring at 160 rpm, the following temperature profile was applied:

- 1. Hold at  $50^{\circ}$ C for 50 s
- 2. Heat to 95°C in 450 s using a linear ramping function
- 3. Hold at  $95^{\circ}$ C for 300 s



Figure 2.4: Schematic diagram of starch stirrer measuring system [5]

- 4. Cool to 50°C in 450 s using a linear ramping function
- 5. Hold at  $50^{\circ}$ C for 120 s

For each type of starch, the test was done three times. Using the rheometer software, the pasting temperature, peak viscosity, breakdown viscosity, final viscosity, and setback viscosity were recorded. This starch stirrer geometry measures the apparent viscosity of sample.

#### 2.2.7 Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed using a DSC 6000 (Perkin-Elmer, Norwalk, CT). Samples consisting of 2.4 mg of starch were weighed into a 30  $\mu$ L aluminum pan, followed by adding 14  $\mu$ L of distilled water. The pan was sealed hermetically and equilibrated at room temperature for 1 h. Samples were then heated from 30°C to 110°C at a rate of 10°C/min. For each type of starch, the test was done twice. Enthalpy, onset temperature, peak temperature, and conclusion temperatures of the gelatinization process were calculated by the software.
### 2.2.8 Mathematical models

In order to analyze the granule swelling process, three models were considered for providing fits to the data. The first model was developed from simple, first-order kinetics [16]:

$$\frac{D(t) - D_0}{D_f - D_0} = \begin{cases} 0 & t < t_0 \\ 1 - e^{-k(T)(t - t_0)} & t \ge t_0 \end{cases},$$
(2.3)

where  $D_0$  is the initial diameter,  $D_f$  is the final diameter, D(t) is the diameter at time t, and  $t_0$  is the time at which the granule begins to swell. According to the Arrhenius equation:

$$k(T) = A \exp\left(\frac{-E_a}{RT(t)}\right)$$
(2.4)

where  $E_a$  is the activation energy, R is the ideal gas constant, and T(t) is the temperature as a function of time. Noting that in our experiments we employ a quadratic temperature ramp, we can write T(t) as:

$$T(t) = T_0 + at^2 + bt (2.5)$$

in accordance with equation 2.2.

The second and third models that were considered are empirical models based on the generalized logistic function. Following Occam's razor, we seek the simplest model (in terms of number of free parameters) that is capable of capturing the features in the data. Therefore, we try the logistic function:

$$D(t) = A_L + \frac{B_L}{[1 + e^{-k_L(t - t_L)}]}$$
(2.6)

and the Gompertz function:

$$D(t) = A_G + B_G \exp\left(-e^{-k_G(t-t_G)}\right)$$
(2.7)

each of which has four free parameters:  $A_L$ ,  $B_L$ ,  $k_L$  and  $t_L$ , and  $A_G$ ,  $B_G$ ,  $k_G$  and  $t_G$  respectively.

Recognizing that the initial and final values of each function must correspond to the initial and final diameters of the granules, these can be rewritten as:

$$\frac{D(t) - D_0}{D_f - D_0} = \frac{1}{1 + e^{-k_L(t - t_L)}}$$
(2.8)

and

$$\frac{D(t) - D_0}{D_f - D_0} = \exp\left(-e^{-k_G(t - t_G)}\right).$$
(2.9)

Written in this way, we can see that two of the parameters have a clear physical interpretation and one can choose to treat them as fitted parameters or extract them directly from the data. The other two parameters relate to the rate of swelling  $(k_L$  and  $k_G)$  and the time at which swelling is rapid  $(t_L \text{ and } t_G)$ . Further, we can non-dimensionalize Gompertz function as follows:

$$\frac{D(t) - D_0}{D_f - D_0} = \exp\left(-e^{-\tau + k_G t_G}\right); \qquad \tau = t k_G.$$
(2.10)

## 2.3 Results and Discussion

#### 2.3.1 Comparison of granule size measurements

To monitor the swelling behavior of individual starch granules, around 180 granules from each type of starch sample were tracked using image analysis. In order to verify that image processing gives reasonable results, the particle size distribution calculated from image processing was compared to that obtained from laser diffraction. The cumulative volume-weighted size distributions from each method are shown in Figure 2.5. Cumulative distributions are shown (rather than probability distributions) to avoid artifacts in plotting the data due to selection of bin size [77].

