

James Patindol*
Xiaofang Gu
Ya-Jane Wang

Department of Food Science,
University of Arkansas,
Fayetteville, AR, USA

Chemometric Analysis of the Gelatinization and Pasting Properties of Long-grain Rice Starches in Relation to Fine Structure

Chemometric tests were carried out to better understand the multidimensional facet of starch fine structure-relationship concerning gelatinization and pasting properties. With Ward's hierarchical cluster analysis 20 long-grain rice starch samples were sorted out into three clusters based on similarities in functional properties, particularly, paste peak (PV) and final viscosity (FV). The three clusters (arbitrarily named Clusters A, B, and C) exhibited a pasting profile trend of $PV < FV$, $PV \sim FV$, and $PV > FV$, respectively. Cluster A samples were also lower in peak temperature, range and enthalpy of gelatinization, and swelling power. These attributes were associated with higher amylose content (AM), β -amylolysis limit, and percentage of B1 chains (DP13-24), but lower amylopectin weight-average molar mass (M_w) and percentage of A chains (DP6-12). A 5-variable linear discriminant function correctly predicted 85% of the Ward's cluster membership of the individual cultivars. The discriminant function included the variables A, B1, and B2 (DP25-36) chains, average chain length (ACL), and gyration radius (R_z). Fine structure variance was fully explained by a total of nine principal components, with the first three components cumulatively accounting for 74%. The leading variables included in the three rotated components pertained to amylopectin chain length distribution (A, B2, and B3+ or $DP \geq 37$ chains, and ACL) and amylopectin molar mass (M_w , R_z , and polydispersity). AM and M_w were loaded most frequently in the 4-variable, best-fit linear regression models for predicting gelatinization and pasting properties. A combination of at least two fine structure variables controls the functionality of rice starch.

Keywords: Rice starch; Fine structure; Pasting; Gelatinization; Chemometrics

1 Introduction

Long-grain rice cultivars comprise the majority of the annual rice production in the United States. About 90% of the dry weight of milled rice is starch. Rice starch has been utilized in a variety of food and industrial applications, such as a thickening, gelling and filling agent, for sugar coating in confectionary, as cosmetic dusting powder, photographic paper powder, and excipient for pharmaceutical tablets [1, 2]. Starch owes much of its functionality to the proportion of its two major components, amylose and amylopectin, besides the architecture of these macromolecules arranged in granular form. Rice starch granules are polygonal and small, about 3-8 μm in diameter, and differ from starch granules of other cereals in size and shape [3]. Rice amylose is a mixture of linear and branched molecules with a degree of polymerization (DP) of 920-1110 glucose units, average chain length

(ACL) of 250-370 glucose units, and a β -amylolysis limit of 73-84% [4]. Rice amylopectin is highly branched and has a DP range of 8200-12,800 glucose units, ACL of 19-23, and β -amylolysis limit of 49-59% [4].

The role of amylose and amylopectin in the gelatinization and pasting properties of rice starch has been widely studied [2, 5-19]. Starch swells irreversibly and its crystalline structure collapses when heated in excess water – a phenomenon known as gelatinization. Starch swelling is a property of amylopectin [5], whereas, amylose has been known to restrict it [5-6, 11-16]. Restricted starch granule swelling results in a lower peak paste viscosity based on measurements with a Brabender Viscoamylograph or a Rapid Visco Analyser [6-17]. Paste breakdown correlates positively with the proportion of short amylopectin chains, negatively with long chains [6-7, 12-14], and also negatively with amylose content [6, 14, 17]. Temperature and enthalpy of gelatinization as measured by differential

Correspondence: Ya-Jane Wang, Department of Food Science, University of Arkansas, Fayetteville, AR 72704, USA. Phone: (+)-1-479-575-3871, Fax: (+)-1-479-575-6936, e-mail: yjwang@uark.edu.

* Current address: USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124, USA

scanning calorimetry correlate negatively with the amount of amylopectin short chains (DP6-12 or A chain), and positively with longer chains (DP12-24 or B1 chain) [6, 9, 12, 18–19]. Amylopectin short chains may destabilize the crystalline lamellar structure of starch granules because at least 10 glucose units are needed to form the double helices of the structure [9, 20]. Amylopectin long chains (DP \geq 37 or B3+) delay gelatinization and correlate positively with starch gelatinization temperature [6, 8, 9], possibly by forming longer double helices within the crystalline lamellae [6, 9]. Double helices from amylopectin long chains require higher temperature for complete dissociation than those from short chains [21]. Literature also associates high temperature and enthalpy of gelatinization with high amylose content [6, 14, 15, 17].

In previous works, starch fine structure-function relationships concerning gelatinization and pasting were mainly described by simple correlation coefficients. The multidimensional relationships among various starch properties are still inadequately studied. Hence, this work used some chemometric tools to better understand the multidimensional facet of starch structure-function relationship. Chemometrics is a specialized discipline for extracting information from multivariate chemical data using tools of statistics and mathematics [22]. Cluster analysis, linear discriminant analysis, principal component factor analysis, and multiple linear regression analysis were employed to classify long-grain rice starches based on similarities and differences in gelatinization and pasting properties, and to identify fine structure variables that most influence the functional properties of starch.

