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Devon K. Cameron[a]
Ya-Jane Wang[a]
Karen A. Moldenhauer[b]

[a] Department of Food Science, University of Arkansas, Fayetteville, Arkansas, USA
[b] Rice Research and Extension Center, University of Arkansas, Stuttgart, Arkansas, USA

Comparison of Starch Physicochemical Properties from Medium-Grain Rice Cultivars Grown in California and Arkansas

The starch molecular structure and physicochemical properties of two medium-grain rice cultivars from Arkansas (Bengal, Medark) and from California (M202, M204) were determined and compared when they were grown in their respective locations and grown together in Arkansas to better understand the impacts of genetics and environment on starch characteristics. Both M202 and M204 contained significantly higher amylose contents (13.2-15.3%) compared with the Arkansas cultivars (11.6-12.4%). Starch from the Arkansas rice cultivars exhibited higher pasting and gelatinization temperatures and higher enthalpy values. Rice amylopectin from the California cultivars consisted of a smaller proportion of intermediate chains (DP 13-24), and had a lower molecular weight and a smaller radius of gyration. When the four cultivars were grown together in Arkansas, the ranges for amylose content narrowed (10.6-12.4%), their differences in thermal and pasting properties became smaller, and the molecular characteristics of amylose and amylopectin changed for all four cultivars. This study demonstrated that genetics, location, and crop year all contributed to variations in rice starch fine structure and physicochemical properties.

Keywords: Medium-grain rice; Physicochemical properties; Starch structure

1 Introduction

Rice quality is primarily determined by its chemical, physical, cooking, and eating characteristics. It is well known that environmental factors under which rice is grown strongly affect its composition and functionality [1], but there is a lack of knowledge regarding the exact physicochemical and biochemical basis for the variability. Our previous work [2] showed that medium-grain rice cultivars from Arkansas and California differed in physical attributes, chemical composition and functionality. However, when all four cultivars were grown together in Arkansas, their differences were significantly reduced, suggesting that the unique environments of Arkansas and California have a considerable impact on the rice composition and properties. Furthermore, variety and crop year were also found to influence medium-grain rice quality.

Starch is the main constituent of rice throughout its growth and consists of two major polymers, namely amylose and amylopectin. Amylopectin is responsible for the swelling of starch granules, while amylose and lipids inhibit swelling [3]. Amylopectin branch chains were first categorized into A, B, and C chains [4], and the B chains were further divided into B1, B2, and B3, etc. [5], depending on how many clusters they span. Amylopectin branch chains are arranged in clusters at intervals of 9 nm, and the clusters organize into a structure of alternating crystalline and amorphous lamellae. *Jenkins* et al. [6] reported the 9 nm repeat to be essentially consistent for a variety of species and cultivars. *Shi* and *Seib* [7] reported that an increase in the proportion of short chains of DP 6-9 reduced the retrogradation rates of waxy rice starch. *Jane* et al. [8, 9] discovered that starches with short average branch chain lengths displayed lower gelatinization temperatures, and those with longer branch chain lengths showed larger enthalpy changes. Very long chains (DP$>$75), if present, appeared to function as amylose to increase pasting temperature and decrease peak viscosity, possibly by interacting with lipids and other branch chains to improve granule integrity [9]. Cooked rice hardness has also been correlated with amylopectin structure, particularly long B chains. It was proposed that the long B chains rendered starch granules strong and rigid through intermolecular interaction [10]. However, *Nakamura* et al. [11] reported a wide variation of onset gelatinization temperature among rice varieties with a similar amylopectin chain-length distribution.

Correspondence: *Ya-Jane Wang*, Department of Food Science, University of Arkansas, Fayetteville, Arkansas 72704, U.S.A. Phone: +1-479-575-3871, Fax: +1-479-575-6936, e-mail: yjwang@uark.edu.

Environments, particularly growth temperature, have been shown to impact rice starch characteristics. *Asaoka* et al. [12] showed that varieties grown under elevated temperatures had reduced chain lengths and narrower molecular-weight distributions of amylose. In contrast, elevated temperatures during grain filling increased the amount of long chains and decreased the amount of short chains of amylopectin [13-16]. *Suzuki* et al. [16] reported that starch pasting and gelatinization temperatures and gelatinization enthalpies increased with elevated environmental temperatures, but swelling factors decreased, which were attributed to the increase in the amount of amylopectin long chains. It was suspected that the unique environments of Arkansas and California have not only changed starch composition but also starch fine structure and physicochemical properties of their medium-grain rice cultivars. This study investigated the starch molecular structure and physicochemical properties of medium-grain rice cultivars from Arkansas and California when grown in their respective locations and when grown together in Arkansas.

