



## Methodologies For The Study Of Morphological And Thermal Properties Of Starch

### Metodologias Para O Estudo De Propriedades Morfológicas E Térmicas De Amido

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#### Resumo

Amido, carboidrato utilizado como reserva de energia na maioria das plantas, é amplamente empregado na indústria alimentícia, seja como componente principal ou como um espessante. Tendo em vista a aplicação deste como um ingrediente na indústria de alimentos, o estudo das propriedades morfológicas e térmicas deste é de grande importância, pois pode prever seu comportamento durante e após o processamento deste. Metodologias como a microscopia eletrônica de varredura, espalhamento de luz e a microscopia ótica são as ferramentas mais utilizadas na caracterização morfológica do grânulo do amido gerando resultados precisos e boa qualidade. Com relação às metodologias para o estudo das propriedades térmicas, o analisador amilografico rápido e a calorimetria diferencial

de varredura, são as mais utilizadas. O conjunto de resultados obtidos com essas metodologias, permite a conhecimento das propriedades tecnológicas do amido, caracterizando-os, e até mesmo identificar a origem deste.

#### Abstract

Starch, a carbohydrate used as a reserve of energy in most plants, is widely used in the food industry, either as a main component or as a thickener. Considering the application of this as an ingredient in the food industry, the study of the morphological and thermal properties of this food is of great importance because it can predict its behavior during and after the processing of this. Methodologies such as scanning electron microscopy, light scattering and optical microscopy are the most used tools in the morphological characterization of the starch granule generating accurate results and good quality. Regarding the methodologies for the study of thermal properties, the rapid amylographic analyzer and the differential scanning calorimetry are the most used. The set of results obtained with these methodologies, allows to know the technological properties of the starch, characterizing them, and even to identify the origin of this one.

#### INTRODUCTION

Starch is a polysaccharide, used as a reserve of energy in all plants, being abundant in seeds, roots and tubers. In contrast to the other types of carbohydrates, starches can be distinguished from

other carbohydrates because they occur in nature, in specific particles called granules, which according to their origin may present different shapes and sizes (Damodaram et al., 2010).

Substances such as lipids, proteins, ashes and mineral salts may be present in small amounts in the starch composition. The amount of these constituents in the starch depends on the method of extraction and the composition of the plant, and the lower the content of these substances, the better the starch quality (Peroni, 2003). Structurally, starch is a homopolysaccharide composed of two polymers: amylose, a linear polymer, and amylopectin, a highly branched polymer.

Amylose is an essentially linear polymer (about 0.3 to 0.5% of the bonds may be branched), composed of  $\alpha$ -linked (1  $\rightarrow$  4) D-glucopyranose units. The amylose molecules tend to form helical or spiral structures due to the axial equatorial coupling position of the D-glucopyranose molecules. It accounts for about 25% of the composition of most starches, except for high amylose maize starches in which it ranges from 52 to 75% (Damodaram et al., 2010).

Amylopectin is an extremely branched molecule and one of the largest, if not the largest, molecule found in nature, consisting of a chain containing only terminal reducing groups in which numerous branching is bound. It is present in all the starches, constituting about 75% of the common starches. Starches consisting entirely of amylopectin are termed waxy starches because they have a vitreous or waxy appearance (Eliasson, 2004).

The amylose and amylopectin content, have a decisive influence on the technological properties of the starch, such as gelatinization, water solubilization, recrystallization (retrogradation), swelling and viscoelastic properties (Eliasson, 2004; Tester e Karkalas, 2004; Denardin e Silva, 2009).

Amylopectin contributes to granule swelling, while amylose and lipids inhibit it (Rocha et al., 2008).

Starch is widely used in the food industry to improve the technological properties in food systems (Peroni, 2003). With this, the study of paste properties, tendency to retrograde, gelatinization temperature, granules morphology, water solubility, viscoelastic properties, paste clarity and is of great interest, since this information facilitates the development of new products, or even their improvement.

The texture of the starch paste is determined by the viscoelastic deformation and depends on the strength of the molecular bonds and the amount of broken granules. The consistency of the starch paste varies, depending on the degree of gelatinization and the swelling power of the bead. The clarity of pulp varies from light to opaque and is related to light scattering resulting from the association of amylose and other components present in the starch (Matsuguma, 2006).