2 Materials and Methods

2.1 Starch samples

Dry rough rice samples (MC~12.0%) from 20 long-grain rice cultivars were provided by the University of Arkansas Rice Research and Extension Center in Stuttgart, Arkansas. The cultivars included: Ahrent, Banks, Bonnet 73, Carolina Gold, Cocodrie, Cybonnet, Cypress, Drew, Francis, Jodon, Katy, L-202, L-205, Labelle, LaGrue, Newrex, Spring, Starbonnet, and Wells. The samples were obtained from different cropping seasons and had been stored at 4°C prior to the experiment. Starch samples were prepared from powdered head rice following the alkaline steeping method of Yang *et al.* [23] with modifications [8]. A 10-g rice flour sample was soaked in 100 mL of 0.1% NaOH for 24 h with stirring. The slurry was filtered through a U.S. standard sieve No. 230 (63 μ m), and then centrifuged at 1,520 $\times g$ for 10 min. The

residue was washed with 0.1% aqueous NaOH and then centrifuged. The top yellowish, curd-like layer was carefully scraped off with a spatula. The residue was dispersed in deionized water and the pH of the slurry was adjusted to pH 6.5 with 0.1 M aqueous HCl. The slurry was centrifuged and then washed with deionized water three times, dried in a convection oven at 40°C for 24 h, ground into powder using mortar and pestle, and passed through a standard 100-mesh sieve. Isolated starch was defatted with water-saturated 1-butanol (WSB, 33:67 water: 1-butanol) as described by Patindol and Wang [8]. A 5-g starch sample was mixed with 25 mL of WSB and shaken at room temperature overnight on a rotary shaker, and then centrifuged at 1,500 $\times g$ for 10 min. The residue was washed with WSB followed by centrifugation, and then washed three more times with deionized water. Defatted starch was dried in a convection oven at 40°C overnight, ground with a mortar and pestle, and passed through a 100-mesh U.S. standard sieve.

2.2 Amylose and β -amylolysis limit

The iodine affinity of defatted starch was determined by potentiometric titration [24] with modifications. Starch (100 mg, dry basis) was dispersed in 10 mL of 1 M aqueous KOH, and the mixture was stirred at room temperature for 20 min prior to measurement. Amylose content (% dry basis) was calculated by dividing iodine affinity by 20%, which is the assumed iodine affinity of purified rice amylose. Beta-amylolysis limit was determined by hydrolyzing a dispersed starch sample (1 mg/mL) with β -amylase (Sigma, St. Louis, MO) at a rate of 15 U/mL substrate in 50 mM acetate buffer (pH 5.0). Reaction time was 3 h at 30°C with constant magnetic stirring (100 rpm) [25]. The amount of maltose produced from the hydrolysis was determined by the colorimetric Nelson-Somogyi method [26, 27].

2.3 Swelling power and soluble solids

Swelling power and soluble solids were evaluated by a 40-mg swelling test [28] with modifications. Starch (40 mg, dry basis) was accurately weighed into a pre-weighed 2.0 mL micro-centrifuge tube. Deionized water (1.5 mL) was added, vortexed for 10 s, and allowed to hydrate at room temperature for 10 min. The sample was heated on a heating block set at 85°C for 30 min with gentle inversion of the tube every 5 min. The sample was cooled in an ice bath for 5 min and then centrifuged at 10,000 $\times g$ for 10 min. The supernatant was carefully transferred into another pre-weighed micro-centrifuge tube using a disposable pipet. The tube with the starch paste residue was wiped dry with a paper towel and then

weighed. The tube with the supernatant was dried in a convection oven set at 40°C to constant weight. Swelling power was calculated as the ratio of paste weight to starch sample weight. Percent soluble solids was obtained by dividing dried supernatant weight by starch sample weight, and then multiplying by 100.

2.4 Pasting properties

The pasting properties of the starch samples were determined with a Rapid Visco Analyser (Newport Scientific, Warriewood NSW, Australia) operated at 160 rpm according to Approved Method 61-02 [29] with modifications. Rice starch slurry was prepared by mixing 3.0 g of rice starch (12% moisture content basis) with 25 mL of deionized water in a canister. The slurry was heated from 50°C to 95°C at 3°C/min, held at 95°C for 10 min, cooled to 50°C at 3°C/min and held at 50°C for 10 min. The initial speed was set at 960 rpm for the first 10 s to thoroughly mix the slurry, and then changed 160 rpm for the rest of the run. The pasting properties measured were: pasting temperature, peak viscosity, peak time, hot paste viscosity (trough), final viscosity, breakdown, and setback. Breakdown viscosity was calculated by subtracting hot paste viscosity from peak viscosity; setback viscosity was taken as final viscosity minus peak viscosity; and paste consistency (total setback) as final viscosity minus hot paste viscosity.

2.5 Gelatinization properties

Gelatinization properties were assessed by a Pyris-1 differential scanning calorimeter (DSC) (Perkin-Elmer Co., Norwalk, CT, USA). Starch (~4.0 mg, dry basis) was weighed into an aluminum DSC pan, and 8 μ L deionized water was added by a microsyringe. The mixture was hermetically sealed and equilibrated at room temperature for at least 1 h before running the thermogram. Thermal scans involved heating the sample from 25°C to 130°C with a temperature increase rate of 10°C/min. An empty pan was used as a reference. Data on onset, peak, and end gelatinization temperatures, and gelatinization enthalpy were calculated from thermograms. Gelatinization range was calculated as the difference of conclusion and onset gelatinization temperature.

