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RESEARCH ARTICLE

Relationship of cooked-rice nutritionally important starch fractions with other physicochemical properties*

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Sixteen rice cultivars representing five cytosine-thymine repeat (CT_n) microsatellite genetic marker groups were analyzed for their cooked rice nutritionally important starch fractions (NISFs, which include rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS)), basic grain quality indices (apparent amylose (AM), crude protein (CP), alkali spreading value (AS), and gel consistency (GC)), pasting characteristics, and thermal properties. Chemometric tools (bivariate correlation, principal component analysis, multiple linear regression, and partial least squares regression) were used to establish the association of NISF with other milled rice physicochemical properties. CT₁₁ was generally associated with high percentages of RS and SDS, and a low percentage of RDS. CT₁₄ was associated with low SDS; whereas, CT₁₇ and CT₁₈ were associated with low RS. The CT₂₀ cultivars were similar to CT₁₁ in SDS and RS; and to CT₁₄, CT₁₇, and CT₁₈ in RDS content. RDS, SDS, and RS were loaded on three different quadrants of the principal component similarity map. RDS was not significantly correlated with any of the physicochemical properties; whereas, SDS was positively correlated with GC. RS was positively correlated with AM, setback (SB) viscosity, total setback (TSB) viscosity, and peak gelatinization temperature; and negatively correlated with breakdown (BD) viscosity. Multivariate techniques indicated lack of robustness in predicting RDS and SDS as the models only explained <50% of the variance. More robust regression models were obtained for RS, explaining >60% of its variation. Basic grain quality indices explained NISF variations better than pasting and thermal properties.

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Abbreviations: AM, apparent amylose; AS, alkali spreading value; BD, breakdown; CP, crude protein; CT, conclusion temperature; CT_n, cytosine-thymine repeat; FV, final viscosity; GC, gel consistency; GE, gelatinization enthalpy; GR,

gelatinization range; HPV, hot paste viscosity; HSD, honestly significant difference; NISF, nutritionally important starch fraction; OT, gelatinization onset; PC, principal component; PLS, partial least square; PT, peak temperature; PV, peak viscosity; RDS, rapidly digestible starch; RMSE, root mean square error; RS, resistant starch; RVA, Rapid Visco Analyzer; RVU, rapid visco-unit; SB, setback; SDS, slowly digestible starch; TS, total starch; TSB, total setback.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

1 Introduction

Anent nutrition and health, starches in food are generally classified into rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS). RDS is the fraction that is hydrolyzed to glucose within 20 min; SDS is converted to glucose between 20 and 120 min; whereas, RS remains undigested after 120 min [1]. SDS goes through a slow but complete hydrolysis in the small intestine and its potential health benefits are linked to a stable glucose metabolism, diabetes management, mental performance, and satiety [2]. RS escapes digestion in the small intestine but is partially or entirely fermented in the colon. It shows promising physiological impact in the prevention of colon cancer, postprandial glycemia and insulinemia, hyperlipidemia, gall stone formation, and cardiovascular diseases [3].

Available methods for the determination of nutritionally important starch fractions (NISFs, *i.e.*, RDS, SDS, and RS) are tedious and time consuming. Developing simple, fast, and accurate estimators will be a valuable innovation. It will also further our understanding of the genetic and chemical factors that influence NISF. In the case of rice, most cooking applications involve the use of whole grains so it is relevant to determine NISF in cooked rice as eaten. Cooked rice quality is primarily gauged by apparent amylose (AM) content [4]. As quality tends to vary among cultivars with similar amylose content, certain secondary parameters have been used for improved differentiation, such as protein content, alkali spreading value (AS), gel consistency (GC), viscosity, and pasting properties with a Brabender or a Rapid Visco Analyzer (RVA), and thermal characteristics with a differential scanning calorimeter [4].

Likewise, the use of microsatellite genetic markers as rice grain quality indicators is becoming popular and has been found useful in categorizing rice according to cooking and processing quality [5–8].

Previous works have shown that RS is positively correlated with amylose content [4, 9–14]. Hu *et al.* [15] identified a high-amylose rice cultivar with low paste viscosity profiles (peak, hot paste, and cool paste) but high in RS. On the contrary, a recent study on rice starch indicated that breakdown (BD) viscosity correlated positively with RDS, negatively with SDS, and insignificantly with RS [16]. Literature is lacking concerning potential associations of cooked rice NISF with secondary rice quality indices (*e.g.*, viscosity and pasting characteristics, thermal properties, and genetic markers). Hence, it will be meaningful to examine such correlations more carefully through the use of chemometrics. Chemometrics is a specialized discipline for extracting information from multivariate chemical data using tools of statistics and mathematics [17]. In the present work, it was hypothesized that aside from amylose, some of the commonly used secondary parameters of rice quality may be valuable predictors of cooked rice NISF.

