RESEARCH ARTICLE

Starch properties of malted barley in relation to real degree of fermentation

James Patindol[1], Guadalupe Mendez-Montealvo[2] and Ya-Jane Wang[2]

[1] USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA
[2] Department of Food Science, University of Arkansas, Fayetteville, AR, USA

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Real degree of fermentation (RDF) is an important measure of brewhouse performance in the production of beer. In this work, the relationship of RDF with malted barley starch properties was investigated. Starches were isolated from 25 malted barley samples and analyzed for molecular size distribution (by HPSEC), AP structural features (by high-performance size exclusion chromatography with multi-angle laser light scattering and RI detectors), and thermal properties (by DSC). Cluster analysis, analysis of variance, principal component analysis, bivariate correlation, and multiple linear regression analysis were used in establishing correlations. RDF, AM, and AP content were 74.0–80.1%, 25.2–34.4%, and 65.6–74.9%, respectively. Starch gelatinization enthalpy was 6.4–8.1 J/g, and onset gelatinization temperature was 58.5–64.3°C. AP and AM content had a positive and negative impact on RDF, respectively. The effect of thermal properties (starch gelatinization and AM-lipid complex melting) on RDF was not clear. Starch properties explained up to 86% of RDF variance to indicate that other malted barley constituents and/or properties also contribute to cultivar variations in RDF.

Keywords:
AM / AP / Malted barley / Real degree of fermentation / Starch

1 Introduction

In the manufacture of beer, real degree of fermentation (RDF) is an important indicator of brewhouse performance. It measures the extent to which the sugars in wort are fermented into alcohol. Percent RDF in the 50's represent full-bodied beers with over 40% of the original extract unfermented; RDF values in the 80's represent highly attenuated beers with $<$20% of their original extract unfermented [1]. Brewers can also use RDF as a guidepost to mouthfeel. The lower the RDF, the fuller is the taste and palate; the higher RDF, the lighter and drier is the beer [2]. The fermentable sugars in wort, which constitute 61–65% of the total extract, are produced from the enzymatic hydrolysis of starch and polysaccharides in the malt and adjunct material during mashing [3]. Hence, the composition of the extract is essentially a function of starch content, although it is also influenced by other characteristics of the barley kernel such as hull content and cell wall thickness, and may be modified by water sorption, enzyme distribution, and other endosperm characteristics [3].

Mashing conditions need to be controlled in order to produce the highest quantity of fermentable sugars such that the final beer will have high alcohol and low carbohydrate concentrations [3, 4]. One important thing to consider is the use of temperatures that are high enough to cause complete starch gelatinization in the malted barley without inactivating its amylolytic enzymes [5–7]. If gelatinization is restricted during the process, incomplete conversion to dextrins, sugars, and ultimately, alcohol, may occur [5]. Gelatinization is affected by starch granule size. Large-granule barley starches gelatinize earlier than small

Correspondence: Professor Ya-Jane Wang, Department of Food Science, University of Arkansas, Fayetteville, AR, USA
E-mail: yjwang@uark.edu
Fax: +1-479-575-6936

Abbreviations: HPSEC-MALLS-RI, high performance size-exclusion chromatography with multi-angle laser light scattering and RI; **HSD,** honestly significant difference; **RDF,** real degree of fermentation; **RMSE,** root mean square error

ones, despite their higher enthalpies [8, 9]. The gelatinization range of small granules is wider and may be attributed to the higher number of granules per unit weight of starch [9]. In relation to fine structure, gelatinization temperature and enthalpy correlate negatively with the percentage of short AP chains, and positively with long chains [10, 11].

The hydrolysis of malted barley starch is not a simple, single process and is affected by multiple factors such as starch structural features, the physical access of enzyme to the starch, substrate viscosity, and the availability of water [12, 13]. Research on the accessibility of starch has included interference by the presence of protein and lipid [12]. Treatments such as heating, while inducing gelatinization, can also lead to physical changes in the starch, rendering it less digestible [12]. Malt enzymes that include α-amylase, β-amylase, α-glucosidase, and limit dixtrinase are involved in the hydrolysis of starch during mashing [14]. As such, the combined activity of these enzymes have been traditionally used as indicator of malt quality through the measurement of diastatic power. However, instances in which barley cultivars and/or lines of similar diastatic power but performing differently in the brewhouse had been reported [15, 16].