The objective of this work was to review the main techniques used in the morphological characterization of starch granules (scanning electron microscopy, light scattering and optical microscopy) and in the study of thermal properties (RVA and DSC).

### **Methods for studying the format of starch granules**

According to Tester et al. (2004) and Eliasson (2004) the shape (round, oval, polyhedral), and the size of starch granules (2 to 100  $\mu\text{m}$ ) may vary, since both depend on the plant species studied (Table 1). The granule size can directly interfere with the granule's technological properties. In addition, Rocha et al. (2008) found that the higher values of

gelatinization temperatures, viscosity and swelling power observed for guava parsley starch of the ASA variety are related to the higher amount of large granules and the lower amylose content of this starch.

**Table 1.** Formats and sizes of starch granules from different plants.

| Starch source | Bead format            | Medium size ( $\mu\text{m}$ ) | Reference              |
|---------------|------------------------|-------------------------------|------------------------|
| Potato        | Oval                   | 50 - 100                      | Damodaram et al., 2010 |
| Ginger        | Rounded and flattened  | 21 - 24                       | Vieira, 2004           |
| Biri          | Circular               | 14 - 17                       | Leonel, 2007           |
|               |                        | 43 – 59                       | Leonel, 2007           |
| Saffron       | Flat Oval              | 35 - 101                      | Santa cruz, 2004       |
|               |                        | 18 - 26                       | Santa cruz, 2004       |
| Wheat         | Flattened triangular   | 2 - 55                        | Damodaram et al., 2010 |
| Common Corn   | Lenticular             | 2 - 30                        | Damodaram et al., 2010 |
| Manioc        | Polyhedral and rounded | 5 – 35                        | Buléon et al., 1998    |
|               |                        | 14 - 17                       | Leonel, 2007           |
| Pinion        | Oval or rounded        | 7 - 20                        | Muccillo, 2009         |

### Scanning electron microscopy

One of the techniques used to study the morphology of starch granules is scanning electron microscopy (SEM) (Amadou et al., 2014, Sun et al., 2014a,b,c; Shi et al., 2012; Chin et al., 2011; Kim et al., 2008; Rocha et al., 2008) in which the external surface of the granules is evaluated. The SEM allows for the differentiation of sizes and shapes of the granules (Figure 2) with good precision.

Scanning electron microscopy analysis can be used in the vast majority of solid materials and is easy to interpret. Another attractive aspect of scanning electron microscopy is the ease of sample preparation. The sample, in general a nonconductive material, must be covered by a thin continuous metal layer (less than 20nm) in order to avoid the accumulation of negative charges on the sample. The most commonly used metals are gold, gold-palladium alloy, platinum, aluminum and carbon (Canevarolo Junior, 2003).

The previously degreased and dewatered samples are sprayed under a double sided adhesive metal tape and placed in stubs. Then, the samples are covered with a gold film, in vacuum metallizer,

and observed in the scanning electron microscope (Amadou et al., 2014; Rodrigues, 2014; Sun et al., 2014a,c; Silva et al., 2013; Leite et al., 2012; Shi et al., 2012). As an alternative, the starch samples can be diluted in 100% ethyl alcohol in the proportion of 1/10 (p.v) and placed in the stubs for metallization (Sun et al., 2014b; Leonel, 2007). Surface micrographs can be enlarged from 20x to 100,000x with a resolution of 10nm (Canevarolo Junior, 2003).

With the digital analysis of the microphotographs obtained by MEV it is possible to determine the average size of the starch granules. Pignatello et al. (2006) point out that it is necessary to use at least 300 electron photomicrographs for posterior image analysis. Qin et al. (2016) performed between 150 and 200 measurements to determine the mean diameter of the starch granules.

### Light scattering

Another methodology used to determine the size distribution of starch granules is the laser light scattering based on the assumption that the granules are spherical. However, Singh et al. (2003) says that laser light scattering may not be accurate, since depending on the type of starch, these may

present slightly oblong, irregular or cuboid (Eliasson, 2004; Tester et al., 2004).

The light scattering is used for the purpose of analyzing the distribution of the particle size of the starch, with light scattering being the most used technique (Qin et al., 2016; Shi et al., 2011; Pignatello et al., 2006). Dynamic light scattering can be applied in the characterization of particles, emulsions and molecules. The principle of this technique is based on the fact that the Brownian motion of particles or molecules suspended in a liquid causes the laser light to be spread with different intensities. The analysis of these intensity fluctuations results in the velocity of the Brownian motion and thus, the particle size can be calculated using the Stokes-Einstein relation (Canevarolo Junior, 2003).