2.6 Amylopectin chain-length distribution

Amylopectin chain-length distribution was characterized by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to *Kasemsuwan* et al. [30] with modifications.

The HPAEC-PAD system consisted of an LC20-1 chromatography organizer, GP50 gradient pump, ED40 electrochemical detector, 4 \times 50-mm CarboPac PA1 guard column, 4 \times 250-mm CarboPac PA1 analytical column, and an AS40 automated sampler (Dionex Corp., Sunnyvale, CA, USA). Deionized water (3.2 mL) was added to 9.0 mg of defatted starch, 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). Isoamylase (5 μ L, 1,180 U, *Pseudomonas isoamylase*, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) was added and the suspension was incubated in a water bath at 40°C and 100 rpm for 2 h. The pH of the mixture was adjusted to 6.5 by adding ~0.22 mL of 2 M aqueous NaOH, heated in a boiling water bath for 15 min, cooled for 5 min and then centrifuged at 5,000 \times *g* for 5 min. The supernatant was injected into the HPAEC-PAD system through an autosampler vial.

2.7 Amylopectin molar mass

Amylopectin weight-average molar mass, z-average gyration radius, and polydispersity (ratio of weight-average and number-average molar mass) were determined by HPSEC-MALLS-RI (high performance size-exclusion chromatography with multi-angle laser light scattering and refractive index detectors). The system consisted of a 515 HPLC pump with a 200 μ L sample loop (Waters, Milford, MA, USA), an inline degasser, a TSKgel PW_{XL} guard column (Tosoh Corp, Tokyo Japan), a series of two size-exclusion columns (TSKgel G5000PW_{XL} and G4000PW_{XL}, Tosoh Corp.), a DAWN-EOS 18-angle light scattering detector (Wyatt Technology, Sta. Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology). The mobile phase consisted of an aqueous solution of 0.15 M NaNO₃ and 0.02% NaN₃ (vacuum-filtered twice through a 0.1 μ m membrane filter) at a flow rate of 0.7 mL/min. The output voltages for RI and LS detectors were collected with the temperature of the columns maintained at 55°C and the RI detector at 35°C, jumpers set at a 21 \times LS detector response, a dn/dc value of 0.146, and then processing the data with ASTRA 5.1.3 software (Wyatt Technology). Samples for injection were prepared following the method of *Patindol* et al. [16].

2.8 Chemometric analysis

A JMP software version 7 (SAS Software Institute, Cary, NC, USA) was used in the chemometric analysis of the experimental data that were all collected from duplicate measurements. Ward's hierarchical cluster analysis was used to sort the 20 samples according to similarities and differences in gelatinization and pasting properties. Linear

discriminant analysis was used to assess the capability of fine structure variables in predicting the cluster membership of each cultivar originally established by Ward's cluster analysis of functional properties. The prediction rate of the degree of association between functional and structural properties. Wilk's lambda was used as an indicator of significance. Principal component factor analysis was performed to obtain a simplified view of the relationship among fine structure variables. Multiple linear regression analysis was used to test how well a particular gelatinization or pasting variable can be predicted, on the basis of multiple and independent fine structure variables. In this chemometric test, both dependent and independent variables are numerical as opposed to discriminant analysis, in which the dependent variable is categorical while the independent variable is numerical. Regression coefficients and root mean square errors were used as indicators of significance of the regressions models.

3 Results and Discussion

3.1 Hierarchical cluster analysis

Cluster analysis is a chemometric tool that sorts objects into groups or clusters, so that the degree of association is strong between members of the same cluster and weak between members of different clusters [22]. Fig. 1 shows a dendrogram obtained from Ward's hierarchical cluster analysis of the gelatinization and pasting properties of the starch samples isolated from 20 long-grain rice cultivars. Cluster analysis grouped the cultivars into three major clusters based on their similarities and differences, and were arbitrarily designated as Clusters A ($n = 5$), B ($n = 7$), and C ($n = 8$). Cluster C was separated from Cluster B by a distance of 4.2 units; whereas Cluster A was separated from the merged Clusters B and C by a distance of 6.6 units. The distance between two clusters is the ANOVA sum of squares between the two clusters added up over all the variables.

Ranges and means of the gelatinization and pasting properties of the starch samples belonging to each of the three clusters are shown in Tab. 1. The clusters noticeably differed in their peak viscosity (PV) and final viscosity (FV), in which the trend was: $PV < FV$ for Cluster A, $PV \sim FV$ for Cluster B and $PV > FV$ for Cluster C. This trend is well represented by the pasting profiles of cultivars Cocodrie (PV < FV), Francis (PV ~ F), and Drew (PV > FV) as shown in Fig. 2. Breakdown viscosity (BV) and setback viscosity (SV) also differed but total setback viscosity (TSV) did not. It was $A < B < C$ for BV, and $A > B > C$ for SV. The samples in Cluster A were also characterized by a lower swelling