The results show minor differences between the two methods for chickpea, green lentil and yellow pea starches and significant differences for red bean starch. The mean values and their standard deviations are given in Table 2.1 and are similar to those found by other researchers [10, 64, 74]. The data show that the measurements of green lentil and chickpea starches are very similar between the two methods for the majority of the distribution, with a only bit more of the sample showing smaller granule sizes in laser diffraction. This is perhaps not very surprising when one notes that the size distributions for green lentil and chickpea are significantly more narrow than than yellow pea and red bean starch, as indicated by the smaller standard deviations and steeper cumulative distribution functions.

On the other hand, laser diffraction showed the presence of larger granules on the upper end of the distribution for yellow pea starch. Also, the size of red bean starch granules measured by image processing is noticeably smaller than that measured by laser diffraction across the entire distribution. One possible explanation for the latter



Figure 2.5: Cumulative size distribution in volume percent of bulk sample measured by a particle sizer (using laser diffraction) and individual granules quantified by image analysis for four starches: chickpea (N = 182), red bean (N = 185), green lentil (N = 179), yellow pea (N = 182). The data in (b) is the same as in (a), but zoomed in on the small particle size region. (c) Representative optical micrographs of chickpea, red bean, green lentil, and yellow pea starch granules. (d) Representative SEM images of chickpea, red bean, green lentil, and yellow pea starch granules.

Table 2.1: Structural and compositional information for each of the four legume starches. The mean diameters are expressed as equivalent spherical diameters (ESD) and were measured using image analysis from optical micrographs and also by laser diffraction. The composition is given by the amylose content.

	Red bean	Green lentil	Yellow pea	Chickpea			
ESD: images (vol. weighted)	$44 \pm 8 \ \mu m$	$25 \pm 3 \ \mu m$	$31 \pm 5 \ \mu m$	$25 \pm 3 \ \mu m$			
ESD: images ( $\#$ weighted)	$38\pm8~\mu\mathrm{m}$	$23 \pm 4 \ \mu m$	$28\pm6~\mu\mathrm{m}$	$23 \pm 4 \ \mu m$			
ESD: laser diffraction	$48 \pm 11 \ \mu m$	$21 \pm 5 \ \mu m$	$30\pm7~\mu{ m m}$	$21 \pm 4 \ \mu m$			
Amylose content $^*$	23.0 - $30.6%$	23.5 - $31.6%$	31.2 - $39.1%$	22.5 - $32.6%$			
*As reported in [64, 78, 10, 74, 73, 79, 80]							

observation is the different morphology of red bean starch granules compared to the others. From micrographs of the starch granules in Figure 2.5 (c), all four types are basically oval in shape, but the red bean starch granules are larger and more irregular in shape. SEM was used to complement the light micrographs in an effort to find differences in the shape, but these only revealed that the surface of all four granules was quite smooth as can be seen in Figure 2.5 (d). It is well known that non-sphericity in particle shape can have a large influence on laser diffraction measurements, with a shift toward larger particle size when the particle becomes more irregular [81].

However, another explanation is possible. Because the discrepancy between the methods is most noticeable for the granules with the largest sizes, it is possible that buoyancy may play a role. Specifically, when loading the ParCS chamber, granules are continually settling in the vial, syringe, and tubing. It stands to reason then that overly large granules could be settling out before being loaded into the viewable portion of the chamber. If that were the case, then minor modifications to the sample loading procedure may be able to remove the discrepancy.

To be conclusive about the reason for the difference in the methods for red bean starch granules requires additional systematic tests. However, we consider this to be beyond the scope of the present manuscript. Based on the minor discrepancies observed between measurements of particle size using image processing in our setup and laser diffraction, we conclude that our measurements are sufficiently accurate for our purposes and that 180 granules is a large enough sample size to represent the full population of granule sizes present in each sample.

#### 2.3.2 Swelling behavior and models

We used around 180 granules from each sample to determine the variability between individual starch granules. Figure 2.6 illustrates granule size changes caused by swelling during gelatinization for each type of legume starch. Starch granules start to swell at a threshold temperature and reach their equilibrium size some time later. The onset time and duration of swelling clearly differs from one granule to another. Such differences in granule swelling behavior during gelatinization have also been reported in other studies using a hot-stage microscope [44, 82, 83, 46].

