Food Hydrocolloids 61 (2016) 36–47

Contents lists available at ScienceDirect

Food Hydrocolloids

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

The extent of maize starch crystal melting as a critical factor in the isolation of amylose via aqueous leaching

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ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form
27 April 2016
Accepted 28 April 2016
Available online 2 May 2016

Keywords:
amylose
Leaching
Crystal melting
Starch fractionation

Chemical compounds studied in this article:
amylose (PubChem CID: 53477771)
amylopectin (PubChem CID: 439207)
maize starch (PubChem CID: 24836924)
water (PubChem CID: 962)

ABSTRACT

Aqueous leaching from granular maize starch is a common technique for amylose (AM) isolation. Leaching was studied in a 60–90 °C temperature range. The leachate yield, degree of polymerization (DP) and purity were related to the extent of melting of the starch crystals at the leaching temperature as determined via differential scanning calorimetry. Annealing increases the amylopectin (AP) crystal stability and hence the remaining crystallinity at a given leaching temperature. Negligible AM leaching occurred at temperatures below the annealing dependent onset of melting. Leaching thus benefits from partial melting. Properties of AM leachates remained constant when the extent of starch melting remained below 80%. Loss of more than 95% of the melting enthalpy resulted in higher leachate DP at the expense of purity. As the crystallinity of annealed starches at a given leaching temperature was higher than for native starches, the leachate purity was higher. Although in no cases residual AP crystals remained at 90 °C, annealed starches yielded more pure AM extracts in higher yields than did native starch. More effective leaching in this case may be due to annealing-induced strengthened AP–AP interactions and AM disentanglement from AP.

1. Introduction

Amylose (AM) is an almost linear glucose polymer. Together with amylopectin (AP), it makes up the bulk of granular starch. Its content in regular starches ranges from 20 to 35% and greatly influences the functionality of starch in aqueous systems (Tester, Karkalas, & Qi, 2004). In a fully gelatinized starch dispersion, AM plays a major role in further gelation (Miles, Morris, Orford, & Ring, 1985). AM network formation starts immediately after starch gelatinization (Putseys, Gommes, Van Puyvelde, Delcour, & Goderis, 2011). Connected cylindrical objects and ultimately fractal structures are formed by aggregation of AM and the outer branches of AP (Putseys et al., 2011; Vermeylen, Derycke, et al., 2006). The aggregate concentration relates to the gel stiffness (Putseys et al., 2011). However, the AM aggregation process calls for further systematic research with pure AM since the earlier reported experimental observations may have been obscured by the presence of (long) AP chains in the assessed systems. Thus, a method for producing pure AM on laboratory scale is needed for studying the mechanisms of AM aggregation. Furthermore, the availability of AM of low polydispersity and high purity may serve other study purposes. For instance, the AM molar mass distribution has been suggested to affect the AM functional properties and behavior also in more complex systems (Chung & Liu, 2009; Eerlingen, Deceuninck, & Delcour, 1993; Gidley & Bulpin, 1989; van Soest, Benes, de Wit, & Vliegenthart, 1996).

The production of AM chains on laboratory scale can be achieved by three different methodologies (Doblado-Maldonado, Gomand, Goderis, & Delcour, 2015). Firstly, AM synthesis with enzymes such as glucanotransferases (Niemann, Saenger, & Pfannemüller, 1992) or phosphorylases (Fujii et al., 2003; Gelders, Goesaert, & Delcour, 2005) provides AM of high purity and low polydispersity. However, such procedures entail high costs. Secondly, AM can be extracted from starch and subsequently be isolated as a complex. In brief, starch is fully solubilized and AM is

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http://dx.doi.org/10.1016/j.foodhyd.2016.04.044
0268-005X/

precipitated by adding a hydrophobic agent (*e.g.* *n*-butanol or thymol) which forms inclusion complexes (Naguleswaran, Vasanthan, Hoover, & Bressler, 2014). Thirdly, AM can be obtained via aqueous leaching. When heating a starch suspension slightly above the gelatinization temperature, AM leaches into the aqueous phase and can be recovered by centrifugation (Banks, Greenwood, & Muir, 1991; Shi, Seib, & Lu, 1991). The effect of starch concentration and heating rate (Shi et al., 1991), leaching temperature (Roger & Colonna, 1996; Shi & BeMiller, 2002; Shi et al., 1991) and time (Shi & BeMiller, 2002) on AM aqueous leaching have been studied. Even though aqueous leaching of AM can be scaled up, a major disadvantage is that the resultant AM is polydisperse. Additionally, leaching at temperatures exceeding 80 °C can also solubilize some AP (Roger & Colonna, 1996; Shi & BeMiller, 2002). To combine the high yields obtained at high leaching temperature with high purity of AM leachates, additional procedures such as ultracentrifugation (Roger, Tran, Lesec, & Colonna, 1996) or AM precipitation (Banks et al., 1991; Mua & Jackson, 1995; Roger & Colonna, 1996) have been suggested.

