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Molecular structure of amylopectin from amaranth starch and its effect on physicochemical properties

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ABSTRACT

The molecular structure of amylopectin and its ϕ,β-limit dextrins from starch of 13 amaranth cultivars was determined by HPAEC-PAD after debranching. Chain length profiles of amylopectins showed bimodal distributions. The molar-based ratios of the average chain lengths of amylopectins (CL_{ap}) ranged from 17.41 to 18.22. The molar-based average chain lengths (CL_{ld}) and average B-chain lengths (BCL_{ld}) of ϕ,β-limit dextrins varied from 7.68 to 8.05, and from 14.10 to 14.73, respectively. Correlation analysis indicated that most structural parameters were positively correlated with thermal properties with few exceptions, whereas the content of fraction fa′ ("′" stands for molar-based chain length ratio) was negatively correlated with the thermal properties. Pasting properties of cold paste viscosity (CPV) and setback were also correlated with amylopectin structural parameters.

1. Introduction

Starch is the main component in grain *Amaranthus* seeds and comprises two major types of biomacromolecules, amylose and amylopectin. Amylose is an essentially linear biopolymer of α-D-(1,4)-glucosyl units with few branches connected by α-D-(1,6)-glucosyl linkage, while amylopectin has much higher density of branching attached by α-D-(1,6)-glucosyl linkage in the main α-D-(1,4)-glucosyl chains. Recently, an "intermediate material" fraction, which is precipitated with isoamyl alcohol and 1-butanol but not with 1-butanol alone and found in normal and high amylose starches, was also defined [1]. Besides, some minor components, such as lipids, proteins, phosphorus, and mineral components such as calcium, potassium, magnesium and sodium in ionic forms, are also usually present in starch granules [2].

Unit chain length profile is an important parameter to characterize amylopectin structure. Basically, the unit chains can be divided into two categories, A-chains (not substituted chains) and B-chains (defined as chains substituted with other chains). On the basis of a comparison between debranched amylopectins from eleven plant sources, Hanashiro et al. suggested that the unit chains could be fractionated into fa (degree of polymerization, DP 6–12), fb_1 (DP 13–24), fb_2 (DP 25–36), and fb_3 (DP ≥37), which constitute a polymodal chain length distribution of amylopectin [3]. The chain distribution can also be obtained from ϕ,β-limit dextrins, in which all A-chains exist as maltosyl stubs and all B-chains are longer than that [4]. The A-chains can be calculated from the amount of maltose of debranched ϕ,β-limit dextrins. The B-chains can be divided into B1-, B2- and B3-chains, and the short B1-chains have sub-groups, which are designated B1a- and B1b-chains, the B1a-chains can be further subdivided into very short B1a(s)- and somewhat longer B1a(l)-chains [5].

Much research has shown that amylose content and the amylopectin structural parameters are important factors affecting the functional properties of starch. Molecular structure characterization of amaranth starch from a limited number of genotypes has been reported [6–9], but there were few studies on amylopectin structure including multiple amaranth starches. More and more studies have shown that chain length distribution and average chain length of amylopectin are correlated with starch functional properties from diverse sources [9–16]. In our previous report we investigated 15 amaranth samples and showed that the amylose content is significantly correlated with physicochemical and functional properties of starch [17]. In this study we have characterized the fine structure of amylopectin included in the same series of amaranth samples and analyzed the relationship between the structural and functional properties.

The objectives of this study were: (i) to characterize the chain length distributions of amylopectins from diverse amaranth cultivars; (ii) to produce ϕ,β-limit dextrins and determine their chain

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length distributions to analyze the internal structure of amylopectin; (iii) to perform correlation analyses among structural parameters and physicochemical properties.

2. Materials and methods

2.1. Materials

Thirteen grain amaranth cultivars, K350, R159, V69, Japan19, M7, Cr064, Cr049, K112, R104, 5293 (*Amaranthus cruentus* L.), Tibet Yellow (*Amaranthus paniculatus* L., abbreviated as TibetY), and NO1, NO2 (*Amaranthus hypochondriacus* L.) were harvested from China. The starch isolation procedure was described previously [17].

2.2. Amylopectin fractionation

Amylopectin fractionation was conducted following a previous report with some modifications [18]. Granular starch (5 g) was dissolved in 100 mL 90% dimethyl sulfoxide (DMSO) by heating the mixture in a boiling water bath with constant stirring for 3 h, making sure that no gelatinous lumps of starch remained. After dispersion, the starch solution was placed at room temperature for 10 min whereafter 200 mL 95% ethanol was added with continuous stirring. A further 200 mL 95% ethanol was added, the solution was left at room temperature for 15 min and then centrifuged at 2500 × *g* for 10 min. The supernatant was discarded and the sediment was washed with 25 mL 95% ethanol and then centrifuged at 2500 × *g* for 10 min. The washing procedure was performed once more using 95% ethanol and once using acetone. The final precipitate was freeze-dried and called non-granular starch.