2 Materials and Methods

2.1 Materials

Rough rice samples of Bengal and Medark were obtained from the 2002 crop grown at the University of Arkansas Rice Research and Extension Center, Stuttgart, Arkansas. Milled rice samples of M202 and M204 were provided by the Rice Experiment Station, California Cooperative Rice Research Foundation, Biggs, CA. Because of disease concern, Arkansas rice varieties are not allowed to grown in California, therefore Bengal, Medark, M202, and M204 were all grown only at the University of Arkansas Rice Research and Extension Center, Stuttgart, Arkansas in 2003. All rough rice samples were milled by multiple passes through a lab-scale continuous, vertical, friction mill (Yamamoto Rice-Pal 31, Tokyo, Japan). The friction mill was set to a medium milling level, and the settings were adjusted appropriately to achieve a similar milling degree for all samples according to the residual surface lipids (0.75-0.89%) determined by near infrared spectroscopy (NIR) using an Infratec 1241 Grain Analyzer (Foss Tecator AB, Höganäs, Sweden). The resulting milled rice was weighed and separated into head rice and broken kernels on a double-tray shaker table (GrainMan Machinery, Miami, FL). Samples were stored in self-sealing plastic bags under ambient conditions. Head rice was ground into flour with a cyclone sample mill (Udy Corp., Ft. Collins, CO) fitted with a 100-mesh sieve. Starch was isolated from milled rice flour by an alkali steeping method [17], and starch moisture content was measured according to Approved Method 44-15A [18].

2.2 Chemical composition

Duplicate samples of 2 g rice starch were placed in aluminum moisture dishes and dried in a 130 °C oven for 60 min according to Approved Method 44-15A [18]. The apparent amylose content was determined by potentiometric titration [19]. Starch lipid content was determined by an enzymatic method (NEFA-C kit, Wako Chemicals, Osaka, Japan). The amount of non-esterified fatty acids (NEFA) was measured by the NEFA-C kit and reported as weight percentage based on initial dry weight. The principle and detailed procedure of the NEFA-C kit were described by *Nakazawa* and *Wang* [20].

2.3 Thermal properties

A differential scanning calorimeter (DSC) (model Pyris-1; Perkin-Elmer Co., Norwalk, CT) was used to evaluate starch thermal properties. Starch (~4 mg, db) was weighed into an aluminum DSC pan and then moistened with 8 μL of deionized water using a microsyringe. The pan was hermetically sealed and equilibrated at room temperature for at least 1 h prior to scanning from 25 to 130 °C at 10 °C/min. The gelatinized sample was stored at 4 °C for 2 weeks and rescanned under the same conditions as in the gelatinization measurement. Retrogradation rate is defined as the ratio of retrogradation enthalpy to gelatinization enthalpy of the same sample. Duplicate measurements were performed for each cultivar.

2.4 Pasting and gelling properties

The pasting characteristics of rice starch were determined at 8% (w/w) concentration in water using a Micro ViscoAmyloGraph (C.W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a 350-cmg and operated at 250 rpm. The starch slurry was heated from 50 °C to 95 °C at 3 °C/min, held at 95 °C for 5 min, and cooled to 50 °C at a rate of 3 °C/min.

The starch paste prepared with the Micro ViscoAmyloGraph was used for the measurement of gelling properties. The paste was poured into three glass dishes (27-mm dia. × 39-mm height); the rims of the dishes were extended with aluminum foil to increase the height of the gel 1 cm above the rim [21]. The starch paste was stored at 5 °C for 24 h and then measured with a TA-XT2 Texture Analyzer (Texture Technologies Corp.,

Scarsdale, NY) by texture profile analysis (TPA). The gel was compressed at a speed of pre-test 1.0 mm/s, test 0.5 mm/s, and post-test 1.0 mm/s to a distance of 5.0 mm with a cylindrical probe (5-mm dia. × 33-mm height) under the TPA test mode. The peak force of the first penetration was termed hardness and the negative peak height during retraction of the probed was termed stickiness. Triplicate measurements were performed on each sample.