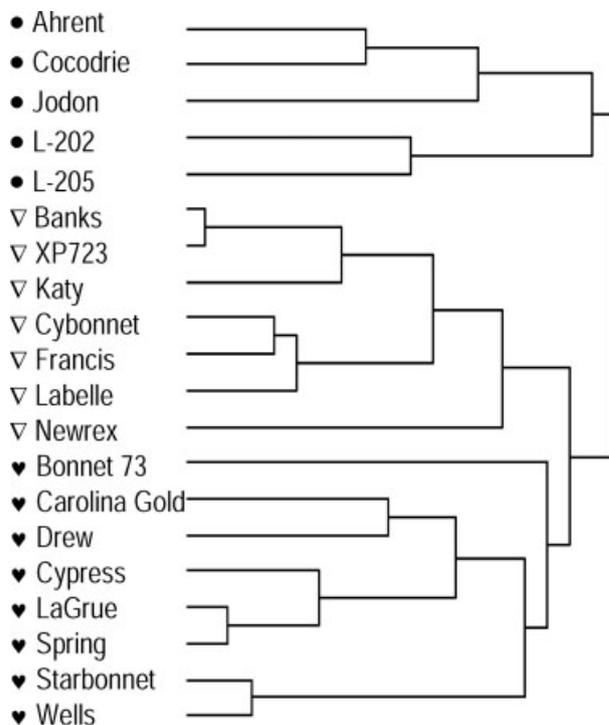


Fig. 1. Dendrogram obtained from the hierarchical cluster analysis of the swelling, gelatinization, and pasting properties of 20 long-grain rice starches (● = Cluster A, ▽ = Cluster B, ♥ = Cluster C).

power (SP) and a larger amount of soluble solids (SS). Samples in Clusters B and C showed similar swelling power. In terms of gelatinization properties, Cluster A was characterized by lower peak temperature, gelatinization range, and gelatinization enthalpy compared with Clusters B and C.

Tab. 2 presents the ranges and means for amylose content, amylopectin chain length distribution, and amylopectin molar mass properties of the three clusters. The Cluster A samples were generally higher in amylose content (AM), β -amylolysis limit (BL), and the percentage of DP13-24 amylopectin chains (B1), but lower in amylopectin molar mass (M_w) and the percentage of DP6-12 amylopectin chains (A) compared with Clusters B and C. The means in Tab. 2 further show that the structural features of Clusters B and C samples were similar, except gyration radius (R_z) and polydispersity (M_w/M_n), in which those of Cluster B were higher.

3.2 Discriminant analysis

Linear discriminant analysis (LDA) is a chemometric tool used to find linear combination of features that best separate two or more classes of objects or events [22]. It

Tab.1. Ranges and means of the swelling, pasting, and thermal properties of 20 starch samples grouped into three clusters by Ward's hierarchical cluster analysis¹.

Property	Cluster A (n = 5)		Cluster B (n = 7)		Cluster C (n = 8)	
	Range	Mean	Range	Mean	Range	Mean
Pasting temperature [°C]	74.5–76.8	75.6 ^a	75.0–75.6	75.4 ^a	73.2–76.2	75.1 ^a
Peak viscosity [mPa s]	2294–3656	2887 ^b	2754–3420	3250 ^a	2925–3534	3226 ^a
Final viscosity [mPa s]	2882–4229	3449 ^a	2830–3423	3262 ^a	2612–3061	2795 ^b
Breakdown viscosity [mPa s]	978–1285	1133 ^c	1422–1711	1574 ^b	1504–2228	1856 ^a
Setback viscosity [mPa s]	270–810	562 ^a	–116–76	12 ^b	–840–162	–431 ^c
Total setback viscosity [mPa s]	1250–1944	1575 ^a	1450–1991	1600 ^a	1280–1560	1425 ^a
Swelling power	8.8–9.6	9.2 ^b	9.3–10.1	9.8 ^a	9.6–10.1	9.9 ^a
Soluble solids [%]	2.5–6.0	4.2 ^a	2.1–4.2	3.2 ^b	2.4–5.3	3.5 ^b
Onset GT [°C]	69.5–73.7	71.5 ^a	71.6–73.2	72.3 ^a	70.5–73.5	72.4 ^a
Peak GT [°C]	74.7–78.4	76.4 ^b	77.0–78.5	77.8 ^a	75.8–79.1	77.6 ^a
Gelatinization range [°C]	8.8–10.7	9.8 ^b	9.1–12.1	10.7 ^a	9.6–12.0	10.7 ^a
Gelatinization enthalpy [J/g]	10.6–11.5	11.0 ^c	11.9–12.2	12.1 ^a	11.1–12.2	11.4 ^b

¹ Means within a row having a common letter are not significantly different based on Tukey's HSD test at 5.0% level of significance.