The equipment used is equipped with a He-Ne laser (helium-neon) of 0.4 mW and wavelength of 633 nm, and a support cell with temperature control. The intensity of the scattered light is detected at a 90° angle to the incident beam (Pignatello et al., 2006). The readings are made in samples diluted in deionized water and analyzed at 25 ° C. The calculation for determining the particle size distribution is described in ISO13321: 1996E standard document of the equipment used (SHI et al., 2011).

### **Optical microscopy**

Optical light microscopy coupled to a digital image analysis system can also be used for the purpose of determining the size distribution of the starch granules (Shi et al., 2011; Rocha et al., 2008; Kim et al., 2008 and Leonel, 2007). It is a technique that can present results with a resolution of up to 200nm and a magnification of 2x up to 2,000x,

depending on the equipment used (Canevarolo Junior, 2003).

In this, a suspension of about 0.1 g of sample should be dripped into 5 ml of 1:1 glycerol / water solution. Then the sample should be placed on the slides and subjected to reading (Rocha et al., 2008; Kim et al., 2008). Rocha et al. (2008) performed the measurement of 300 granules of each slide. According to these authors, size is determined by measuring the largest diameter of the granules.

Leonel (2007) proposes that for the determination of the distribution of the starch granules for the largest and smallest diameter, use an image analysis system. Starch samples should be collected with a platinum grade and mixed on glass slides in two drops of water and glycerol solution (50%) and covered by cover slip. Once the slides have been prepared, they should be observed under an optical microscope and the selected images analyzed by the system.

### **Methodologies for studying the thermal properties of starch**

The behavior of the starch paste properties is determined by the changes occurring in the starch granules during the gelatinization and retrogradation process. These changes can be measured mainly by changes in viscosity during heating and cooling of starch dispersions (Peroni, 2003; Eliasson, 2004; Damodaram, 2010). The starch paste properties are affected by the amylose, lipid, sugar and phosphorus contents, as well as by the branched chain length distribution of amylopectin. Amylopectin favors swelling of starch granule and impasto, while amylose and lipids inhibit them (Chantaro and Pongsawatmanit, 2010). In addition, interactions of the starch with gums can cause

increase of paste viscosities and reduce the tendency for starch retrogradation due to the increase of water retention (Leite et al., 2012).

### **Rapid Visco Analyser**

Equipment such as the Brabender visco-amylograph and more recently, the rapid visco-amylograph or rapid viscosity analyzer (RVA) are widely used for the study of the behavior of starch paste (Sun et al., 2014b,c; Amadou et al., 2014; Chantaro et al., 2013; Oro et al., 2013; Chantaro and Pongsawatmanit, 2010), as it evaluates the changes in the viscosity of the starch solution during heating, followed by a cooling.

According to Peroni (2003), the parameters usually determined for the interpretation of the paste properties by means of the filling curve obtained with the RVA (Figure 3) are:

- Temperature of paste: temperature in °C, corresponding to the point where the formation of the viscosity curve begins. It occurs when the starch granules begin to swell. At this point, low molecular weight polymers, particularly amylose molecules, begin to be leached from the granules (Chantaro et al., 2013);

- Viscosity peak: value of maximum viscosity of the starch during the heating cycle. Occurs when most granules are fully swollen, intact granules and the molecular alignment of any solubilized polymer has not yet occurred within the instrument's field of attrition (Chantaro et al., 2013; Iromidayo et al., 2011);

- Breakdown: The difference between the maximum viscosity value and the minimum viscosity at constant temperature (95 ° C) is associated with the stability of the starch granules in relation to the

heating (Oro et al., 2013; Chantaro and Pongsawatmanit, 2010);

- Final viscosity: viscosity value at final cooling temperature (50°C). During the cooling phase, the increase in viscosity is due to the rapid rearrangement of the amylose molecules, forming a gel matrix. (Iromidayo et al., 2011; Gunaratne and Corke, 2007);

- Retrogradation: calculated by the difference between the final viscosity and the minimum viscosity at constant temperature, represent a measure of the tendency to retrograde, and is related to the reorganization of the starch molecules (Sun et al., 2014c; Oro et al., 2013).

