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The effect of high hydrostatic pressure treatment on the molecular structure of starches with different amylose content

Artur Szwengiel [a], Grażyna Lewandowicz [b], Adrian R. Górecki [c], Wioletta Błaszczak [c,*]

[a] Institute of Food Technology of Plant Origin, Poznań University of Life Sciences, Poznań, Poland
[b] Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Poznań, Poland
[c] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland

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ABSTRACT

The effect of high hydrostatic pressure processing (650 MPa/9 min) on molecular mass distribution, and hydrodynamic and structural parameters of amylose (maize, sorghum, Hylon VII) and amylopectin (waxy maize, amaranth) starches was studied. The starches were characterized by high-performance size-exclusion chromatography (HPSEC) equipped with static light scattering and refractive index detectors and by Fourier Transform Infrared (FTIR) spectroscopy. Significant changes were observed in molecular mass distribution of pressurized waxy maize starch. Changes in branches/branch frequency, intrinsic viscosity, and radius of gyration were observed for all treated starches. The combination of SEC and FTIR data showed that α-1,6-glycosidic bonds are more frequently split in pressurized amaranth, Hylon VII, and waxy maize starch, while in sorghum and maize starches, the α-1,4 bonds are most commonly split. Our results show that the structural changes found for pressurized starches were more strongly determined by the starch origin than by the processing applied.

1. Introduction

Starch is a biodegradable polymer with simple and well-defined chemical properties. There are two major starch constituents: amylose, which is primarily linear with a few long-chain branches, and amylopectin, a highly branched molecule. Both the molecular mass of amylose/amylopectin and the amylose/amylopectin ratio can be considered the most important structural factors directly affecting the technological properties of a starch (Kurzawska et al., 2014; Yoo & Jane, 2002). It is generally known that waxy starches with trace amount of amylose require higher energy to achieve gelatinization, as a result of their higher crystallinity, than do starches with regular amylose content (Singh, Inouchi, & Nishinari, 2006; Van Hung, Maeda, & Morita, 2006). The differences in the physicochemical properties of starches such as swelling, solubility, and thermodynamic properties (melting temperature and melting enthalpy) are directly assignable to the differences in amylopectin molecular structure (molecular mass, *M* and its distribution) (Guo et al., 2015).

Various techniques have been used to study the structures of starch macromolecules with an emphasis on the branch structure of amylopectin—for example, high-performance size-exclusion chromatography (Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016; Yoo & Jane, 2002) and high-performance anion-exchange chromatography (Kong, Corke, & Bertoft, 2009; Raghunathan, Hoover, Waduge, Liu, & Warkentin, 2017). High-performance size-exclusion chromatography (HPSEC), equipped with multiangle laser-light scattering (MALLS) and refractive index (RI) detectors, is a powerful technique that allows the absolute molecular mass of starch macromolecules to be characterized (Yoo & Jane, 2002). However, determining amylopectin molecular mass is challenging, due to the fact that it is an extremely high molecular mass polymer. It is thus difficult using any available technique to obtain a solution with a molecular dispersion of such starch without it undergoing degradation. On the other hand, physical treatment preceding the dissolution of starch granules could affect the analysis of molecular mass distribution and hydrodynamic parameters. For this reason, the results of molecular structure studies of starch may be considered and compared only if the same methods of granule dissolution and separation (of amylopectin from amylose) were used (Kurzawska et al., 2014; Yoo & Jane, 2002).

As mentioned, knowledge of the molecular architecture of starch is crucial, since as well as permitting its technological use-

* Corresponding author at: Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, 10-748 Olsztyn, Poland.
E-mail addresses: artursz@up.poznan.pl (A. Szwengiel), gralew@up.poznan.pl (G. Lewandowicz), a.gorecki@pan.olsztyn.pl (A.R. Górecki), w.blaszczak@pan.olsztyn.pl (W. Błaszczak).

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fulness to be predicted, it also allows control of granules behavior under physical, chemical, and enzymatic treatment.

Although the high hydrostatic pressure (HHP) process has been described in the literature as a "mild technology", it can result in significant changes in the physicochemical properties of starch granules (Hu, Zhang, Jin, Xu, & Chen, 2017; Pei-Ling, Qing, Qun, Xiao-Song, & Ji-Hong, 2012). Intensive HHP treatment is expected to affect not only the morphology/properties of granules, but also of starch molecules (amylose and/or amylopectin). It was found that the HPLC profile of molecular mass distribution of waxy maize starch (composed mainly of amylopectin) pressurized at 650 MPa for 9 min differed significantly from that of native starch (Błaszczak, Fornal, Valverde, & Garrido, 2005). A HPSEC-MALLS-RI analysis performed by Guo et al. (2015) revealed a distinct decrease in the values of weight molar mass, number molar mass, and dispersity index of lotus starch (40% of amylose) subjected to HHP treatment (100–600 MPa/30 min). The effects of HHP treatment on the structural properties of starch have been widely documented in the literature, (Oh, Pinder, Hemar, Anema, & Wong, 2008; Yang et al., 2016), but very little is known about their molecular structure, in particular the structure of starches with varied amylose content.

