RESEARCH ARTICLE

Chemometric analysis of cooked rice texture in relation to starch fine structure and leaching characteristics

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Cluster, correlation, and multivariate regression analyses were used to rationalize the effects of grain composition, starch fine structure, and leaching characteristics on cooked rice texture (hardness and stickiness). The head rice grain composition of 23 U.S. long-grain cultivars was evaluated in terms of apparent amylose content, crude protein, and surface lipids. Starch samples were prepared by extraction with dilute alkali and amylopectin fine structure was characterized by high-performance anion-exchange chromatography with pulsed amperometric detection. Hardness and stickiness of head rice samples cooked in optimum water were measured with a texture analyzer. The amylose–amylopectin ratio (AAR) of the material that leached out of the grains on cooking was evaluated by high-performance size-exclusion chromatography (HPSEC). Simple correlation and multivariate linear regression analyses pointed to AAR as the main indicator of cooked rice hardness and stickiness. Cluster analysis showed that the leached starch from soft-cooking, high-amylose cultivars (*e.g.*, Jodon and L-202) generally had a higher proportion of amylopectin than amylose (AAR$<$1). In contrast, dry-cooking, high-amylose cultivars (*e.g.*, Newrex and L-205) leached out starch with a higher proportion of amylose than amylopectin (AAR$>$1) during cooking. The amount of leached materials itself was also higher for the soft-cooking cultivars than the dry-cooking counterparts. Cultivar differences in leaching characteristics were attributed to variations in apparent amylose content, crude protein, and amylopectin chain-length distribution.

Keywords:
Amylopectin / Amylose / Leaching / Rice / Texture

1 Introduction

The textural attributes of cooked milled rice are of prime importance to its eating quality. Texture is a multiparameter sensorial property [1], with hardness and stickiness as the most commonly determined parameters for cooked rice [2]. Besides sensory evaluation, texture can be measured directly by instruments such as a textural analyzer or be assessed indirectly by pertinent physicochemical properties. For cooked rice texture, amylose content is considered the most important determinant [3–6]. Nevertheless, rice cultivars with similar amylose contents may still differ in textural properties. Therefore certain secondary parameters have been used for improved differentiation, such as protein content, alkali spreading value (ASV), gel consistency, amylograph viscosity profile, and amylopectin fine structure [7–19]. Protein content correlates negatively with cooked rice adhesiveness [7, 8] and positively with hardness [9]. ASV, which is an indicator of gelatinization temperature [10], is useful in discriminating the cooked rice texture of intermediate and high-amylose cultivars [11]. Gel consistency test is primarily used to

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Abbreviations: AAR, amylose–amylopectin ratio; **ACL,** average chain length; **AM,** apparent amylose; **ASV,** alkali spreading value; **CP,** crude protein; **DP,** degree of polymerization; **HSD,** honestly significant difference; **LM,** leached material; **RMSE,** root mean square error; **SL,** surface lipid.

differentiate the eating quality of high-amylose rices [12, 13]. Amylograph viscosity profiles are used to differentiate soft-cooking from hard-cooking U.S. long-grain cultivars [14]. Amylopectin long chains are positively associated with cooked rice hardness [15–19]. The long chains of amylopectin may strengthen starch granules through intermolecular interaction, which in turn contribute to firmer texture [15].

Rice textural properties are also affected by cooking method [2, 20, 21]. The two most common methods are cooking in excess boiling water and cooking with optimum water to rice ratio [2]. When cooked with optimum water level, rice grains absorb water, swell to a great extent, some materials leach into the cooking water, and the leached materials (LMs) are left on the surface of the cooked grains as the water is boiled off. The amount of LMs from cooking rice at 85°C ranged from 0.8–1.3% with more than 93% being carbohydrates [22]. It was initially thought that only amylose leaches out of the starch granule during cooking whereas amylopectin remains in the gelatinized granule [2]. However, more recent works reported that both amylose and amylopectin leach out during cooking [18, 19, 22–24]. A greater amount of leached amylose was associated with higher cooked rice hardness [23] and lower adhesiveness [22].

This work investigated the leaching characteristics of 23 U.S. long-grain cultivars when cooked by the optimum rice-to-water ratio method in a household-type rice cooker. Cluster, correlation, and multivariate linear regression analyses were used to verify the importance of the LMs during cooking to cooked rice texture. The role of starch fine structure on rice leaching characteristics and textural properties were also examined.

2 Materials and methods

2.1 Rice samples

Rough rice samples (~12% moisture content) from 23 long-grain rice cultivars were provided by the University of Arkansas Rice Research and Extension Center in Stuttgart, Arkansas. The samples were either seed-increase or foundation seeds harvested from different cropping years as follows: Ahrent (2002), Banks (2003), Bonnet73 (2003), Carolina Gold (2004), Cybonnet (2003), Cypress (2002), Cocodrie (2003), Drew (2004), Francis (2002), Jodon (1997), Katy (2003), L-202 (2004), L-205 (2004), Labelle (2004), LaGrue (2002), Newrex (2000), RU0401096 (2004), RU9201127 (2004), Spring (2004), Starbonnet (2000), Wells (2003), XL8 (2004), and XP723 (2004). All samples were stored at 4 °C until analysis.

2.2 Preparation of head rice

Rough rice samples were removed from the cold room and allowed to equilibrate at room temperature for 2 h prior to milling. A 150-g sample of each cultivar was dehulled with a Satake THU-35 dehusker (THU-35, Satake, Hiroshima, Japan). The recovered brown rice was milled in a friction mill for 30 s (McGill Miller 2, Rapsco, Brookshire, TX, USA). Degree of milling was determined by near-infrared spectroscopy (Infratec 1241 Grain Analyzer, Foss Tecator AB, Hoganas, Sweden) to ensure that the residual lipid content was in the range of 0.45–0.65% (as-is basis). Milled rice was then separated into head rice and broken kernels using a double-tray shaker table (GrainMan Machinery, Miami, FL, USA). Head rice samples were stored at 4°C and equilibrated at room temperature for 24 h before analysis.