Three different mathematical models (see section 2.2.8) were used to fit the granule size vs. time. Figure 2.7 shows the fitted curves for each function applied to three representative granules. Others have used the logistic function to model the change in size change of individual starch granules [47, 48], but one limitation of the logistic function is that it is anti-symmetric about the vertical line that passes through  $\frac{D(t)-D_0}{D_f-D_0} = 0.5$ . In contrast, our data does not show this anti-symmetry since the initial rise in the sigmoidal curve is faster than the final decay as shown in Figure 2.6. For this reason, the fitting results using the logistic function deviated from the data at the initial log phase and final equilibrium phase (Fig. 2.7).

Next, we tested the first-order kinetic function, which was used by others studying



Figure 2.6: Granule size as a function of time for a representative subset of granules from (a) chickpea, (b) red bean, (c) green lentil, and (d) yellow pea starch. (e) shows the same data, but for all yellow pea starch granules measured for reference.



Figure 2.7: Example data for starch granule size as a function of time showing the best fits of the Gompertz function, the logistic function, and a first-order kinetic model. The data in (b) is the same as in (a), but zoomed in on the region when swelling begins. Note that the logistic function over-predicts the initial swelling and the first-order kinetic model under-predicts it, while the Gompertz function fits the data quite well at all times. (c) The same data as in Figure 2.6 (a) - (d), but with a dimensionless diameter. For clarity, the data have been artificially shifted vertically by 0.33, 0.67, and 1 for chickpea, green lentil, and chickpea respectively. (d) The same data as in (c) but with the time also nondimensionalized according to equation 2.10.

gelatinization under isothermal conditions [84]. Our results show that the first-order kinetic model fits the data quite well after granules have begun to swell rapidly and reach equilibrium, but fail to capture initiation of swelling. Mathematically speaking, the rate of swelling in the first order model can only decrease with time, in contrast to the data for which the rate initially increases before decreasing. Because of this, the first-order kinetic model is not suitable for capturing the onset of swelling in our experiments.

The Gompertz function, on the other hand, performed much better than the other two models (as determined by least-squares fitting). This is because the Gompertz function inherently maps out a sigmoidal curve where the initial rise is faster than the final decay. Further, when the data from Figures 2.6 (a - d) are non-dimensionalized according to equation 2.10 and plotted in Figure 2.7c) and d), we see that the size evolution of granules overlaps. This suggests the existence of a master curve for this describing the physics of this process and that the Gompertz function provides a close approximation.

It is worth noting that we did not explore the use of a first-principles model in this study. For example, Narsimhan et al. have used chemical potentials along with the governing equations for heat and mass transfer to describe the swelling of starch granules with great success [51, 85]. However, application of this model currently requires the solution of a complex set of partial differential equations and requires material property data that we do not have available for these starches. Therefore, implementation of this model was too complex to be useful for the present study, but we do plan to explore its use in future work.

## 2.3.3 New parameters for characterizing swelling behavior of individual starch granules

In order to make quantitative comparisons between gelatinization data for different granules and different starches, it is essential to be able to reduce the swelling curve to one or two parameters. The Gompertz function provides a convenient way to do this and here we propose two novel parameters for characterizing gelatinization of starch based on ParCS data (defined below). In addition, the ParCS data allows us to compute the swelling ratio which has already been defined previously [86, 87]. Using the equivalent spherical diameter, the swelling ratio is simply calculated by  $D_f^3/D_0^3$ .

The first parameter that we define is a granule-swelling temperature. Conceptually, the swelling temperature is intended to represent the temperature at which the starch begins to gelatinize, in analogy with the pasting temperature from starch pasting experiments. To define this parameter quantitatively, we must determine an exact point in time at which the granule swelling has begun and then find the temperature of the ParCS chamber at that time. This can be relatively challenging due to the noise in the data and a systematic approach is required to avoid subjectivity.

One option for doing this is to follow an algorithmic approach as done in starch pasting experiments. This approach is illustrated in Figure 2.8 a), and consists of fitting the steepest portion of the swelling curve to a line and then extrapolating to find the time when the line intersects the initial granule size. If the initial rise in granule diameter is very rapid, then this may work reasonably well, but otherwise it is likely to predict a later time and higher temperature than one might determine "by eye". Another option is to simply use the best fit Gompertz function to find the point in time when the granule size (according to the Gompertz function) exceeds a certain threshold value. For example, we computed swelling temperatures based on threshold values where the granule size increased by 5%, 10%, 15%, and 20% of the initial granule size (i.e.,  $\frac{D(t)-D_0}{D_0} = 0.05$ , 0.1, 0.15, 0.2). A comparison of the values from the two different methods is shown in Figure 2.8 b) and Table 2.2. As expected, the average swelling temperatures increased as the threshold was increased. Interestingly, the swelling temperatures obtained from the linear fitting method were similar to those between a threshold of 15% and 20%, but showed less variation between starch types.



