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Evaluation of variations in amylose–iodine absorbance spectra ☆

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Abstract

Evaluation of the variations in spectra of the amylose–iodine complex of starches and amyloses from various botanical sources provide a useful aid for identification and characterization of these substances. Variations in absorptivity, wavelengths of maximum absorbance, and the slopes of the absorbance peaks provide characteristic data for starches as a result of variations in amylose molecular weight. This data can be used to provide a convenient and relatively rapid means for detecting major differences, as between starches from normal and high amylose maizes, or between raw and modified starches and to evaluate effect of processing conditions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Variations in spectra; Amylose–iodine complex; Processing conditions

1. Introduction

Determination of amylose content is an important aspect of starch characterization from both a fundamental and applied point of view. Amylose content affects the functional properties of starch in its many uses in the food industry as well as other applications.

Amylose is commonly determined by the classical reaction between α-1,4-glucans and iodine to form a blue complex, which is measured either spectrophotometrically or potentiometrically. Although potentiometric titration offers the most definitive measure of the iodine binding capacity of the sample, spectrophotometric determinations are more widely used because of convenience and simplicity of use.

Spectrophotometric methods for amylose determination in maize starch have been studied at NCAUR for many years, originally in cooperative research projects to develop high amylose corn varieties. The method of Wolf, Melvin, Garcia, Dimler and Kwolek (1970) utilizes the superior starch-solvent properties of 90% DMSO. A modification of that procedure by Knutson (1986) takes advantage of the fact that the tri-iodide (I_3^-) ion forms spontaneously when iodine is dissolved in DMSO. This ion is necessary for initiation of amylose–iodine complex formation. In this method, samples are simply dissolved in an I_2-DMSO solution and diluted with water to form the amyloseiodine complex, absorbance of which is measured at 600 nm. This method has the further advantage that interference from lipids is minimal. More recently, this procedure was further modified using low temperature gelatinization and sonication to rapidly dissolve starch from ground corn samples, thereby reducing total assay time to 1 h or less, including grinding, dissolution and measurement (Knutson & Grove, 1994). These modifications are currently in use in many laboratories, as evidenced by numerous requests received at this laboratory each year for assistance in assays, advice on methodology and for standard samples. These methods have been satisfactorily applied to starches from other sources as well, and there has been increased interest for such use. The method has also been applied to the determination of amylose content and estimation of DP of synthetic amyloses synthesized by the action of cyclodextrin glucanotransferase (CGTase) on α- and β-cyclodextrins (Rendleman & Knutson, 1998).

A recurring problem for those attempting to use this or any other, method for amylose analysis is that high purity amyloses are not widely available for use as standards, and commercially available amyloses are generally inconsistent in quality. Additionally, there has been no definitive study of the variability that might be encountered when using this method to analyze starches or amyloses from other sources. It is therefore important to obtain definitive absorbance data