2.3 Chemical composition

A portion of the head rice was ground into flour with a UDY cyclone sample mill (UDY, Ft. Collins, CO, USA) fitted with a 0.50-mm sieve. Moisture content was determined by the AACC Approved Method 44-15A [25]. A 1.0 g sample was placed in an aluminum dish and dried in a convection oven at 135°C for 2 h. Crude protein (CP) content was measured by a micro-Kjeldahl apparatus according to AACC Approved Method 46-13 [25]. Apparent amylose (AM) content was determined by iodine colorimetry [3]. Surface lipids (SLs) measurement followed the rapid extraction method with isopropanol by Lam and Proctor [26]. All measurements were done in duplicate.

2.4 Amylopectin chain-length distribution

Starch was isolated following the alkaline steeping method of Yang *et al*. [27] with modifications [28]. Isolated starch was defatted with water-saturated 1-butanol (WSB, 33:67 water/1-butanol) as described by Patindol and Wang [28]. Amylopectin chain-length distribution was characterized by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to Kasemsuwan *et al*. [29] with modifications. A 9-mg defatted rice starch sample was added with 3.2 mL of deionized water, heated in a boiling water bath for 30 min, cooled to room temperature, and the pH was adjusted with 0.4 mL of 0.1 M acetate buffer (pH 3.5). Twenty microliters of isoamylase (1,180 U, Pseudomonas isoamylase, Hayashibara Biochemical Laboratories, Okayama, Japan) was added and the suspension was incubated in a water bath shaker at 40°C and 125 rpm for 48 h. The enzyme–substrate reaction was terminated by heating the solution in a boiling water bath for 30 min, and impurities were removed by centrifugation

Table 1. AM, CP, SL, and ASV of 23 U.S. long-grain rice cultivars[a)]

Cultivar (code)	AM content (%, db)	Protein content (%, db)	SL content (%, db)	ASV
Ahrent (ARNT)	25.0^{i-j}	9.0^{a-c}	0.29^{d-j}	3.9^{c-d}
Banks (BANK)	25.0^{i-j}	7.6^{b-e}	0.41^{a}	2.0^{g}
Bonnet73 (BONN)	22.7^{k}	6.8^{d-e}	0.26^{g-k}	2.8^{e-g}
Carolina Gold (CGOL)	25.3^{h-i}	6.8^{d-e}	0.28^{e-j}	3.5^{c-f}
Cybonnet (CBON)	26.3^{f-h}	8.0^{a-e}	0.31^{c-h}	2.2^{g}
Cypress (CPRS)	22.1^{k-l}	8.1^{a-e}	0.31^{c-h}	2.9^{d-g}
Drew (DREW)	26.7^{f-g}	9.2^{a-c}	0.35^{a-c}	4.2^{b-c}
Francis (FRAN)	26.8^{f-g}	8.7^{a-d}	0.25^{h-l}	2.1^{g}
Katy (KATY)	27.2^{f}	8.7^{a-d}	0.23^{j-k}	2.5^{f-g}
Labelle (LABL)	19.2^{n}	9.9^{a}	0.31^{c-g}	2.7^{e-g}
Starbonnet (STAR)	20.2^{m}	7.7^{b-e}	0.34^{b-d}	2.6^{f-g}
Wells (WELS)	24.3^{j}	7.5^{b-e}	0.27^{f-k}	2.1^{g}
XP723 (XP23)	28.5^{e}	8.4^{a-d}	0.22^{k}	2.3^{g}
Cluster mean	24.6	8.2	0.29	2.8
Cocodrie (COCD)	28.4^{e}	8.5^{a-d}	0.25^{h-k}	3.0^{d-g}
L-205 (L205)	29.6^{c-d}	8.2^{a-e}	0.38^{a-b}	5.8^{a}
LaGrue (LAGR)	21.5^{l}	7.9^{b-e}	0.28^{e-k}	2.4^{g}
Newrex (NWRX)	30.6^{b}	8.2^{a-e}	0.25^{h-k}	3.0^{d-g}
Cluster mean	27.5	8.2	0.29	3.6
Jodon (JODN)	35.2^{a}	7.3^{b-e}	0.25^{i-k}	5.0^{a-b}
L-202 (L202)	30.4^{b-c}	6.3^{e}	0.30^{c-i}	5.9^{a}
RU0401096 (RU96)	26.0^{g-i}	8.3^{a-d}	0.32^{c-f}	3.7^{c-e}
RU9201127 (RU27)	27.2^{f}	8.7^{a-d}	0.26^{f-k}	3.0^{d-g}
Spring (SPRG)	25.3^{h-i}	9.3^{a-b}	0.33^{b-e}	2.1^{g}
XL8 (XL08)	29.3^{d-e}	7.4^{b-e}	0.25^{i-k}	5.1^{a-b}
Cluster mean	28.9	7.9	0.29	4.1

a) In a column, means of duplicate measurements with common superscript letter(s) are not significantly different at $p<0.05$ based on Tukey's HS\D test.

at 4500 $\times$ *g* for 5 min. The supernatant (containing the products of debranching) was injected into the HPAEC-PAD system through an autosampler.

