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Note

Improved colorimetric determination of amylose in starches or flours

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The quantitative determination of amylose by means of its color reaction with iodine has been practiced for many years. Several procedures have been described, but there are many reports in the literature about the inconsistency, inaccuracy, and lack of reproducibility of these procedures[1–8].

The frequently employed methods of McCready and Hassid[3], Wu *et al.*[4], Wolf *et al.*[5], or Williams *et al.*[6] used neutral or alkaline solutions to develop the colored amylose–iodine complex. Juliano[7] used acetic acid for neutralization, and a large excess of iodine to develop the color. Recently, Morrison and Laignelet[8] attempted to minimize the poor reproducibility of the previous methods by solubilizing lipid-free, purified starches in urea–Me_2SO. We have found, however, that these methods are sensitive to the pH, the temperature, and the concentration of amylose and of iodine.

We now report, for the colorimetric quantitation of amylose, an improved method which is reproducible and results in a stable color of the amylose–iodine complex.

EXPERIMENTAL

Materials. — All chemical compounds were obtained from the J. T. Baker or Sigma Chemical Companies, and were reagent grade or of the highest purity obtainable from them.

Lipid extraction. — Flour (10–20 mg) was weighed on a microbalance. The samples were extracted in test tubes with 85% methanol (5 mL) for 30 min at 60° with occasional mixing. The samples were centrifuged, the supernatant liquors were discarded, and the extractions were repeated. The third extract contained only traces of lipids, as determined by t.l.c. Usually, two extractions were sufficient.

Solubilization. — M NaOH (2 mL) and water (4 mL) were added to the lipid-free samples, and the test tubes were capped, and heated for 30 min in a waterbath at 95°, with occasional mixing (solution I). Other samples were solubilized for 30

min at 100° with 6 mL of urea–Me_2SO (0.6M urea in 90% Me_2SO) as described by Morrison and Laignelet[8].

Determination of amylose. — Solution I (0.1 mL) was added to 5 mL of 0.5% trichloroacetic acid (TCA) in a separate test-tube. The solutions were mixed, and 0.05 mL of 0.01N I_2–KI solution (1.27 g of I_2 per L + 3 g of KI per L) was added, and mixed in immediately. The blue color was read at 620 nm after 30 min at 25 ±1° *vs.* H_2O on a Shimadzu 260 double-beam spectrophotometer. The absorbance of the reaction blanks with water was zero, and with pure amylopectin (corn amylopectin from Sigma Chem. Co., purified by precipitation with acetone), <0.03.

RESULTS AND DISCUSSION

The colored amylose–iodine complex was sensitive to changes of pH in the alkaline or the neutral region (see Table I). The reaction was also sensitive to temperature and to dilution. Alkaline or neutral buffers (glycine, borate, or Tris buffers) inhibited the reaction, or reduced the color stability, or both.

On the other hand, the blue amylose–iodine complex was stable in acidic medium. However, hydrochloric, sulfuric, and nitric acids could not be used, because they precipitated the amylose–iodine complex. Using dilute TCA, no precipitation of the colored complex occurred, even after long standing at room temperature. An additional advantage of using TCA was the precipitation of proteins which otherwise interfered with the color reaction. Under these conditions, the colored amylose–iodine complex remained in the supernatant liquors. The color was more stable, and less sensitive to experimental conditions, than that developed in neutral or alkaline medium.

When the color was developed by using TCA solutions, the color intensity was independent of the TCA concentration in the range 0.2–1.0% TCA (see Table II). When the color was developed at higher or lower temperatures, and the mix-

TABLE I

ABSORBANCE (AT 635 nm) OF COMPLEX OF IODINE WITH POTATO STARCH AT DIFFERENT pH VALUES[a]

pH	*(0.1 mL sample)*		*(0.2 mL sample)*	
	A_{635}	λ_{max}	A_{635}	λ_{max}
6.5	0.260	593	0.516	600
7.5	0.271	605	0.530	606
8.5	0.284	601	0.558	601
9.5	0.200	625	0.380	622
10.5	0.000	—	0.002	—

[a]The color was developed by the method described by Morrison and Laignelet[8]; it was scanned from 800–400 nm, to obtain A and λ_{max}. The starch (20 mg) was solubilized in urea–Me_2SO (see text). Values in the Table are the average of triplicate determinations.