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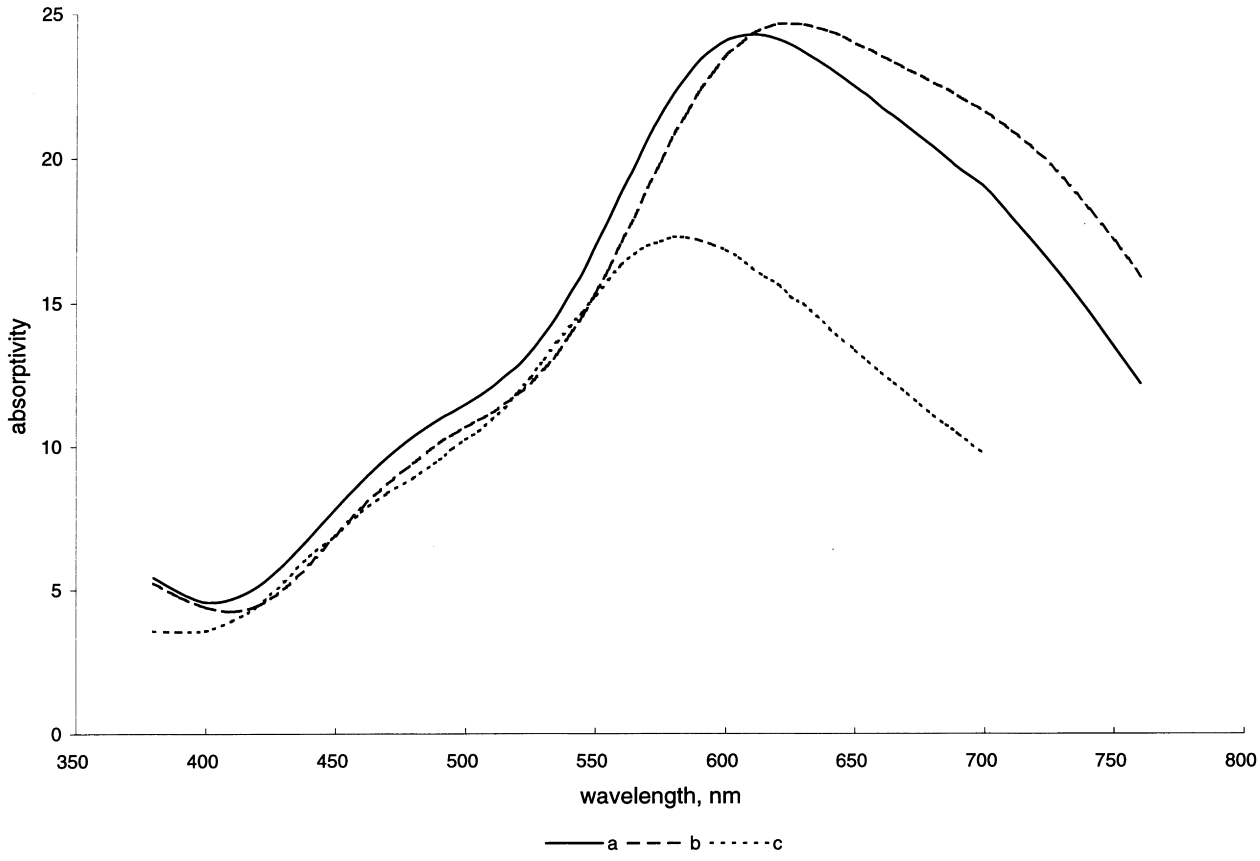

Fig. 1. Spectrum of amylose iodine complexes: absorptivity of (a) high purity maize amylose; (b) high purity potato amylose; and (c) synthetic amylose, λ_{max} 580 nm.

using high purity amyloses, in order to develop uniform parameters, which might be applicable to a wide range of starches.

Beyond the difficulties that may occur due to incomplete dissolution of starch or use of inappropriate standards, problems can arise from variation in the extent of complex formation with amylose from varying starch sources. This variation primarily results from the nature of the amylose–iodine complex itself. All α-1,4-glucans form helical inclusion complexes with iodine to some degree; the extent of the iodine binding depends upon the degree of helix formation which can be attained. A single helix, capable of supporting complex formation, has been shown to require six glucose molecules per turn (Gidley & Bulpin, 1987). As the length of the glucan chain, and hence the number of helical turns, increases, the number of iodine molecules which can be accommodated also increases, so that the iodine binding capacity is enlarged. This increased binding has been shown to result in a shift in the wavelength of maximum absorbance; $1/\lambda_{max}$ has been shown to be directly proportional to 1/DP up to a DP of about 100 (Banks, Greenwood & Khan, 1971; Bailey & Whelan, 1961). The limit of λ_{max} has been estimated to be 642 (Banks et al., 1971) to 650 (John, Schmidt & Kneifel, 1983).

