Carbohydrate Polymers 108 (2014) 216–223

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Molecular weight, chain profile of rice amylopectin and starch pasting properties

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ARTICLE INFO

Article history:
Received 6 August 2013
Received in revised form 12 February 2014
Accepted 24 February 2014
Available online 11 March 2014

Keywords:
Amylopectin
Average molecular weight
Fine structure
Pasting properties
Rice starch

ABSTRACT

Differences in fine structure, average molecular size of amylopectin (AP) as well as clarity of the AP solution from indica waxy rice and high amylose (HAM) rice were examined. Despite similar amylose content (AM), rice starches displayed different pasting properties. Waxy APs had higher values of both number–average and weight–average molecular weight ($\overline{M_n}$ and $\overline{M_w}$) but lower values of intrinsic viscosity [η], compared to HAM APs. HAM APs had higher values of average chain length ($\overline{CL}$), average external chain length ($\overline{ECL}$), and a proportion of DP $\geq$ 37. Statistical correlations of mol proportions of debranched AP, branching parameters, and molecular weight of AP were calculated. The study showed that starch pasting properties and clarity of AP solutions were influenced by molecular weight and branching characteristics of AP.

1. Introduction

Amylopectin (AP) fine structure and its influences on starch functional properties are topics of interest and have been extensively investigated in the past decades (Han & Hamaker, 2001; Jane & Chen, 1992; Jane et al., 1999; Patindol, Gu, & Wang, 2009). Chain length (CL) distribution, branching characteristics, and intramolecular organization of the macromolecules are the principal subjects of AP chain profile that have been explored (Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008; Hanashiro, Abe, & Hizukuri, 1996; Hizukuri, 1986; Jane & Chen, 1992; Jane et al., 1999; Vandeputte, Derycke, Geeroms, & Delcour, 2003). Using different enzymes based on their selectivity to hydrolyze AP molecules is a major approach used in the studies.

Compared to chain profile investigations, studies related to the molecular weight of AP are rather scarce (Chung, Han, Yoo, Seib, & Lim, 2008; Ma et al., 2007; Wang & Wang, 2002; Yoo & Jane, 2002; Zhong, Yokoyama, Wang, & Shoemaker, 2006). Unlike the particular means and specific parameters used in the study of chain profiles, there are several ways to determine the average molecular weight (MW) of polymers. Number–average molecular weight ($\overline{M_n}$) represents MW of the most prevalent polymer molecules and small polymer molecules generally have a major influence on the value. Weight–average molecular weight ($\overline{M_w}$) emphasizes large molecules more than small molecules whereas $\overline{M_v}$ indicates the molecular volumes of polymers in a solution.

Numerous studies (Jane et al., 1999; Shi & Seib, 1995; Vandeputte et al., 2003; Yuan, Thompson, & Boyer, 1993) have emphasized the influence of AP chain profiles on starch functional properties. In regard to pasting properties, debranched medium-amylose (AM) rice starch with a higher amount of the B_3 fraction ($\overline{DP}37-60$) displayed higher peak viscosity, hot paste viscosity, and breakdown viscosity than did others (Patindol & Wang, 2002). Paste breakdown of low-AM rice starches was negatively and positively correlated with long-chain ($\overline{DP}_n > 100$) and short-chain ($\overline{DP}_n \sim 17$) fractions of debranched starch, respectively (Han & Hamaker, 2001). In contrast, the findings about the significance of average molecular weight of AP on functional properties of starch are somewhat contradictory. $\overline{M}_n$ of AP was reported to be positively correlated with peak viscosity of wheat starch (Shibanuma, Takeda, & Hizukuri, 1996), swelling power of waxy rice (Wang & Wang, 2002), and induction time of retrogradation of normal rice starches (Lai, Lu, & Lii, 2000). $\overline{M}_w$ was negatively correlated with peak viscosity and setback of normal rice starches (Patindol et al., 2009). Mufumbo et al. (2011) reported a negative correlation between $\overline{M}_w$ and setback but not between $\overline{M}_w$ and peak viscosity of cassava

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http://dx.doi.org/10.1016/j.carbpol.2014.02.081
0144-8617/

starches. Lin et al. (2013) reported no correlations between $\overline{M_w}$ and pasting properties of Taiwanese waxy rice starches.

Besides the inconclusive results of the effects of MW on starch functional properties, few studies have simultaneously covered both AP chain profile and its average molecular weight (Lu, Chen, & Lii, 1997; Takeda, Hizukuri, & Juliano, 1987; Vilaplana & Gilbert, 2010; Wang & Wang, 2002). Additionally, to the best of our knowledge, no studies have been conducted on the various kinds of average MW and the chain profile of the same AP sample. It is worthwhile to elucidate disagreement in the effects of MW on starch functional properties due to measurements of different kinds of average MW performed on AP from various starches. Thus, the objectives of the present study were to investigate the different kinds of average MW, the chain profile, solution clarity of identical AP samples as well as their starch pasting properties. Any indications of relationships between the AP chain profile and average MW as well as effects of the structural parameters on starch pasting properties and clarity of the AP solutions were also examined. Thai rice starches were used as the model for this study.