2.5 Alkali spreading value

ASV was determined following the method of Little *et al*. [10]. Six whole rice kernels were spaced evenly in a 60 mm $\times$ 15 mm transparent plastic culture dish. Ten milliliter of 1.7% potassium hydroxide was added to submerge the grains. The dish was covered and left undisturbed at room temperature for 23 h. A 7-point numerical scale was applied as follows: 1-grain not affected; 2-grain swollen; 3-grain swollen, with incomplete and narrow collar; 4-grain swollen, with complete and wide collar; 5-grain split or segmented, with complete and wide collar; 6-grain dispersed and merging with collar; and 7-grain completely dispersed and intermingled. The gelatinization temperature classes corresponding to the seven alkali spreading scores were: 1-2, high; 3, high-intermediate; 4-5, intermediate; and 6-7, low.

2.6 Cooking of head rice

Before cooking, residual bran, and other adhering particles were removed from head rice kernels with an aspirating device (Seedburo Equipment, Chicago, IL, USA). A 10-g cleaned head rice sample was soaked in 20 g of deionized water (1:2 rice/water ratio) in a 100-mL beaker for 15 min and thereafter cooked in a household rice cooker (Aroma, model ARC-707, San Diego, CA, USA) containing 350 mL water for 30 min. Six samples were cooked at a time. Cooked rice was kept at ''warm'' setting before textural properties were measured within 30 min.

2.7 Cooked rice texture

Cooked rice hardness and stickiness were analyzed with a texture analyzer (TA-XT2 Plus, Texture Technologies, Scarsdale, NY, USA) by the uniaxial single compression method of Sesmat and Meullenet [30] with modifications. Ten intact cooked rice kernels were placed on a flat aluminum plate (100-mm diameter) and compressed to 90% of their

original height using a 50-kg load cell. The crosshead speed, test speed, and post-test speed were set at 10, 5, and 0.5 mm/s, respectively. Texture data were obtained and processed with Texture Exponent software (Stable Microsystems, version 1.0.0.92, 2000, Surrey, England). The maximum compression force (N) was used as an indicator of cooked rice hardness, while the adhesion energy measured during the upward travel of the compression plate (area under the curve expressed in N · s) was used as an indicator for cooked rice stickiness. Measurements were repeated six times for each replicate sample.

2.8 Leaching properties

A 5-g head rice sample was soaked and cooked following the aforementioned method. The LMs on the surface of the cooked rice were recovered by rinsing with 25 mL of hot deionized water (∼80°C) with gentle stirring using a spatula for 5–10 s before filtering through a Whatman no. 4 filter paper under vacuum. The rinsing procedure was repeated again with 25 mL of hot deionized water, and then small amounts of water (∼1 mL) were used to rinse the sample until the filtrate became clear. The filtrates were pooled, and the amount of LM was determined by drying 25 g of the filtrate in a convection oven at 40°C until constant weight, and calculated based on the total amount of extract and head rice weight.

A 10-mg portion of the dried leached solids was dissolved in 2 mL DMSO in a boiling water bath with stirring for 30 min, filtered through a syringe fitted with a 0.45-μm nylon membrane filter, and then injected into an high-performance size-exclusion chromatography (HPSEC) system (Waters, Milford, MA, USA) for leached carbohydrate profile analysis [24]. The amylose–amylopectin ratio (AAR) was calculated as the ratio of amylose and amylopectin peak area. Percent leached amylose was obtained using the formula, % Leached Amylose = (% LM × % Amylose Peak Area) ÷ 100. An exponential regression curve obtained by plotting the logarithm of dextran molecular weight (log M_w) *versus* elution time was used to estimate the degree of polymerization (DP). Dextran standards ranging from DP 1 to 61,250 (Polymer Standards Service-USA, Warwick, RI) were used in the calibration.

2.9 Data analysis

A JMP Version 6 software (SAS Software Institute, Cary, NC, USA) was used in the statistical analyses of the experimental data that were all collected from duplicate measurements. Analysis of variance was used to evaluate the effects of cultivar on the different physicochemical properties examined. Significantly different means were identified by Tukey's honestly significant differences (HSDs) test. Hierarchical cluster analysis was done by the Ward's minimum variance method. Pairwise correlation was carried out by the Pearson-product moment approach. A stepwise regression technique was employed for the multivariate regression analysis.

3 Results and discussion

3.1 Cluster analysis of physicochemical and textural data

Tables 1–3 list the physicochemical properties, amylopectin fine structure, and cooking characteristics, respectively, of the 23 U.S. long-grain cultivars. Ward's cluster analysis of these data sets grouped the cultivars into three main clusters and a dendogram obtained from the analysis is shown in Fig. 1. The three major clusters were arbitrarily designated as Wells, Jodon, and Newrex. The "Wells Cluster" consisted of Wells and 12 other regular long-grain cultivars, Ahrent, Banks, Bonnet73, Carolina Gold, Cybonnet, Cypress, Drew, Francis, Katy, Labelle, Starbonnet, and XP723. The "Jodon Cluster" consisted of Jodon, L-202, and four other cultivars (XL8, Spring, RU0401096, and RU9201127). Jodon and L-202 have been commercially released as soft-cooking type, high-amylose specialty rice

