Food Chemistry 121 (2010) 980–989

Contents lists available at ScienceDirect

Food Chemistry

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

Formation of starch spherulites: Role of amylose content and thermal events

Jaspreet Singh [a,*], Charline Lelane [a], Robert B. Stewart [b], Harjinder Singh [a]

[a] Riddet Institute, Massey University, Palmerston North, New Zealand
[b] Institute of Natural Resources, Massey University, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 4 November 2009
Accepted 21 January 2010

Keywords:
Starch
Amylose
Crystallinity
Thermal
Spherulites

ABSTRACT

Commercial maize starches and potato starches of two cultivars differing in physicochemical composition (granule size distribution; amylose to amylopectin ratio) and crystallinity were heated to 180 °C and then cooled by fast quench using a differential scanning calorimeter (DSC), in order to produce spherulitic starch morphologies. Among the raw maize starches, waxy maize starch had highest relative crystallinity (49%) whereas a lowest crystallinity of 33–39% was calculated for high-amylose maize starches. Potato starches showed a relative crystallinity of 50%. The temperatures and enthalpies of gelatinisation and melting varied among all the starches. High-amylose maize starches showed higher transition temperatures of gelatinisation (T_{gel}), whereas waxy maize starch had lowest T_{gel} and enthalpy of gelatinisation (ΔH_{gel}). Similarly, a considerable variation in parameters related with crystalline melting (T_{m1}, T_{m2} and ΔH_{m1}, ΔH_{m2}) was observed for different starches. The superheated gels of different starches treated using DSC were subjected to polarised microscopy, to confirm the formation of spherulites. Both the high-amylose starch gels showed the presence of spherulites exhibiting birefringence and a weak crystalline pattern. No birefringence was observed for waxy maize starch gel, while potato starch gels had some birefringence. The particle size distribution of high-amylose maize starch gels analysed through Zetasizer showed the sizes of spherulitic particles fall in the range of 300 nm–900 nm. The scanning electron micrographs of the dried high-amylose maize starch gels showed the presence of round spherulites consisting of several aggregated spherulitic particles. Amylose content and melting of crystallites during heating play an important role during recrystallisation of amylose (spherulite morphologies).

1. Introduction

Starch is the cheapest and most abundant food biopolymer worldwide. It occurs in a variety of botanical sources, including potato, wheat and maize, and has found diverse applications, ranging from breakfast cereals, snacks and thickeners in the food industry to binders for drug delivery systems, packaging, paper and adhesives in the non-food industry. Native starches from different botanical sources vary widely in structure and composition, but all granules consist of two major molecular components, amylose and amylopectin, both of which are polymers of α-D-glucose units in the $^4C_1$ conformation (Singh, Kaur, & McCarthy, 2007). In the starch granules, amylose and amylopectin molecules are organised in granules as alternating semi-crystalline and amorphous layers that form growth rings. The semi-crystalline layer consists of ordered regions composed of double helices formed by short amylopectin branches, most of which are further ordered into crystalline structures known as the crystalline lamellae. Crystallinity of the starch is mainly attributed to the regular arrangement of the short amylopectin chains, which form double helices packed into one of the well-known A, B or C polymorphs (as distinguished by X-ray diffraction). The amorphous regions of the semi-crystalline and the amorphous layers are composed of amylose and non-ordered amylopectin branches (Jenkins & Donald, 1995; Singh et al., 2007). The crystallinity of different native starches varies from 15% to 45%, depending on the origin of the starch, its hydration level, and the characterisation method (Buléon, Véronèse, & Putaux, 2007).

Ungelatinised starch granules exhibit birefringence in the form of a "Maltese" cross, when viewed between crossed polarizers under a light microscope. Heating of water–starch mixtures leads to the loss of birefringence (Maltese cross) and melting of ordered structures (crystallites) contained in the native starch granules. This process is known as a starch gelatinisation, which occurs due to the collapse (disruption) of molecular order within starch granules. Collapse of crystalline order within the starch granules manifests itself as irreversible changes in properties, such as granule swelling, pasting, loss of optical birefringence, loss of crystalline order, uncoiling and dissociation of the double helices, and starch solubility (Atwell, Hood, Lineback, Varriano-Marston, & Zohel, 1988; Hoover, 2001; Stevens & Elton, 1971). The extent of crystals' perfection within the granule, the length of amylopectin chain involved in the crystalline domain, or the amorphous phase

* Corresponding author. Tel.: +64 6 3505062.
E-mail address: j.x.singh@massey.ac.nz (J. Singh).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.01.032

behaviour have a strong influence on starch gelatinisation (Whittam, Noel, & Ring, 1990). Indeed, the parameters of the melting process of starch depend on several factors, such as heating regime, and the particular character of supermolecular structure of the native granules, which in turn depends on the biological origin of starch and the water content in gelatinisation systems (Donovan, 1979). Differential scanning calorimetery (DSC) has been applied in many studies of thermal properties of starch, since its first use by Stevens and Elton (1971).