Figure 2.8: (a) Example data showing one method for determining the granule swelling temperature by a linear fit to the steepest portion of the data (green dashed line) and taking the point where it intersects with the initial size (lower dashed horizontal line). The vertical dashed line shows the intersection with the temperature curve and the corresponding temperature is indicated by the upper dashed horizontal line. (b) Swelling temperatures obtained using different methods: the linear method shown in (a) and a threshold method described in the text with difference values of the threshold.

Because the Gompertz function decays exponentially with decreasing time, mathematically the function only approaches the initial size and it does not make sense to

		Green	Yellow	Chickpea
	bean [°C]	lentil [°C]	$\mathbf{pea} \ [^{\circ}C]$	$[^{\circ}\mathbf{C}]$
Linear fit	$65.6\pm2.5$	$68.4\pm2.3$	$66.4 \pm 1.9$	$66.4\pm2.5$
5%	$61.6\pm3.7$	$66.4\pm3.2$	$62.8\pm2.8$	$62.7\pm2.3$
10%	$63.4\pm3.4$	$67.8\pm2.8$	$64.9\pm2.8$	$64.8\pm2.1$
15%	$64.7\pm3.0$	$68.6\pm2.6$	$66.4\pm2.6$	$66.2 \pm 1.9$
20%	$65.8\pm2.6$	$69.2\pm2.5$	$67.1\pm2.1$	$67.2 \pm 1.8$

Table 2.2: Swelling temperatures computed by different algorithms

use an arbitrarily small value for the threshold (e.g.,  $D(t) = D_0$  only when  $t = -\infty$ ). Therefore, we recommend that the threshold be based on the amount of noise in the data to avoid non-physical results. To quantify the noise in our data, we analyzed the first 8 measurements of granule size in the experiments (i.e., before gelatinization has began) to get a constant baseline. We then define the noise for a single granule as the largest absolute difference between a single point and the average of those 8 points. The average noise across all granules from each type of starch was found to be 6.3%, 5.4%, 6.1%, and 4.4% for red bean, green lentil, yellow pea, and chickpea starch data respectively. Based on these values, a threshold of 5% was used in all subsequent analysis.

The second parameter that we define is the time scale for granule swelling. Conceptually, this is intended to capture the time scale over which the swelling occurs as that is relevant to setting processing times. There are a number of ways that this could be done, but it is most convenient to again leverage the Gompertz function. Specifically, the parameter  $k_G$  is a rate constant with units of inverse time and so we chose  $1/k_G$  for this parameter, as it represents the characteristic time scale for gelatinization. From Figure 2.7d) we see that gelatinization is essentially complete for all granules when  $t \approx 4.5/k_G$  (i.e., when  $\tau - k_G t_G \approx 3.5$ ) and the measured values are given in Table 2.3.

### 2.3.4 Comparison of techniques

Because we are proposing the use of a new method for characterizing gelatinization of starch granules, we must compare it quantitatively to standard methods. As mentioned previously, to two main methods used are starch pasting experiments and DSC. Pasting curves and DSC curves (with all replicates shown) for each of the four types of starch are shown in Figure 2.9. The pasting temperatures of our samples are slightly different from those reported in other studies [10, 65] as shown in Table 2.3 and these differences are likely the result of using different sources for the starch samples since the protocol was the same and sample concentration was similar (8 wt% vs. 12 wt%).

In contrast to pasting temperatures that are based on the viscosity, DSC measurements are based on physicochemical changes in the starch molecules and the small, but distinct, peak in the DSC curves (Figure 2.9) corresponds to the gelatinization process [86, 88]. The DSC onset temperatures measured in our study were somewhat different than those reported in previous studies as shown in Table 2.3. However, as with the pasting curves, the results can be expected to vary with the source of the sample. In addition, the precise amount of water added and the heating ramp rate are also known to influence the measurement to some extent [89].