2.5 Purification of amylopectin from starch

Fractionation of amylopectin from all starch samples was done by following the procedure of Takeda et al. [22] with modifications. Approximately 200 mg of defatted starch was stirred into 12 mL of 0.2 M aqueous NaOH in a 25-mL capped test tube at 65 °C for 18 h to achieve complete dissolution. The starch solution was then neutralized with 1 M aqueous HCl, 2.4 mL of 1-butanol added, and stirred at 100 °C in an oil bath for 3 h under nitrogen. The heated dispersion was slowly cooled down to room temperature over 24 h by immersing the sample test tube in a sealed 2-L Dewar flask (model Thermo-flask; Lab-line instruments Inc., Melrose Park, IL) filled with hot water to allow the formation of amylose-butanol complexes. The cooled dispersion was then kept at 4 °C for 48 h. The amylose-butanol complex was separated from the mixture by centrifugation at 12,100 × g and 4 °C for 45 min. The supernatant containing mostly amylopectin was further purified for another recrystallization cycle with the addition of 1 mL of 1-butanol. At the end of the second recrystallization, the purified amylopectin was precipitated with 100 mL of methanol at room temperature for 24 h. The precipitated amylopectin was then collected by centrifugation at 1,520 × g for 15 min at room temperature, washed with 30 mL of methanol, and dried at 40 °C for 24 h.

2.6 Amylopectin chain-length distribution

The amylopectin chain-length distribution was analyzed by high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan et al. [23]. The HPAEC-PAD (Dionex DX500) system consisted of the following components: GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, 4 × 50-mm CarboPac PA1 guard column, 4 × 250-mm CarboPac PA1 analytical column, and AS40 automated sampler. Nine mg of purified amylopectin was stirred into 3.2 mL of deionized water in a boiling water bath for 1 h, cooled, and 0.4 mL of 0.1 M acetate buffer (pH 3.5) and 20 μL of isoamylase (1,180 U; Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) were added. The enzymatic reaction was carried out in a shaking water bath at 40 °C and 140 rpm for 48 h. Enzyme activity was terminated by heating the mixture in a boiling water bath for 30 min. The sample was centrifuged at 4500 × g for 5 min, and the supernatant was placed into the automated sampler. The gradient system consisted of two eluents: eluent A was 150 mM aqueous NaOH and eluent B was 500 mM $NaNO_3$ in 150 mM aqueous NaOH. Starting at 0 min, the gradient was 94% A and 6% B. At 31 min, the gradient changed to 87% A and 13% B. At 81 min, the gradient was changed to 80% A and 20% B. At 101 min, the gradient changed to 75% A and 25% B. From 105.1 min to 130 min, the end of test, the gradient changed back to 94% A and 6% B.

2.7 Amylopectin and amylose molecular structures

The weight-average molecular weight (M_w), z-average radius of gyration (R_z), and polydispersity of amylopectin and amylose were determined from native and isoamylase-debranched starch, respectively, by high-performance size exclusion chromatography (HPSEC) with multiangle laser light scattering (MALLS) and refractive index (RI) detectors. The HPSEC-MALLS-RI system consisted of a Waters 515 HPLC pump (Milford, MA) with an injector of 100 μL sample loop, in-line degasser, one guard column (TSKgel PW_{XL} 6.0 mm × 4.0 cm, Tosoh Corp., Tokyo, Japan), two size exclusion columns (TSKgel G4,000 and G5,000 PW_{XL}, 7.8 mm × 30 cm), a multiangle laser light scattering detector equipped with a gallium arsenate laser light source with wavelength of 690 nm and jumpers set at 21 × (MALLS, model DAWN-EOS; Wyatt Technology, Santa Barbara, CA), and a refractive index detector (model Optilab rEX; Wyatt Technology, Santa Barbara, CA). The mobile phase was aqueous 0.15 M $NaNO_3$ with 0.02% NaN_3, which was filtered through a 0.1 μm membrane filter (Supor-100; Pall Corp., Ann Arbor, MI) at a flow rate of 0.7 mL/min. The column temperature was maintained at 55 °C.

The native starch sample for amylopectin structure characterization was prepared by heating 24.0 mg of defatted starch in 5 mL of 90% DMSO in a boiling water bath for 1 h, and then the dispersion was stirred overnight at room temperature. One milliliter of the starch dispersion was precipitated with 10 mL methanol, centrifuged at 1520 × *g* for 10 min, and then redissolved in 5 mL distilled water by boiling for 30 min. The cooled sample was centrifuged at 7000 × *g* for 5 min, and the supernatant was ready for injection.