In addition to the λ_{max} increase with increased DP, the increase in iodine binding capacity of the complex has been demonstrated with potentiometric and spectrophotometric measurements. Banks et al. (1971) measured the increase in iodine uptake with increased DP potentiometrically, and Bailey and Whelan (1961) and Pfannemuller, Mayerhofer and Schulz (1969) demonstrated that absorptivity of the complex increases with increased molecular weight of the α-1,4-glucan. The amount of that increase is large at low DP but becomes progressively smaller above DP of 100. Knutson, Cluskey and Dintzis (1982) and Knutson, Khoo, Cluskey and Inglett (1982) showed in a study of potentiometric titration in the presence of excess iodine that the binding capacity varies inversely with the quantity of triiodide ion (I_3^-) in the reaction system. Although I_3^- is necessary to initiate complex formation, increasing the ratio of $I_3^- - I_2$ in the reaction system limits the chain length of the iodine species available for complexing, and therefore limits the degree of binding. Yu, Houtman and Atalla (1996) have demonstrated that the primary structures of the polyiodide chains are composed of I_3^- and I_5^- subunits, combined to form four dominant polyiodide chains (I_9^{3-}, I_{11}^{3-}, I_{13}^{3-} and I_{15}^{3-}) which give different absorbance spectra when complexing with amylose. Absorbance maxima of those spectra are 480–510, 610–640, 690–720 and 730–760 nm, respectively. These spectra overlap to

produce the characteristic spectrum of the amylose–iodine complex. Variation in the iodide concentration changes the relative population of these chains and their substructures. In addition, the length of the 1,4 α-glucan chains available for complex formation influences the relative proportions of the individual spectra.

Thus, the equilibrium of the complex formation is highly complicated, depending upon the interaction of both amylose chain length and iodine chain length. The result of these complexities can be seen in a typical absorbance spectrum of the amylose iodine complex in Fig. 1. The absorbance peaks are not symmetrical around the maximum, but are skewed toward higher wavelengths. Because amyloses contain a distribution of molecular size, and absorptivity of low DP amyloses increases as DP and λ_{max} increase, the absorbance will increase more rapidly below λ_{max} than it decreases above λ_{max}, even if distribution of chain lengths is normal. In essence, the absorbance spectrum is actually a compilation of overlapping individual spectra, each with individual λ_{max} and absorptivity values characteristic of a molecule of a specific DP.

The complexity of the iodine binding process makes interpretation of amylose-iodine data difficult. Using the classical method for potentiometric titration, it has been determined that pure amylose can accommodate 19–22% of its weight in iodine (Banks et al., 1971); this figure has been used as a standard for amylose determination for a long time. However, it has been shown that by reverse potentiometric titration, i.e. addition of amylose to a system containing an excess quantity of iodine, this amount is increased by half, to about 30% of the weight of the amylose (Knutson et al., 1982). Interestingly, this increase in iodine uptake was not reflected in an increase in the measured absorbance of the amylose–iodine complex. One may speculate that this is because spectrophotometric measurements at a single wavelength (i.e. at or near the wavelength of maximum absorbance) only reflect the absorbance of the most abundant molecular size in the sample, and variations in the number of chains above or below that size may be observed as a change in the shape of the absorbance curve, but not necessarily in the peak absorbance.

Examinations in this laboratory of the visible spectrum of the amylose–iodine complex of various starches led to the observation that differences in spectra of amyloses from different sources are constant, significant and quantifiable, even though they might be relatively small. Therefore, an investigation was carried out, using available synthetic amyloses (Rendleman & Knutson, 1998), highly purified laboratory preparations of naturally occurring amylose from different sources, and several laboratory-isolated and commercially available starches, to define these differences precisely. In the course of this study it became apparent that comparative evaluations of spectra of amylose–iodine complexes from varying sources might provide a useful aid for starch identification and for estimating variations in size and dispersity of amylose molecules based on characteristic absorbance profiles.

2. Materials and methods

Synthetic, low molecular weight amyloses with λ_{max} between 556 and 589 nm were prepared by the action of CGTase on cyclodextrins as described by Rendleman and Knutson (1998). Estimated DP of these amyloses ranged from 44 to 76.