Because of the difference in detection mechanisms (viscosity vs. molecular interactions), one might anticipate that the pasting temperature and DSC onset temperature would be correlated, but not necessarily equal. In fact, from Figure 2.10a) we can see that – based on the values reported by the instruments – they are certainly not equal, but also do not appear to be strongly correlated. This apparent lack of correlation caused us to look more closely at each of the two measurements in order to provide



Figure 2.9: (a) Starch pasting curves showing the sample viscosity as a function of time. Three replicates are shown for each starch type indicating a high degree of repeatability. (b) The same data as in (a), but zoomed-in on the region of initial increase in viscosity and with the y-axis as a log scale. The two dashed vertical lines in (a) and (b) indicate time when the temperature reaches 65 °C and 72 °C. (c) Differential scanning calorimetry profiles. Two replicates are shown for each type of starch and the small, but distinct peak in each curve corresponds to gelatinization.

Table 2.3: Pasting temperature, DSC onset temperature, and granule swelling temperature, and granule swelling rate constant of four legume starches. Note that the temperature range for ParCS indicates the intra-sample variability rather than the uncertainty in the measurement. Three different pasting temperatures and two different DSC onset temperatures are provided:

- <sup>a</sup> reported by the instrument software in our experiments
- $^{b}$  reported in [10, 78, 74]
- $^{c}$  corrected based on the raw data from our experiments (see Fig. 2.9)

	$\mathbf{Pasting}^a$	$\mathbf{Pasting}^b$	$\mathbf{Pasting}^{c}$	$\mathbf{DSC}^{a}$	$\mathbf{DSC}^b$	ParCS	$k_G \times 10^3$
	$[^{\circ}C]$	$[^{\circ}C]$	$[^{\circ}C]$	$[^{\circ}C]$	$[^{\circ}C]$	$[^{\circ}C]$	$[s^{-1}]$
Red bean	$71.6\pm0.2$	75.6	$63.8\pm0.2$	$59.9\pm0.5$	61.2	$61.7\pm3.7$	$27.5\pm12$
Yellow pea	$72.3\pm0.1$	70.5	$64.3\pm0.4$	$61.1\pm0.0$	58.2	$62.8\pm2.8$	$33.5\pm9.1$
Chickpea	$72.7\pm0.2$	73.1	$65.8\pm0.3$	$62.7\pm0.2$	58.7	$62.7\pm2.3$	$36.0\pm13$
Green lentil	$72.4\pm0.1$	72.0	$66.3\pm0.0$	$65.1\pm0.1$	60.7	$66.4\pm3.2$	$45.6\pm13$

an explanation.

On possible explanation for the discrepancy between pasting and DSC measurements is related to the limitations of heat transfer in the starch pasting cell [90, 91]. Specifically, the temperature reported is that of the cell, rather than the sample, one can expect some time lag in transferring the heat from the cell to the sample. However, if we observe that the pasting temperatures are about 10 degrees higher than than the DSC temperatures, the time lag would have to be almost two minutes to account for the difference! Since the sample volume is relatively small and is being continuously stirred heat transfer be much quicker than that, so it does not seem reasonable to blame the lack of correlation on heat transfer alone.

Another explanation that we considered is that might be some problem with how the instrument software calculates the temperatures. Looking first at DSC, the numbers reported by the software are in good agreement with visual inspection of the data in Figure 2.9 c). Next, for the pasting curves we plotted two vertical lines at the times with the cell temperature was equal to 65C and 72C in Figure 2.9 a). From



Figure 2.10: Comparisons between (a) DSC onset temperature and pasting temperature, (b) DSC onset temperature and average swelling temperature, (c) pasting temperature and average swelling temperature of 4 types of starch. The filled symbols in (a) and (c) correspond to the temperatures reported by the starch pasting instrument software, and the open symbols correspond to recalculated values of the pasting temperature using a method proposed in this manuscript.

Figure 2.9 a), one would also clearly agree with the values reported by the software based on visual inspection. However, there is an important subtlety that must be addressed.

When one is a gelatinization temperature using viscometric analysis, it is important to consider the end goal. For example, for food processing applications one would likely be interested in knowing at what temperature the viscosity of the sample begins to rapidly increase relative to the *final* viscosity. In that case, the pasting temperatures reported by the software give a clear indication of this. However, if one is interested in knowing the temperature at which gelatinization begins to alter the granules, from a material property perspective, then it is the change in viscosity relative to the *initial* viscosity that matters.