The debranched starch sample for amylose structure characterization was prepared following the same method as for the previous debranched sample, except that 24.0 mg of defatted starch was added with 3.5 mL of deionized water. The mixture was heated in a boiling water bath with stirring for 1 h, cooled, and then 0.5 mL of 0.1 M acetate buffer (pH 3.5) and 20 μL of isoamylase (1,180 U) were added. The mixture was incubated in a shaking water bath at 40 °C and 140 rpm for 48 h. The enzyme was inactivated by boiling, and the sample was filtered while still hot through a 5.0 μm syringe filter (Whatman Inc., Clifton, N.J.) before injection. The eluted materials were separated into three fractions with division made at minimum RI response. Fraction I (Fr. I) was composed mostly of amylose, fraction II (Fr. II) included long B chains of amylopectin, and fraction III (Fr. III) contained A and short B chains of amylopectin [5].

2.8 Statistical analysis

Experimental data were analyzed by using the general linear models procedure, the ANOVA procedure, and Duncan's multiple range test (1999 version; SAS Software Institute, Inc., Cary, NC). Least significance differences were computed at $p < 0.05$. Data were also analyzed by using the correlation procedure (Pearson's correlation coefficients) in SAS.

3 Results and Discussion

3.1 Chemical composition

The apparent amylose, lipid and crude protein contents of the isolated rice starches are presented in Tab. 1. The Arkansas rice cultivars were significantly lower in apparent amylose content when grown separately in 2002 compared with the California cultivars. California typically has cooler temperatures than Arkansas, and a lower growing temperature has been associated with higher amylose content in rice cultivars [24, 25]. Because of the small amount of residual lipid in starch, non-esterified free fatty acids (NEFA) were measured and used to represent the total lipid content. The starch lipid content was not significantly different when the cultivars were grown separately, but a marked increase in lipid content was observed for all cultivars and most notably in the California cultivars when they were all grown in Arkansas. A similar increase in the total crude lipid content was also observed in these rice cultivars when grown in Arkansas [2]. The residual protein content in the isolated starches was less than 1% for all cultivars, yet starches from the 2003 crop contained slightly higher residual protein.

Tab. 1. Chemical composition of starch from medium-grain rice cultivars Bengal, Medark, M202, and M204 grown in 2002 and 2003[a].

Crop year	Cultivar	Location	Apparent amylose	Lipid [%, db]	Crude protein [%, db]
2002	Bengal	AR	11.6d	0.51cd	0.22c
	Medark	AR	12.4c	0.45cd	0.22c
	M202	CA	13.2b	0.41d	0.36b
	M204	CA	15.3a	0.43cd	0.37b
2003	Bengal	AR	10.6e	0.67b	0.68a
	Medark	AR	11.7d	0.52c	0.58a
	M202	AR	11.3d	0.87a	0.35b
	M204	AR	12.4c	0.80a	0.48ab

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$.

3.2 Thermal, pasting, and gelling properties

The gelatinization properties of starches followed a similar trend as the flour samples (Tab. 2) [2]. When grown in respective locations in 2002, Arkansas cultivars exhibited significantly higher onset and peak gelatinization temperatures and enthalpy values than California cultivars. When the California cultivars were grown in Arkansas in 2003, their onset and peak gelatinization temperatures and enthalpy values greatly increased as compared to the same cultivar grown in California in 2002. Arkansas cultivars had similar enthalpy values for both years, but showed slightly lower onset and peak gelatinization temperatures in 2003. The retrogradation rates of Medark from 2002 and M204 from 2003 were slightly higher than that of M204 from 2002.

When grown in their respective locations in 2002, the California cultivars displayed lower pasting temperatures and lower peak and breakdown viscosities (Tab. 3), which were attributed to their higher apparent amylose contents (Tab. 1). When all four cultivars were grown in Arkansas in 2003, most of their peak and breakdown viscosity values increased; however, the California cultivars still showed lower peak and breakdown viscosities. The changes in starch pasting properties might be correlated with their changes in apparent amylose and lipid because starch pasting properties are influenced by its amylose and lipids contents.

The gelling properties of the rice starch pastes prepared from the Micro ViscoAmylograph are listed in Tab. 4. All cultivars had similar gel hardness, except M202, which exhibited a softer gel in 2002. The gel stickiness of

Tab. 2. Thermal properties of starch from medium-grain rice cultivars Bengal, Medark, M202, and M204[a].

Crop year	Cultivar	Location	Gelatinization			Retrogradation rate [%]
			Onset temperature [°C]	Peak temperature [°C]	Enthalpy [J/g]	
2002	Bengal	AR	70.3a	74.7a	12.4ab	26ab
	Medark	AR	69.3b	74.0ab	11.8bc	33a
	M202	CA	64.8d	70.6e	10.9c	22ab
	M204	CA	64.7d	70.4e	10.6c	20b
2003	Bengal	AR	68.1c	73.7bc	13.3a	25ab
	Medark	AR	68.5bc	73.1cd	12.8ab	27ab
	M202	AR	68.3c	72.8d	12.6ab	28ab
	M204	AR	68.5bc	74.1ab	13.4a	30a

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$.