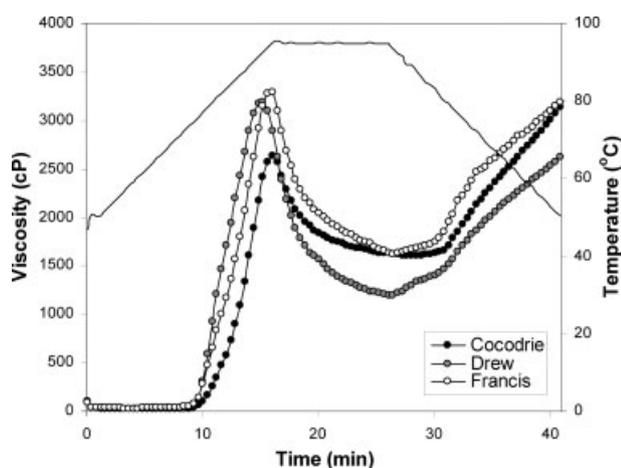


Fig. 2. Representative cultivars showing the PV>FV, PV~FV, and PV<FV trends in starch pasting profiles as measured with a Rapid Visco Analyser (PV = peak viscosity, FV = final viscosity).

makes use of a qualitative (categorical) dependent variable and several quantitative (numerical) independent variables. Linear discriminant analysis was used to validate the sorting of cultivars into Clusters A, B, and C by the Ward's cluster analysis of gelatinization and pasting properties. In the analysis, the clusters assigned to each cultivar were used as the categorical dependent variable and the structural features (Tab. 2) as numerical independent variables. The best discriminant function was extracted by a stepwise procedure (backward selection). It was selected based on high F Ratios and low Prob>F values. It consisted of five variables that pertain to amylopectin chain length (A, B1, B2, CL, R₂), and two canoni-

cal components that explained 100% of the variance. Its prediction rate for the cluster membership of each cultivar was 85% correct (Tab. 3). The same prediction rate was obtained when all fine structure variables were included in the discriminant function. Only three (15%) out of the 20 samples were misclassified. These were Cybonnet, Francis, and Starbonnet. Cybonnet and Francis belonged to Cluster B in terms of gelatinization and pasting properties, but were classified as Cluster C in terms of structural features. On the other hand, Starbonnet was similar to Cluster B in gelatinization and pasting properties but similar to the samples in Cluster C in terms of fine structure (Tab. 1, 2, and 3). The results of the LDA substantiated the importance of starch fine structure to gelatinization and pasting properties.

3.3 Principal component factor analysis

Principal component analysis (PCA) is a powerful visualization tool for reducing data dimensionality and finding linear combinations of the original independent variables that are used to explain the maximal variance of the data [22]. A total of nine principal components (Tab. 4) explained the variation in amylose content and amylopectin fine structure data presented in Tab. 2. Principal component 1 (PC1) and principal component 2 (PC2) accounted for 37% and 23% of the variation, respectively. Score (cultivar) and factor loading (fine structure variable) plots of PC1 and PC2 are shown in Fig. 3. The results of the PCA of starch structural features paralleled those obtained with the cluster analysis of gelatinization and pasting properties data. As shown in Fig. 3A, the

Tab. 2. Ranges and means of amylose content and amylopectin fine structure of the starched samples grouped by hierarchical cluster analysis of their swelling, pasting and thermal properties¹.

Property ²	Cluster A (n = 5)		Cluster B (n = 7)		Cluster C (n = 8)	
	Range	Mean	Range	Mean	Range	Mean
Amylose [%]	17.5–24.2	22.1 ^a	16.9–19.7	18.0 ^b	16.3–21.3	18.6 ^b
β-Amylolysis limit [%] ³	53.1–56.6	54.1 ^a	48.2–54.3	52.7 ^b	50.2–56.4	53.9 ^{ab}
DP6–12 [%]	23.3–24.6	23.8 ^b	24.0–24.6	24.4 ^a	23.6–24.6	24.2 ^a
DP13–24 [%]	52.1–53.8	53.1 ^a	51.7–52.5	52.1 ^b	51.8–52.8	52.2 ^b
DP 25–36 [%]	13.1–13.4	13.2 ^a	12.8–13.5	13.2 ^a	12.8–13.4	13.2 ^a
DP 37+ [%]	9.4–10.3	9.8 ^b	9.8–10.7	10.2 ^{ab}	9.8–11.1	10.4 ^a
Average chain length ⁴	20.2–20.6	20.3 ^a	20.2–20.6	20.4 ^a	20.2–20.8	20.5 ^a
Molar mass [$\times 10^8$ g/mol]	0.93–1.17	1.21 ^b	1.13–1.85	1.51 ^a	1.21–2.05	1.53 ^a
Gyration radius [nm]	191–320	242 ^{ab}	212–352	250 ^a	194–290	239 ^b
Polydispersity [M_w/M_n]	1.47–1.76	1.68 ^b	1.44–2.47	1.85 ^a	1.28–1.95	1.60 ^b

¹ Means within a row having a common letter are not significantly different based on Tukey's HSD test at 5.0% level of significance.

² Amylopectin-associated property, unless otherwise specified.

³ Measured based on total starch and not amylopectin.

⁴ Glucose molecules (units) per chain.

Tab. 3. Prediction of starch sample clusters by a linear discriminant function consisting of five fine structure variables (A, B1, B2, CL, and R_z).