When considering changes in viscosity, it is important to remember that the initial value of the viscosity is not zero and that it increases by nearly a factor of more than 500 in a typical pasting experiment. Because of this the changes relative to the initial viscosity may not be noticeable when plotted on a linear axis. However, when plotted on a logarithmic axis as shown in Figure 2.9 b), it becomes clear that the temperatures reported by the software are better tuned to capture changes relative to the maximum viscosity and fail to capture changes relative to the initial viscosity. Fortunately, this problem is easily remedied by reanalyzing the raw data from the pasting experiments.

In order to extract a gelatinization temperature from the viscosity data, we introduce the following procedure. First, only the initial portion of the viscosity data, where the viscosity  $\mu$  is less than 10× the initial viscosity  $\mu_0$ , is used. This portion of the data is then fit to the following power-law function:

$$\mu = \begin{cases} \mu_0 & t < t_0 \\ \mu_0 + At^n & t \ge t_0 \end{cases},$$
(2.11)

where  $t_0$ , A, and n are fitting constants. Following the same logic as in the procedure above for granule swelling temperature (see Figure 2.8) we find the point in time when the viscosity has increased by 5% (i.e.,  $\mu/\mu_0 = 0.05$ ). The values calculated in this way are reported in Table 2.3 and plotted as open symbols in Figure 2.10.

After recalculating the gelatinization temperature from the pasting data, we see much better agreement between pasting and DSC measurements, though they are still not equal. In contrast, the granule swelling temperature agrees remarkably well with the gelatinization onset temperature from DSC as shown in Figure 2.10b). Interestingly, the granule swelling temperature also agrees reasonably well with the recalculated temperatures from pasting data. From this we can conclude that the ParCS method of characterizing starch gelatinization using granule swelling is able to accurately capture the type of physicochemical change detected by DSC as well as correlate to measurable changes in viscosity.

It is important to note here that we do not wish to imply that either of the two methods for calculating a gelatinization temperature from viscometic analysis is right or wrong. In fact, we propose that researchers consider using both methods as each clearly has a different meaning and potential for application. As such, we suggest that the two temperatures may be differentiated in terms of nomenclature by referring to the temperature determined from the standard software method as the "pasting temperature" and the one determined by our method as the "gelatinization temperature".

Using a ParCS method has the additional advantage that it can easily be combined with polarized light microscopy to observe changes in the crystallinity of the starch granules (via changes in birefringence) [3]. The ParCS method could also be considered more accurate than pasting for determining the material properties (as opposed to processing properties) of the starch and is much less expensive than DSC testing. Finally, the ParCS method is able to provide detailed data on intra-sample variability in gelatinization properties that are not possible with either pasting or DSC measurments.

#### 2.3.5 Correlations between size and gelatinization

Because we have clearly shown that different granules within a starch sample have different gelatinization properties, it makes sense to determine if one can separate the granules to obtain fractions with different, or more consistent, properties. It is known that the gelatinization temperature of starch is related to the composition of the granule, e.g., the ratio of amylose and amylopectin content [92]. For example, the double helices of amylopectin form the crystallites of starch which is also related to the branch chain-length of amylopectin [8, 93], and high amylose starch has been found to have a higher gelatinization temperature [94]. If the gelatinization properties were correlated to the size of the granules, then this would provide a good basis for separation and others have found evidence of a correlation for some starches [95, 96, 68].

In order to determine if there is a correlation between gelatinization properties and granule size, we have created scatter plots showing the size versus the swelling temperature, swelling rate constant, and swelling ratio for every single granule measured. These plots are shown in Figure 2.11 a) to c), and visually it does not appear that there are any obvious correlations. Among all of the correlations, the data for the  $1/k_G$  and the size appear to have the strongest correlation and the Pearson correlation coefficient was highest at 0.32 for yellow pea, but the rest were all less than 0.3 and as low as 0.01. In the case of yellow pea, this is high enough that it *might* be possible to split the granules into two groups with slightly different swelling rates, but not in a way that would be useful for processing.

In contrast to legume starch granules, wheat starch has two distinct types of granules with different shapes and compositions: A-type with a higher gelatinization onset temperature and B-type with lower crystallinity [62]. In contrast again, the pasting properties of size-fractionated potato starch granules showed the contradictory trends in two separate studies [9, 63]. It is possible that the source of the sample and/or differences in fractionation method are responsible for this contradiction, but a more detailed study (e.g., using ParCS) would be needed to resolve it. In any case, the lack of a strong correlation in our data suggests that for these four legume starches, size is not a valid basis for separating granules with different gelatinization parameters.