High molecular weight amyloses were from laboratory-isolated starch products previously collected at NCAUR. Corn amyloses, designated C1–C4, potato amyloses, designated P1 and P2, which had been purified by 3–5 crystallizations from butanol according to Schoch (1942), were chosen as standards. Molecular weights of samples C2 and C3 have previously been reported; M_w of C2 was determined to be 1.76×10^5 by intrinsic viscosity measurements, (Knutson et al., 1982), and that of C3 was 2.78×10^5 by intrinsic viscosity and 2.56×10^5 by light scattering measurements (Griffin, unpublished data). Two laboratory-isolated amylose samples, from corn and tapioca, with an indeterminate purification history, and a commercial sample of amylose isolated from potatoes by butanol crystallization, were studied for comparison to the standard samples. The amylopectin standard was a sample of 'Amioca' waxy maize starch from Cerestar, which gave the lowest absorbance at 600 nm among samples used in this study. Laboratory-isolated amylopectins from corn and potato were also evaluated.

Starches were either laboratory-purified samples from the NCAUR collection or commercially available samples, used as received. Cornstarches were from: normal dent corn (2 samples); unfractionated amylomaize V, and large (14 μm) and small (6 μm) granule fractions (Cluskey, Knutson & Inglett, 1980); and unfractionated amylomaize VII (3 samples), and large (11 μm) and small (5 μm) granule fractions (Cluskey et al., 1980). Also, mixtures of amylose and amylopectin (designated 'C', 'V', and 'VII') that duplicated the proportions of amylose and amylopectin in normal corn, amylomaize V and amylomaize VII starches were prepared with standard amylose C1 (above) and 'Amioca' waxy maize starch. Other cereal starches were from barley, oats and rye (1 sample of each), sorghum (2 samples) and wheat (3 samples). In addition to the cereal starches, 2 samples of native and one sample of lintnerized potato starch were evaluated.

Amylose analysis was done by the method of Knutson (1986). Samples were dissolved in a mixture of 90% dimethylsulfoxide and 10% water containing 6×10^{-3} Molar iodine. Samples were allowed to dissolve at room temperature overnight. Assay was performed by diluting an appropriate volume of sample solution with eight volumes of water. Blue color developed immediately; samples were allowed to stand at room temperature 1 h to ensure maximum complex formation and color development.

Total carbohydrate content of samples was determined using the phenol-sulfuric acid method of Dubois, Gilles,