Considering that starch gelatinization and hydrolysis are key factors for efficient mashing and brewing, it is of the essence to study in detail the relationship of malt starch composition and structural features with RDF, which is an important indicator of brewhouse performance in the production of beer. The objective of this study was to identify starch properties that will be valuable in predicting the brewing performance of malted barley. Specifically, correlations of RDF with starch content, composition, fine structure, and thermal properties were established by chemometrics.

2 Materials and methods

2.1 Malted barley

Twenty-five malted barley samples of both commercial and experimental lines (2007 cropping season, with moisture content of $\sim$8.0%) were provided by Busch Agricultural Resources Inc. (Fort Collins, CO). The samples were: 1. M02A, 2. M03A, 3.M05A, 4. M06A, 5.M07A, 6. M09A, 7. M10A, 8. M11A, 9. M12A, 10. M13A, 11. M14A, 12. M15A, 13. M17A, 14. W01B, 15. W02B, 16. W03B, 17. W04B, 18. W05B, 19. W06B, 20. W07B, 21. W08B, 22. W09B, 23. W10B, 24. W11B, and 25. W12B. Samples were kept at 4°C prior to sample preparation, starch isolation, and analyses. RDF was determined using the Wort-5B method and expressed as percentage of extract [17]. Flour samples were prepared by grinding with a UDY cyclone sample mill (UDY Corp., Ft. Collins, CO) fitted with a 0.50-mm sieve. Moisture content was determined by AACC Approved Method 44-15A (AACC International). Starch content of ground barley was assayed by AACC Approved Method 76-13 [18] with a Megazyme total starch assay kit (Megazyme International, Wicklow, Ireland). Total soluble sugars was determined by the phenol-sulfuric acid method [19].

2.2 Starch isolation

Starch from malted barley was prepared following the procedure of Andersson et al. [20] with modifications. The method consisted of soaking, wet milling, sieving, pH adjustment, repeated centrifugation and decantation, and drying. Wet milling was done with a locally fabricated centrifugal mill that consisted of a cone-shaped rotor and a stator for regulating the shear gap required for suitable size reduction by intense friction. Isolated starches were dried in a convection oven at 40°C to a moisture content of $\sim$10%, ground with mortar and pestle to pass through a 100-mesh sieve, and kept in airtight vials.

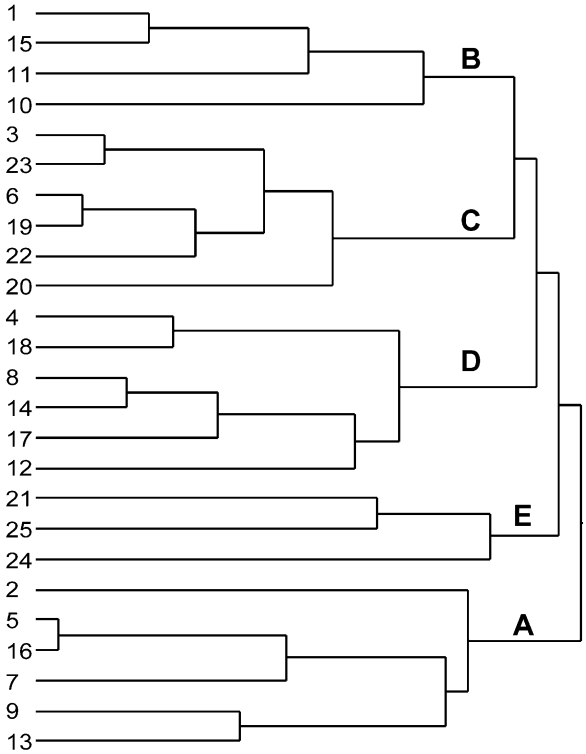

Figure 1. Dendogram obtained from Ward cluster analysis of real degree of fermentation data (RDF means for A, B, C, D, and E clusters were 79.4, 78.1, 77.4, 76.5, and 74.6%, respectively. Numbers on the cluster represent the 25 different barley samples).

2.3 Thermal properties

Thermal properties were evaluated with a Pyris-1 differential scanning calorimeter (Perkin–Elmer Co., Norwalk, CT). Starch (∼4.0 mg, dry basis) was weighed into an aluminum pan and was added with 8 μL deionized water. The pan was hermetically sealed and equilibrated at room temperature for 1 h before running the thermogram. Thermal scans involved heating the sample from 25°C to 130°C at a rate of 10°C/min. An empty pan was used as reference. Enthalpy, onset, peak, and conclusion temperatures were calculated from the endothermic transition peaks corresponding to starch gelatinization (onset temperature of <70°C) and AM-lipid complex melting (onset temperature of >90°C). Gelatinization/melting temperature range was calculated as difference of conclusion and onset temperature.