As a final point in analyzing our data, we also created scatter plots comparing the correlation between individual swelling parameters in Figure 2.11 d) to f). From the data we see that again there is no strong correlation between any of two of the three parameters. This lack of correlation is actually rather noteworthy because it implies that each of the three parameters can vary independently. In contrast, if each of the parameters was controlled simply by granule composition, one would expect to see a strong correlation between the parameters. This suggests that the underlying physics and/or chemistry that determine each parameter may be more complex than



Figure 2.11: Starch granule size vs. (a) swelling temperature ( $\rho = -0.05, -0.28, -0.17, -0.07$ ), (b)  $1/k_G$  ( $\rho = -0.11, 0.23, -0.32, -0.11$ ), and (c) swelling ratio ( $\rho = -0.31, 0.11, -0.08, -0.01$ ). Swelling ratio vs. (d)  $1/k_G$  ( $\rho = -0.19, 0.02, 0.11, 0.09$ ) and (e) swelling temperature ( $\rho = 0.06, -0.01, 0.18, -0.03$ ), (f) swelling temperature vs.  $1/k_G$  ( $\rho = -0.37, -0.29, -0.18, -0.15$ ). For each plot the Pearson correlation coefficient is proved in the parentheses for each starch in this order: (chickpea, green lentil, yellow pea, Ted bean).

one might guess.

### 2.4 Conclusion

ParCS was used to track the size evolution of approximately 180 individual starch granules during gelatinization for four different legume starches. This data clearly and quantitatively show the intra-sample variability for these starches and the size of the data set will be a useful reference for future studies. In analyzing the data we found that the empirical, Gompertz function is extremely effective for describing the granule size as a function of time for all the granules studied. The data also allowed us to measure two new gelatinization parameters that we define here for the first time: a granule swelling temperature and granule swelling rate constant. From our results we reject our hypothesis that there is a correlation between starch granule size and gelatinization temperature. Further, we find that a similar lack of correlation between swelling temperature, welling rate, and swelling ratio suggests that each parameter can be independent for a given starch granule.

On the other hand, the data supports our hypothesis that granule swelling temperature is is correlated to DSC measurements since we see very good agreement with gelatinization onset temperatures measured by DSC. The pasting temperature as reported by the instrument did not agree well with either DSC measurements or ParCS measurements. However, we were able to propose a modified way of calculating the pasting temperature that is more consistent with the onset of gelatinization and that agrees with the other measurements much better. From this we conclude that ParCS as a method has significant potential for augmenting or replacing other gelatinization characterization tools, and is uniquely capable of quantifying the variance in addition to the mean gelatinization properties.

# Chapter 3

# Conclusion

In this work, we studied the swelling process of around 180 starch granules isolated from four types of legume starch by using ParCS apparatus and image analysis. The hypothesis of this study was that there could be some significant variabilities between individual starch granules. We compared the size distribution of individual granules with results obtained from a laser diffraction method. We fitted the granule size evolution data to a first-order kinetic model, the Logistic function, as well as the Gompertz function, and proposed three swelling parameters including swelling temperature, swelling ratio, and  $k_G$ . Starch pasting results and DSC thermal properties showed good agreement with these swelling parameters from individual granules.

The size distributions of individual granules are similar to the results of laser diffraction except for red bean starch which shows a smaller size average size when viewing individual granules. The Gompertz function is more effective than the other two models for fitting the granule swelling size. We extracted the swelling temperature from Gompertz function as a new parameter to characterize swelling granule. The results indicate that the legume starch granules have a low variability from granule to granule and no significant dependence on granule size. This new method can characterize starch at the micro scale and has potential to improve the optimization process of food products involving starch gelatinization.

## Chapter 4

# **Future work**

Future research into other types of starch, such as potato and wheat starches whose variabilities in size and composition are more obvious, is a great opportunity to learn the swelling behaviors of more species. The correlation between granules size and swelling behavior requires data from more starches. Currently, the semiautomated method of image analysis is not very efficient, and upgrading it to an automatic analysis tool is needed to improve the efficiency in the future. There are other models that have not been tested in this study, such as the Weibull model and the Peleg model. Finally, Narsimhan et al. have proposed a model for swelling of starch granules based on first-principles theory that might be of great use for determining fundamental material properties of starch using data from individual granules [51].

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