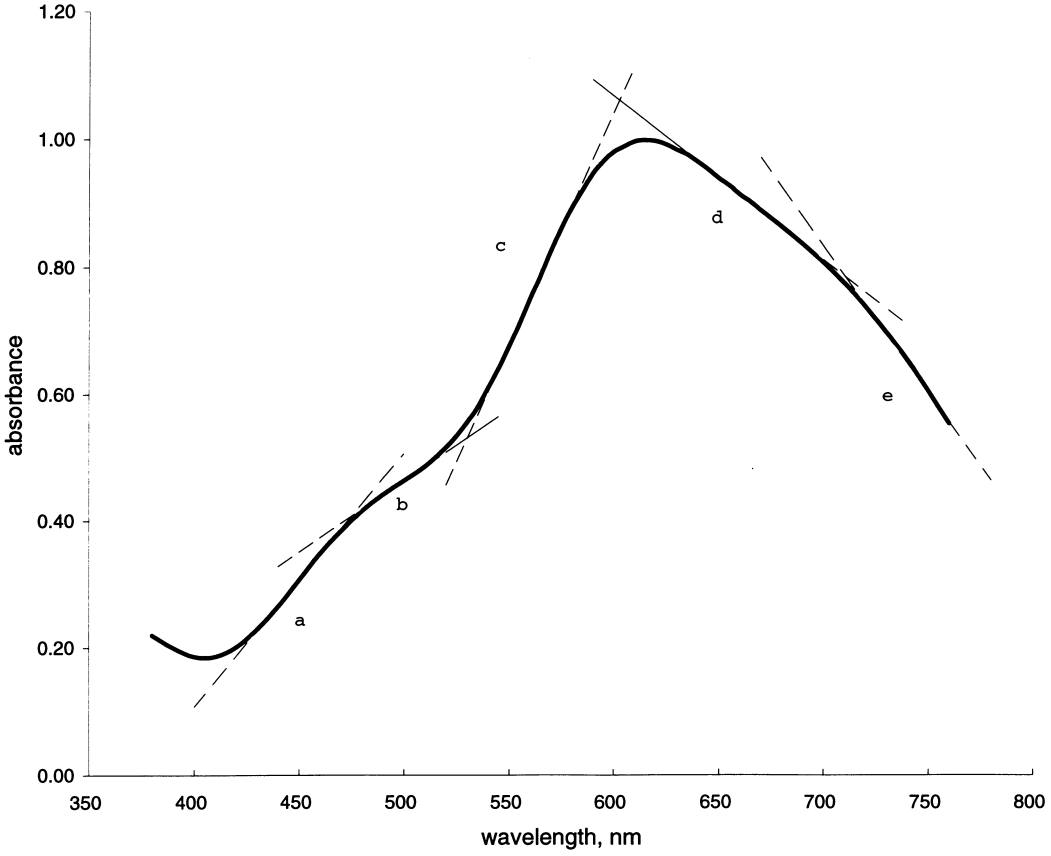

Fig. 2. Composite spectrum of high purity maize amyloses, showing slopes of regions between inflection points.

Hamilton, Rebers and Smith (1956). The value thus obtained was considered equivalent to starch content to determine absorptivity of amylose and amylose content of starches.

Spectrophotometric measurements were made using a Sequoia-Turner model 690 UV-visible spectrophotometer. Absorbance measurements were made at 5 nm intervals from 340 to 760 nm to obtain spectra. For each of the 6 standard amyloses, at least 4 spectra were obtained. The spectrum of each starch sample was measured at least twice.

3. Results and discussion

Variation in spectra of amyloses is demonstrated in Fig. 1, which shows absorptivity, calculated as absorbance per g/ml/cm, of a synthetic amylose with λ_{max} at 580 nm, and the high-purity maize and potato amyloses, with λ_{max} at 610 and 625 nm, respectively.

The variation in spectra of amylose–iodine complexes shown here was representative of that seen with starches from different species, i.e. substantial variation in λ_{max} and in the overall shape of their spectra; within species, variations were not significant. The changes were interrelated, i.e. slope of the ascending portion (below λ_{max}) increased as λ_{max} increased, while slope of the descending portion (above λ_{max}) decreased less rapidly. Spectra of the synthetic amyloses, with λ_{max} below 600 nm, were nearly symmetrical, whereas spectra of the high molecular weight amyloses were skewed. To describe these changes quantitatively, slopes of linear portions of the spectra were evaluated as described below.

3.1. Pure amyloses

Fig. 2 shows a composite spectrum of the 4 standard maize amyloses, normalized to an A_{max} of 1.0. The spectrum was typical for high molecular weight amylose, with inflection points near 420, 480, 530, 600 and 710 nm. Between those inflection points, five regions could be readily defined by their slopes, which were determined by linear regression analysis. The slopes of these regions (designated a, b, c, d and e) for the composite maize amylose, calculated as 10^{-3} absorbance units per nm relative to A_{max}, were 3.98, 2.26, 7.30, −2.56 and −4.62, respectively. Points of inflection, as determined by the intersection of the extension of the slopes, were 479, 530, 603, and 710 nm. Interestingly, these inflections corresponded fairly well with the absorbance maxima reported by Yu, Houtman and Atalla (1996) for amylose complexes with I_9^{3-}, I_{11}^{3-}, I_{13}^{3-} and I_{15}^{3-}.

As λ_{max} varied with molecular weight of the amylose, so too did the ascending and descending slopes of regions ‘c’