2 Materials and methods

2.1 Rice samples

Sixteen rice cultivars were evaluated as part of the 2007 Uniform Regional Rice Nursery conducted in Stuttgart, Arkansas and Beaumont, Texas (Table 1). The field study was conducted as a randomized complete block design

Table 1. Grain type, genotype (CT_n repeat), and amylose class of the rice samples

Cultivar	Grain type	CT _n repeat	^{a)} Amylose class
Arborio	Medium	18	Low (12.9)
Bengal	Medium	18	Low (10.0)
Bowman	Long	11	High (27.3)
Cheniere	Long	20	High (28.4)
Cocodrie	Long	20	High (28.6)
Dixie Belle	Long	11	High (28.9)
HB-1	Long	17	Waxy (0.3)
Hidalgo	Long	18	Low (10.5)
Jupiter	Medium	17	Low (12.6)
L205	Long	11	High (27.1)
Rondo	Long	11	High (26.4)
Sabine	Long	11	High (28.3)
Sierra	Long	11	High (31.4)
Tesanai 2	Medium	11	High (26.7)
Wells	Long	14	Intermediate (21.8)
XP723	Long	14:20	Intermediate (20.1)

a) Value in parenthesis is average amylose content (% dry basis) of Texas sample.

with two field replications. Head rice samples were produced from 125 g of rough rice harvested from the plots as they approached 18–22% MC. Samples were genotyped according to cytosine-thymine repeats (CT_ns) using the whole kernel alkali DNA extraction method [5–7] and Waxy RM190 microsatellite CT_n markers [5]. Grain type was determined based on grain length/width ratios of ≥ 3.0 for long, 2.0–2.9 for medium, and < 2.0 for short measured using a WinSeedle Pro 2005a™ image analysis system (Regent Instruments, Sainte-Foy, Quebec, Canada).

2.2 Basic grain quality indices

Head rice was ground into flour with a cyclone sample mill (Udy Corp., Fort Collins, CO, USA) fitted with a 0.50-mm screen. AM content on flour samples was determined by iodine colorimetry [18]. Samples were classified based on AM as follows: 0–2% (waxy), 2–12% (very low), 12–20% (low), 20–25% (intermediate), and 25–33% (high). Crude protein (CP) content was measured by combustion method on a nitrogen analyzer (FP-428, LECO, St. Joseph, MI, USA). A factor of 5.95 was used for converting nitrogen content to protein. Moisture content was determined by the AACC Method 44-15A [19]. AM and CP were expressed as percent dry basis. GC was determined according to Cagampang *et al.* [20] with modifications. A 25-mg rice flour sample was weighed into a 10 mm $\times$ 75 mm test tube, wetted with 50 μ L of thymol blue solution (0.025% thymol blue in 85% ethanol), and vortexed for 10 s. It was immediately added with 0.5 mL of 0.2 N KOH, covered with marble, and heated in a boiling water bath for 7 min. The water level of the bath was maintained to cover the lower 1/3 of the tube. The tube was taken out, cooled at room temperature for 5 min, and then chilled in an ice water bath (8–9°C) for 10 min. The chilled test tube was laid horizontally on a table for 30 min and the length of gel was measured (in mm) from the bottom of the tube to the top of the gel. AS was determined following the method of Little *et al.* [21]. 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 immerse the grains. The dish was covered and left undisturbed at room temperature for 23 h. The extent of spreading was rated using a 7-point numerical scale 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.

2.3 Thermal properties

Thermal properties were assessed with a multi-cell differential scanning calorimeter (TA Instruments, New Castle,

DE, USA). In a stainless steel ampoule, a 300-mg head rice sample (~ 15 grains) was added with 600 μ L deionized water, tightly sealed, equilibrated for 30 min at room temperature, and heated from 25 to 140°C at a rate of 2°C/min. Water was used in running the baseline and baseline subtractions were made on the thermal curves of the samples. Thermal transitions were defined in terms of gelatinization enthalpy (GE), range (GR), onset (OT), peak (PT), and conclusion (CT) temperatures. GR was obtained as the difference between CT and OT. The software Universal Analysis 2000 (TA Instruments) was used in the data acquisition.

2.4 Pasting properties

Pasting properties were measured with an RVA (Model 4, Newport Scientific, Warriewood, New South Wales, Australia) according to AACC Approved Method 61-02 [19]. Rice flour (3.0 g, 12% moisture) was weighed into an RVA aluminum canister and 25 g of distilled water was added. The sample was first held for 1.5 min at 50°C, heated to 95°C at 12°C/min, held for 2.0 min at 95°C, cooled to 50°C at 12°C/min, and finally held for 1.5 min at 50°C. The temperature corresponding to the initial increase in viscosity was designated as pasting temperature. Viscosity values were recorded in rapid visco-units (RVUs), in which 1 RVU is equivalent to 12 centipoise. The viscosity variables measured were: peak viscosity (PV), hot paste viscosity (HPV or trough), final viscosity (FV), BD, setback (SB), and total setback (TSB). BD was calculated as PV minus HPV; SB as FV minus PV; and TSB as FV minus HPV.