TABLE II

ABSORBANCE AT 620 nm OF IODINE COMPLEX WITH A MIXTURE OF 40% AMYLOSE AND 60% AMYLOPECTIN AT DIFFERENT CONCENTRATIONS OF TCA[a]

TCA (%)	*Solubilized in NaOH*	*Solubilized in urea–Me_2SO*
0.1	0.545	0.547
0.2	0.550	0.548
0.4	0.548	0.550
0.6	0.552	0.549
0.8	0.550	0.553
1.0	0.548	0.550
2.0	0.535	0.532

[a]The mixture (10 mg) of amylose (40%) and amylopectin (60%) was solubilized by means of two methods (see Experimental section), the color was developed in different concentrations of TCA. Values are the averages of triplicate determinations.

ture kept for 60 min at 25°, the color intensity always approached the same value (see Table III). In the dark, the color intensity was constant for at least 24 h; after that, the color intensity decreased, but the change was slow. The presence of glutelin or albumin (up to 10%) did not influence the color intensity.

The same color intensity was obtained after solubilization of the samples by NaOH, Me_2SO, or urea–Me_2SO (see Table II), and the choice of NaOH, Me_2SO, or urea–Me_2SO depended only on the solubility of the sample. Although the advantages of Me_2SO were addressed by Leach and Schoch[9,10], rice and sorghum starches were found in this study to be more soluble in NaOH than in Me_2SO or urea–Me_2SO. The choice of solubilizing agent had no significant influence on the final color reaction.

The improved colorimetric determination described herein is accurate and reproducible. The coefficient of variation between sample triplicates was ~4%, and was caused, unevenly, by different steps of the analytical procedure: weighing, 0.03%; first pipetting (2 + 4 or 6 mL), 2%; solubilization, 3% (with both NaOH

TABLE III

ABSORBANCE (AT 620 nm) OF IODINE COMPLEX[a] WITH A MIXTURE OF 40% AMYLOSE AND 60% AMYLOPECTIN AT DIFFERENT TEMPERATURES

Temperature (°C)	*Time (min) at 25°*		
	0	*30*	*60*
4	0.535	0.550	0.549
25	0.540	0.551	0.552
60	0.515	0.531	0.550

[a]The color was developed in TCA as described in the Experimental section. The samples were kept at the designated temperature before addition of the iodine reagent. The absorbance was then read at 620 nm immediately and after 30 and 60 min at 25°. Values are the average of triplicate determinations.

or urea–Me_2SO); second pipetting (5 mL TCA), 2%; third pipetting (0.1 mL of sample + 0.05 mL of iodine solution), 2%; temperature changes (temperature was always within 24–26°), 0.05%; and color reading, 0.2%. Thus, the final s.t.d. variation was $s\% = [(0.03)^2 + (2)^2 + (3)^2 + (2)^2 + (2)^2 + (0.05)^2 + (0.2)^2]^{1/2} = 4.6\%$. When 10 replicates were analyzed under the same conditions, the coefficient of variation was reduced to 2%. The largest sources of error were the solubilization step and the pipetting. The influence of gluten or albumin (up to 10% of the total starch or amylose) on color intensity was small, normally <2%.

The absorbance of impure amylopectin blanks at 620 nm could be between 0.1 and 0.2 absorbance units. Very pure amylopectin blanks were 0.02 to 0.04 absorbance units. Because the error caused by amylopectin at 620 nm could be higher than, for example, the error caused by solubilization or pipetting, it was essential to include the approriate corrections in the calculations of amylose content. Thus, assuming that the starch is a mixture of just two pure components, amylose and amylopectin, and also assuming that other starch or flour components do not absorb light at 620 nm, the amylose content was approximated by the following formula.

$$A \times 45.8 = \text{mg of amylose per L in cuvet},$$

where A is the absorbance of the sample.

The standard curve was obtained from triplicate determinations. The standards contained from 10–100% of amylose, the difference being made up with pure amylopectin. The standard curve was linear to 1.5 absorbance units, with a slope of 0.02185 and y-intercept through the origin. Blank determinations are not necessary, because blanks without amylose, or amylopectin, or both, show zero absorbance.

The method gives reproducible results, not only with different starches but

TABLE IV

AMYLOSE CONTENT OF SOME FLOURS[a]

Flour	*Amylose (% dry wt.)*[b]			
	a	*b*	*c*	*d*
Rice (long grain)	20.8	30.9	40.8	20.6
Rice (medium grain)	12.2	22.0	32.4	12.2
Rice (short grain)	13.9	24.1	33.9	13.9
Rice (waxy)	3.2	13.2	23.4	3.3
Corn	25.3	35.0	45.1	25.6
Wheat	27.6	37.5	47.9	27.3

[a]Flours were solubilized by NaOH, and amylose was determined in TCA as described in the Experimental section. [b]a = flour; b = flour + 10% of pure amylose; c = flour + 20% of pure amylose; d = flour + 10% of pure amyulopectin. Amylose or amylopectin (or both) was added to the flour before solubilization. Values are the average of duplicate deteminations.

also with different flours, and the addition of pure amylose or amylopectin to the flours does not influence the accuracy of the method (see Table IV). The method can readily accommodate small samples for microdeterminations, if necessary.

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