Food Hydrocolloids 69 (2017) 97–102

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Structural characterization of aroid starches by means of chromatographic techniques

Javier D. Hoyos-Leyva [a], Luis A. Bello-Pérez [a], J. Alvarez-Ramirez [b], Edith Agama-Acevedo [a, *]

[a] Instituto Politécnico Nacional, CEPROBI, Yautepec, Morelos, Mexico
[b] Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa, Iztapalapa, Ciudad de México, 09340, Mexico

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form
20 January 2017
Accepted 28 January 2017
Available online 3 February 2017

Keywords:
Aroid starches
Structure
Chromatography
Chain conformation

ABSTRACT

Starch functional properties are largely determined by the molecular structure. The weight average molar mass and radius of gyration can be calculated by the analysis of solubilized polymers using AF4-MALLS-DRI systems. The chain length distribution can be determined by analysing debranched starches by gel permeation chromatography (GPC) and high performance anion exchange chromatography (HPAEC-PAD). Aroid starches were used as study case for structure analysis with the abovementioned chromatographic techniques. Amylose and amylopectin chains from aroid starches exhibited weight-average molar mass (Mw) higher than 2.5×10^7 g mol^{-1} and 5.7 to 13 $\times$ 10^8 g mol^{-1}, respectively. The relatively high values of Mw are correlated to high radius of gyration to both amylose and amylopectin molecules (253–291 nm and 311–410 nm, respectively). The hydrodynamic coefficient (υ_G) of amylose and amylopectin was 0.6–0.7 and 0.28–0.35, respectively, suggesting random coil conformation for amylose and spherical conformation for amylopectin. The conformation of amylopectin was confirmed by GPC and HPAEC-PAD with the presence of high population of short amylopectin chains (DP 6–12–30% and DP 13–24–50%).

1. Introduction

It is a well-accepted fact that starch functional properties (e.g., solubility, light transmittance, retrogradation rate and freeze–thaw stability) are largely determined by the underlying molecular structure (Annison & Topping, 1994; Eliasson, 2004; Hoover, 2001; Mua & Jackson, 1997). Starch has a semi crystalline granular structure conformed by amylose (linear, α-1,4 glycosidic linkages) and amylopectin (branched, α-1,4 and α-1,6 glycosidic linkages) chains (Buléon, Colonna, Planchot, & Ball, 1998). The organization of the starch polysaccharides into granules is displayed at different levels, from the packing of double helices formed from amylose to the clustered branches of amylopectin molecules (French, 1972; Gallant, Bouchet & Baldwin, 1997). The analysis of starch molecular structure (e.g., molecular weight and chain length distribution) is an important issue to understand the relationship between starch biosynthesis, functional and physicochemical properties, and digestibility with its structural characteristics (Cave, Seabrook, Gidley, & Gilbert, 2009). Small and subtle differences between external and/or internal structure of starch from different sources can explain large differences in its functional properties. In turn, these differences can be linked to the role of longer internal segments and very short chains. The former bring on interactions of starch molecules in solution, while the latter can interfere with such interactions providing additional effects on starch functionality (Bertoft et al., 2016).

The high complexity of starch chains hampers the accurate characterization of the molecular structure. Nowadays, a number of methods are available for research purposes. Recently, Harding Adams and Gillis (2016) provided a critical discussion of the methods available for the characterization of starch molecular structure. The merits and limitations of size-exclusion chromatography or field flow fractionation coupled to multi-angle light scattering, viscosimetry, sedimentation velocity and sedimentation equilibrium in the analytical ultracentrifuge were outlined. Asymmetrical flow field flow fractionation (AF4) and size-exclusion chromatography are two methods to separate starch chains and components (amylose and amylopectin). Coupled to multi-angle

* Corresponding author.
E-mail address: eagama@ipn.mx (E. Agama-Acevedo).

http://dx.doi.org/10.1016/j.foodhyd.2017.01.034
0268-005X/

laser light scattering (MALLS) and refractive index detector (RID), AF4 is used to quantify the molecular weight and gyration radii of polymeric chains. Limitations such as shear and adsorption on the column packing materials can be largely reduced and even eliminated.

The fine structure of amylopectin is reflected in its chain length distribution (Koizumi, Fukuda, & Hizukuri, 1991). Such structure can be characterized by analysing the number distribution of the linear glucan chains released by enzymatic debranching of starch α-1, 6 glycosidic bonds for subsequent characterization by techniques such as high performance anion exchange chromatography (HPAEC), size exclusion chromatography (HPSEC), etc. On the other hand, gel permeation chromatography (GPC) is a traditional used technique for chain length distribution (Biliaderis, Grant, & Vose, 1979; Suzuki, Hizukuri & Takeda, 1981; Takeda, Takeda, & Hizukuri, 1986) in which the size exclusion allows the separation of polysaccharide chains with different polymerization degree. However, GPC is unable to separate individual molecules for characterization purposes (Koizumi et al., 1991). In this regard, HPAEC coupled to pulsed amperometric detector (PAD) has proven to be useful for the determination of the chain length distribution by analyzing the polymerization degree of individual chains (Hanashiro, Abe, & Hizukuri, 1996; Koizumi, Kubota, Tanimoto, & Okada, 1989; Wong & Jane, 1997). Given the specific limitations of individual chromatographic techniques, it seems that the use of a sole method would lead to incomplete characterization of the molecular and granular starch structure. In this regard, the suitable combination of several chromatographic techniques should provide a more accurate view of the starch structure to gain insights in functional properties and potential applications.

