Comparison of Two Models to Predict Amylose Concentration in Rice Flours as Determined by Spectrophotometric Assay[1,2]

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ABSTRACT

A spectrophotometric assay was used to test two models for predicting amylose in 16 solutions containing known concentrations of amylose and amylopectin and in 10 rice flours of unknown composition. The simple linear regression model based on an amylose-only standard curve overpredicted amylose in all solutions (relative bias of 7-329%). The model used for simultaneous estimation of amylose and amylopectin was more accurate with some slight overprediction or underprediction of amylose (<6%). The amylose-only method predicted amylose content to be 7.4% for waxy flour and 16.4-25.4% for nonwaxy varieties. Defatted flours were measured at 8.0% (waxy) and 18.4-29.6% (nonwaxy). Lower amylose content was measured by the simultaneous estimation method. Predictions for the flours were 0% (waxy) and 6.6-14.2% (nonwaxy). Defatted flours had 0% (waxy) and 9.8-22.0% (nonwaxy). This method resulted in greater accuracy, although use of laboratory time and resources was not changed.

Amylose content is considered to be the single most important indicator for predicting the cooking and processing behavior of rice varieties (Sanjiva Rao et al 1952; Williams et al 1958; Halick and Keneaster 1956; Juliano 1979, 1985). The most widely used method for amylose determination has been a colorimetric assay in which iodine binds with amylose to produce a blue color that is measured spectrophotometrically at one wavelength (Halick and Keneaster 1956; Hall and Johnson 1966; Juliano 1971, 1979; Juliano et al 1968, 1981; Perez and Juliano 1978; Shanthy et al 1980; Sowbhagya and Bhattacharya 1971, 1979; Williams et al 1958).

Amylopectin also produces a colored reaction with iodine, interfering with the direct measurement of the color produced by the amylose-iodine complex. Attempts have been made to reduce the interference of the amylopectin-iodine complex by use of amylose-amylopectin mixtures to form a standard curve (Perez and Juliano 1978, Juliano et al 1981).

In a dual-component system, where no region can be found in which only one component absorbs, it is still possible to determine the amounts of the two substances by making measurements at two wavelengths and solving simultaneously for the concentration of each component (von Elbe and Schwartz 1984, Sawyer et al 1984, Williard et al 1965). Simultaneous determinations rest on the assumption that the substances concerned contribute additively to the total absorbance at an analytical wavelength. This method is well known in analytical chemistry but has only recently been applied to starch research (Hovenkamp-Hermelink et al 1988).

In this study, two methods (an amylose-only standard curve and simultaneous estimation of amylose and amylopectin assuming additivity) were used to determine the amylose content in mixtures of known amylose-amylopectin concentrations and in rice flours of unknown starch composition.

MATERIALS AND METHODS

Amylose

Highly purified amylose was obtained from the Agricultural Research Service (Midwest Area Northern Regional Research Center, Peoria, IL) and had been prepared according to Knutson's (1986) method. Using Juliano's (1971) method for solubilization and color development, a standard curve was prepared with 19 replicate samples of amylose in concentrations ranging from 2 to 22.5 μg/ml (dry weight basis). Absorbance was measured at 620 and 560 nm. The absorbances at 620 nm and the concentrations were used to develop the amylose-only standard curve. The absorbance data were also used to determine absorptivity values for amylose at 620 and 560 nm for use in the simultaneous estimation method.

Amylopectin

The waxy maize Amioca from American Maize-Products (Hammond, IN) was used to determine the absorptivity values for amylopectin. Twenty-seven samples with concentrations ranging from 4 to 60 μg/ml were randomly solubilized. Color was developed according to Juliano's (1971) method, and the absorbance values measured at 620 and 560 nm were used to determine absorptivity values for use in the simultaneous estimation method.

Simultaneous Estimation of Amylose and Amylopectin

Since amylose and amylopectin are polysaccharides of various degrees of polymerization, it was not possible to determine a molar absorptivity (ϵ). Instead, absorptivity values (a) for amylose and amylopectin were calculated and expressed as unit absorptivity (μg/ml). The wavelengths 620 and 560 nm were chosen because these were the absorption maxima for amylose and amylopectin, respectively. Absorptivity constants were determined for amylose and amylopectin at both 560 and 620 nm by dividing absorbance by concentration (μg/ml). The resulting absorptivity values were averaged to obtain the value used in the equations.