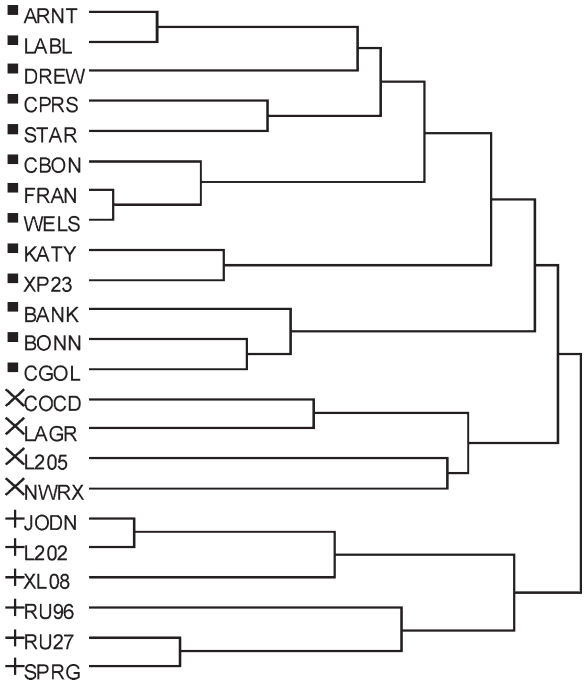

Figure 1. A dendogram obtained from the Ward cluster analysis of the chemical composition, starch fine structure, and cooking properties of 23 long-grain rice cultivars. (The three major clusters are designated as: ■, Wells Cluster; X, Newrex Cluster, and +, Jodon Cluster, with the complete cultivar names listed in Table 1.)

[31]. The "Newrex Cluster" had only four cultivars, including Newrex, Cocodrie, L205, and LaGrue. Newrex and L-205 are processing-type cultivars [31, 32], which are generally high-amylose, dry-cooking, and well-suited for canned soups, par-boiling, and noodle making [31, 33].

As shown in Table 1, head rice AM ranged from 19.2% (Labelle) to 35.2% (Jodon). In general, the AM of the cultivars in the Wells cluster was intermediate (20–25%), with a cluster mean of 24.6%. Except for LaGrue, the AM of the cultivars in both the Jodon and Newrex clusters was high (25–35%), with cluster means of 28.9 and 27.5%, respectively. CP ranged from 6.3 (L-202) to 9.9% (Labelle). The Jodon cluster had a slightly lower CP mean (7.9%) compared with the Wells and Newrex cluster (8.2% for both). SLs ranged from 0.22 (XP723) to 0.41% (Banks), but the means were the same for the three clusters (0.29%). Banks had the lowest ASV of 2.0, which corresponded to a "high" gelatinization temperature of 74.5–80°C. L-202 showed the highest ASV of 5.9 and was equivalent to a "low" gelatinization temperature of <70°C. ASV varied noticeably among cultivars within the same cluster (2.0–4.2, 2.1–5.9, and 2.4–5.8 for Wells, Jodon, and Newrex cluster, respectively), and this reflects the importance of ASV as a secondary indicator of rice functionality in addition to AM content [10, 11].

Variations in amylopectin chain length distribution among cultivars were quite minimal and generally did not exceed 1.0% (Table 2). Cluster-wise, the cultivars in the Jodon tended to have a slightly higher percentage of the short chains (A and B1) and lower percentage of the long chains (B2 and B3+). The cultivars in the Newrex cluster showed the opposite trend, *i.e.*, lower percentage of the short chains and higher percentage of the long chains.

3.2 Leaching characteristics during cooking

Some distinct differences in the appearance of the cooked rice samples from the 23 cultivars were noted. Newrex,

Table 2. Amylopectin fine structure of the starches from 23 U.S. long-grain rice cultivars[a)]

		DP (%)				
		A	B1	B2	B3+	
Cultivar	ACL	(DP6-12)	(DP13-24)	(DP25-36)	(DP37-65)	A/B1
Ahrent	20.3^{b-c}	24.7^{a}	52.1^{a-c}	11.6^{a-d}	11.7^{a-b}	0.47^{a-b}
Banks	20.6^{a-b}	24.0^{a-e}	51.8^{b-c}	12.0^{a}	12.2^{a-b}	0.46^{a-c}
Bonnet73	20.6^{a-b}	24.2^{a-e}	51.8^{b-c}	11.9^{a-b}	12.2^{a-b}	0.47^{a-b}
Carolina Gold	20.8^{a}	24.0^{a-e}	51.4^{c}	11.9^{a-b}	12.7^{a}	0.47^{a-b}
Cybonnet	20.5^{a-c}	24.4^{a-c}	51.7^{b-c}	11.6^{a-d}	12.3^{a-b}	0.48^{a}
Cypress	20.6^{a-b}	24.2^{a-e}	52.0^{b-c}	11.5^{b-d}	12.3^{a-b}	0.47^{a-b}
Drew	20.5^{a-c}	24.4^{a-c}	51.9^{b-c}	11.6^{a-d}	12.0^{a-b}	0.48^{a}
Francis	20.5^{a-c}	24.2^{a-e}	52.1^{a-c}	11.3^{d}	12.3^{a-b}	0.46^{a-c}
Katy	20.3^{b-c}	24.4^{a-c}	52.4^{a-c}	11.8^{a-c}	11.4^{a-b}	0.46^{a-c}
Labelle	20.3^{b-c}	24.4^{a-c}	52.5^{a-c}	11.7^{a-d}	11.5^{a-b}	0.46^{a-c}
Starbonnet	20.2^{b-c}	24.6^{a-b}	52.4^{a-c}	11.6^{a-d}	11.4^{a-b}	0.47^{a-b}
Wells	20.4^{a-c}	24.5^{a-c}	52.4^{a-c}	11.3^{d}	11.9^{a-b}	0.47^{a-b}
XP723	20.2^{b-c}	24.7^{a}	52.2^{a-c}	11.7^{a-d}	11.4^{a-b}	0.47^{a-b}
Cluster mean	20.4	24.3	52.0	11.7	11.9	0.47
Cocodrie	20.6^{a-b}	23.3^{e}	53.0^{a-c}	11.8^{a-c}	11.9^{a-b}	0.44^{c-d}
L-205	20.3^{b-c}	23.7^{b-e}	53.3^{a-b}	11.9^{a-b}	11.2^{b}	0.44^{c-d}
LaGrue	20.4^{a-c}	23.6^{c-e}	52.8^{a-c}	11.9^{a-b}	11.7^{a-b}	0.45^{b-d}
Newrex	20.3^{b-c}	24.5^{a-c}	52.1^{a-c}	11.8^{a-c}	11.6^{a-b}	0.47^{a-b}
Cluster Mean	20.4	23.8	52.8	11.9	11.6	0.45
Jodon	20.2^{b-c}	23.5^{d-e}	53.8^{a}	11.7^{a-d}	11.0^{b-c}	0.43^{d}
L-202	20.2^{b-c}	23.9^{a-e}	53.3^{a-b}	11.8^{a-c}	11.1^{b-c}	0.45^{b-d}
RU0401096	19.8^{d}	24.7^{a}	53.8^{a}	11.7^{a-d}	9.8^{c}	0.46^{a-c}
RU9201127	20.1^{c-d}	24.2^{a-e}	53.4^{a-b}	11.4^{c-d}	11.0^{b-c}	0.45^{b-d}
Spring	20.2^{b-c}	24.3^{a-d}	52.8^{a-c}	11.5^{b-d}	11.4^{a-b}	0.46^{a-c}
XL8	20.3^{b-c}	23.7^{b-e}	53.1^{a-c}	11.6^{a-d}	11.5^{a-b}	0.45^{b-d}
Cluster mean	20.1	24.0	53.4	11.6	11.0	0.45