2.5 Cooking rice and starch digestibility assay

Head rice (5 g) was weighed into a long-type 150 mL beaker, added with 10 mL of deionized water, and allowed to stand for 15 min. The beaker was transferred into a home-style rice cooker with the inner pan containing 175 mL of deionized water, and then steam-cooked until automatic shutoff (about 25 min). Only four samples (in separate beakers) were cooked at a time to minimize variation in the subsequent analyses due to time factor. Cooked rice samples were allowed to cool inside the rice cooker for 15 min prior to analyses. To simulate a well-chewed sample, cooked rice was minced by passing it through a garlic press (GRIP-EZ®, Norpro, Everett, WA, USA) that has 61 holes (2.0 mm in diameter) on its base, three times. The different starch fractions (total starch (TS), RDS, SDS, and RS) were analyzed *in vitro* according to the Englyst *et al.* [1] method with modifications.

A 0.50 g minced freshly cooked rice sample (in duplicate) was weighed into a 50-mL graduated conical, screw-cap centrifuge tube, and 25 mg of guar gum, a

12.7 mm × 3 mm magnetic stir bar, and 10 mL of acetate buffer (0.1 M, pH = 5.2) were added. The tube was capped and incubated in a water bath at 37°C with stirring at 160 rpm for 10 min, and then treated with 2.5 mL of amylase cocktail (3800 U/mL pancreatin, 188 U/mL invertase, 13 U/mL amyloglucosidase from Sigma–Aldrich, St. Louis, MO, USA). The water bath set-up consisted of a 190 × 100 crystallizing dish half-filled with deionized water and fitted with a centrifuge tube rack to hold the samples in place. The dish was heated atop a digital hot plate/stirrer (Dataplate PMC 720 Series, Barnstead International, Dubuque, IA, USA) equipped with a temperature probe to regulate the water temperature. RDS and SDS were determined on 0.25 mL aliquots as the glucose released by enzymatic hydrolysis after 20 and 120 min, respectively. The remaining mixture was heated in a boiling water bath, treated with 7 M KOH, and hydrolyzed further with amyloglucosidase (50 U/mL) to determine TS. RS, which is the starch fraction that remained unhydrolyzed after 120 min, was obtained by subtracting RDS and SDS from TS ($RS = TS - [RDS + SDS]$) [1]. The amount of glucose released at a given period of hydrolysis was determined with a D-glucose oxidase-peroxidase assay kit (Megazyme, Wicklow, Ireland).

2.6 Statistical analysis

JMP® software version 7 (SAS Software Institute, Cary, NC, USA) was used in the chemometric analysis of the experimental data. The 16 samples from Arkansas and 16 samples from Texas consisted of the same set of cultivars but were considered as different data points due to the well known influence of growing environment on rice quality [22]. As a result, the total number of observations (n) used in the analyses was 32 instead of 16. Tukey's honestly significant difference (HSD) test was used to identify significantly different means between groups. Bivariate correlation was carried out by the Pearson-product moment approach. Principal component (PC) analysis was performed to obtain a simplified view of

the relationship among samples, and between NISF and rice quality indices. A stepwise regression approach was employed in the multiple linear regression analysis, and cross-validation was chosen for the partial least squares (PLSs) regression analysis. Coefficients of determination (R^2), correlation coefficients (R), and root mean square errors (RMSEs) were used as indicators of significance for the regressions models.

3 Results and discussion

3.1 Sample genotype and NISF

Table 1 presents the grain type, CT_n and amylose class of the cultivars used in this study. The samples were associated with 5 CT_n alleles of the *Waxy* microsatellite RM190 (CT_{11} , CT_{14} , CT_{17} , CT_{18} , and CT_{20}) and the number of samples (n) belonging to each CT_n marker was as follows: $CT_{11} = 14$, $CT_{14} = 4$, $CT_{17} = 4$, $CT_{18} = 6$, and $CT_{20} = 4$. The CT_n microsatellite markers were also used in designating the amylose class of each cultivar as reported by previous workers [5–7]. CT_{11} and CT_{20} were associated with high AM; CT_{14} with intermediate AM; CT_{18} with low AM; and CT_{17} with low AM or waxy (0–5% AM). The hybrid cultivar XP723 was actually associated with both CT_{14} and CT_{20} alleles but was included in the CT_{14} group because its AM content was intermediate (Table 2). Typical US cultivars having the CT_{20} allele are also intermediate-AM. However, the CT_{20} samples used in this study (Cheniere and Cocodrie) were high-AM type due to the presence of single nucleotide polymorphic (SNP) sites in exon 10 of the *waxy* gene [7, 8]. A more detailed report on the association of rice AM classes with microsatellite genetic markers has been described elsewhere [7]. Grain type was derived based on milled rice grain length/width ratio (data not shown). Eight samples were medium-grain type, while the remaining 24 were long-grain. Table 2 shows the cooked rice NISF of the samples grouped according to CT_n . Considering the ranges on Table 2 and the NISF