In general, 3.5 to 4.0 g of starch sample (moisture content of 11 to 14%) are weighed directly into the RVA vessel and 25 ml of distilled water is added to give a sample weight of 28.0 g (Newport Scientific, 2010). The slurry should then be homogenized to avoid lump formation prior to RVA analysis. The slurry is heated at a rate of 12 °C/min from 50 °C to 95 °C and maintained at 95 °C for 2.5 min. On cooling, the same rate is used until the temperature of 50 °C is reached, maintained at 50 °C for 2 min (Sun et al., 2014b,c; Amadou et al., 2014; Chantaro et al., 2013; Oro et al., 2013; Chantaro and Pongsawatmanit, 2010).

### **Differential scanning calorimetry**

Differential scanning calorimetry (DSC) has proved to be an extremely valuable tool in the study of the thermal properties of starch, and according to Donovan (1979, cited by Singh et al., 2003), it can be used to quantify crystallinity in native and retrograded starches, to determine the kinetics of retrogradation, and to study the effects of factors that influence retrogradation. Studies by Qin et al.

(2016), Amadou et al. (2014), Sun et al. (2014a, b, c), Shi et al. (2012), Chin et al. (2011), Chantaro & Pongsawatmanit (2010), and Kim et al. (2008) has been using differential scanning calorimetry to determine the thermal transitions of samples in the gelatinization, characterized by  $T_o$  (on set temperature),  $T_p$  (peak temperature),  $T_c$  (conclusion temperature), and  $\Delta H$  (gelatinization enthalpy).

DSC is a technique in which the temperature difference between the sample and a reference material is measured, while both are subjected to controlled temperature programming. The temperature measurement is made by means of thermocouples attached to the base of the sample holder and the reference. Temperature changes of the sample are proportional to the enthalpy change, the heat capacity and the total heat resistance to the caloric flow (Canevarolo Junior, 2003).

The thermal events that cause modifications in the DSC curves may be basically of first order and second order transitions. The enthalpy changes ( $\Delta H$ ) are called first-order transitions (fusion, crystallization, vaporization, solidification and adsorption) and may be either endothermic or exothermic and represented by the area under the peak in the DSC cuvette. The second order transitions are accompanied by variation of the heat capacity of the sample, along with dimensional and viscoelastic variations (such as the glass transition  $T_g$ ), but they do not present variations of enthalpy, generating no peaks in the DSC curves, changes in baseline (Canevarolo Junior, 2003).

The methodology used by Qin et al. (2016), Amadou et al. (2014), Sun et al. (2014a, b, c), Shi et al. (2012), Chin et al. (2011), Chantaro & Pongsawatmanit (2010) and Weber et al. (2009),

suggests that about 2-5 mg (b.s) sample are weighed in aluminum sample port, suitable for DSC equipment. Distilled water (6 $\mu$ L) should be added, and the sample port sealed in a specific press. Thereafter, they should be kept for 12 h at room temperature to standardize the water distribution. It is also recommended that the analysis be carried out in two stages: in the first stage they should be heated from 20 to 180 °C and in the second, they should be cooled from 180 to 20 °C, and at all stages should be carried out at a rate of 10 °C/min. An empty aluminum sample port can be used as a reference.

The choice of material to calibrate the equipment should be based on the temperature range to be explored in the experiment, with the most used material in this step being Indium (melting temperature of 156.6 °C and enthalpy of 28.5 J/g) . An instrumental factor that interferes with DSC curves is the type of fluent gas, since some (air, O<sub>2</sub>, H<sub>2</sub>) may interact with the sample (Canevarolo Junior, 2003). Seetapan et al. (2013) and Shi et al. (2012) used nitrogen gas as the entrainment gas. Although data may interfere with the results of the analysis, few papers present these in the description of the methodology.

## CONCLUSION

The methodologies used in the determination of the morphology of the starch granules, can both make an analysis of the external surface, when the internal structure of these. These present results with high quality of increase and resolution (SEM) to results with inferior quality (OM), more with the advantage of lower cost. They are analyzes that, in a complete work, certainly must be present, since the morphology of the granules, can identify the species of origin, besides being a

property of technological interest. Regarding the methodologies presented for the determination of the thermal properties of the starch, these present accurate results and corroborate for the understanding of the behavior of starch emulsions during the industrial thermal processing, as well as the behavior of these during the storage.

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