Tab. 3. Pasting properties of starch from medium-grain rice cultivars Bengal, Medark, M202, and M204[a].

Crop year	Cultivar	Location	Pasting temperature [°C]	Peak viscosity [BU[b]]	Breakdown [BU]	Total setback [BU]	Final viscosity [BU]
2002	Bengal	AR	68.8b	810bcd	415b	−110c	700bc
	Medark	AR	69.0b	824bc	432b	−138cd	686bc
	M202	CA	67.5d	759ef	343cd	−45b	715b
	M204	CA	67.1e	721f	301d	23a	744a
2003	Bengal	AR	68.8b	876a	478a	−174d	702bc
	Medark	AR	68.0c	832b	446ab	−139cd	693bc
	M202	AR	68.8b	785cde	363c	−112c	674c
	M204	AR	69.5a	774de	359c	−94c	680c

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$. [b]BU, Brabender unit.

Tab. 4. Hardness and stickiness of starch gels of medium-grain rice cultivars Bengal, Medark, M202, and M204[a].

Crop year	Cultivar	Location	Hardness	Stickiness
			(g-force)	
2002	Bengal	AR	4.9a	1.9bc
	Medark	AR	5.2a	1.6c
	M202	CA	4.1b	1.9bc
	M204	CA	5.0a	2.2ab
2003	Bengal	AR	5.3a	2.3a
	Medark	AR	5.3a	2.0abc
	M202	AR	4.9a	1.9bc
	M204	AR	5.0a	2.0abc

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$.

California rice samples remained the same for both crop years, although they were grown at different locations.

3.3 Molecular structure of rice amylopectin and amylose

The average chain length and chain-length distribution of purified amylopectin of all rice samples are summarized in Tab. 5. When grown in their respective locations, the Arkansas cultivars consisted of a greater percentage of B1 chains but a smaller percentage of B3+ chains. A correlation of higher amylose contents and increased percentages of long-branch chains in amylopectin for corn starch has been reported [26, 27]. This is supported by the present results that the California cultivars had significant higher apparent amylose contents and greater

Tab. 5. Average chain length and chain-length distribution of amylopectin from medium-grain rice cultivars Bengal, Medark, M202, and M204[a].

Crop year	Cultivar	Location	Average Chain Length	A (DP[b] 6-12)	B1 (DP 13-24)	B2 (DP 25-36)	B3+ (DP > 37)
2002	Bengal	AR	18.43e	31.37a	49.97a	11.18ab	7.48e
	Medark	AR	18.78d	30.92bc	49.55b	10.88bc	8.65d
	M202	CA	19.09a	30.76c	48.55e	11.13ab	9.51a
	M204	CA	18.85d	31.20ab	48.94d	10.76c	9.10bc
2003	Bengal	AR	18.96bc	30.70c	49.05cd	11.05ab	9.15bc
	Medark	AR	19.04ab	30.20d	49.40bc	11.30a	9.15bc
	M202	AR	18.88cd	30.85c	49.15cd	11.10ab	8.95cd
	M204	AR	19.04ab	30.05d	49.65ab	11.05ab	9.30ab

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$. [b]Degree of polymerization.

proportions of long-branch chains with DP (degree of polymerization) > 37. The Arkansas cultivars, Bengal and Medark, showed a marked increase in percentage of B3+ chains from 2002 to 2003 and decreased percentages of B1 and B2 chains in 2003. In contrast, M202 showed a slightly decreased proportion of branch chains of B3+ chains, and both M202 and M204 increased in B1 chains from 2002 to 2003. In general, the amylopectin average chain lengths increased in 2003 relative to those in 2002, with the exception of M202. All amylopectin samples had the highest peak at DP 12, a slight shoulder at DP 18, and a second peak at DP 42 (profiles not shown). The shoulder at DP 18-21 has been reported by Hanashiro et al. [28] and Jane et al. [9], and the relative intensity of this shoulder was suggested to positively correlate with defects in amylopectin crystallites [9].