Table 2. Ranges and means ± SD for cooked rice NISFs of rice samples group according to CT_n repeat^{a)}

Genetic marker group	Amylose class	RDS (%)		SDS (%)		RS (%)	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
CT_{11} ($n = 14$)	High	53.9–63.0	59.9 ± 2.5 ^b	11.0–23.2	18.2 ± 4.1 ^a	3.7–7.9	6.1 ± 1.3 ^a
CT_{14} ($n = 4$)	Intermediate	58.3–64.0	61.8 ± 2.7 ^{ab}	12.4–16.3	14.7 ± 1.6 ^b	3.2–5.1	4.3 ± 0.9 ^b
CT_{17} ($n = 4$)	Waxy/low	60.0–67.6	63.5 ± 3.3 ^a	15.7–19.8	17.9 ± 2.0 ^a	2.2–6.0	3.5 ± 1.7 ^{bc}
CT_{18} ($n = 6$)	Low	56.1–65.6	60.7 ± 3.6 ^{ab}	12.8–24.2	17.7 ± 5.3 ^a	1.2–3.9	2.5 ± 0.9 ^c
CT_{20} ($n = 4$)	High	58.6–62.3	60.3 ± 1.5 ^{ab}	16.2–22.7	18.8 ± 3.2 ^a	5.5–7.7	6.4 ± 1.0 ^a

^{a)} Means in a column with a common superscript letter(s) are not significantly different from one another based on Tukey's HSD test ($p > 0.05$).

Figure 1. SDS and RS of the rice samples grouped according to CT_n s. (The 32 data points represented 16 cultivars grown in 2 environments.)

profiles of the different samples in Fig. 1, overlapping occurred for RDS, SDS, and RS among CT_n groups and among samples in each group. Taking the means into account (despite unequal n among CT_n groups), the CT_{11} samples (high-AM type) were generally associated with higher percentages of SDS and RS, and a lower RDS. The CT_{20} samples (also high-AM type) were comparable to the CT_{11} group in SDS and RS but their comparatively higher RDS was more similar to the other CT_n groups (CT_{14} , CT_{17} , and CT_{18}). The CT_{17} and CT_{18} samples (waxy to low-AM type) were generally characterized by a lower percentage of RS, but with SDS and RDS that were both comparable to the CT_{20} group. On the other hand, both RS and SDS of the CT_{14} samples (intermediate-amylose type) tended to be lower.

3.2 Bivariate correlations

Tables 3–5 list the ranges and means of the basic grain quality indices, pasting characteristics, and thermal properties, respectively, of the different rice samples grouped according to CT_n . AM widely varied from 0 to 32.6% and the values were in total agreement with the amylose class based on CT_n marker (Table 3). The AM range among CT_n groups did not overlap contrary to the other physico-chemical properties. CP ranged from 6.6 to 10.4%, but on average was not different for all the CT_n groups. On average, the AS of the 5 CT_n groups did not differ as well. As to the GC, the noticeable difference was between CT_{14} and CT_{20} groups, in which it was higher for the latter (Table 3). For pasting properties (Table 4), it is interesting to note that the high-amylose CT_n groups (CT_{11} and CT_{20}) were differentiated from each other by lower PV, FV, SB, and TSB of the latter. Furthermore, the waxy to low amylose groups (CT_{17} and CT_{18}) were differentiated by the lower PV of the former (Table 4). A more detailed report on the association of rice pasting properties with microsatellite genetic markers has been described elsewhere [8]. In terms of thermal properties, the OT, PT, GR, and GE of the samples in the CT_{18} group were noticeably lower compared to the CT_{20} group; whereas, those of CT_{11} , CT_{14} , and CT_{17} group were either similar to CT_{18} or CT_{20} (Table 5). It is well known that low-amylose cultivars, such as those in the CT_{18} group, tend to gelatinize at lower temperatures, although cultivars of similar amylose content may differ in thermal properties due to differences in amylopectin fine structure [23, 24].

Bivariate correlations between NISF and the 16 variables (AM, AS, BD, CP, FV, GC, GE, GR, OT, PV, PT, RDS, RS, SB, SDS, and TSB) given in Tables 3–5 are presented in Table 6. RS was positively correlated with AM, FV, SB, TSB, and PT (peak gelatinization temperature). RS also showed significant inverse relationship with BD. The positive linear correlation between RS and AM has been reported in previous works [4, 9–14]. On the other hand,

Table 3. Ranges and means $\pm$ SD for the basic grain quality indices of rice samples grouped according to CT_n repeat^{a)}