The aim of this work was to explore the use of different chromatographic techniques (AF4-MALLS-RID, GPC and HPAEC-PAD) to study starch molecular structure. The motivation relies from the fact that an accurate knowledge of the molecular characteristics provides valuable guidelines for assessing starch potential applications. As a case study, starch from aroids cultivars was considered. Aroids cultivars (e.g., *Xanthosoma sagittfolium*, *Colocassia esculenta* and *Colocassia esculenta*) are important dietary energy sources in many regions of Africa, Asia, Central and South America. Those food crops have been entered in the transition of special food markets systems, which has an increasing interest for fresh and/or processed products to the development of its value chains.

2. Materials and methods

2.1. Starch isolation

Starches were isolated from corms of three aroid cultivars produced and commercialized in the Southern region of Mexico by local farmers (Integradora R y T de la Cuenca del Papaloapan, Tuxtepec, Oaxaca, Mexico). The samples were identified as *Colocassia esculenta* var. Esculenta (V_1); *Colocassia esculenta* var. Schott (V_2) and *Xanthosoma sagittifolium* (V_3). The corms were manually cleaned and peeled, cut in 2 mm slides and dried in a convection oven at 40 °C for 48 h. The dried slides were milled to obtain flour. Starch isolation was carried out following the procedure described by Whistler (1998). Briefly, aroid flours were suspended in sodium bisulfite (1% w/v) solution (ratio 1:8) with continuous magnetic stirring for 4 h at room temperature. The mixture was liquefied in a commercial blender and then filtered through mesh 100, 200 and 325 US. The residue was washed many times with distilled water. Afterwards, the filtrate was centrifuged at 10,800*g* to obtain the starchy precipitate. The starch was dried in a convection oven at 40 °C for 24 h and stored in hermetic bags for subsequent analysis.

2.2. AF4 coupled to MALLS and RID

Samples were treated with dimethylsulphoxide (DMSO) (Bello-Pérez, Roger, Baud, & Colonna, 1998). Starch (0.025 mg mL^{-1}) was suspended in DMSO (95%) during 4 days with continuous magnetic stirring. The starch suspension was added to a beaker with pre-cooled (4 °C) ethanol (95%). The mixture was stored overnight at 4 °C with magnetic stirring. The starch-DMSO-ethanol mixture was centrifuged at 13,000 rpm for 10 min and three washing cycles with ethanol were applied. An additional washing with 20 mL acetone and 20 mL of ethylic ether was carried out. The sample was dried in a convection oven at 40 °C for 48 h. The starch solubilisation was estimated by following the methodology proposed by Bello-Pérez et al. (1998), consisting in aqueous starch solubilisation within pressure vessel by microwave heating. The time of microwave heating was 40 s. Heating time over this value triggered amylopectin degradation, resulting in biased high values of amylose fraction. The carbohydrate concentration after filtration and the injected mass were determined using phenol-sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The mass recovery of all samples from AF4 system was over to 90%.

The AF4 system consisted in a short channel (Wyatt Technology Corporation, Santa Barbara, USA) with a 10 kDa membrane, coupled to a multiangle laser light scattering-MALLS (Dawn Heleos 8, Wyatt Technology Corporation, Santa Barbara, USA) and refractive index detector-RID (1100 Generic RI, Agilent Technologies, Santa Clara, CA, USA). The flow conditions were 1 mL min^{-1} to detector, focus flow and cross flow, 1 min of inject time and 32 min of elution. The eluent was HPLC water with 0.02% sodium azide. Average molecular weight (Mw) and gyration radii (Rz) were calculated using ASTRA® Version 5.3.1.5 software (Wyatt Technology Corporation, Santa Barbara, USA), Berry method and second order polynomial were used.

2.3. Debranching and chromatographic techniques

The aroid starches were submitted to debranching by the procedure reported by Chávez-Murillo, Wang, and Bello-Pérez (2008). The debranched starches were analyzed by gel permeation chromatography (GPC) on two-system columns (Superdex 200 pg and Superdex 30 pg) connected in tandem, equipped with a 515 HPLC pump (Waters Corporation, Milford, Massachusetts, USA) and fractions collector Frac 920 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The descendent flow was 0.4 mL min^{-1} with sodium acetate (0.1 M, 0.02% sodium azide, pH 4.7) as eluent. Fractions of 4 mL were collected during 1400 min of running time. The total carbohydrates of each fraction were determined using phenol-sulfuric method (Dubois et al., 1956). Recovery percentage of the column was up to 95%. Debranched starch and collected fractions (three mix of fractions of amylopectin chains) from GPC were analyzed by HPAEC-PAD using a Dionex ICS 5000 instrument equipped with a Dionex AS-AP auto-sampler (Thermo Scientific, Waltham, Massachusetts, USA), CarboPac PA200 (3 × 250 mm) column and CarboPac PA200 guard column (3 × 50 mm). The potential and time periods for the pulsed amperometric detection were: E_1, +0.10 V for 0.4 s; E_2, -2.0 V for 0.02 s; E_3, +0.60 V for 0.01 s; E_4, -0.10 V for 0.07 s. Two eluents were used as mobile phase: eluent A, 150 mM sodium hydroxide; and eluent B, 150 mM sodium hydroxide containing 500 mM sodium acetate. The flow was 0.5 mL min^{-1} and an eluent gradient was used as follow: 95% of eluente A for 5 min, 60% to 18 min, 15% to 55 min and 95% to 75 min. The data were processed with the Chromeleon v. 6.80 SR11 software (Thermo Scientific, Waltham, Massachusetts, USA). The degree of polymerization (DP) was reported as % area. Maltotriose and maltopentose were used as reference for the determination of