Hydrothermal treatment of starch affects AM leaching (Zavareze & Dias, 2011), but has up to now hardly been considered in the context of optimizing AM leaching in terms of quality and yield. Apparently, only Shi et al. (1991) explored annealing in the context of AM harvesting via leaching procedures. When comparing the native starch with its annealed counterpart, AM yield (based on starch) increased from 21 to 22% and from 18 to 23% for leaching from respectively wheat and maize starches at 95 °C (Shi et al., 1991). However, this study only highlighted the increase in yield and provided no information on the purity and molecular weight distribution of the material leached from annealed starch. Annealing is a hydrothermal treatment in which starch in excess water is heated at a temperature between the glass transition and gelatinization temperatures. It has more profound effects when carried out closer to the gelatinization temperature (Jacobs & Delcour, 1998). The most noticeable effect of annealing is an increased gelatinization temperature and a narrowed gelatinization range, which in turn has been associated with crystal stabilization.

Although only slight crystal modifications occur during starch annealing (Gomand et al., 2012), there seem to be non-negligible effects on AM leaching (Shi et al., 1991), the systematics of which to the best of our knowledge have not been disclosed so far. We here annealed starch to vary the thermal stability of its crystals and studied leaching at different relevant leaching temperatures (i.e., near or within the range of melting events). This way, we addressed the importance of the remaining crystallinity and characteristics of the semi-crystalline structure on AM leaching in terms of yield and quality. The quality of the AM extracts refers to the AM polydispersity, degree of polymerization (DP) and presence of AP contaminants.

2. Materials and methods

2.1. Samples

Maize (*Zea mays* L.) starch was from Tereos Syral (Aalst, Belgium). Moisture content was determined in triplicate using an air-oven method [Approved Method 44–19.01; (AACCInternational, 2013)]. Using the iodine binding procedure (Kaufman, Wilson, Bean, Herald, & Shi, 2015), its AM content was found to be 28%. Its lipid content (0.19%) was reported earlier (Dries, Gomand, Goderis, & Delcour, 2014). All used reagents, solvents, and chemicals were of at least analytical grade and obtained from Sigma–Aldrich (Bornem, Belgium) unless indicated otherwise.

2.2. One and two step annealing of starch

Starch suspensions (3.0% w/w) were prepared in bottles. Their headspace was flushed with nitrogen and the bottles were closed. Samples were then annealed in a water bath with mild agitation for 24 h at 57 °C, which is below the onset of the gelatinization endotherm as measured by differential scanning calorimetry (DSC, Fig. 1). At the chosen annealing temperature, excessive crystal melting is avoided (Gomand et al., 2012). This starch is referred to as one step annealed starch. A second incubation of one step annealed starch was carried out at 60 °C for an additional 24 h. The resultant starch is referred to as two step annealed starch. The second temperature was selected based on the thermal properties of the one step annealed starch as also done for native starch (Fig. 1). After the treatments, starches were Büchner-filtered and air-dried at room temperature overnight.

2.3. Analysis of starch damage

The degrees of starch damage of native and annealed starches were determined in triplicate according to AACC International Approved Method 76–31.01 with a starch damage assay kit (Megazyme, Bray, Ireland).

2.4. Microscopic analysis

Birefringence of the starches was studied using a Nikon (Melville, NY, USA) Eclipse 80i epifluorescence microscope equipped with a charge-coupled device camera. The light micrographs (in bright field and polarization modes) were analyzed using the NIS-Elements BR software (Nikon). A Jeol (Tokyo, Japan) JSM7401F field emission scanning electron microscope was operated at an accelerating voltage of 2 kV and working distance of 15 mm to monitor changes in morphology. Starch samples were placed on a stub with carbon sticker and sputter coated with platinum (Agar auto sputter coater).

2.5. Analysis of crystallinity

Native or annealed starches were equilibrated in a humidifier to 13.0 ± 1.0% moisture content for 48 h. Aluminum DSC pans (Perkin–Elmer, Waltham, MA, USA) were then filled with the samples and hermetically sealed. Wide angle X-ray diffraction (WAXD) measurements were performed with a XeuSS X-ray camera (Xenocs, Sassenage, France). The Mo Kα radiation (wavelength, $\lambda = 0.71$ Å) source was operated at 50 kV and 1 mA. Diffracted photons were collected on a 2D area image plate detector (MAR research, Norderstedt, Germany). Reflections of silver behenate and polyethylene were used to calibrate the scattering angles. Data were azimuthally averaged and converted into one-dimensional scattering patterns using ConeX 1.5 (Gommes & Goderis, 2010). These patterns were background corrected using the empty holder scattering and taking into account transmission differences. The scattering angles 2θ, with θ being half the scattering angle, were converted into scattering angles as if Cu Kα radiation ($\lambda = 1.54$ Å) would have been used to facilitate comparison with literature data. An amorphous sample of native maize starch was prepared by gelatinizing a sample in excess water, freeze drying and adjusting it to a moisture content of 13.0 ± 1.0% as above. Degrees of crystallinity (X_c) were calculated with equation (1) as in Dries et al. (2014).