Genetic marker group	AM (%)		CP (%)		AS		GC (mm)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
CT_{11} ($n = 14$)	26.4–32.6	29.2 $\pm$ 2.0 ^a	6.6–10.3	8.3 $\pm$ 1.0 ^a	4.0–7.0	4.9 $\pm$ 1.3 ^a	14.0–73.5	41.4 $\pm$ 27.0 ^{ab}
CT_{14} ($n = 4$)	19.6–22.7	21.3 $\pm$ 1.3 ^b	7.2–9.3	8.2 $\pm$ 1.0 ^a	3.1–4.0	3.5 $\pm$ 0.4 ^a	14.5–34.5	24.2 $\pm$ 8.4 ^b
CT_{17} ($n = 4$)	0.0–12.8	6.3 $\pm$ 6.7 ^d	6.7–9.1	7.9 $\pm$ 1.1 ^a	2.5–6.0	4.4 $\pm$ 1.9 ^a	37.0–50.5	46.1 $\pm$ 6.2 ^{ab}
CT_{18} ($n = 6$)	7.9–11.7	10.0 $\pm$ 1.5 ^c	7.3–10.4	8.8 $\pm$ 1.2 ^a	2.0–6.0	4.7 $\pm$ 2.0 ^a	28.5–72.5	46.2 $\pm$ 16.8 ^{ab}
CT_{20} ($n = 4$)	28.4–31.8	29.9 $\pm$ 1.7 ^a	7.2–9.3	8.3 $\pm$ 1.0 ^a	3.6–4.0	3.9 $\pm$ 0.2 ^a	37.0–74.5	57.0 $\pm$ 16.9 ^a

a) Means in a column with a common superscript letter(s) are not significantly different from one another based on Tukey's HSD test ($p > 0.05$).

Table 4. Ranges and means $\pm$ SD for the pasting properties of head rice samples grouped according to CT_n repeat^{a)}

Genetic marker group	PV (RVU)		FV (RVU)		BD (RVU)		SB (RVU)		TSB (RVU)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
CT ₁₁ (n = 14)	258–348	295 $\pm$ 25 ^{ab}	321–542	383 $\pm$ 53 ^a	53–113	96 $\pm$ 20 ^{bc}	43–194	88 $\pm$ 35 ^a	120–280	184 $\pm$ 39 ^a
CT ₁₄ (n = 4)	279–318	294 $\pm$ 18 ^{ab}	266–307	284 $\pm$ 18 ^b	124–171	142 $\pm$ 20 ^a	–43–8	–11 $\pm$ 24 ^c	123–142	131 $\pm$ 8 ^b
CT ₁₇ (n = 4)	228–298	264 $\pm$ 31 ^b	150–249	200 $\pm$ 49 ^c	118–130	125 $\pm$ 6 ^{ab}	–83–42	–63 $\pm$ 20 ^d	38–88	61 $\pm$ 26 ^c
CT ₁₈ (n = 6)	240–356	307 $\pm$ 40 ^a	213–295	242 $\pm$ 31 ^{bc}	76–203	148 $\pm$ 49 ^a	–121–14	–65 $\pm$ 54 ^d	74–93	82 $\pm$ 8 ^c
CT ₂₀ (n = 4)	162–211	188 $\pm$ 20 ^c	190–261	230 $\pm$ 37 ^{bc}	74–94	85 $\pm$ 9 ^c	23–68	42 $\pm$ 21 ^b	110–142	127 $\pm$ 16 ^b

a) Means in a column with a common superscript letter(s) are not significantly different from one another based on Tukey's HSD test ($p > 0.05$).

Table 5. Ranges and means $\pm$ SD for the thermal properties of head rice samples grouped according to CT_n repeat^{a)}

Genetic marker group	Onset GT (°C)		Peak GT (°C)		GR (°C)		GE (J/g)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
CT ₁₁ (n = 14)	82.5–88.1	85.5 $\pm$ 1.5 ^{ab}	96.8–111.0	104.9 $\pm$ 3.5 ^{ab}	35.6–41.8	40.1 $\pm$ 1.8 ^{ab}	3.1–10.3	8.3 $\pm$ 2.0 ^b
CT ₁₄ (n = 4)	82.1–87.3	85.7 $\pm$ 2.4 ^{ab}	104.0–108.9	106.3 $\pm$ 2.1 ^{ab}	42.0–44.3	43.0 $\pm$ 1.0 ^a	10.5–12.3	11.5 $\pm$ 0.9 ^{ab}
CT ₁₇ (n = 4)	81.8–87.2	84.2 $\pm$ 2.5 ^b	97.6–104.8	101.8 $\pm$ 3.4 ^{bc}	38.4–43.8	41.7 $\pm$ 2.5 ^{ab}	3.9–14.5	9.3 $\pm$ 5.2 ^{ab}
CT ₁₈ (n = 6)	82.1–87.2	84.5 $\pm$ 1.9 ^b	92.8–106.8	99.9 $\pm$ 4.8 ^c	30.8–41.7	39.0 $\pm$ 4.1 ^b	4.2–12.0	8.2 $\pm$ 3.5 ^b
CT ₂₀ (n = 4)	86.9–88.8	87.6 $\pm$ 0.9 ^a	107.1–108.2	107.5 $\pm$ 0.5 ^a	37.4–45.1	41.1 $\pm$ 4.0 ^{ab}	9.8–13.1	11.9 $\pm$ 1.4 ^a

a) Means in a column with a common superscript letter(s) are not significantly different from one another based on Tukey's HSD test ($p > 0.05$).