2.4 Starch fractions

Twenty milligrams of defatted starch [21] was placed in a 50-mL screw-cap test tube that contained a magnetic stir bar (12.7 × 3 mm), added with 3.2 mL of millipore water, capped, and heated in a boiling water bath for 30 min with magnetic stirring. The suspension was allowed to cool to 45°C, added with 0.4 mL of 0.1 M acetate buffer, followed by 5 μL of isoamylase (Hayashibara Biochemical Laboratories, Okayama, Japan), and then incubated at 45°C for 2 h with stirring. After hydrolysis, the suspension was neutralized with 0.2 mL of 0.2 M NaOH, heated in a boiling water bath for 5 min and cooled soon after. A milliliter of the hydrolysate was pipeted into an effendorf tube, added with 10 μL of 2 M barium acetate to precipitate out anions that interfere in running the chromatogram, and centrifuged at 5 rcf for 10 min. A portion of the supernatant was injected right way into the HPSEC system. The HPSEC system (Waters, Milford, MA) consisted of a 515 HPLC pump with a 100-mL sample loop injector, an in-line degasser, a Shodex OHpak SB-G guard column (Shoko Co., Kanagawa, Japan), a series of size exclusion columns (Shodex OHpak KB-804 and KB-802, Shoko Co.) maintained at 55°C, and a 2410 RI detector maintained at 40°C. Degassed 0.1 M $NaNO_3$ containing 0.02% NaN_3 was used as eluent at a flow rate of 0.7 mL/min.

2.5 AP molecular properties

AP weight-average molar mass (M_w), *z*-average gyration radius (R_z), and polydispersity (ratio of weight-average and

Table 1. RDF and starch properties of the five malted barley clusters[a)]

Property (unit)	Cluster A ($n = 6$)	Cluster B ($n = 4$)	Cluster C ($n = 6$)	Cluster D ($n = 6$)	Cluster A ($n = 3$)
Real degree of fermentation (%)	79.4 ± 0.1[a]	78.1 ± 0.2[b]	77.4 ± 0.1[c]	76.5 ± 0.1[d]	74.6 ± 0.2[e]
Total starch (%)	58.7 ± 0.5[b]	58.8 ± 0.6[b]	59.6 ± 0.5[ab]	58.9 ± 0.6[b]	60.2 ± 0.7[a]
Total soluble sugars (%)	9.9 ± 0.2[a]	9.8 ± 0.3[a]	9.7 ± 0.2[a]	9.6 ± 0.2[a]	9.4 ± 0.4[a]
Debranched-starch fraction					
Fraction I (%)	29.4 ± 0.8 [b]	31.3 ± 1.0[ab]	31.6 ± 0.8[a]	30.9 ± 0.8[ab]	32.9 ± 1.1[a]
Fraction II (%)	19.5 ± 0.4[a]	18.8 ± 0.5[ab]	17.3 ± 0.4[b]	17.6 ± 0.4[b]	17.7 ± 0.6[b]
Fraction III (%)	51.5 ± 0.9[a]	49.9 ± 1.2[b]	50.8 ± 1.0[ab]	51.1 ± 1.0[a]	49.7 ± 1.1[b]
Fraction II + Fraction III (%)	71.0 ± 0.9[a]	68.7 ± 1.0[ab]	68.1 ± 0.8[ab]	69.1 ± 1.0[a]	67.4 ± 1.1[b]
Fraction III/Fraction II	2.7 ± 0.2[a]	2.6 ± 0.1[a]	2.9 ± 0.1[a]	2.9 ± 0.2[a]	2.8 ± 0.1[a]
AP fine structure					
Molar mass ($\times 10^8$ g/mole)	1.4 ± 0.1[a]	1.4 ± 0.1[a]	1.5 ± 0.0[a]	1.4 ± 0.0[a]	1.5 ± 0.1[a]
Gyration radius (nm)	153.3 ± 3.2[a]	152.9 ± 3.9[a]	155.0 ± 3.2[a]	155.3 ± 3.2[a]	155.5 ± 4.1[a]
Polydispersity (M_w/M_n)	1.5 ± 0.0[a]	1.4 ± 0.1[a]	1.5 ± 0.0[a]	1.5 ± 0.1[a]	1.4 ± 0.1[a]
Starch gelatinization					
Onset temperature (°C)	59.4 ± 0.4[a]	60.7 ± 0.5[a]	59.8 ± 0.4[a]	59.9 ± 0.4[a]	59.7 ± 0.6[a]
Peak temperature (°C)	63.7 ± 0.2[a]	63.2 ± 0.2[a]	63.5 ± 0.1[a]	63.5 ± 0.2[a]	63.2 ± 0.2[a]
Gelatinization range (°C)	8.3 ± 0.4[a]	6.8 ± 0.5[b]	7.7 ± 0.4[ab]	7.8 ± 0.4[ab]	7.7 ± 0.6[ab]
Enthalpy (J/g)	7.4 ± 0.1[a]	7.0 ± 0.2[ab]	6.9 ± 0.2[b]	7.2 ± 0.1[ab]	6.9 ± 0.2[b]
AM-lipid complex melting					
Onset temperature (°C)	94.7 ± 0.7[a]	93.9 ± 0.8[a]	95.0 ± 0.7[a]	95.5 ± 0.7[a]	94.1 ± 1.0[a]
Peak temperature (°C)	102.2 ± 0.4[a]	102.6 ± 0.6[a]	102.3 ± 0.4[a]	101.5 ± 0.4[a]	101.7 ± 0.6[a]
Gelatinization range (°C)	13.3 ± 0.8[a]	14.2 ± 1.0[a]	12.3 ± 0.8[a]	12.6 ± 0.8[a]	13.2 ± 1.2[a]
Enthalpy (J/g)	1.7 ± 0.1[a]	1.9 ± 0.2[a]	2.0 ± 0.1[a]	1.9 ± 0.1[a]	1.5 ± 0.2[a]