a) In a column, means of duplicate measurements with common superscript letter(s) are not significantly different at $p<0.05$ based on Tukey's HSD test.

Table 3. Cooking and textural properties of 23 U.S. long-grain rice cultivars[a)]

Cultivar	LM (%)	Leached amylose (%)	AAR[b)]	Hardness (N)	Stickiness (N · s)
Ahrent	3.0^{e-g}	1.3^{f-g}	0.91^{b-h}	88.8^{f-g}	9.3^{d-e}
Banks	3.8^{c}	1.6^{c-f}	0.88^{c-h}	95.3^{c-f}	8.9^{d-g}
Bonnet73	4.8^{b}	2.2^{a-c}	0.95^{a-h}	95.2^{c-f}	11.0^{a-b}
Carolina Gold	3.3^{d-e}	1.4^{d-g}	0.87^{d-h}	97.2^{b-d}	9.2^{d-e}
Cybonnet	2.8^{f-g}	1.3^{f-g}	1.03^{a-g}	98.7^{a-d}	9.0^{d-h}
Cypress	1.8^{j}	0.9^{g}	1.12^{a-b}	93.0^{d-g}	6.6^{j-k}
Drew	3.0^{e-g}	1.2^{f-g}	0.89^{c-h}	92.1^{d-g}	7.1^{h-k}
Francis	3.2^{d-e}	1.3^{f-g}	0.84^{e-h}	95.1^{c-e}	9.2^{d-e}
Katy	3.1^{e-f}	1.3^{e-g}	0.88^{c-h}	87.4^{g}	8.1^{e-i}
Labelle	2.9^{e-g}	1.2^{f-g}	0.86^{d-h}	89.2^{e-g}	7.8^{f-j}
Starbonnet	2.1^{i-j}	1.0^{g}	1.04^{a-f}	92.9^{d-g}	6.2^{k-l}
Wells	2.9^{e-g}	1.3^{f-g}	0.94^{a-h}	95.9^{b-e}	10.0^{b-d}
XP723	3.2^{d-e}	1.3^{f-g}	0.88^{c-h}	93.1^{d-g}	12.2^{a}
Cluster mean	3.1	1.3	0.92	93.4	8.8
Cocodrie	3.0^{e-g}	1.6^{c-f}	1.17^{a}	105.3^{a}	7.5^{g-k}
L-205	2.3^{h-i}	1.1^{f-g}	1.11^{a-c}	104.2^{a-b}	4.3^{m}
LaGrue	2.3^{h-i}	1.1^{f-g}	1.07^{a-e}	98.5^{b-d}	9.1^{d-f}
Newrex	2.6^{g-h}	1.1^{f-g}	1.08^{a-d}	105.9^{a}	5.3^{l-m}
Cluster mean	2.7	1.3	1.11	103.5	6.6
Jodon	5.9^{a}	2.4^{a}	0.75^{h}	98.3^{b-d}	10.0^{b-d}
L-202	5.5^{a}	2.2^{a-c}	0.77^{h}	101.1^{a-c}	8.6^{d-h}
RU0401096	4.9^{b}	1.9^{a-e}	0.75^{f-h}	87.7^{g}	6.8^{i-k}
RU9201127	5.6^{a}	2.3^{a-b}	0.81^{h}	86.7^{g}	6.5^{j-l}
Spring	4.8^{b}	2.1^{a-c}	0.91^{b-h}	90.0^{d-g}	9.5^{c-d}
XL8	5.0^{a}	2.0^{a-d}	0.75^{h}	92.1^{d-g}	10.9^{a-c}
Cluster mean	5.3	2.1	0.79	92.7	8.7

a) In a column, means of duplicate measurements with common superscript letter(s) are not significantly different at $p<0.05$ based on Tukey's HSD test.
b) Ratio of the area of the amylose and amylopectin peaks on the HPSEC chromatogram of LM.

L-205, and Cocodrie gave separate, intact, and dry cooked grains. The cooked grains from Jodon, L-202, RU9201127, XL8, XP723, and Ahrent were noticeably frayed, clumpy, and moist. Wells, Bonnet73, Cypress, Katy, and Starbonnet showed separate and slightly frayed cooked grains. These visual observations coincided well with the groupings based of Ward cluster analysis (Fig. 1).