Simultaneous Equation

The equations (Williard et al 1965) used to solve simultaneously for the concentrations of amylose (A) and amylopectin (AP) were

$$\mu g/ml\ A = \frac{(a_{AP560})(A_{620}) - (a_{AP620})(A_{560})}{(a_{A620})(a_{AP560}) - (a_{AP620})(a_{A560})}$$

$$\mu g/ml\ AP = \frac{(a_{A620})(A_{560}) - (a_{A560})(A_{620})}{(a_{A620})(a_{AP560}) - (a_{AP620})(a_{A560})}$$

where, for example, a_{AP560} represents the absorptivity value of amylopectin at 560 nm and A_{620} represents the absorbance at 620 nm.

Mixtures of Known Amylose and Amylopectin Content

To determine how well the amylose-only standard curve and the simultaneous equations predicted the amount of amylose in a starch solution, it was necessary to have solubilized starch solutions of known concentrations of amylose and amylopectin. Using the amylose and amylopectin samples described above, 16 mixtures were prepared that ranged from 100% AP, 0% A (37.7 μg/ml AP, 0 μg/ml A) to 46% AP, 54% A (17.8 μg/ml AP,

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20.8 μg/ml A). The mixtures were solubilized and color was developed by iodine according to Juliano's (1971) method. Absorbance was read at 620 and 560 nm.

Rice Flours

Ten rice varieties were chosen from the laboratory inventory as unknowns to test the predictive models. The rice varieties were Mochi Gomi, waxy grain; Nortai and S201, short grain; Nato, M201, and Mars, medium grain; and IR36, Tebonnet, Newbonnet, and Lebonnet, long grain. The grains were hulled in a Satake rice machine and milled in a McGill no. 2 rice mill. After grinding in a Udy cyclone mill and sieving to pass a 100-mesh screen in an Alpine siever, the flours were defatted with petroleum ether (bp 35–60°C) on a Goldfisch solvent extractor for 5 hr, followed by a 4-hr 95% ethanol extraction in the same extractor. All flours were then resieved. Triplicate samples of the flours and defatted flours were solubilized. Color was developed according to Juliano's (1971) method, and the absorbance was read at 620 and 560 nm.

Statistical Analysis

Regression analysis of the absorbance at 620 nm on the amylose concentration was used to obtain the amylose-only standard curve. Since the variability in absorbance readings increased as the amylose concentration increased, the weighted least squares method was used. Statistical details relating to the calculation of estimated concentrations and their estimated standard errors for the simultaneous equation method can be found in Gbur et al (1990). Computer analyses utilized SAS (SAS Institute 1985).

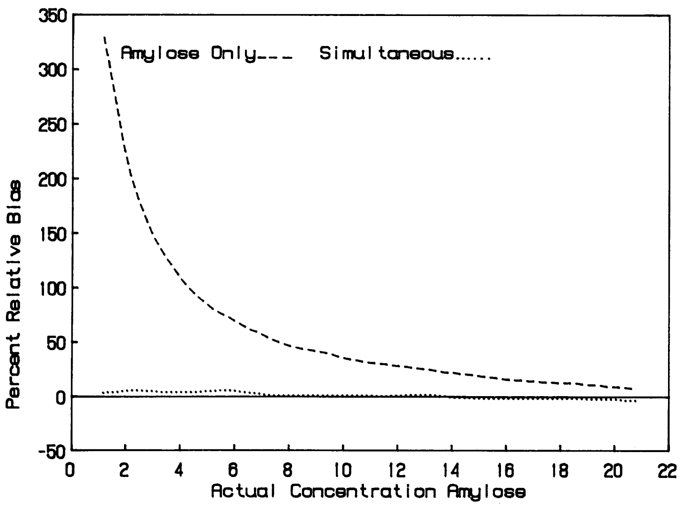

Fig. 1. Relative bias for amylose-only and simultaneous methods for estimating amylose content.

