Journal of Food Engineering 152 (2015) 65–71

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Loss of birefringence and swelling behavior in native starch granules: Microstructural and thermal properties

Loreto A. Muñoz [a,*], Franco Pedreschi [a], Angel Leiva [b], José Miguel Aguilera [a]

[a] Pontificia Universidad Católica de Chile, Escuela de Ingeniería, Departamento de Ingeniería Química y Bioprocesos, 7860230 Santiago, Chile
[b] Pontificia Universidad Católica de Chile, Facultad de Quimica, Departamento de Química-Física, 7860230 Santiago, Chile

ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form 28 October 2014
Accepted 2 November 2014
Available online 10 December 2014

Keywords:
Starch gelatinization
Degree of gelatinization
Birefringence

ABSTRACT

Starch granules imbibe water and swell when exposed to abundant water and temperatures above their gelatinization point. The degree of gelatinization of four native starches, wheat, potato, cassava and corn, was determined by the enthalpic transitions and simultaneous events between loss of birefringence and swelling to quantify the process *in situ* and in real time. In all cases, the following three stages were identified: low granular swelling, with little water absorption and 100% birefringence; gradual leading to complete loss of birefringence with the absorption of a large amount of water (approximately 50%); and complete granular swelling to equilibrium. A clear gap between the beginning and end of the loss of birefringence and swelling was observed. When the birefringence reached zero value, 50% swelling was reached at 55.7, 62.0, 68.7 and 70.6 °C for wheat, potato, cassava and corn, respectively. A good correlation between the degree of gelatinization measured by differential scanning calorimeter and microscopy was found for potato, corn and cassava with r^2 values of 0.98, 0.99 and 0.98, respectively, and an r^2 of 0.70 for wheat. Therefore, the loss of birefringence and swelling does not characterize gelatinization in equivalent ways.

1. Introduction

Starch represents between 70% and 80% of the calories consumed by people worldwide (Bertolini, 2010b), and it is one of the primary components in several foods because it is responsible for many desirable functional and nutritional properties in the food and other industries. Starches from different botanical sources vary in composition, morphology, molecular structure as well as in arrangement and content of amylose and amylopectin (Hoover, 2010). The content of amylopectin has been suggested as responsible for granule swelling while the presence of an amylose–lipid complex may retard it (Schirmer et al., 2013). Starches from cereals are known to contain higher quantities of lipids complexed with amylose, while most starches from tubers have little or no lipids (Tester et al., 2004). Most of cereal starches show A-type crystallinity, whereas tuber starches shows B-type X-ray diffraction patterns (Tester et al., 2004). Starch is a widely studied food material, and the characterization of its granules and gelatinization has been studied with atomic force microscopy (Simao and Cordenunsi, 2010). Its functional properties have been assessed through thermal rheological analysis (Kim et al., 2012; Mishra and Rai, 2006) and scanning electron microscopy (Jane et al., 1994). The swelling and water absorption properties of starch have also been evaluated (Choi and Kerr, 2004; Hellman et al., 1952). Starch has many roles in the food industry. For example, it has been used as adhesive, binder, emulsion stabilizer, encapsulation agent, expansion agent, fat replacement, foam stabilizer and thickening agent (Mason, 2009). The gelatinization process is a primary issue in food processing. The starch gelatinization process has been studied in different food matrices using modern techniques, such as enthalpic transitions (Ratnayake et al., 2009), crystallinity disruption patterns (Cai and Wei, 2013) and behavior with isolated components (Beleia et al., 1996; Biliaderis, 1991; Kaur et al., 2008; Zhou et al., 2008). The gelatinization process occurs when starch is heated

Abbreviations: T_{gel}, gelatinization temperature; W_s, weight of sediment; W_l, weight of the dried supernatant; WSI, water soluble index; SP, swelling power; SEM, scanning electron microscopy; $T_{o\text{-}m}$, onset temperature measured by hot-stage and image analysis; $T_{p\text{-}m}$, peak temperature by hot-stage and image analysis; DG_m, degree of gelatinization by microscopy; DSC, differential scanning calorimeter; $T_{o\text{-}D}$, onset temperature measured by DSC; $T_{p\text{-}D}$, peak temperature measured by DSC; ΔH, heat of gelatinization; DG_D, degree of gelatinization by DSC; r^2, correlation coefficients; SPP, swelling power progression; T_o, onset temperature; T_p, peak temperature; T_e, endset temperature; T/T_p, relationship between a determined temperature and peak temperature.

* Corresponding author.
E-mail address: lmunoz@ing.puc.cl (L.A. Muñoz).

http://dx.doi.org/10.1016/j.jfoodeng.2014.11.017
0260-8774/

above its gelatinization point in the presence of excess water, which disrupts the native crystalline structure (Parker and Ring, 2001). Biliaderis (2009) reported that when the granules are heated progressively to higher temperatures in the presence of excess of water, there is a point at which the Maltese cross of the native granules disappears and the granules begin to irreversibly swell. Colonna and Buleon (2010) describe the gelatinization as several events that occur simultaneously, and one event is the predominant swelling of the granule after the loss of birefringence. Singh et al. (2004) report that starch swelling occurs concomitantly with loss of birefringence and precedes solubilization. Ratnayake et al. (2009) identified the following three distinct stages in the gelatinization process: (i) granular swelling by slow water absorption; (ii) followed by a rapid loss of birefringence via the absorption of large amounts of water by the granules; and (iii) finally, leaching of the soluble portion into the solution, transforming the granules into formless sacs.