a) Means ± SE in a row with a common superscript letter are not significantly different from one another based on Tukey's HSD test at 5% level of significance.

number-average molar mass, M_w/M_n) were determined by high performance size-exclusion chromatography with multi-angle laser light scattering and RI (HPSEC-MALLS-RI) detectors. The system consisted of a 515 HPLC pump with a 200 mL sample loop (Waters, Millford, MA), an inline degasser, a TSKgel PWXL guard column (Tosoh Corp, Tokyo, Japan), a series of two size exclusion columns (TSKgel G5000PWXL and G4000PWXL, Tosoh Corp.), a DAWN-EOS 18-angle light scattering detector (Wyatt Technology, Santa Barbara, CA), and an Optilab rEX RI detector (Wyatt Technology). The conditions used in preparing the samples and running the chromatograms were described by Patindol et al. [22].

2.6 Statistical analysis

JMP® software version 8 (SAS Software Institute, Cary, NC) was used in the chemometric analysis of the experimental data. Hierarchical cluster analysis of RDF data was done by the Ward's minimum variance method. Tukey's honestly significant difference (HSD) test was used to identify significantly different means among clusters. Principal component analysis was performed to obtain a simplified view of the relationship of RDF with various malted barley starch properties. Bivariate correlation was carried out by the Pearson-product moment approach. A stepwise regression approach was employed in the multiple linear regression analysis. Coefficient of determination (R^2) and root mean square error (RMSE) were used as indicators of significance for the regressions models.

3 Results and discussion

3.1 Cluster analysis

Ward Cluster analysis grouped the 25-malted barley samples into five clusters based on percent RDF that ranged from 74.0 to 80.1% (Fig. 1). The clusters were arbitrarily named as Clusters A, B, C, D, and E, with Cluster A having the highest mean RDF and Cluster E the lowest (Table 1). The number of samples in each cluster (*n*) was 6, 4, 6, 6, and 3 respectively, for Cluster A, B, C, D, and E. Total starch content was 57.1–62.1% and was significantly higher for Cluster E. Total soluble sugars was 8.8–11.4% and did not vary among the five clusters. In Table 1, Fractions I, II, and III represent the three fractions in the chromatogram eluted when isoamylase-debranched starch was injected into the HPSEC column (Fig. 2). Fraction I is essentially AM; whereas, II and III are the long- and short-chain AP fractions, respectively [23]. The