Starch sample	Ward's cluster	Probability to each cluster		
		A	B	C
Ahrent	A	0.51	0.21	0.28
Banks	B	0.01	0.58	0.41
Bonnet 73	C	0.00	0.47	0.52
Carolina Gold	C	0.00	0.44	0.56
Cocodrie	A	0.98	0.00	0.02
Cybonnet	B*	0.00	0.44	0.56*
Cypress	C	0.00	0.24	0.76
Drew	C	0.00	0.39	0.61
Francis	B*	0.01	0.19	0.80*
Jodon	A	1.00	0.00	0.00
Katy	B	0.02	0.43	0.37
L-202	A	1.00	0.00	0.00
L-205	A	0.97	0.01	0.02
Labelle	B	0.00	0.66	0.34
LaGrue	C	0.01	0.44	0.55
Newrex	B	0.00	0.82	0.18
Spring	C	0.06	0.37	0.57
Starbonnet	C*	0.06	0.51*	0.43
Wells	C	0.00	0.43	0.57
XP723	B	0.00	0.80	0.20
Number of misclassified samples		0 (0%)	2 (10%)	1 (5%)

* Misclassified sample.

majority of the Cluster A cultivars were positioned at the upper left quadrant of the similarity map to indicate their

resemblance in properties. Cluster B cultivars were confined at the lower quadrant close to the PC2 axis, whereas the Cluster C cultivars were scattered both at the upper and lower right quadrants. About four outlier cultivars are also evident in the similarity map. As to the loading plot (Fig. 3B), amylose content (AM) and amylopectin B1 chain (B1) were laid on the upper left quadrant, opposite to the quadrant where amylopectin molar mass (M_w), gyration radius (R_z), polydispersity (M_w/M_n), A chain (A) were positioned. This indicates that the variables AM and B1 are inversely related with M_w , R_z , M_w/M_n , and A. Amylopectin average chain length (CL), B2 chain (B2), B3 chain (B3), and β-amylolysis limit (BL) were positioned at the upper right quadrant of the loading plot. CL and B3 are the major factors of PC1, whereas, BL and B2 are the major factors of PC2.

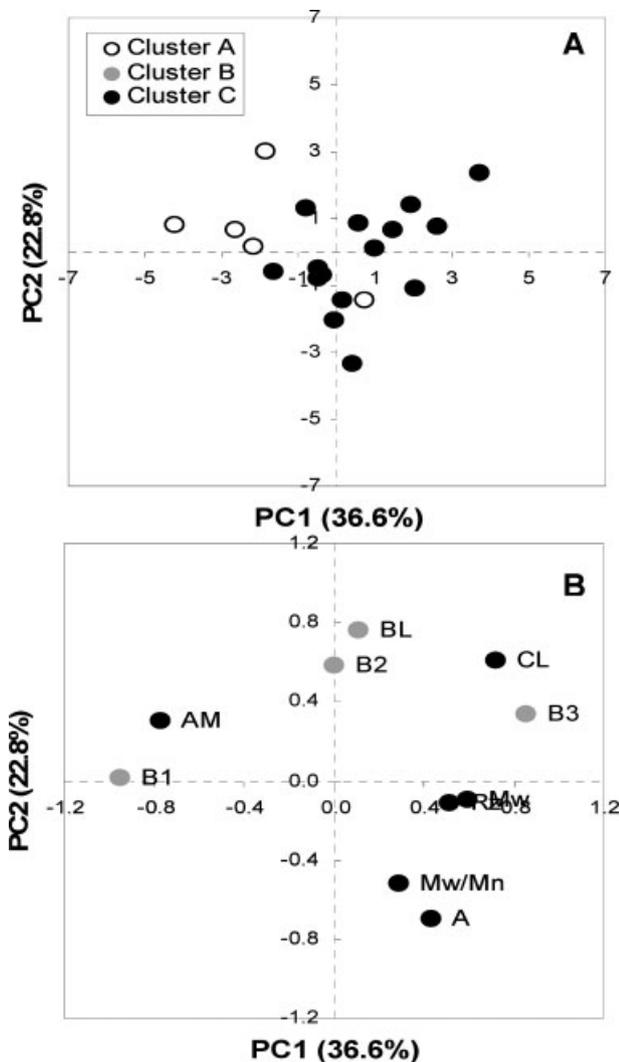
Factor analysis was applied to obtain a simplified view of the relationship among starch fine structure variables. A Varimax rotation was employed to minimize the number of variables that influence the original principal PCs. Only the first three PCs were considered in the rotation because these were the PCs that showed an eigenvalue of >1.0 (Tab. 4), and therefore, explained the variation in structural properties better than the other components. The factor matrix of the three PCs rotated by Varimax is presented in Tab. 5. Except R_z , all the fine structure variables were well represented by the three rotated PCs considering that the final communality estimates listed in Tab. 5 were greater than 0.6. Rotated PC1 explained 32% of the variance and is related to amylopectin chain length

Tab. 4. Properties of the nine principal components obtained from the principal component analysis of starch fine structure variables.

Principal component	Eigenvalue	Percent of variation	Cumulative percent
1	3.658	36.58	36.58
2	2.282	22.82	59.39
3	1.403	14.03	73.43
4	0.923	9.23	82.65
5	0.685	6.85	89.50
6	0.503	5.03	94.53
7	0.342	3.42	97.95
8	0.204	2.04	99.98
9	0.002	0.02	100.00

Tab. 5. Factor analysis matrix of three principal components rotated by Varimax method.