Gelatinization properties have been found to be predominantly controlled by amylopectin structure. The significantly higher onset and peak gelatinization temperatures of Arkansas cultivars from 2002 crop year can be ascribed to their greater percentages of B1 chains relative to the California cultivars. California cultivars increased in gelatinization temperatures and enthalpies in 2003, and a significant increase in their amylopectin B1 chains was also observed. The maximum temperatures for Arkansas and California in 2002 and 2003 were similar, ranging from 30 to 35 °C, but California was consistently lower in the minimum temperatures [2]. The higher growing temperature has been reported to increase the amount of long chains and decrease the amount of short chains of amylopectin [14-16], agreeing with the changes in M204 amylopectin. *Jiang* et al. [29] reported temperature altered gene expression and activities of soluble starch synthase (SS) and starch branching enzymes (BE), which in turn had an impact on chain-length distribution in amylopectin. They reported that the activity of SS increased at higher temperatures, maintaining the elongation of A and B chains, whereas the activity of BE decreased, lowering the branching frequency of amylopectin. As a result, there was an increase in the average chain length of amylopectin in rice grains at high temperatures. However, the impact of growth temperature was not obvious on M202 amylopectin structure. Nonetheless, the amorphous regions have also been demonstrated to be important in the melting of starch crystallites [30-32]. It is possible that the amorphous regions were altered from growing at different locations, resulting in changes in starch gelatinization properties.

Tab. 6 lists weight-average molecular weight (M_w), *z*-average radius of gyration (R_z), and polydispersity (M_w/M_n) of amylopectin and amylose, and ratio of Fr. III/Fr. II in amylopectin. The ratio of Fr. III/Fr. II was used as an index of the extent of branching of amylopectin; the higher the ratio, the higher the degree of branching [33]. The Arkansas cultivars had a larger R_z and a lower degree of branching than the California cultivars in 2002, implying that less branching and fewer short-branch chains were present in the amylopectin of Arkansas cultivars in 2002. These results agree with previous report that the activity of BE can decrease at higher temperatures, thus lowering the branching frequency of amylopectin [29]. Medark and M202 showed a significant increase in R_z from 2002 to 2003, suggesting a decreased amount of branches in their amylopectins. The extent of branching in amylopectin (Fr. III/ Fr. II) increased for Medark but decreased for M204 from 2002 to 2003. The M_w and polydispersity (M_w/M_n) were not different among the cultivars, except M202 from 2002, and did not change from 2002 to 2003.

Bengal amylose had the largest M_w among all cultivars for both crop years. When grown separately in 2002, Medark

Tab. 6. Weight-average molecular weight (M_w), *z*-average radius of gyration (R_z), and polydispersity (M_w/M_n) of amylopectin and amylose, and ratio of Fr III/Fr II in amylopectin from medium-grain rice cultivars Bengal, Medark, M202, and M204[a].

Crop year	Cultivar	Location	Amylopectin				Amylose		
			M_w ($\times 10^8$)	R_z [nm]	M_w/M_n	Ratio of Fr III/ Fr II	M_w ($\times 10^5$)	R_z [nm]	M_w/M_n
2002	Bengal	AR	2.50a	375.2a	1.17a	3.6b	4.91a	57.0b	1.97e
	Medark	AR	2.28ab	324.7b	1.13a	3.7b	3.11c	58.5ab	2.05de
	M202	CA	1.83b	296.9c	1.15a	3.8a	3.88b	38.4e	2.62bc
	M204	CA	2.17ab	296.1c	1.14a	3.9a	3.67bc	49.8cd	2.35cd
2003	Bengal	AR	2.61a	387.0a	1.23a	3.7b	5.34a	46.2d	3.13a
	Medark	AR	2.78a	395.0a	1.13a	3.8a	3.36bc	38.9e	2.86ab
	M202	AR	2.38ab	340.9b	1.17a	3.8a	3.59bc	63.8a	2.31cd
	M204	AR	2.25ab	310.8c	1.19a	3.5b	3.88b	52.9bc	2.70b

[a]Mean values of duplicate measurements in a column followed by the same letter are not significantly different at $p < 0.05$.

amylose had a relatively small M_w, but a large R_z. The polydispersity of the Arkansas cultivars was significantly lower than that of the California cultivars in 2002, indicating that the amylose molecules in the Arkansas cultivars were more uniform in molecular weight. In 2003, Bengal amylose decreased in R_z but increased in polydispersity. Medark amylose also increased in M_w and polydispersity, but decreased in R_z. Amylose M_w did not correlate well with R_z, indicating the wide variation in M_w and/or the presence of branched structure in amylose. The results also suggest that both amylose M_w and the extent of branching were affected by genetics and environment.