Spherulites are semi-crystalline entities with some degree of radial symmetry, displaying birefringence (Abo el Maaty, Hosier, & Bassett, 1998). Starch-based spherulites are semi-crystalline particles formed after heating of starch slurry at fairly high temperatures (>140 °C) in the presence of some impurity or complexing agent, such as fatty acids, and then cooled through a fast quench. DSC can be an efficient tool for the study of starches and the production of spherulites because of its high sensitivity and ease of use (Ziegler, Nordmark, & Woodling, 2003). Furthermore, analyses are generally carried out using a sealed pan, which prevents the loss of water from the starch suspension during heating. Ziegler et al. (2003) produced spherulites using a DSC, whereas Shogren, Fanta, and Felker (2006) used a steam jet cooker to produce spherulites from different starch sources, and reported their characteristics. The spherulite formation has been reportedly influenced by the type of crystallinity; starches exhibiting B- or C-type crystallinity form spherulites more easily than A-type starches (Ziegler et al., 2003).

Identification of native starch sources is required for desired functionality and unique properties (Kaur, Singh, McCarthy, & Singh, 2007). Starches obtained from different sources vary in physicochemical and other functional characteristics. During spherulite formation, the interaction of the starch with the guest molecules, such as fatty acids, is influenced greatly by starch source and the thermal events. For example, potato starch contains a small quantity of native lipids (Singh, McCarthy, Singh, & Moughan, 2008). The major objective of this investigation was to study the suitability of starch source and especially the role of amylose and different thermal events, such as gelatinisation and melting, on the spherulite formation.

2. Material and methods

2.1. Materials

Hylon VII (high-amylose maize starch I), Crispfilm (high-amylose maize starch II), and waxy maize starch were procured from National Starch Ltd., Green Mount, Auckland, New Zealand. In addition, potato starch was isolated (as described below) from tubers of the cultivar Nadine (potato starch I), and Moemoe (potato starch II), from the 2006 harvest. All the reagents used in the study were of analytical grade.

2.2. Potato starch isolation

Potato starch was isolated from both the potato cultivars, as described by Singh, McCarthy, and Singh (2006). Potatoes were washed, brushed in warm water and peeled. The eyes and all bruises were pitted out. Immediately after peeling, the potatoes were cut into small pieces (4 cm^2) and dipped in water containing a small amount of sodium metabisulfite (0.35 g/l). Pieces with dark spots were discarded. The juice was extracted from the potato pieces using a laboratory scale juicer. The juice was filtered through muslin cloth. The residue left on the muslin cloth was washed with distilled water, until only a small amount of starch was passing through the cloth. The filtrate was collected in a glass beaker and the residue left on the muslin cloth was discarded. The filtrate was passed through fine sieves (200- and 100-μm mesh size, respectively) and was left undisturbed for four hours. A solid layer of starch settled. The supernatant was decanted, the starch layer was reslurried in distilled water and, again, the starch was allowed to settle. This procedure was repeated for 4–5 times until the supernatant became transparent. The starch cake was collected and dried at 40 °C in a hot-air cabinet drier.

2.3. Moisture content

The moisture content of the starches in dry powder form was calculated from the weight loss upon overnight heating at 105 °C in an oven (AACC method 44-15 A, 1995).

2.4. Spherulite formation and thermal properties

A Perkin–Elmer DSC 7 operated by Pyris software (Perkin–Elmer Instruments, Norwalk, CT), with an internal coolant and nitrogen purge gas, was used in the experimental work. Large-volume stainless steel capsules (0319-0218) with O-ring were used to study the thermal behaviour of test samples and for spherulite formation. Aqueous samples (30% (w/w); approximately 15 mg wet weight) were prepared in sealed stainless steel differential scanning calorimetry (DSC) pans (60 μl; Perkin–Elmer Instruments) and stored for at least one hour at ambient temperature to facilitate moisture equilibration. Samples were heated in the DSC from 10 °C to 180 °C at 5 °C/min with a holding time of 1 min at maximal temperature, and then quenched to 10 °C at 150 °C/min. The slow heating rate (5 °C/min) was used to minimise any temperature lag due to the large mass of the steel pans. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH_{gel}) and melting (ΔH_{mel}) were calculated. Enthalpies were calculated on a dry starch basis through the DSC software. The DSC pans were opened carefully with the help of pliers to transfer the spherulitic material for characterisation.

2.5. Morphological properties

2.5.1. Size distribution of native starches

The starch granule size distribution was determined with a laser diffraction particle size analyzer (Malvern Mastersizer, Malvern Instruments Ltd., Malvern, UK). The starch sample (0.1125 g, dry weight basis) was mixed with 150 ml distilled water. The suspension was agitated at a very slow speed, using a magnetic stirrer for one hour at room temperature. The starch suspension was then filled into the small volume sample presentation unit of the Mastersizer to obtain an obscuration level of ~20%. Refractive indices of 1.530 and 1.330 were used for the starch and liquid phases, respectively, while the starch granule absorption was 0.1.

2.5.2. Polarised microscopy of native starches and spherulites

For the birefringence observations, an aliquot of starch suspension (raw or treated starch) was transferred on to a glass slide and a cover slip was placed on top of the slide. A polarised light microscope (Nikon Eclipse E600 Pol, Nikon Corporation, Tokyo, Japan) was used to observe the birefringence pattern of starch granules and the spherulites.

2.5.3. Size distribution of spherulites

The particle size distribution of the spherulite samples was measured by using a Malvern Zetasizer Nano ZS (ZEN 3600) instrument (Malvern Instruments Ltd.). Immediately after opening the DSC pans, an aqueous suspension (~0.005% w/v) of spherulite sample was prepared and stirred for one hour, and was put in a Zetasizer cell. The temperature of the cell was maintained at 25 °C.