Structural feature	Communality estimate	Principal component		
		1	2	3
Amylose	0.72	-0.56	-0.55	-0.31
β -Amylolysis limit	0.66	0.52	-0.42	-0.47
A chain	0.82	-0.14	-0.87	0.20
B1 chain	0.94	-0.85	0.38	-0.28
B2 chain	0.63	0.14	0.76	0.18
B3 chain	0.88	0.93	0.06	0.12
Average chain length	0.90	0.90	0.28	0.08
Molar mass	0.62	0.33	0.05	0.72
Gyration radius	0.56	0.24	0.07	0.70
Polydispersity	0.61	-0.12	-0.21	0.74

**Fig. 3.** Cultivar similarity map (A) and fine-structure variable loadings (B) for the principal component analysis of starch fine structure data (PC1 = first principal component, PC2 = second principal component).

distribution (particularly CL and long branch-chains, B3). Rotated PC2 explained 21% of the variance and was attributed to the contrasting effects of amylopectin short (A) and long branch-chains (B2). Rotated PC3, which accounted 20% of the variance, was associated with amylopectin molar mass (M_w , R_z , and M_w/M_n).

3.4 Multiple linear regressions

Regression models obtained from the multiple linear regression analysis of pasting, gelatinization, and structural properties of the 20 rice starch samples are presented in Tab. 6. A total of 1,024 possible regression models can be obtained in modeling each pasting/gelatinization property. Tab. 6 only shows the 4-variable models that gave the highest regression coefficient and smallest residual mean square error (RMSE). The models for pasting temperature, final viscosity, and total setback were excluded in the list due to insignificant regression coefficients. Results of the multiple linear regression analysis complemented those obtained with other the chemometric tools discussed in previous sections. The models highlighted the contribution of amylose content, amylopectin molar mass, and amylopectin chain length distribution to starch pasting and gelatinization properties. AM and M_w were the two most frequently included variables in the 4-variable, best-fit models. The cultivars in Cluster A exhibited a PV<FV pasting profile, which was associated with higher AM and lower M_w (Tab. 1 and Tab. 2). Clusters B and C were similar in AM and M_w but showed a PV~FV and PV>FV pasting pattern, respectively. Lower R_z and M_w/M_n made Cluster C different from Cluster B; and this possibly contributed to the difference in pasting profiles between these two clusters. It is inter-

Tab. 6. Four-variable linear regression models for the gelatinization and pasting properties of long-grain rice starch¹.

Functional property	Multiple linear regression equation	R	RMSE
Peak viscosity	29802.36 – 94.50BL – 413.71B1 – 435.84M _w + 2.89R _z	0.79**	248.90
Breakdown viscosity	52319.17 – 38.91AM + 1163.65B3 – 3008.08CL – 268.93M _w /M _n	0.73*	261.31
Setback viscosity	–11683.26 + 113.91AM + 692.90B2 – 344.07M _w + 477.05M _w /M _n	0.76**	325.50
Swelling power	262.84 – 1.04A + 2.35B2 + 5.71B3 – 15.54CL	0.84***	0.62
Soluble solids	–6.64 + 0.19AM + 0.29BL – 1.07B3 + 1.28M _w	0.82**	0.59
Onset gelatinization temperature	100.08 – 0.32AM – 0.18BL – 0.74B2 – 1.49M _w	0.73*	0.40
Peak gelatinization temperature	80.87 – 0.43AM – 0.21BL + 1.35B2 – 1.17M _w	0.89***	0.57
Gelatinization range	135.17 – 1.32B1 + 3.21B2 – 4.84CL + 0.63M _w	0.88***	0.49
Gelatinization enthalpy	7.06 – 0.12AM + 0.27A – 0.04R _z + 0.75M _w /M _n	0.74*	0.42

¹*** Significant at $p < 0.001$; ** significant at $p < 0.01$; * significant at $p < 0.05$.

esting to note that R_z and M_w/M_n improved the fit of the regression models for pasting properties listed in Tab. 6. BL was next to AM and M_w in terms of frequency of inclusion in the best-fit models. Amylopectin chain length distribution variables (A, B1, B2, B3 and CL) were also included in the regression models but to a lesser frequency.

4 Conclusions

Ward's hierarchical cluster analysis was found helpful in classifying 20 long-grain rice starch samples based on similarities and differences in swelling, pasting, and gelatinization properties. The three identified clusters (arbitrarily named Clusters A, B, and C) exhibited a pasting profile trend of $PV < FV$, $PV \sim FV$, and $PV > FV$, respectively. The samples in Cluster A were also lower in peak temperature, range and enthalpy of gelatinization, and swelling power, but higher in soluble solids than those in Clusters b and C. The functional attributes of Cluster A samples were associated with higher AM, BL, B1, but lower M_w and A. Linear discriminant analysis, with fine structure variables as discriminants, complemented the results of cluster analysis, considering that the cluster membership of each cultivar was 85% correctly predicted. Principal components analysis indicated that A, B2, B3+, ACL, M_w , R_z , and M_w/M_n significantly contributed to the starch fine structure data variance. AM and M_w were important in formulating multiple linear regression models for predicting starch swelling, pasting, and thermal properties. Overall, present findings indicate that the functionality of rice starch is controlled not by a single fine

structure variable but by a combination of two or more variables.