The amount of LM extracted from the cooked grains ranged from 1.8 (Cypress) to 5.9% (Jodon) (Table 3). The cultivars in the Jodon cluster had the highest average LM (5.3%), followed by the Wells cluster (3.1%) and then the Newrex cluster (2.7%). Figure 2 shows the saccharide molecular-size distribution of the LM from Wells, Jodon, and Newrex cooked rice as determined by HPSEC. The profiles noticeably differed from that of a native starch isolated from the cultivar, Wells. Native rice starch typically consists of three components: amylopectin, intermediate material, and amylose [28, 34]. Nevertheless, the two peaks eluted from the LM samples were designated as amylopectin (DP $\sim$ 11 000) and amylose (DP $\sim$ 2100),

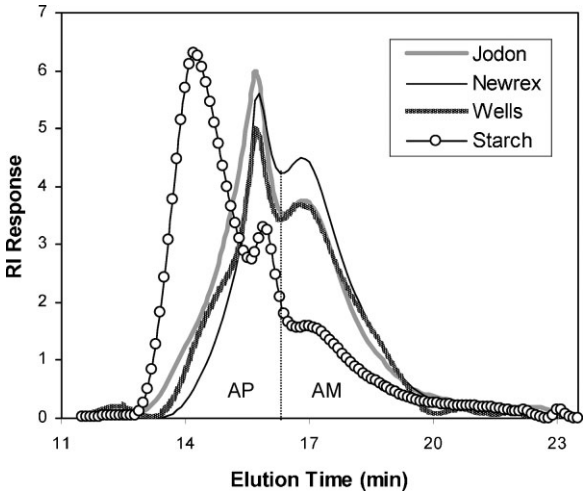

Figure 2. HPSEC molecular size distribution of the LM from Jodon, Newrex, and Wells cooked rice, in comparison with Wells native starch (AP, amylopectin; AM, amylose).

respectively [24]. The DP of purified rice amylose and amylopectin has been reported to be 900–1700 and 5000–15 000, respectively [34]. The HPSEC profiles confirmed previous findings that both amylose and amylopectin leached out of rice grains during cooking [15–19, 22, 24, 35]. The AAR of the starch fraction in the LM ranged from 0.75 (Jodon, RU0401096, and XL8) to 1.17 (Cocodrie). AAR cluster means were 0.91, 0.79, and 1.11 for Wells, Jodon, and Newrex Cluster, respectively (Table 3). LM positively correlated with head rice apparent AM (Table 4). This correlation was attributed mainly to the cultivars in the Jodon and Wells Cluster as shown in Fig. 3, and Tables 1 and 3. Some cultivars from the Newrex cluster (Newrex, Cocodrie, and L-205) and Cybonnet (Wells cluster) deviated the LM-AM positive relationship (Fig. 3). These cultivars had a relatively high AM but low LM.

Multivariate regression analysis (Table 5) showed that AM explained 32% ($R^2 = 0.32$) of the LM data variance. Among the single-variable regression models, it was the one with AM that gave the highest coefficient of determination (R^2) and lowest root mean square error (RMSE). The inclusion of secondary variables (particularly CP and amylopectin fine structure) in the regression models improved the predictability of LM up to 47%, although the decrease in RMSE was not remarkable. Amylopectin average chain length (ACL) and the percentage of DP37-65 chains (B3+) negatively correlated with LM (Tables 2 and 4, and Fig. 3A), which is in agreement with previous works about the importance of long amylopectin chains on the leaching behavior of rice (15–19, 24, 35). Mizukami *et al*. [35] proposed that amylopectin molecules with extended long chains restrict leaching, possibly by complexing with lipids or anchoring deeply inside the crystalline domains. Similarly, Ong and Blanshard [16, 23] inferred that long amylopectin chains (presently described as B3+ chains), through its interaction with amylose, promoted the formation of double helices in several crystallites, and consequently minimized the leaching of materials on cooking. Regression analysis also showed the importance of protein to leaching, which might be attributed to protein bodies. It was reported that some protein bodies were bound tightly to starch granules and remained intact during cooking [36].

Overall, only 45% ($R^2 = 0.45$) of the LM variance was explained by all the physicochemical variables evaluated in this work (Table 5). Other grain attributes such as fissures, and grain-to-grain variations in length, width, thickness, and chronological age may also influence the leaching behavior of rice during cooking.

Table 4. Correlation coefficients (R) from the pairwise correlation analysis of leaching characteristics with chemical composition and amylopectin fine structure

Variable	% LM	AAR
AM Content	0.49[a)]	−0.30
CP	−0.26	0.01
SL	−0.06	0.15
A chain (DP6-12)	−0.25	−0.19
B1 chain (DP13-24)	0.56[b)]	−0.24
B2 Chain (DP25-36)	−0.02	0.18
B3+ chain (DP37-65)	−0.45[a)]	0.37[a)]
ACL	−0.38[a)]	0.40[a)]
A/B1 chain ratio	−0.42[a)]	0.02
AAR	−0.66[b)]	1.00[b)]

a) Significant at $p<0.05$, $n = 23$.
b) Significant at $p<0.01$, $n = 23$.