Table 1
Absorbance profiles of amyloses and amylopectin

Sample	λ_{max}	DP	Absorptivity	'c' slope	'd' slope	Ratio
Amylopectin	551		1.50	3.841	−4.621	0.833
Synthetic amyloses						
	556	44	11.81	5.077	−5.216	0.973
	570	54	13.73	5.319	−4.913	1.083
	575	59	17.32	5.782	−4.689	1.234
	578	62	17.71	6.161	−4.530	1.361
	580	64	18.16	6.219	−4.505	1.385
	582	66	18.79	6.424	−4.289	1.498
	584	69	21.22	6.017	−4.304	1.398
	585	70	21.24	6.272	−4.366	1.437
	586	71	20.29	6.299	−4.350	1.448
	588	74	20.40	6.357	−4.317	1.473
	589	76	20.65	6.531	−4.113	1.588
Amylose standards						
Maize						
C 1	609		27.20	7.468	−3.040	2.458
C 2	616		25.71	7.380	−2.783	2.659
C 3	620		27.42	7.385	−2.578	2.865
C 4	618		24.13	7.382	−2.560	2.884
Average	616		26.28	7.404	−2.740	2.716
Potato						
P 1	624		28.21	7.373	−2.453	3.007
P 2	620		23.67	7.367	−2.432	3.032
Avg	622		25.94	7.370	−2.443	3.019
Average, all standards:	618		26.18	7.403	−2.698	2.766
Other amyloses						
Corn	615		21.08	7.403	−3.095	2.392
Potato	620		19.58	6.861	−2.222	3.087
Tapioca	612		20.99	7.327	−2.924	2.506

and 'd' adjacent to λ_{max}. Slopes of regions 'a' 'b' and 'e' were less subject to molecular size and remained essentially constant. For the low molecular weight synthetic amyloses, slope of the 'd' region approached that of the 'e' region as the molecular weight decreased, until only one slope was detectable.

Table 1 lists the spectral characteristics of complexes of the representative synthetic amyloses of low molecular weight, the individual maize and potato standard amyloses, and the uncharacterized amylose samples. Those characteristics were: wavelength of maximum absorbance (λ_{max}); absorptivity at λ_{max}; slopes of regions 'c' and 'd' of the spectra (as 10^3 absorbance units per nm); and the absolute value of the ratios of region 'c' to region 'd'. This latter value provided an index of the symmetry of the spectra near λ_{max}; i.e. a ratio of 1 indicates a symmetrical peak. Additionally, this ratio provided an indication of the DP of the amylose; the ratio increased with increasing DP. Together, these values provided a profile of the spectra of individual amyloses which was constant and reproducible and therefore useful for characterization of the amyloses. For region 'c', slopes were calculated from absorbance values between 0.7 and 0.9 of A_{max}; for region 'd', slopes were calculated from values between 0.9 and 0.7 A_{max} where possible; but for most high molecular weight samples, the inflection at 710 nm interfered with linearity of the slope before 0.7 was reached. For those samples, the lower limit was arbitrarily set at 710 nm. Linear correlation in the region of 'c' and 'd' slopes was 0.998 or higher.

For low DP amyloses, in which c/d slope ratio approached unity at the lowest molecular weight, the slopes presumably represented that of a sample with a normal molecular weight distribution at constant absorptivity. As DP and absorptivity increased, slope of the 'c' region increased rapidly; slope of the 'd' region decreased, but less rapidly than the 'c' slope increase, resulting in an increase in the c/d ratio with increase in molecular size. For high molecular weight amyloses, slopes of the 'c' regions showed relatively little variation, indicating that the maximum attainable slope, given the contributing factors of increased DP and increased absorptivity, was 7.4×10^{-3} au/nm. The 'shoulder' region, represented by slopes 'a' and 'b' in Fig. 2, was the result of formation of complex with the shorter chains in the amylose molecules, as described by Takeda, Maruta and Hizukuri (1992). Interestingly, λ_{max} of amylopectin was similar to that of the lowest DP synthetic amylose (551 vs. 556 nm), but its absorptivity was much lower. This demonstrated the steric effects interfering with complexing of amylopectin segments with iodine, as complex formation can occur only with free branches, i.e. A chains and ends of B chains, that are long enough to form helices. The fact that the

Table 2
Comparison of absorbance profiles of cornstarches, amylose–amylopectin mixtures