3.4 Statistical analysis

The effects of variety, location, and crop year on rice starch molecular structure and physicochemical characteristics were analyzed by ANOVA, and results are listed in Tab. 7. Although the data were limited (four varieties, two locations, and two years), location was found to be an influential factor affecting starch chemical composition and pasting and gelatinization properties. Variety and crop year, on the other hand, exerted more influence on molecular structure of amylopectin and amylose. Differences were observed between varieties; nevertheless these results demonstrated that location and crop year had a strong influence on molecular structure and physicochemical composition of rice starch as well. Correlation analysis revealed a strong impact from location on pasting and thermal properties, apparent amylose content, and amylopectin chains of DP 13-24 and R_z. On the other hand, peak, breakdown, and setback viscosities were more affected by variety.

Tab. 7. F values for the effects of variety, location, and crop year on the chemical composition, physicochemical characteristics and molecular structure of starch[a].

	Variety	Location	Crop year
Apparent amylose content	39.28**	150.18**	58.25**
NEFA content	2.17	38.53**	2.96
Pasting temperature	2.28	55.33**	3.83
Peak viscosity	18.97**	7.16*	6.28*
Breakdown viscosity	20.97**	7.62*	6.40*
Setback viscosity	17.27**	27.59**	3.43
Final vscosity	2.99	42.00**	0.31
Onset temperature	16.86**	67.52**	10.67*
Peak temperature	14.48**	55.37**	4.82
Enthalpy	2.76	19.44**	2.64
Regrogradation rate	1.28	4.32	1.08
Amylopectin average chain length	3.90*	0.03	18.00**
Amylopectin DP 6-12	1.12	3.51	5.98*
DP 13-24	6.02*	14.17**	9.98*
DP 25-36	1.46	1.13	1.52
DP >37	5.88*	0.54	19.26**
Amylopectin M_w	3.08	2.96	2.65
Amylopectin R_z	15.58**	5.2	10.19*
Amylose M_w	38.53**	0.03	3.43
Amylose R_z	0.22	12.24**	13.98**

[a]Values with * are significant at $p<0.05$ and values with ** at $p<0.01$.

4 Conclusion

Genetics, environment, and crop year were found to have major impacts on the molecular structure and physicochemical properties of medium-grain rice starch. Growing

environment affected starch apparent amylose content, which subsequently modified the starch pasting properties. Variations in amylopectin chain-length distribution were observed among cultivars and between crop years, but these variations alone could not fully account for their differences in thermal properties. Significant differences in weight-average molecular weight and radius of gyration of amylopectin and amylose among the cultivars were noted, and they were more affected by genetics than by growing location. However, their impacts on starch physicochemical properties are not clear, and further research is needed to fully elucidate their relationships.

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References

[1] B. O. Juliano: *Criteria and tests for rice grain qualities, in Rice: Chemistry and Technology, 2nd ed.* (Ed. B. O. Juliano) American Association of Cereal Chemists, St. Paul, MN, **1985**, pp. 443–524.

[2] D. K. Cameron, Y.-J. Wang, K. Moldenhauer: Comparison of physical and chemical properties of medium-grain rice cultivars grown in California and Arkansas. *J. Food Sci.* (accepted).

[3] R. F. Tester, W. R. Morrison: Swelling and gelatinization of cereal starches I: Effects of amylopectin, amylose, and lipids. *Cereal Chem.* **1990**, *67*, 551–557.

[4] S. Peat, W. J. Whelan, G. J. Thomas: The enzymatic analysis of weights, lengths, distribution of side-chain. *J. Chem. Soc*. **1956**, 44–53.

[5] S. Hizukuri: Polymodal distribution of the chain lengths of amylopectins and its significance. *Carbohydr. Res.* **1986**, *147*, 342–347.

[6] P. J. Jenkins, R. E. Cameron, A. M. Donald: A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke* **1993**, *45*, 417–420.

[7] Y.-C. Shi, P. A. Seib: The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.* **1992**, *227*, 131–145.

[8] J. Jane, J.-F. Chen: Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* **1992**, *69*, 60–65.

[9] J. Jane, Y. Y. Chen, L. F. Lee, A. E. McPherson, K. S. Wong, M. Radosavljevic, T. Kasemsuwan: Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* **1999**, *76*, 629–637.

[10] K. Radhika Reddy, R. Subramanian, S. Z. Ali, K. R. Bhattacharya: Viscoelastic properties of rice-flour and their relationship to amylose content and rice quality. *Cereal Chem.* **1994**, *71*, 548–552.

[11] Y. Nakamura, A. Sakurai, Y. Inaba, K. Kimura, N. Iwasawa, T. Nagamine: The fine structure of amylopectin in endosperm from Asian cultivated rice can be largely classified into two classes. *Starch/Stärke* **2002**, *54*, 117–131.