The gelatinization process has been characterized by many chemical, physical and enzymatic methods, but there is not clear consensus as to when the process starts and ends. Currently, several physical properties, such as the loss of birefringence and swelling, are described as separate events. Therefore, it is necessary to implement methodologies that allow for *in situ* and real time observation to monitor the gelatinization process under controlled conditions that allow for the understanding of the importance of the degree of gelatinization. The objective of this study was to identify the morphological and thermal changes during gelatinization of four of the most important starches in foods (two from tubers and two from grains), all of them widely used in the food industry.

2. Materials and methods

2.1. Materials

Four native food grade starches were used in this study. Wheat, potato, corn and cassava native starch were purchased from the local market.

2.2. Swelling power progression (%)

Swelling power progression was determined in triplicate using the modified method proposed by Li and Yeh (2001). Nine samples of 0.5 g each starch were weighed into a centrifuge tube with a screw cap. Distilled water (10 ml) was added and the tubes were heated at 55, 65, 75, 85 and 95 °C in a shaking water bath. At 10, 20 and 30 min, each set of three tubes was cooled in an ice water bath until they reached room temperature (20 °C) and then centrifuged at 8000*g* for 20 min. The supernatant was decanted from each tube and the weight of the sediment was recorded (W_s). The weight of the dried supernatant at constant weight was also measured (W_l). The water soluble index (WSI) and swelling power (SP) were calculated using the following equations:

$$\mathrm{WSI} = \frac{W_l}{0.5} \times 100 \tag{1}$$

$$\mathrm{SP} = \frac{W_s}{0.5(100 - \mathrm{WSI})} \tag{2}$$

2.3. Morphology and particle size distribution

The morphology of the four native starches was qualitatively assessed using scanning electron microscopy (SEM). Corn, potato, wheat and cassava native starches were sputter coated with gold and examined with a LEO 1420 VP scanning electron microscope (Cambridge, UK), operated at an acceleration voltage of 25 kV. The images were analyzed with the software LEO 32 (Soft Imaging System GmbH 1986–2003, Münster, Germany).

The particle size distribution was measured in sextuple using a Malvern Mastersizer 2000 laser diffraction particle-size analyzer (Malvern, Worcestershire, UK) with distilled water at 18 °C as a solvent according to the method of Stoddard (1999). The particle size distribution and average granule diameter were calculated using the software Hydro Application 5.60 (Malvern, Worcestershire, UK.).

2.4. Hot-stage light microscopy and the progression of the gelatinization process

The progression of the gelatinization process was determined for the four starches as parallel events of the loss of birefringence and swelling using image analysis. Starch granule suspensions were prepared prior to observation by resuspending 1 mg each starch in 1 ml distilled water using a vortex mixer. The suspensions were equilibrated for 2 h, and several drops were deposited in a concave slide and covered with a coverslip. Each specimen was observed under normal and polarized light. To determine the progression of the process, a Hot-stage Linkam (model THMS350V, Linkam Scientific Instruments, Surrey, UK) adapted to an Olympus BX61 light microscope (Olympus Optical Corporation, Tokyo, Japan) equipped with polarized filter and analyzer was used. The hot-stage is also equipped with a temperature controller (PE95/T95 System controller), which allows for heating from 20 to 80 °C at rate of 2 °C/min. A sequence of images were captured with a digital camera (Cool Snap Pro Colour, Photometrics Roper Division, Inc., Tucson, AZ, USA) every 2 min between the temperatures T_{gel} −10 °C and T_{gel} +10 °C.

Based on Ovalle et al. (2013), after image acquisition each image was processed by enhancement and filtering, where the contrast and brightness were adjusted. Then the noise was reduced by applying a median filter and the borders were enhanced using a sharpening filter with the Image Pro Plus 4.5 Program (Media Cybernetics, Inc.). Subsequently, the images were segmented selecting the threshold manually in order to binarize (black and white) the images. Thereafter, to each binarized granule the Feret diameter was measured in order to identify and count each one. Finally, the area of the granules was obtained. The onset temperature ($T_{o\text{-}m}$) at which the Maltese cross starts to disappear, and the peak temperature ($T_{p\text{-}m}$) at which the granule stops swelling and remains in equilibrium were determined. Next, the birefringence area and the total area of the granules in pixels were calculated. The degree of gelatinization by microscopy (DG_m) was determined as the percentage of birefringence in pixels reached from T_o to a specific temperature.

2.5. Thermal properties

The thermal characteristics of each starch were studied in triplicate using a Mettler Toledo Star System 821e differential scanning calorimeter (DSC) at heating rate of 25–90 °C at 2 °C/min to determine the thermal transitions. Approximately 10 mg of each starch solution (10% starch and 90% distilled water) was loaded into a 160 μL capacity aluminum pan. The samples were hermetically sealed and each specimen was equilibrated for 2 h at 20 °C for prior analysis. An empty crucible was used as the reference. The analyses were performed under a nitrogen atmosphere. The onset temperature ($T_{o\text{-}D}$), peak temperature ($T_{p\text{-}D}$) and heat of gelatinization (ΔH) were determined using the data analysis system software $Star_e$. The degree of gelatinization by DSC (DG_D) was