Starch	λ_{max}	%Amylose	'c' slope	'd' slope	Ratio
Waxy maize	551	0.0	3.841	−4.621	0.833
Common					
CS1	610	23.07	6.369	−2.735	2.328
CS2	610	21.81	6.398	−2.737	2.337
Average	610	22.44	6.383	−2.736	2.332
Mixture 'C'	610	22.08	6.601	−3.080	2.143
Class V amylomaize					
Unfractionated	601	47.85	6.328	−3.205	1.976
14 μ	596	38.86	6.116	−3.323	1.841
6μ	600	49.56	6.472	−3.359	1.927
Mixture 'V'	610	46.53	7.283	−3.116	2.338
Class VII amylomaize					
Amy1, unfractionated	604	64.73	6.685	−3.208	2.085
Amy1, 11 μ	599	52.65	6.725	−3.511	1.915
Amy1, 5 μ	605	69.62	6.859	−3.248	2.112
Amy2	605	67.63	6.852	−3.183	2.153
Amy3	600	63.89	6.686	−3.265	2.048
Average, unfractioned	603	65.42	6.741	−3.219	2.095
Mixture 'VII'	610	68.69	7.458	−2.881	2.589
Class VIII amylomaize	600	73.51	6.956	−3.472	2.003

absorptivity of the amylopectin is 13% that of the corresponding amylose is consistent with the estimate of 15–20% crystallinity that can be achieved in amylopectin (Zobel, 1988), in that crystallinity also requires the freedom of branches to form helices.

Difference in the spectra of the four maize amyloses demonstrated the effect of molecular size on the parameters of the amylose iodine complex. Molecular weights of samples C2 and C3, have previously been reported. C2, with a M_w of 1.76×10^5 (DP 1100) by intrinsic viscosity measurements, (Knutson et al., 1982), was consistent with the value of 1.08×10^5 (DP 667) reported by Jane and Chen (1992) and 1.59×10^5 (DP 980) reported by Hizukuri, Takeda and Yasuda (1981) for maize amylose isolated in the same manner; C3 was 2.78×10^5 by intrinsic viscosity and 2.56×10^5 by light scattering measurements (Griffin, unpublished data), equivalent to a DP of 1600–1700. The differences in the parameters of their spectra, especially the slope of the 'd' region and the corresponding effect on slope ratios, demonstrated a significant and measurable effect of size on amylose–iodine complex formation at DP levels above 100, for which the linear relationship between 1/DP and $1/\lambda_{max}$ reported by Banks et al. (1971) and Bailey and Whelan (1961) cannot be measured accurately.

The slope and λ_{max} values for potato amylose indicated molecular weights higher than those for the maize amylose samples, consistent with the reported values for potato amylose 972×10^5 (DP 6000) by Jane and Chen (1992) and 1030×10^5 (DP 6360) by Hizukuri and Takagi (1984). It thus appears feasible to make preliminary molecular weight estimations from these values.

Absorptivity of high purity maize and potato amyloses at λ_{max} did not vary substantially. Overall average was 26.18 with a standard deviation of 2.17.

Absorptivity values of other amylose samples (tapioca amylose, commercial potato amylose and the uncharacterized laboratory-purified maize amylose) were substantially lower than that of the highly purified potato or maize amyloses, indicating lower purity; that assumption needs to be verified. The slope ratio of the commercial amylose was equivalent to that of the laboratory-purified samples, suggesting that an estimation of amylose molecular weight can be attained even if samples are not completely pure.

3.2. Corn starches

The same parameters were calculated for normal maize starches and amylomaizes, and for mixtures of amylose and amylopectin in proportions corresponding to those of the native starches. These mixtures were analyzed to attempt to define the spectral parameters for 'ideal' starches. The results are shown in Table 2.

Values for amylose–amylopectin mixtures varied significantly from those of starches with corresponding proportions. The 'c' slope of the 25% amylose mixture, corresponding to normal maize starch, was lower than that of high molecular weight maize amylose, due to the presence of amylopectin, but higher than the slopes for native starches, indicative of larger molecular size for the amylose in the mixture. However, the 'd' slope of the mixture was significantly more negative than either the purified amyloses or native starches. These variations appeared to indicate that the normal maize starches had a broader distribution of molecular size than did the highly purified amylose fraction, as would be expected. Absorbance maxima were identical for mixtures and native starches.