The non-granular starch (2 g) was dispersed in 56 mL 90% DMSO as above. 1-Butanol (23.5 mL) and isoamyl alcohol (23.5 mL) were diluted in 345 mL deionized water and mixed (giving 6% 1-butanol and 6% isoamyl alcohol) whereafter it was added to a 500 mL Erlenmeyer flask containing the non-granular starch–DMSO solution and sealed with aluminum foil. The mixture was stirred and placed in a 95 °C water bath for 1 h, and then the entire system was cooled for about 18 h to 28 °C. The mixture was agitated to resuspend any precipitation and centrifuged (10,000 × *g*, 15 min, 4 °C). The supernatant was concentrated under vacuum to 100 mL at 60 °C using a rotary evaporator (R114 Rotovapor with a B480 water bath, Büchi, Switzerland). The concentrated fraction was precipitated and dried with the same procedure as for the preparation of non-granular starch. The dried sample was fractionated amylopectin.

The purity of fractionated amylopectins was checked by gel permeation chromatography (GPC) on Sepharose CL 6B (Pharmacia, Uppsala, Sweden) with debranched samples and making sure no peak resembling debranched amylose fraction was present.

2.3. Production of *ϕ,β-limit dextrin (phosphorolysis beta-amylolysis limit dextrin)*

The production of ϕ,β-limit dextrin was performed according to a previous description with minor modifications [19]. The purified amylopectin (150 mg) was dissolved in 2.5 mL 100% DMSO with constant stirring for two days and diluted with 47.5 mL hot MilliQ water. To the solution was added 5 mL 1.1 M sodium phosphate buffer (pH 6.8), 2.5 mL 2.8 mM EDTA and 12.5 mL freshly prepared phosphorylase *a* (from rabbit muscle, ~25 U/mg, EC 2.4.1.1, Sigma–Aldrich, Deisenhofen, Germany; 1.25 mg dissolved in 12.5 mL MilliQ water when used), then stirred overnight at room temperature. The reaction was terminated in a boiling water bath for 5 min and the solution was centrifuged. The α-D-glucose 1-phosphate produced was removed by tangential flow filtration using Omega 10 K membrane (Minimate^{TM} TFF System, Pall Life Sciences, Ann Arbor, MI, USA). The phosphorolysis was repeated and the α-D-glucose 1-phosphate was removed.

The volume of the filtrate from above was adjusted to 25 mL, and 2.5 mL 0.1 M sodium acetate buffer (pH 6.0) and 30 μL β-amylase (from barley, 10,000 U/mL, EC 3.2.1.2, Megazyme, Wicklow, Ireland; ~4 U/mg substrate) was added. The reaction was incubated at room temperature overnight with constant stirring and terminated by boiling for 5 min. Maltose produced from β-amylolysis was removed by filtration as above. The β-amylolysis was repeated once and maltose was removed completely.

The ϕ,β-limit dextrin produced was freeze-dried and sealed in capsule bottle for later use.

2.4. Unit chain length profile determination

Amylopectin or ϕ,β-limit dextrin (3 mg) was dissolved in 150 μL 100% DMSO with constant stirring overnight. The solution was then diluted with 750 μL MilliQ water and 100 μL 0.1 M sodium acetate buffer (pH 5.5), 1 μL isoamylase (from *Pseudomonas amyloderamosa*, 250 U/mL, EC 3.2.1.68, Hayashibara Shoji Inc., Okayama, Japan) and 1 μL pullulanase (from *Klebsiella pneumoniae*, 429 U/mL, EC 3.2.1.41, Hayashibara Shoji Inc.) were added. The debranching reaction was conducted at room temperature with constant stirring overnight and terminated by heating. The sample was centrifuged and filtrated (pore size 0.45 μm) before injection to the HPLC system.

The chain length distribution of debranched sample was analyzed with a method modified from a previous report by using a high-performance anion-exchange chromatography (HPAEC) system (Series 4500i, Dionex Corp., Sunnyvale, CA, USA) coupled with a BioLC gradient pump and a pulsed amperometric detector (PAD) [20]. PAD signal was recorded by PowerChrom software (eDAQ Pty Ltd, Australia) and corrected to carbohydrate content [21]. Prior to loading the sample, the column (250 mm × 4 mm, Carbo-Pac PA-100 with a guard column) was eluted at a rate of 1 mL/min with 150 mM NaOH for 20 min, then with a mixture of two eluents: 150 mM NaOH (eluent A, 93%) and 150 mM NaOH containing 1 M NaOAc (eluent B, 7%) for another 20 min. The elution gradient with a rate of 1 mL/min was as follows: from 0 to 1.3 min, 93% eluent A; from 1.3 to 10 min, eluent A changed from 93 to 82% linearly; from 10 to 19 min, from 82 to 78%; from 19 to 111 min, from 78 to 50%; from 111 to 113 min, from 50 to 93% (returned to start mixture). The sample was injected during the initial 1.2–1.3 min. For the debranched ϕ,β-limit dextrin sample (containing high content of maltose), the PAD signal was recorded at 10 times less sensitivity output range (3000 nA instead of 300 nA).

2.5. Physicochemical properties analyses

Analysis of the physicochemical properties of the amaranth starch was described previously [17].

2.6. Statistical analysis

The chain-length distributions were determined at least twice, the reported data are average of two close duplicate analyses. The data analysis was conducted using SigmaPlot 9.01 integrated with SigmaStat 3.11 (Systat Software, Inc., CA, USA).

3. Results and discussion

3.1. Chain-length profiles of amylopectins from amaranth starches

The molar- and weight-based chain-length distributions of debranched amylopectins grouped into four fractions according to