TABLE I
Prediction of Amylose (A) Concentration and Relative Bias for 16 Mixtures of Known Concentration Using the Amylose-Only Standard Curve

Actual Concn of A (μg/ml)	Mean Predicted Concn of A[a] (μg/ml)	95% Confidence Interval Mean Predicted A Concn Lower (μg/ml)	Upper (μg/ml)	Relative Bias (%)
0.0	4.04	3.87	4.19	...[b]
1.16	4.98	4.83	5.12	329.23
2.32	6.70	6.59	6.81	189.50
3.47	7.87	7.78	7.96	126.77
4.62	8.83	8.76	8.91	91.26
5.78	9.92	9.87	9.98	71.70
6.93	10.89	10.85	10.94	57.17
8.09	11.80	11.76	11.84	45.84
9.24	12.97	12.92	13.02	40.38
10.40	13.85	13.78	13.91	33.15
11.55	14.91	14.83	14.98	29.05
12.71	15.93	15.84	16.03	25.36
13.86	16.88	16.76	16.99	21.77
16.17	18.65	18.51	18.80	15.36
18.48	20.70	20.52	20.89	12.03
20.79	22.32	22.10	22.54	7.35

[a]There were either two or three replications at each concentration of amylose.
[b]Relative bias is not defined since bias cannot be divided by zero.

RESULTS AND DISCUSSION

Amylose-Only Standard Curve

Due to the presence of unequal variances, weighted least squares regression of the absorbance at 620 nm on the concentration of amylose was used to yield parameter estimates for the intercept and slope (absorptivity) and their standard errors of 0.0037 (SE = 0.00240) and 0.0276 (SE 0.00026), respectively. From the above regression, given an absorbance at 620 nm, the estimated concentration of amylose is calculated by

$$(A_{620} - 0.0037) \,/\, 0.0276$$

Spectrophotometric data from the 16 mixtures of known amylose and amylopectin concentrations were used to test the amylose-only regression equation. Relative bias, which adjusts the difference for the magnitude of concentration, was then calculated for each mixture as

$$\text{Relative bias} = \frac{\text{estimated} - \text{true concentration of amylose} \times 100}{\text{true concentration of amylose}}$$

The relative bias (as a percentage) in predicting amylose increased as the concentration of amylose decreased (Fig. 1). Summary statistics for the prediction of amylose concentration and estimation of relative bias (using the amylose only standard curve) for each of the 16 mixtures of known amylose content are listed in Table I.

Simultaneous Estimation of Amylose and Amylopectin

The mean absorptivity values (a) for amylose and amylopectin, their standard errors and the sample sizes are shown in Table II.

The correlation between absorptivity at 560 and 620 nm was 0.97 for amylose and 0.72 for amylopectin. The absorptivity values were used to solve the simultaneous equations to predict the concentrations of amylose and amylopectin (in micrograms per milliliter) in the 16 mixtures of known concentration.

Summary statistics for the prediction of amylose concentration and relative bias (using simultaneous estimation) for each of the amylose concentrations in the 16 known mixtures are listed in Table III. Relative bias as a function of the true amylose concentration is graphically illustrated in Figure 1.

Figure 2 illustrates the prediction of amylose by both methods. The determinations from the simultaneous estimation method (Table III) were great improvements over the corresponding estimates from the amylose-only standard curve (Table I). In the amylose-only prediction model, no confidence interval contained the true concentration of amylose for a known mixture, whereas 13 of 14 (93%) of the confidence intervals for the simultaneous prediction model (Table III) contained the actual amylose concentration.

Estimation of Amylose in Rice Flours

The weighted least squares regression developed from the amy-

TABLE II
Absorptivity (a) at 560 and 620 nm

Parameter	Amylose a_{A620}	Amylose a_{A560}	Amylopectin a_{AP620}	Amylopectin a_{AP560}
Mean	0.0281	0.0215	0.0033	0.0048
Standard error	0.0002	0.0002	0.0001	0.0001
Sample size	19	19	27	27

TABLE III
Amylose (A) Concentration and Relative Bias for 88 Mixtures of Known Concentration (Simultaneous Estimation)

Actual Concn of A (μg/ml)	Mean Predicted Concn of A[a] (μg/ml)	95% Confidence Interval Mean Predicted A Concn Lower (%)	Upper (%)	Relative Bias (%)
0.00	0.00	0.00	0.52	...[b]
1.16	1.20	0.69	1.71	3.53
2.32	2.44	1.98	2.90	5.52
3.47	3.61	3.08	4.14	4.16
4.62	4.81	4.37	5.25	4.13
5.78	6.12	5.62	6.62	5.89
6.93	7.08	6.55	7.61	2.11
8.09	8.13	7.60	8.66	0.50
9.24	9.32	8.89	9.75	0.90
10.40	10.49	10.02	10.96	0.91
11.55	11.58	11.21	11.95	0.25
12.71	12.97	12.53	13.41	2.04
13.86	13.82	...[c]	...[c]	−0.27
16.17	15.86	15.36	16.36	−1.89
18.48	18.13	17.82	18.44	−1.92
20.79	20.10	...[c]	...[c]	−3.30

[a]There were either two or three replications at each concentration of amylose.
[b]Relative bias is not defined since bias cannot be divided by zero.
[c]There was no variability in A620 readings for this set of replications.