Profiles for high amylose starches were significantly different from those of normal maize starches, and varied

Table 3
Absorbance profiles of other starches

Starch	λ_{max}	%Amylose	'c' slope	'd' slope	Ratio
Barley	604	22.49	6.491	−3.091	2.104
Oats	598	18.68	6.420	−3.561	1.805
Potato, native					
PS1	617	24.55	6.508	−2.629	2.477
PS2	613	23.83	5.819	−2.501	2.326
Average	615	24.19	6.163	−2.565	2.401
Potato, lintnerized	600	20.56	6.136	−3.187	1.925
Rye	600	21.34	7.029	−3.684	1.908
Sorghum					
SS1	600	20.05	6.656	−3.574	1.864
SS2	605	21.24	6.420	−2.944	2.181
Average	603	20.64	6.538	−3.259	2.022
Wheat					
WS1	618	28.01	6.655	−2.528	2.633
WS2	613	24.52	6.562	−2.491	2.635
Average	615	26.27	6.609	−2.509	2.634

even more from the profiles of their corresponding mixtures than did the normal maize starch. The 'c' slopes of unfractionated amylomaize V and VII were similar to that of normal maize starch, but λ_{max} and 'd' slopes were significantly lower, indicative of lower molecular size. This finding is consistent with the numerous reports in the literature demonstrating that high amylose starches are of lower molecular weight than normal maize starches (Jane & Chen, 1992) and contain long-chain amylopectins and intermediate material with lower DP than true amylose (Wang, White, Pollak & Jane, 1993). Chain lengths of those components, while large enough to engage in increased iodine binding, are still relatively short compared to normal amylose chains, and would therefore cause an apparent increase in the distribution of amylose molecular size, with a shift of the overall spectrum to lower wavelength and a more negative 'd' slope.

Samples of granule fractions of varying sizes (Cluskey et al., 1980) of high amylose maizes were also evaluated. For both amylomaize V and VII, the large granules, which have been previously shown to have reduced amylose content (Knutson et al., 1982), had lower slope ratios than the small granules or the unfractionated samples, indicative of more long chain amylopectin and intermediate material in the large granules; the difference, however, was not great enough to be statistically significant, given the small sample population involved.

3.3. Starches from other sources

Parameters for other starches are listed in Table 3. Each starch had a characteristic absorbance profile, providing parameters useful for characterization or identification of these starches. The profile of lintnerized potato starch vividly demonstrated the effect of molecular weight reduction on the 'd' slope and consequently on the slope ratio.

Comparison of pure amylose profiles in Table 1 with those of the corresponding corn and potato starches in Tables 2 and 3 showed that the presence of amylopectin substantially reduced the 'c' slope and had a small effect on λ_{max}, but did not affect the 'd' slope significantly.

Examination of 'd' slope values for starches other than corn and potato predict relatively low DP for amyloses of barley, oats, rye and sorghum, and a value for wheat starch comparable to that of potato starch.

Because of the absorptivity of high purity amyloses was found to vary only slightly, and absorbance curves in the vicinity of λ_{max} were relatively broad, variations in spectra caused only minor discrepancies in amylose estimations based on spectrophotometric measurement of the complex. For example, error in amylose content caused by a fluctuation of 10 nm in λ_{max} was only 1%.

4. Conclusions

Amyloses vary substantially in the characteristics of their complex formation with iodine, depending upon molecular size. In addition to determination of absorptivity and λ_{max}, variations in spectra can be simply and conveniently evaluated by measuring the slopes of the absorbance curve above and below λ_{max}. Although these variations are small, they are quantitatively reproducible, making it possible use these values to obtain additional information about samples being assayed. Measurement of the complete visible spectrum of the amylose–iodine complex thus provides a convenient and relatively rapid means for detecting major differences between starches from normal and high amylose maizes or from other botanical sources, or between raw and modified starches, and for evaluating effect of processing conditions. For starches with unusual composition, e.g. the amylomaizes, these characteristics can be used for positive identification. Further investigation is continuing to determine feasibility of identification of starches from other maize genotypes by this technique.

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