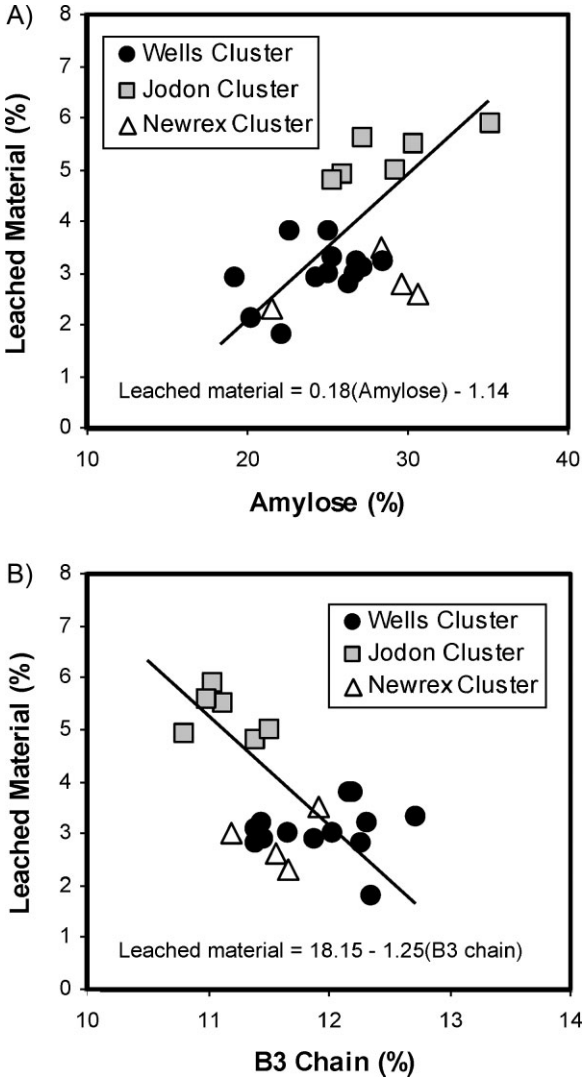

Figure 3. Plots of the relationship between LM during cooking and head rice AM content (A), and between LM and amylopectin B3+ chains (B).

Table 5. Best-fit regression models for explaining cooking properties variance

Number of variables	Variable (regression coefficient)[a)]	Intercept	R^2	RMSE
LM				
1	AM (0.18)	−1.1	0.32	1.03
2	AM (0.130, B3 + (−0.67)	8.0	0.34	1.03
3	AM (0.08), CP (−0.35), B1 (0.83)	−39.0	0.44	0.98
4	AM (0.07), CP (−0.52), B1 (0.82), B2 (−1.26)	−22.6	0.46	0.98
5	AM (0.08), CP (−0.62), B2 (−2.90), B3 + (−2.57), ACL (4.91)	−29.6	0.47	1.00
9	All variables	−23.7	0.45	1.14
Cooked rice hardness				
1	AAR (22.73)	74.1	0.29	4.85
2	AAR (30.40), AM (0.94)	42.6	0.63	3.59
3	AAR (29.33), AM (0.80), CP (−2.62)	69.0	0.76	2.96
4	AAR (28.29), AM (0.80), CP (−2.55), SL (19.11)	63.7	0.79	2.87
5	AAR (19.18), AM (0.78), CP (−2.69), A (−3.12), LM (−1.36),	159.7	0.81	2.82
13	All variables	−1461.9	0.87	3.22
Cooked rice stickiness				
1	AAR (−6.57)	14.4	0.21	1.74
2	AAR (−9.06), B3 + (1.47)	−0.4	0.40	1.56
3	AAR (−8.43), SL (−16.62), B3 + (1.61)	2.4	0.56	1.36
4	AAR (−8.50), SL (−17.50), A (−4.81), A/B1 (156.39)	65.8	0.63	1.28
5	AAR (−9.76), ASV (−0.45), SL (−14.40), A (−4.93), A/B1 (137.31)	79.2	0.68	1.23
13	All variables	437.5	0.89	1.01

a) A (% A chains in amylopectin); AAR (LM AAR); A/B1 (A chain-B1 chain ratio); ACL (amylopectin ACL); AM (% AM content); ASV; B1 (% B chains in amylopectin); B3+ (% B3+ chains in amylopectin); CP; LM (% LM); SL (% SLs).

3.3 Cooked rice texture

Cooked rice hardness and stickiness ranged from 86.6 (RU9201127) to 105.9 N (Newrex), and 4.3 (L-205) to 12.2 N · s (XP723), respectively. The cultivars in the Newrex cluster were characterized by high hardness (103.5 N) and low stickiness (6.6 N · s). Hardness and stickiness overall means for the Wells and Jodon cluster were comparable, although some noticeable textural differences were also observed among the cultivars within these clusters. Correlation analysis (Table 6) showed that hardness positively correlated with the AAR ratio of the LM but not with the LM itself. Hardness also correlated positively with AM, B2, and ACL; and negatively with A chain and CP. Stickiness negatively correlated with AAR and SL but again not with LM. This is in accord with the report of Hanashiro *et al*. [22] that sticky rice tended to leach less amylose than non-sticky rice. Upon cooling of rice cooked in optimum water, leached amylose may retrograde on the surface of the grain and subsequently contribute to a firm, hard coating. The cultivars in the Newrex cluster leached out more amylose than amylopectin (AAR mean of 1.11), hence, resulting in harder and less sticky cooked rice. This agrees with the findings of Ong and Blanshard [16] that parboiled, hard-texture rice cultivars leached out relatively higher amounts of amylose than their soft-texture counterparts. In contrast, the cultivars in the Jodon cluster leached out more amylopectin than amylose (AAR mean of 0.79), making cooked rice less hard but more sticky (Table 3). Overall, an AAR of <1 was associated with low hardness and high stickiness; an AAR of >1 was associated with high hardness and low stickiness.