2. Materials and methods

2.1. Materials

Three waxy rice varieties – RD6, Hang Yee 71 (HY71) and Sew Mae Jan (SMJ) and three high amylose (HAM) rice varieties – Prajeenburi 1 (PJ1), Chainart 1 (CN1), and Suphanburi 1 (SP1) – were obtained from the Rice Research Center (Thailand). β-Amylase from sweet potato was purchased from Sigma–Aldrich (St. Louis, MO), and isoamylase was obtained from Hayashibara (Okayama, Japan). Analytical-grade chemicals were used in the study unless otherwise noted.

2.2. General methods

Moisture, protein, lipid and ash contents of the starches were determined using AACC methods (AACC methods 08-01, 30-10, 44-15A and 46-11A). The conversion factor of N × 5.95 was applied to convert nitrogen to crude protein content. Total amylose (AM) content of starch was measured by the method of Chrastil (1987). Pasting properties of all starches were determined by a rapid visco analyzer (Newport Scientific, Narrabeen, Australia) (Lumdubwong & Seib, 2000). The clarity of AP solution (1%, w/w) was measured by the method of Craig, Maningat, Seib, and Hoseney (1989). Swelling power and water solubility (%) of all starches (1% w/w) were determined by the method of Holm, Björck, Asp, Sjöberg, and Lundquist (1985).

2.3. Preparation of AP samples

All starches were isolated using the alkaline steeping method (Lumdubwong & Seib, 2000). All waxy AP samples in this study were used in the form of non-granular (NG) starch. Waxy AP solutions were prepared by the method of Yoo & Jane (2002) but the solutions were stirred for 48 h instead of 24 h. The starch solutions were then precipitated with absolute ethanol, followed by centrifugation at 3000 × *g* for 10 min and the supernatant was discarded. After washing three times with ethanol, the NG starches were dried in a vacuum oven.

For HAM AP samples, the starch was first prepared in the form of NG starch in the same manner as waxy samples. The NG starch was then re-dissolved in distilled water and boiled for 1 h. The HAM AP was further extracted from the NG starch solution using the alcohol precipitation method of Takeda et al. (1987) and the precipitated AP samples were dried in a vacuum oven at ambient temperature. The purity of HAM AP samples was verified using high-performance size-exclusion chromatography (HPSEC). For further analyses, all powdered AP samples were re-dissolved in deionized water and boiled for 1 h prior to uses.

2.4. Number–average molecular weight measurement

Number–average molecular weight measurement ($\overline{M_n}$) was obtained by multiplying the number–average degree of polymerization ($\overline{DP_n}$) by 162. $\overline{DP_n}$ was measured using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and a modified Park–Johnson procedure (Takeda et al., 1987).

2.5. Weight–average molecular weight measurement

Weight–average molecular weight ($\overline{M_w}$) of AP was measured by both a laser light scattering (LLS) instrument and by high-performance size-exclusion chromatography with multi-angle laser light scattering coupled with refractive index detection (HPSEC–MALLS–RI).

For static LLS, AP was dissolved in 90% DMSO at a concentration of 0.2 mg/ml. The solution was simultaneously gently stirred and heated for 1 h in a boiling water bath, and diluted with 90% DMSO to five concentrations ranging from 0.004 to 0.012 mg/ml. All solutions were filtered with a 5 μm PTFE filter before LLS measurement. The scattered light intensities were measured from 5 to 18 angles by a MALLS instrument with a He–Ne laser source (λ = 690 nm) and a K-5 flow cell.

The AP solution (0.2 mg/ml) using water as dispersing medium was prepared in the same manner as with 90% DMSO, and was injected into a HPSEC–MALLS–RI system consisting of: a 515 HPLC pump (Waters, Milford, MA) equipped with an injection valve (200 μl sample loop, model 7725i; Rheodyne); a MALLS detector (DAWN® EOS; Wyatt Technology, Santa Barbara, CA) with a He–Ne laser source (λ = 690 nm) and K-5 flow cell; and a refractive index detector (Optilab rEX; Wyatt Technology). The SEC columns were composed of a guard column (OHpak SB-G; Shodex, Tokyo, Japan) and two analytical columns (OHpak SB-804HQ and SB-806HQ; Shodex). The columns were maintained at 55 °C and the temperature of the RI detector was maintained at 30 °C. The mobile phase was deionized water (18.2 MΩ) and the flow rate was 0.6 ml/min.

A dextran standard ($\overline{M_w} = 2.50 \times 10^4$, 0.4 mg/ml) was used for normalization of the multi-angle photodiode detector used in MALLS and LLS. Astra software was used for data acquisition and analysis. The curve fitting method and calculation of $\overline{M_w}$ were based on the second-order Berry method (Yoo & Jane, 2002; Zhong et al., 2006).

2.6. Intrinsic viscosity measurement

Waxy starches and NG HAM AP samples were dispersed in different solvents, 90% DMSO and water, at a concentration of 3×10^{-3} g/ml. When dispersed in 90% DMSO, the samples were heated in a boiling water bath for 15 min and stirred overnight at room temperature. When dispersed in water, the samples were gently stirred and boiled for 1 h. The final concentrations of the solutions were 0.5–3.0 × 10^{-3} g/ml. Intrinsic viscosity measurements were obtained using a Cannon-Ubbelohde glass capillary viscometer (Cannon Instrument Co., State College, PA) immersed in a water bath at 25 ± 1 °C for 90% DMSO and at 40 ± 1 °C for water. The intrinsic viscosity [η] was calculated from the *y*-axis intercept of the Huggins plot of η_{sp}/c against *c* and the Kramer plot of $\ln \eta_{rel}/c$ against *c*.