[12] M. Asaoka, K. Okuno, Y. Konishi, H. Fuwa: The effects of endosperm mutations and environmental temperature during development on the distribution of molecular weight of amylose in rice endosperms. *Agric. Biol. Chem.* **1987**, *51*, 3451–3453.

[13] M. Asaoka, K. Okuno, H. Fuwa: Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). *Agric. Biol. Chem.* **1985**, *49*, 373–379.

[14] T. Umemoto, Y. Nakamura, H. Satoh, K. Terashima: Differences in amylopectin structure between two rice varieties in relation to the effects of temperature during grain filling. *Starch/Stärke* **1999**, *51*, 58–62.

[15] N. Inouchi, H. Ando, M. Asaoka, K. Okuno, H. Fuwa: The effect of environmental temperature on distribution of unit chains of rice amylopectin. *Starch/Stärke* **2000**, *52*, 8–12.

[16] Y. Suzuki, Y. Sano, T. Ishikawa, T. Sasaki, U. Matsukura, H.-Y. Hirano: Starch characteristics of the rice mutant du2-2 Taichung 65 highly affected by environmental temperatures during seed development. *Cereal Chem.* **2003**, *80*, 184–187.

[17] C. C. Yang, H. M. Lai, C. Y. Lii: The modified alkaline steeping method for the isolation of rice starch. *Food Science* **1984**, *11*, 158–162 (in Chinese).

[18] *American Association of Cereal Chemists. Approved methods of the AACC, 10th ed.; Methods 44-15A, 46-13 and 30-20*; The Association, St. Paul, MN. **2000**.

[19] T. J. Schoch: Starch fractions, iodimetric determination of amylose, potentiometric titration: standard method, in *Methods in Carbohydrate Chemistry Vol. IV* (Ed. R. L. Whistler), Academic Press: New York, **1964**, pp. 157–160.

[20] Y. Nakazawa, Y.-J. Wang: Effect of annealing on starch-palmitic acid interaction. *Carbohydr. Polym.* **2004**, *57*, 327–335.

[21] S. Takahashi, C. C. Maningat, P. A. Seib: Acetylated and hydroxypropylated wheat starch: Paste and gel properties compared with modified maize and tapioca starches. *Cereal Chem.***1989**, *69*, 328–334.

[22] Y. Takeda, S. Hizukuri, B. O. Juliano: Purification and structure of amylose from rice starch. *Carbohydr. Res.* **1986**, *148*, 299.

[23] T. Kasemsuwan, J.-L. Jane, P. Schnable, P. Stinard, D. Robertson: Characterization of the dominant mutant amylose-extender (ae1-5180) maize starch. *Cereal Chem.* **1995**, 72, 457–464.

[24] M. Asaoka, K. Okuno, Y. Sugimoto, J. Kawakami, H. Fuwa: Effect of environmental temperature during development of rice plants on some properties of endosperm starch. *Starch/Stärke* **1984**, *36*, 189–193.

[25] Y. Sano, M. Maekawa, H. Kikuchi: Temperature effects on the Wx protein level and amylose content in the endosperm of rice. *J. Heredity* **1985**, *76*, 221–222.

[26] Y. Takeda, S. Hizukuri, C. Takeda, A. Suzuki: Structures of branched molecules of amyloses of various origins, and molar fractions of branched and unbranched molecules. *Carbohydr. Res.* **1987**, *165*, 139–145.

[27] J. P. Mua, D. S. Jackson: Fine structure of corn amylose and amylopectin fractions with various molecular weights. *J. Agric. Food Chem.* **1997**, *5*, 3840–3847.

[28] I. Hanashiro, J. Abe, S. Hizukuri: A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydr. Res.* **1996**, *283*, 151–159.

[29] H. Jiang, W. Dian, P. Wu: Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochem*. **2003**, *63*, 53–59.

[30] J. W. Donovan: Phase transitions of the starch-water system. *Biopolymers* **1979**, *18*, 263–275.

[31] J. W. Donovan, C. J. Mapes: Multiple phase transitions of starches and Naegeli amylodextrins. *Starch/Stärke* **1980**, *32*, 190–193.

[32] Y.-C. Shi, P. A. Seib: Fine structure of maize starches from four wx-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydr. Polym.* **1995**, *26*, 141–147.

[33] C. G. Biliaderis, D. R. Grant, J. R. Vose: Structural characterization of legume starches. I. Studies on amylose, amylopectin, and beta-limit dextrins. *Cereal Chem.* **1981**, *58*, 496–502.

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