Table 6. Correlation coefficients (R) from the pairwise correlation analysis of cooked rice texture with chemical composition, amylopectin fine structure, and LM

	Hardness	Stickiness
AM	0.40[a)]	0.04
CP	−0.49[a)]	−0.19
SL	0.28	−0.42[a)]
ASV	0.25	−0.20
A chain (DP6-12)	−0.54[b)]	−0.04
B1 (DP13-24)	−0.01	−0.19
B2 (DP25-36)	0.40[a)]	−0.07
B3+ (DP37-65)	0.26	0.24
ACL	0.38[a)]	0.16
LM	−0.19	0.34
AAR	0.54[b)]	−0.46[a)]

a) Significant at $p<0.05$, $n = 23$.
b) Significant at $p<0.01$, $n = 23$.

Thirteen variables were used in the stepwise regression analysis of cooked rice texture, yielding a total 8192 regression models. The regression models (with 1–5 variables) that gave the highest R^2 and lowest RMSE are presented in Table 5. The models explained 21–81% of the texture data variance. It appears that regression analysis primarily highlighted the importance of AAR to cooked rice texture. AAR was part of all the regression models that gave the highest R^2 and lowest RMSE. Figure 4 shows the relationship between cooked rice texture and AAR, and depicts the position of the individual cultivars on the one-variable regression line. Other variables included in the best-fit, five-variable regression models for hardness were AM, CP, SL, LM, and A chain.

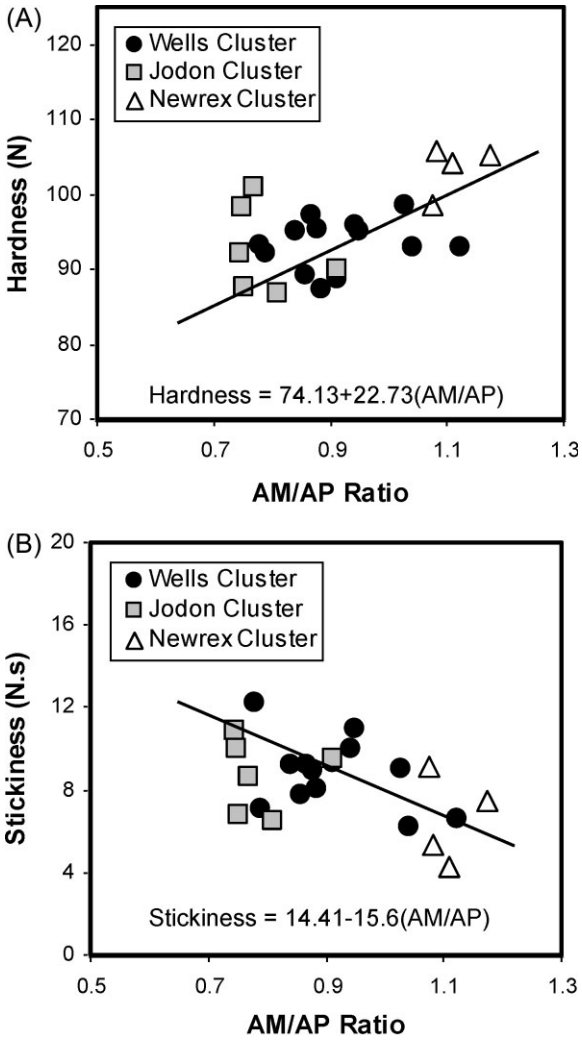

Figure 4. Plots of the relationship between cooked rice hardness and LM AAR (A), and between cooked rice stickiness and LM AAR (B).

For stickiness, the other variables included in the best-fit regression models were associated with amylopectin structure (*i.e.*, A, A/B1, B3 + chains), as well as SL and ASV. The role of amylopectin long chains to cooked rice texture had been emphasized in previous works [15, 16]. However, the effect of amylopectin long chains was not as prominent as that of AAR in the present study according to the correlation and multivariate regression results. This could be possibly due to the method used in cooking. A fixed optimum cooking water to rice ratio of 2:1 was employed in the current work, whereas, Radhika-Reddy *et al*. [15] and Ong and Blanshard [16] cooked rice in excess water. In the optimum water method, the LMs are left on the surface of the grains, whereas, in the excess water method, the LMs are essentially removed from the grains. In addition, the samples used in this work did not come from the same cropping season (described in the Section 2) and this may also affect cooked rice texture–amylopectin fine structure relationship. It was reported that amylopectin chain-length distribution shifted to shorter branch chains as a result of aging rough rice [37].

4 Conclusions

Ward's cluster analysis of the physicochemical properties, amylopectin chain-length distribution, and cooking characteristics of 23 U.S. long-grain cultivars resulted in a dendogram with three major clusters: Wells, Jodon, and Newrex. The Wells cluster consisted mainly of the regular-type, intermediate-amylose, long-grain cultivars characterized by soft to moderate cooked rice hardness; the Jodon cluster had the soft-cooking, high-amylose cultivars; whereas, the Newrex cluster had the dry-cooking, high-amylose cultivars. Correlation and multivariate regression analysis highlighted the relevance of the AAR of the LMs during cooking in explaining the variation in cooked rice texture data. AAR may be used as a simple index of texture, as it can assume values of either <1, $=1$, or >1. In general, the starch fraction that leached out from soft-cooking, sticky cultivars on cooking had a higher proportion of amylopectin than amylose (AAR$<$1), whereas, dry-cooking cultivars leached out more amylose (AAR$>$1). The amount of LMs *per se* was also higher for the soft-cooking cultivars than those of the dry-cooking ones. The present data also indicate that cooked rice hardness is a function of amylose; whereas, stickiness is attributable to amylopectin. Cultivar differences in leaching characteristics were affected by head rice AM content, CP, and amylopectin chain length distribution, particularly the relative proportion of long and short branch chains.

The authors have declared no conflict of interest.

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