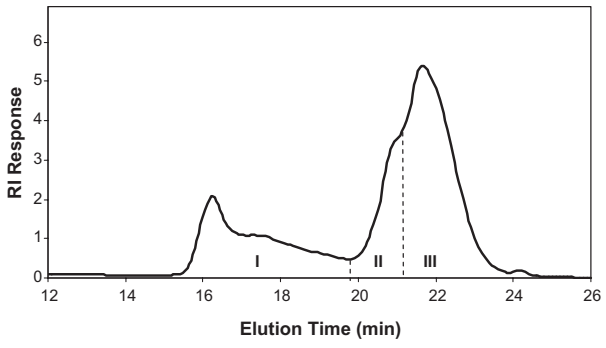

Figure 2. Chromatogram of debranched starch (I, AM; II, long AP chain; III, short AP chain)

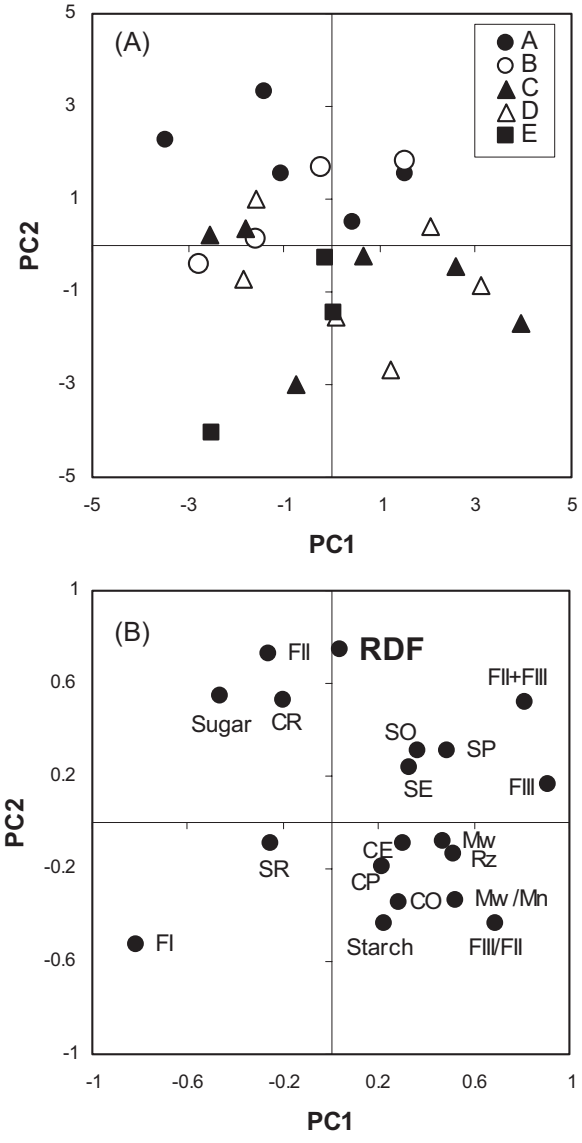

Figure 3. Similarity map obtained by principal component analysis. (A) Sample sore plot with A, B, C, D, and E as sample clusters; (B) Variable loading plot.

sum of Fractions II and III (or simply II + III) is an index for total AP, and the ratio of III/II is indicative of DB [23]. Cluster A samples were evidently lower in Fraction I but higher in II, III, and II + III compared to the Cluster E samples. The III/II did not vary significantly. The samples in Clusters B, C, and D were similar to Cluster E in I and II but related to Cluster A in terms of III. The ranges of AP molecular properties determined by HPSEC-MALLS-RI were quite narrow: 1.30–1.62 $\times$ 10^8 g/mole for M_w, 143.7–164.8 nm for R_z, and 1.37–1.53 for M_w/M_n. The five clusters were comparable in M_w, R_z, and M_w/M_n as shown in Table 1. In terms of thermal properties, Cluster A had a wider gelatinization range and a higher enthalpy of gelatinization. Onset and peak gelatinization temperature as well as the variables associated with the melting of AM-lipid complex were basically the same for all five clusters.