Table 6. Bivariate correlation matrix^{a),b)}

Property	RDS	SDS	RS
Basic quality indices			
AM	–0.312 ^{ns}	0.106 ^{ns}	0.788 ^{***}
Total protein	0.188 ^{ns}	–0.347 ^{ns}	0.024 ^{ns}
AS	0.054 ^{ns}	–0.227 ^{ns}	0.144 ^{ns}
GC	–0.019 ^{ns}	0.459 [*]	0.241 ^{ns}
Thermal properties			
Onset GT	–0.145 ^{ns}	0.134 ^{ns}	0.233 ^{ns}
Peak GT	–0.132 ^{ns}	0.115 ^{ns}	0.395 [*]
GR	–0.277 ^{ns}	0.164 ^{ns}	0.096 ^{ns}
GE	–0.016 ^{ns}	0.155 ^{ns}	–0.032 ^{ns}
Pasting properties			
PV	–0.061 ^{ns}	–0.083 ^{ns}	–0.284 ^{ns}
FV	–0.124 ^{ns}	–0.196 ^{ns}	0.501 ^{**}
BD viscosity	0.130 ^{ns}	0.087 ^{ns}	–0.526 ^{**}
SB viscosity	–0.111 ^{ns}	–0.174 ^{ns}	0.744 ^{***}
TSB	–0.070 ^{ns}	–0.183 ^{ns}	0.687 ^{***}

a) Statistical significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns not significant, $p > 0.05$.

b) RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

Benmoussa *et al.* [16] reported a significant positive correlation between RDS and BD and a negative correlation between SDS and BD but such associations were not observed in this work. It should be pointed out that Benmoussa *et al.* [16] used rice starch samples in assaying NISF and pasting characteristics; whereas, in this work whole grains were used in the NISF assays and rice flour for the viscosity and pasting tests. RDS did not show significant correlations with any of the physicochemical variables, whereas, SDS was significantly correlated only with GC. The lack of significant correlations between these physicochemical variables with RDS and SDS imply that these variables are not useful by themselves for predicting RDS or SDS.

3.3 Principal component analysis

PC analysis was carried out to show the interrelationships among the rice samples and physicochemical variables in a visually intuitive manner [17]. A total of 14 PCs completely explained the variance of the 16-variable dataset. The first five PCs (PC1–5) had Eigen values ≥ 1 and accounted for 83.3% of the data variation. PC1 and PC2, with respective Eigen values of 4.8 and 3.8, accounted for 30.2 and 23.8% of the variation, respectively. A similarity map based on score and loading plots for PC1 and PC2 is shown in Fig. 2. The score plot (Fig. 2A) shows that the CT₁₁ and CT₂₀ samples were mainly positioned on the first (+x, +y) and fourth quadrants (+x, -y) of the similarity map, whereas, the CT₁₄, CT₁₇, and CT₁₈ were distributed on the second (-x, +y) and third quadrants (-x, -y). Hence, the sit-together rule of PCs analysis points to the close resemblance in physicochemical properties among CT₁₁ and CT₂₀ samples, and among CT₁₄, CT₁₇, and CT₁₈ samples. Considering the samples within a CT_n group, those with the CT₂₀ marker appeared to be more related in properties than the samples from other groups as they were loaded more close to each other on the similarity map. The three NISF variables were positioned on three different quadrants as follows: RS on the first, SDS on the second, and RDS on the third (Fig. 2B). RS and RDS were loaded on opposite quadrants indicating that these two variables are inversely related. SDS had some direct association with RS owing to their common >0 PC2 load. In addition, SDS was directly associated with RDS to some extent due to their common <0 PC1 load.

RS was loaded on the first quadrant together with AM, TSB, SB, OT, and PT and by the sit-together rule, these variables are closely associated. Such associations nearly matched those obtained with bivariate correlation analysis in which RS significantly correlated with AM, FV, SB, TSB, and PT (Table 6). On the other hand, SDS was loaded on the second quadrant along with GC, GR, and GE. This association is somewhat different from that obtained with

Figure 2. A similarity map based on score (A) and variable (B) loadings of the first (PC1) and second components (PC2) derived by PC analysis.

bivariate correlation analysis in which SDS was significantly correlated with GC only. PV, FV, and AS were scattered on the fourth quadrant and positioned opposite SDS to indicate their inverse relationship. RDS was positioned on the third quadrant all by itself to imply that its direct association with other physicochemical properties was weak. It should be recalled that RDS did not show significant correlation with any of the physicochemical variables when bivariate correlation analysis was used (Table 6). PC analysis is not a quantitative modeling tool so that other multivariate techniques are necessary to further verify the relation of the different physicochemical variables with NISF.