Therefore, the aim of this study was to determine the effect of HHP processing (650 MPa/9 min, 30 ± 2 °C) on molecular mass distribution, hydrodynamic and structural parameters (intrinsic viscosity (IV), gyration radius (R_g), hydrodynamic radius (R_h) and number of branches) of starches with varied amylose content (maize, sorghum, Hylon VII) and pure amylopectin starches (waxy maize, amaranth starch). The HHP-treated starches were characterized by HPSEC equipped with static light scattering (SLS) and RI detectors. Fourier Transform Infrared (FTIR) spectroscopy was additionally performed to identify the relationship between the hydrodynamic and FTIR parameters of the starches.

2. Materials and methods

2.1. Materials

The experimental materials were commercial maize starch (20.5% of amylose; donated by the Department of Food Concentrates, Institute of Agricultural and Food Biotechnology, Poznań, Poland), Hylon VII starch (68% amylose; donated by the National Starch & Chemical Co.), and waxy maize starch (with trace amounts of amylose; Sigma, S-9679).

The other materials, sorghum starches (19.2% amylose) and amaranth starches (pure amylopectin starch), were isolated in our laboratory from sorghum grains and amaranth seeds, respectively.

The grains of *Sorghum bicolor* (*v. Rona 1*) were purchased from the Kutno Centre for Sugar Beet Breeding in Straszkow, Poland, and the seeds of *Amarantus cruentus* L. were donated by the Szarłat Metro Industrial Centre (Łomża, Poland).

The isolation procedure described by Olayinka, Adebowale, and Olu-Owolabi (2008) was used to obtain starch from sorghum grains; the starch from the amaranth seeds was isolated and purified according to the method developed by Walkowski, Fornal, Lewandowicz, and Sadowska (1997).

For clarity, the following preparation codes have been proposed for the starches: maize starch (MS), sorghum starch (SS), Hylon VII (Hylon), waxy maize starch (WMS), and amaranth starch (AS).

The chemical composition of these starches has been fully characterized in our previous work (Błaszczak, Misharina, Fessas, Signorelli, & Górecki, 2013); the results are not presented here.

Pullulan standards were purchased from Showa Denko K.K. (Tokyo, Japan). Dimethyl sulfoxide (DMSO, HPLC grade) and sodium nitrate (reagent plus grade), were obtained from Sigma–Aldrich (USA).

Other chemicals were reagent grade and used without further treatment.

2.2. High hydrostatic pressure treatment of starch granules

Our previous study demonstrated that starch processing at 650 MPa for 9 min in excess water (3 g d.m./10 mL) may considerably influence starch crystallinity and/or the molecular mass distribution profile of the analyzed material already at ambient temperature (Błaszczak et al., 2005). In view of the above, starch was pressure-processed under the same conditions in this study.

The starch–water suspensions (3 g d.m./10 mL) were thoroughly mixed and homogenized with a Polytron Ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, USA) for 1 min at 12,000 rpm (revolutions per minute). The homogenized samples were closed into 50 mL Teflon tubes, thoroughly mixed, deaerated, sealed tightly, and subjected to HHP treatment using a high pressure Unipress U-303 device (Warsaw, Poland).

The Teflon tubes were placed into a high pressure chamber (capacity approximately 100 mL) filled with pressure-transmitting medium, which also minimized adiabatic heating. The samples were then pressure-treated at 650 MPa for 9 min. The time taken to reach the working pressure was 2 min. The temperature inside the pressure chamber averaged 30 ± 2 °C. The pressure treatment was performed in two repetitions for each combination.

After pressure treatment, the starch material was frozen in liquid nitrogen, freeze-dried, ground in a laboratory grinder (Sadkiewicz Instruments, Poland), and sieved through a screen with a mesh of 170, so as to unify the diameters. This homogeneously granulated material was sealed in tubes and stored until analysis in a dark, cold, dry place.

2.3. Molecular mass distribution and hydrodynamic parameters of native and HHP-treated starches, as determined by SEC

Following Jackson (1991), an aqueous DMSO solution (90:10, v/v) was used to obtain the starch dispersion, as the most effective procedure for achieving maximum dispersibility of starch granules. Starch samples of 30 mg were dissolved in 5 ml of DMSO/H_2O mixture at 100 °C with gentle stirring at 125 rpm in a Reacti-Therm heating system and stirring modules (Thermo Fisher Scientific, USA). The samples were further diluted with DMSO (the final concentration of the samples being 2.4 mg/mL) and filtered through 5 μm filters prior to analysis (Han & Lim, 2004a, 2004b). SEC equipment (Malvern, TX, USA) with triple detection (Viscotek 305 TDA, Triple Detector Array) was used for starch separation. Conventional dual cell refractometer, viscometer (VIS), and light scattering (low-angle light scattering, LALS, and right-angle light scattering, RALS) detectors were employed to act in concert. The aqueous SEC analysis was performed using three aqueous SEC columns (Shodex OHpak SB-800HQ series) with a guard SB-G type column (Showa Denko, Japan). The chromatography parameters were described by Kurzawska et al. (2014). The starch was analyzed in 0.1 M aqueous sodium nitrate with a 0.3 mL/min flow rate. The RI of the solvent was 1.3340, while that of the sample was 0.160 (In, Ibanez, & Shoemarker, 2007). Intrinsic viscosities (IV) were calculated based on the viscosity signals obtained from the viscometric detector. The calculations of molar mass average (M_n – number-average molar mass; M_w – weight-average molar mass; M_z – Z average molar mass), polydispersity index (M_w/M_n), R_h and the Mark–Houwink *a* value were performed using OmiSEC 4.7 software (Malvern, TX, USA). The amylopectin fraction was coded as AP and the amylose fraction as A.