3.2 Principal component analysis

A total of 17 components completely explained the variance of the 19-variable dataset, with principal components 1 and 2 (PC1 and PC2), explaining 23.9 and 16.7%, respectively. A similarity map based on the sample score and variable-loading plots for PC1 and PC2 is presented in Fig. 3. The score plot (Fig. 3A) shows that the Cluster A samples were positioned on the upper two quadrants of the similarity map (quadrants 1 and 2); the Cluster B, C, and D samples were scattered on three different quadrants; whereas, the Cluster E samples were confined on the lower left quadrant (quadrant 3). Overall, the score plot was able to visually discriminate the Cluster A samples from Cluster E, but not those in Clusters B, C, and D. On the variable loading plot (Fig. 3B), RDF was positioned on the first quadrant. Based on the sit-together rule of principal component analysis, the variables that were closely related to RDF were: soluble sugar content, Fraction II, CR (AM-lipid complex melting temperature range), and II + III (total AP) (PC2 $>$ 0.5). RDF was loaded opposite Fraction I (AM) and SR (starch gelatinization temperature range) to indicate the antagonistic effect of the latter two variables. The variables whose values did not vary considerably among the five clusters (III/II, AM-lipid complex melting temperature and enthalpy, and AP molecular properties) were loaded on the lower right quadrant (quadrant 4) together with starch content. The impact of the aforementioned variables on RDF was quite ambiguous based on the results of principal component analysis.

3.3 Multiple linear regression

Cluster and principal component analyses are not quantitative modeling tools so that multiple linear regression analysis was used to further verify the relation of RDF with malted barley starch properties. It is a rule of thumb that 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 ($n = 25$ in this work) in order to minimize the possibility of chance correlations [24]. The regression models (consisting of 1–5 variables) that gave the highest R^2 and lowest RMSE are presented in Table 2 and Fig. 4. The models were able to explain up to 68% of RDF variance. Present data

Table 2. Best-fit linear regression models[a)]

Number of variables	Variable (coefficient of prediction)	Intercept	R^2	RMSE
1	II(0.76)	63.50	0.41^{**}	1.19
1	III/II (−2.38)	84.11	0.19^{*}	1.41
1	I (−0.22)	84.24	0.15^{ns}	1.46
2	I(−0.88) + III(−0.72)	141.30	0.50^{***}	1.11
2	I(−0.35) + III/II(−3.66)	98.66	0.49^{***}	1.12
2	I(−0.35) + III/II(−3.66)	98.66	0.48^{***}	1.13
3	I(−0.94) + III(−0.72) + M_w(−4.76)	150.00	0.59^{***}	1.04
3	I(−9.31) + III/II(−3.44) + II + III(−9.03)	998.77	0.58^{***}	1.05
3	I(−0.42) + III/II(−3.607) + M_w(−4.62)	107.22	0.58^{***}	1.06
4	I(−7.34) + III/II(−3.45) + M_w(−3.52) + II + III(−6.99)	802.57	0.64^{***}	1.00
4	I(−7.25) + II(−6.40) + III(−7.08) + M_w(−3.74)	783.84	0.63^{***}	1.01
4	I(−7.25) + III(−0.68) + M_w(−3.74) + II + III(−6.40)	783.84	0.63^{***}	1.01
5	I(−13.30) + III/II(−3.34) + SP(−1.08) + SE(1.15) + II + III(13.01)	1457.98	0.68^{***}	0.97
5	I(−9.12) + III/II(−3.54) + SP(−0.95) + M_w(−4.06) + II + III(−8.70)	1036.52	0.66^{***}	0.99
5	I(−13.40) + II(−12.40) + III(−13.29) + SP(−1.09) + SE(1.18)	1459.12	0.66^{***}	0.99

Abbreviations: I, II, III represent debranched starch fractions; M_w, amylopectin weight average molar mass; SP, starch gelatinization peak temperature; SE, starch gelatinization enthalpy; R^2, coefficient of determination; RMSE, root mean square error.
a) Statistical significance: $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; ^{ns}not significant, $p > 0.05$.