Table 7. Multiple linear regression matrix^{a),b)}

Number of variables	^{a)} Predictor and regression coefficient	Intercept	R^2	R	RMSE	Prob > F
RDS						
1	AM (−0.090)	62.77	0.10 ^{ns}	0.32	2.76	0.0818
2	AM (−0.195), TSB (0.024)	61.84	0.18 ^{ns}	0.42	2.68	0.0585
3	AM (−0.0271), PV (−0.021), TSB (0.038)	67.53	0.26*	0.51	2.59	0.0345
4	AM (−0.262), GR (−0.177), PV (−0.023), TSB (0.037)	75.02	0.33*	0.57	2.52	0.0260
5	AM (−0.231), AS (0.813), FV (−0.043), GR (−0.198), TSB (0.086)	71.59	0.40*	0.63	2.41	0.0147
13	All variables	66.09	0.49 ^{ns}	0.70	2.53	0.1261
SDS						
1	GC (0.081)	14.19	0.21**	0.46	3.45	0.0083
2	GC (0.073), CP (−1.026)	23.08	0.28**	0.53	3.34	0.0079
3	AM (0.216), GC (0.070), TSB (−0.041)	15.48	0.35**	0.59	3.24	0.0066
4	AM (0.309), GC (0.082), PV (0.027), TSB (−0.058)	7.55	0.42**	0.65	3.11	0.0039
5	AM (0.274), AS (−1.454), FV (0.063), GC (0.105), TSB (−1.130),	12.24	0.53***	0.73	2.85	0.0008
13	All variables	73.12	0.62*	0.79	2.93	0.0170
RS						
1	AM (0.152)	1.58	0.62***	0.79	1.19	<0.0001
2	AM (0.150), GC (0.019)	0.78	0.67***	0.82	1.14	<0.0001
3	AM (0.079), GC (0.026), SB (0.011)	1.81	0.72***	0.85	1.05	<0.0001
4	AM (0.074), GC (0.022), PV (−0.006), SB (0.011)	3.62	0.74***	0.86	1.04	<0.0001
5	AM (0.077), GC (0.018), OT (−0.217), PV (−0.016), TSB (0.017)	23.23	0.77***	0.88	1.01	<0.0001
13	All variables	26.27	0.77***	0.88	1.13	0.0002

a) Statistical significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns not significant, $p > 0.05$.

b) AM, apparent amylose; AS, alkali spreading value; CP, crude protein; FV, final viscosity; GC, gel consistency; GR, gelatinization range; OT, onset gelatinization temperature; PV, peak viscosity; SB, setback viscosity; and TSB, total setback viscosity; R^2 coefficient of determination; R , regression coefficient; RMSE, root mean square error.

3.4 Multiple linear regression

Stepwise multiple linear regression analysis was used to create models for each NISF (as response variable) regressed against 13 predictor variables. Only the best-fit regression models, with 1–5 predictor variables that gave the highest R^2 and lowest RMSE are presented in Table 7. As proposed by several statisticians, the number of predictor variables to consider in generating multiple linear regression models should not be more than 1/5 of the total number of observations ($n = 32$ in this work) in order to minimize the possibility of chance correlations [25]. The best-fit models shown in Table 7 explained 10–77% ($R^2 = 0.10$ – 0.77) of the variation in NISF (RDS, SDS, and RS) among the 32 data points. The models for predicting RDS showed a relatively low R^2 (0.1–0.5) and a high RMSE (2.4–2.8). In fact, only three models that consisted of 3–5 predictor variables were

significant at $p \leq 0.5$. It was observed that both AM and TSB were common to the three models; AS, GR, FV, and PV were also included but with a lower frequency. The selected models for predicting SDS were all significant, with AM, GC, and TSB as the common variables. The other variables included in the model but to a lower frequency were AS, CP, FV, and PV. Higher R^2 (0.6–0.8) and lower RMSE (1.0–1.2) were obtained in the models for predicting RS, with AM and GC as the common variables. Overall, the results of the multiple linear regression analysis implicate the importance of AM, GC, and TSB in predicting NISF.

3.5 Partial least squares regression

PLSs regression is typically used when the independent variables are correlated, or the number of the independent variables exceeds the number of observations. PLS regression differs from standard multiple linear regression

as it accounts the variation of both response and predictor variables [17]. Bivariate correlation analysis showed that collinearity tended to exist among pasting variables (PV, FV, BD, SB, and TSB) as these variables were all derived from the same pasting profile/curve measured by a single instrument, the RVA (data not shown). The same is true for the thermal variables (GE, GR, OT, and PT), which were all determined with a multi-cell differential scanning calorimeter. Hence, PLS regression analysis appeared to be more appropriate to use in modeling NISF (as response variable) with pasting and thermal properties (as predictors). The PLS regression models were derived by regressing the 13 predictor variables in groups as follows: basic quality indices (AM, AS, CP, and GC), pasting properties (BD, FV, PV, SB, and TSB), and thermal properties (GE, GR, OT, and PT). Deriving the models involved the extraction of minimum number of latent vectors that gave the lowest prediction RMSE upon cross-validation. A summary of the coefficients and other important parameters obtained from PLS regression analysis is shown in Table 8. Figures 3 and 4 show the plots for actual and predicted SDS and RS, respectively, using the PLS regression models. The prediction models for RDS generally lacked robustness, explaining only 2–12% ($R^2 = 0.02$ – 0.12) of the variation of the response variable. The models were also characterized by high prediction RMSE (1.09–1.15). Even then, the predictor variables that comprised the basic quality indices group turned out as a better predictor for