References

- [1] E. T. Champagne: Rice starch composition and characteristics. *Cereals Food World* **1996**, *41*, 833–838.
- [2] N. Singh, L. Kaur, K.S. Sandhu, J. Kaur, K. Nishinari: Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocoll.* **2006**, *20*, 532–542.
- [3] R. P. Ellis, M. P. Cochrane, M. F. B. Dale, C. M. Duffus, A. Lynn, I. M. Morrison, R. D. M. Prentice, J. S. Swanston, S. A. Tiller: Starch production and industrial use. *J. Food Sci. Agric.* **1998**, *77*, 182–187.
- [4] Y. Takeda, S. Hizukuri, B. O. Juliano: Structures of rice amylopectins with low and high affinities for iodine. *Carbohydr. Res.* **1987**, *168*, 79–88.
- [5] 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.
- [6] J.-L. 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.
- [7] X. Z. Han, B. R. Hamaker: Amylopectin fine structure and rice starch paste breakdown. *J. Cereal Sci.* **2001**, *34*, 279–284.
- [8] J. Patindol, Y.-J. Wang: Fine structures of starches from long-grain rice cultivars with different functionality. *Cereal Chem.* **2002**, *79*, 465–469.
- [9] G. E. Vandeputte, V. Derycke, J. Geeroms, J. A. Delcour: Rice starches. I. Structural aspects provide insight into crystallinity characteristics and gelatinization behavior of granular starch. *J. Cereal Sci.* **2003**, *38*, 43–52.
- [10] G. E. Vandeputte, V. Derycke, J. Geeroms, J. A. Delcour: Rice starches. II. Structural aspects provide insight into swelling and pasting properties. *J. Cereal Sci.* **2003**, *38*, 53–59.

- [11] T. Horibata, M. Nakamoto, H. Fuwa, N. Inouchi: Structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan. *J. Appl. Glycosci.* **2004**, *51*, 239–346.
- [12] N. Inouchi, H. Hibi, T. Li, T. Horibata, H. Fuwa, T. Itani: Structure and properties of endosperm starches from cultivated rice of Asia and other countries. *J. Appl. Glycosci.* **2005**, *52*, 303–313.
- [13] J. Patindol, Y.-J. Wang: Structure-functionality changes in starch following rough rice storage. *Starch/Stärke* **2005**, *57*, 197–207.
- [14] J. Patindol, A. Flowers, M. Kuo, Y.-J. Wang, D. Gealy: Comparison of physicochemical properties of starches from red rice and cultivated rice. *J. Agric. Food Chem.* **2006**, *54*, 2712–2718.
- [15] I.-M. Park, A. M. Ibanez, F. Zhong, C. F. Shoemaker: Gelatinization and pasting properties of waxy and non-waxy rice starches. *Starch/Stärke* **2007**, *59*, 388–396.
- [16] J. Patindol, B. Gonzalez, Y.-J. Wang, A. M. McClung: Starch fine structure and physicochemical properties of specialty rice for canning. *J. Cereal Sci.* **2007**, *45*, 209–218.
- [17] Y. Li, C. F. Shoemaker, J. Ma, K. J. Moon, F. Zhong: Structure-viscosity relationships of starches from different rice varieties during heating. *Food Chem.* **2008**, *106*, 1105–112.
- [18] 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*, 363–376.
- [19] T. Noda, Y. Nishiba, T. Sato, I. Suda: Properties of starches from several low-amylose rice cultivars. *Cereal Chem.* **2003**, *80*, 193–197.
- [20] M. J. Gidley, P. V. Bulpin: Crystallization of maltotriooligosaccharides as models of the crystalline forms of starch: minimum chain length requirement for the formation of double helices. *Carbohydr. Res.* **1985**, *161*, 291–300.
- [21] F. F. Yamin, M. Lee, L. M. Pollak, P. L. White: Thermal properties of starch in corn variants isolated after chemical mutagenesis of inbred line B73. *Cereal Chem.* **1999**, *76*, 75–181.
- [22] P. C. Jurs: Chemometrics and multivariate analysis in analytical chemistry, in *Reviews in Computational Chemistry, Volume 1* (Eds. K. B. Lipkowitz, D.B. Boyd). VCH Publishers, New York, **1990**.
- [23] C. C. Yang, H. M. Lai, C. Y. Lii: The modified alkaline steeping method for isolation of rice starch. *Food Sci.* **1984**, *11*, 158–162.
- [24] T. J. Schoch: Iodimetric determination of amylose. Potentiometric titration: standard method, in *Methods in Carbohydrate Chemistry* (Eds. R.L. Whistler, R. J. Smith, J. N. BeMiller). Academic Press, New York, NY, **1964**.
- [25] S. Hizukuri, Y. Takeda, M. Yasuda, A. Suzuki: Multibranched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* **1981**, *94*, 205–213.
- [26] N. Nelson: A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **1944**, *153*, 375–380.
- [27] M. Somogyi: Notes on sugar determination. *J. Biol. Chem.* **1952**, *195*, 19–23.
- [28] C. M. Konik-Rose, R. Moss, S. Rahman, R. Appels, F. Stoddard, G. McMaster: Evaluation of the 40 mg swelling test for measuring starch functionality. *Starch/Stärke* **2001**, *53*, 14–20.
- [29] AACC: *Approved methods of the AACC*, American Association of Cereal Chemists, St. Paul, MN, **2000**.
- [30] 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.

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