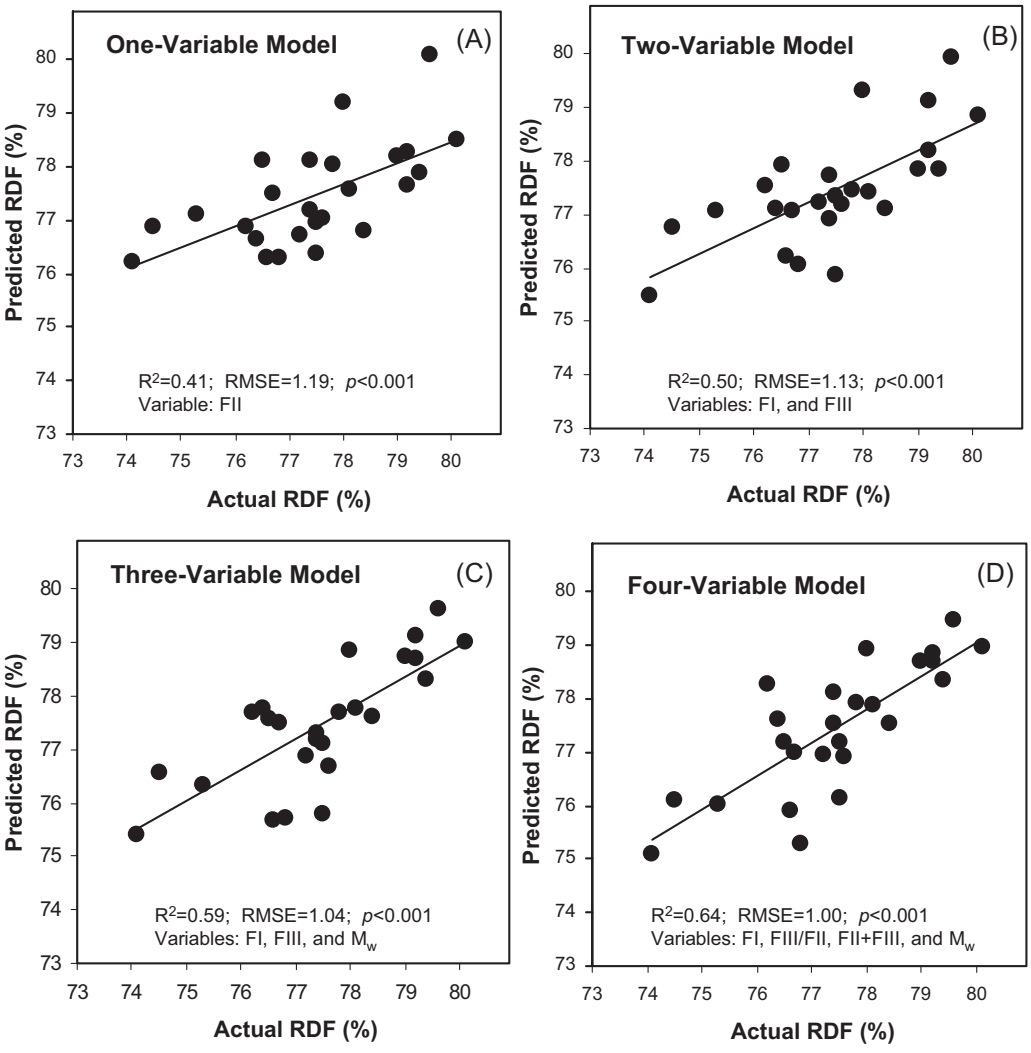

Figure 4. Actual and predicted RDF based on multiple linear regression. (A) One-variable model; (B) Two-variable model; (C) Three-variable model; (D) Four-variable model.

indicates that the single most important starch property in predicting RDF is Fraction II (AP); it explained 41% percent of the variance. The other one-variable models lack robustness in predicting RDF (data not shown). Fraction I (AM), when used in combination with other starch properties improved the predictability of RDF. Aside from debranched starch properties (I, II, III, III/II, and II + III), the other variables that were found relevant in predicting RDF included AP M_w, peak gelatinization temperature (SP), and gelatinization enthalpy (SE). When all starch properties (18 variables) were included in a regression model, up to 86% of RDF variance was explained. This indicates that aside from starch, there are other constituents and/or properties of malted barley that contribute to variations in RDF among different cultivars. The contribution of the yeast strain used in converting fermentable sugars to alcohol and the conditions used for fermentation should also be considered [25].

4 Conclusions

This work focused on the determination of molecular size distribution, AP structural features, and thermal properties of starches from 25-malted barley samples. Chemometric tools were used to understand the importance of these starch properties in predicting RDF. AP (debranched starch fractions II and III) and AM (debranched starch fraction I) exhibited positive and negative impact on RDF, respectively. Starch properties explained up to 86% of RDF variance to indicate that aside from starch, other malted barley constituents and/or properties

also contribute to variations in RDF among different cultivars.

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