RDS compared with the pasting and thermal property groups. For SDS, the models explained 4–31% ($R^2 = 0.04$ – 0.31) of its variation, and only the model derived with the basic grain quality indices as predictors was noteworthy (Fig. 3). On the other hand, RS was adequately predicted by the basic quality indices, pasting, and thermal properties, with 38–75% ($R^2 = 0.30$ – 0.75) of its variation explained (Fig. 4). Among the three groups of predictor variables, the basic quality indices also appeared as better predictors for RS.

4 Conclusions

Chemometrics elucidated the relationship of NISF with other rice physicochemical properties. CT₁₁ was associated with higher amount of RS and SDS, and lower amount of RDS; CT₁₄ was associated with low SDS; and CT₁₇ and CT₁₈ with low RS. The CT₂₀ cultivars were similar to CT₁₁ in SDS and RS contents, and to CT₁₄, CT₁₇, and CT₁₈ with respect to RDS. PC analysis provided a simplified view of the interrelationship among samples, among variables, and between samples and variables. Bivariate correlation showed that RDS had no significant correlation with any of the physicochemical properties; whereas, SDS correlated positively with GC. RS correlated positively with AM, SB, TSB, and PT; and negatively with BD. Except GC, no single predictor variable could decently estimate SDS and RDS.

Table 8. PLSs regression matrix^{a)}

Response variable	Predictor variable and coefficient	Intercept	Latent vectors	R^2	R	RMSE
RDS	AM (−0.082), AS (0.100), CP (0.479), GC (−0.002)	58.26	1	0.12	0.35	1.09
RDS	BD (0.004), FV (−0.002), PV (−0.001), SB (−0.001), TSB (−0.001)	61.42	1	0.02	0.14	1.09
RDS	GE (−0.010), GR (−0.137), OT (−0.134), PT (−0.057)	83.86	1	0.05	0.22	1.08
RDS	All 13 variables	76.28	1	0.12	0.35	1.15
SDS	AM (0.032), AS (−0.483), CP (−1.008), GC (0.063)	24.82	1	0.31	0.56	0.95
SDS	BD (0.003), FV (−0.003), PV (−0.002), SB (−0.003), TSB (−0.004)	19.42	1	0.04	0.20	1.06
SDS	GE (0.087), GR (0.065), OT (0.114), PT (0.046)	−0.26	1	0.04	0.20	1.05
SDS	All 13 variables	7.33	1	0.23	0.48	1.04
RS	AM (0.147), AS (0.191), CP (0.043), GC (0.021)	−0.41	1	0.68	0.82	0.66
RS	BD (0.004), FV (0.001), PV (−0.018), SB (0.009), TSB (0.016)	6.58	3	0.66	0.81	0.72
RS	GE (−0.442), GR (−0.045), OT (0.059), PT (0.412)	−37.21	3	0.38	0.62	0.89
RS	All 13 variables	2.26	3	0.75	0.87	0.63

a) AM, apparent amylose; AS, alkali spreading value; BD, breakdown viscosity; CP, crude protein; FV, final viscosity; GC, gel consistency; GE, gelatinization enthalpy; GR, gelatinization range; OT, onset gelatinization temperature; PV, peak viscosity; PT, peak gelatinization temperature; RDS, rapidly digestible starch; RS, resistant starch; SB, setback viscosity; SDS, slowly digestible starch; TSB, total setback viscosity; R^2 coefficient of determination; R , regression coefficient; RMSE, root mean square error.

Figure 3. Actual and predicted SDS by PLS regression models with basic quality indices (A), thermal properties (B), and pasting properties (C) as predictor variables.

Moreover, multivariate techniques (multiple linear regression and PLSs regression) indicated that RS was better predicted by the measured variables than SDS and RDS. Regression models for RDS and SDS generally lacked robustness, were characterized by low R^2 and high prediction RMSE, and explained less than 50% of the

Figure 4. Actual and predicted RS by PLS regression models with basic quality indices (A), thermal properties (B), and pasting properties (C) as predictor variables.

response variable's variation. In contrast, regression models for RS explained 62–77% of its variance. Variables that comprised the basic quality indices were better estimators of cooked rice NISF than pasting or thermal properties. It appears that starch properties other than those included in this study may better predict SDS and RDS.

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5 References

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