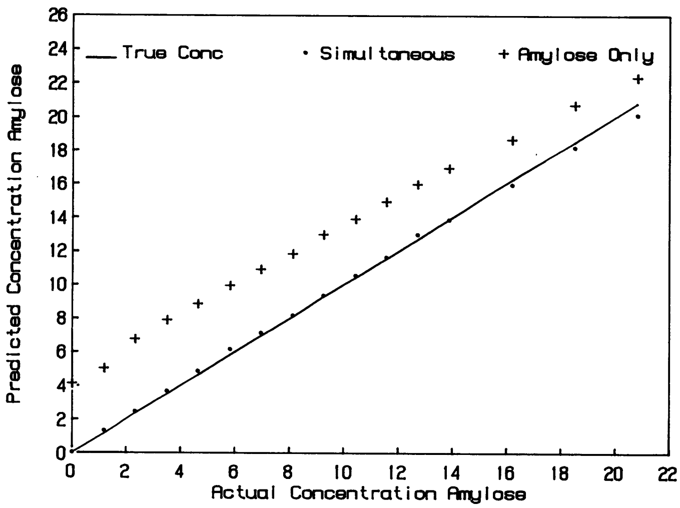

Fig. 2. Predicted vs. actual amylose concentrations for the two models.

lose-only standard curve and the simultaneous estimation method were applied to predict the amylose concentration in 10 flours. Percentage values listed in Table IV are estimates calculated on the basis of 85% starch in the flours. In both methods, the defatted flours had higher amylose contents than their corresponding full-fat flours. These differences are likely due to formation of amylose-lipid complexes in the full-fat flour (Karkalas and Raphaelides 1986, Perez and Juliano 1978, Williams et al 1958) that interfere with the formation of the amylose-iodine complex. The values for percent amylose based on the amylose-only prediction model are consistent with other published values.

CONCLUSIONS

Two methods were used to predict the concentration of amylose in 16 mixtures of known amylose and amylopectin concentration and in 10 flours of unknown composition. The weighted least squares regression based on an amylose-only standard curve overpredicted amylose in all known mixtures (relative bias 7–329%). The simultaneous spectrophotometric method was a more accurate predictor of true amylose content, with relative biases being scattered about zero (−3 to 6%). This study showed that the

TABLE IV
Estimates of Amylose Content in Whole and Defatted Rice Flours

Rice-Type Cultivar	Concentration (μg/ml) in Flour: Whole A[a]	Whole S[b]	Defatted A	Defatted S	Percent in Flour: Whole A	Whole S	Defatted A	Defatted S
Waxy								
Mochi Gomi	3.7	0	4.0	0	7.4	0	0	0
Nonwaxy								
Short grain								
Nortai	10.3	5.4	11.6	8.4	20.6	10.8	23.2	16.8
S201	9.6	4.2	11.4	7.4	19.2	8.4	22.8	14.8
Medium grain								
Mars	8.8	3.9	10.1	6.1	17.6	7.8	20.2	12.2
M201	8.2	3.3	9.2	4.9	16.4	6.6	18.4	9.8
Nato	8.7	3.8	9.8	5.7	17.4	7.6	19.6	11.4
Long grain								
IR36	12.7	6.9	14.4	10.7	25.4	13.8	28.8	21.4
Lebonnet	12.5	6.7	14.4	10.9	25.0	13.4	28.8	21.8
Newbonnet	11.8	6.6	13.8	10.1	23.6	13.2	27.6	20.2
Tebonnet	12.7	7.1	14.8	11.0	25.4	14.2	29.6	22.0

[a]A denotes amylose-only standard curve.
[b]S denotes simultaneous estimation of amylose and amylopectin.

most commonly used method to determine amylose content in rice flours is systematically biased toward overprediction. Researchers can improve accuracy by use of the simultaneous estimation method. Results are closer to the true concentration, especially at low amylose levels. Moreover, use of the simultaneous method involves no increase in use of laboratory resources.

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