Determination of Starch and Amylose in Vegetables

Application to Peas

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Sugars are extracted from peas with 80% ethyl alcohol and the starch is solubilized with dilute perchloric acid. Starch is then determined colorimetrically, without previous acid hydrolysis, by means of the sugar-anthrone–sulfuric acid reaction. Amylose is estimated colorimetrically with iodine. Both total starch and amylose can be determined in about 30 minutes in sugar-free solutions. The accuracy and precision are at least equal to those of standard methods involving acid hydrolysis. Analyses of smooth and wrinkled dried peas and fresh wrinkled peas are presented.

IN CONNECTION with work on the starch and amylose content of peas, it was necessary to analyze many samples, some of which were available in only small amounts.

Various methods for starch were examined. Those based upon optical rotation were unsatisfactory because of turbidity and the relatively large quantities of material required. Enzymatic hydrolyses are unreliable with starches of varying amylose-to-amylopectin ratios, while methods involving acid hydrolysis followed by reducing-sugar determinations are slow and require much of the analyst's working time.

A more expeditious method for starch and amylose in peas, applicable to small amounts of material, was required. Morse (*13*), Morris (*12*), Viles and Silverman (*19*), and Seifter *et al.* (*18*) used Dreywood's (*2*) color reaction of sugars with anthrone in sulfuric acid to determine sucrose, glycogen, and mixtures of pure dry starch and cellulose. None of these methods is directly applicable to the determination of starch and amylose in vegetables, but they offer a means of solving the problem.

This paper describes a method based upon the anthrone reaction, after removal of sugars with alcohol and solubilization of starch with dilute perchloric acid (*14, 15, 17*). Amylose is separately determined on an aliquot of the perchloric acid solution by its formation of an iodine-blue complex.

REAGENTS

Glucose Standard. Dissolve 0.100 gram of anhydrous glucose in 100 ml. of water. Preserve with 0.1% benzoate. Dilute 10 ml. of the stock solution to 1000 ml., and use 5 ml. for the standardization at 50 micrograms of glucose. Prepare the dilute standard daily.

Anthrone–Sulfuric Acid. Dissolve 2 grams of anthrone in 1 liter of cold 95% sulfuric acid. Store near 0° C. and prepare fresh every 2 days. This reagent is unstable and gives high blanks and variable results when too old. [Anthrone can be synthesized (*4*) or purchased. The Paragon Division, Matheson Company, Inc., Joliet, Ill.; the Panrone Chemical Company, Farmington, Conn.; and the National Biochemical Company, 3106 West Lake St., Chicago 12, Ill., are sources of anthrone known to the authors.]

Ethyl Alcohol–Water. Dilute 1680 ml. of 95% ethyl alcohol to make 2 liters of 80% alcohol.

Iodine–Potassium Iodide. Dissolve 2 grams of iodine in 20 ml. of a solution containing 20 grams of potassium iodide and dilute to 1 liter.

Perchloric Acid, 52%. Add 270 ml. of 72% perchloric acid to 100 ml. of water. Store in glass-stoppered containers.

PROCEDURE

Extraction of Sugars and Starch. DRIED PEAS. After grinding a composite sample of peas to pass a 60- to 80-mesh screen, weigh 0.200 gram of the flour into a 50-ml. centrifuge tube. Add a few drops of 80% alcohol to wet the flour and prevent clumping, add 5 ml. of water, and stir thoroughly. Add 25 ml. of hot 80% ethyl alcohol, stir thoroughly, and centrifuge after 5 minutes' standing. Decant and discard the alcoholic solution. Add 30 ml. of fresh hot 80% ethyl alcohol, stir, and centrifuge as before. Discard the alcoholic solution. Repeat this washing treatment twice more for a total of four washing treatments or until a test with anthrone is negative.

To the residue after the final centrifugation add 5 ml. of water, cool in ice water, and while stirring add 6.5 ml. of diluted perchloric acid reagent. Stir for about 5 minutes with a glass rod and occasionally thereafter for 15 minutes, keeping the mixture cold. Add 20 ml. of water and centrifuge. Pour the aqueous starch solution into a 100-ml. volumetric flask, add 5 ml. of water to the residue, cool in ice water, and stir while adding 6.5 ml. of diluted perchloric acid reagent. Solubilize as before for 30 minutes at 0° C. with occasional stirring and wash the contents of the tube into the 100-ml. flask containing the first extract. Dilute the combined solutions to 100 ml. and filter, discarding the first 5 ml. of solution. Cooling during solubilization is unnecessary if amylose is not determined.

FRESH PEAS. Blend 100 grams of fresh peas, or an amount sufficient to prevent sampling errors, with an equal weight of water in a mechanical blender for 5 minutes. Weigh a 5.00-gram sample of this slurry into a 50-ml. centrifuge tube and extract four times with 30-ml. portions of hot 80% alcohol by centrifugation and decantation or until a qualitative test with anthrone is negative.

After the final extraction add water to the sugar-free residue to make 10 ml. Cool the tubes and contents in an ice water bath and stir while adding 13 ml. of perchloric acid reagent. Stir occasionally for 15 minutes. Add 20 ml. of water, stir, and centrifuge. Pour the solution into a 100-ml. volumetric flask. To the residue add water to 5 ml., cool as before, and add 6.5 ml. of perchloric acid reagent. Solubilize as before for 15 minutes and wash the contents of the tube into the 100-ml. flask. Dilute the combined solutions to 100 ml. and filter, discarding the first 5 ml. of solution.

Determination of Starch. Dilute 5 to 10 ml. of the filtered starch solution to 500 ml. or to contain 25 to 100 micrograms of starch per 5 ml. of solution. Pipet 5 ml. of the diluted solution into a 25 × 250 mm. borosilicate glass tube, cool in a water bath, and add 10 ml. of fresh anthrone reagent. After the anthrone has been added to all of a series of sample tubes cooled in water, mix each one thoroughly and heat them together for 7.5 minutes at 100° C. Cool the tubes rapidly to 25° C. in a water bath and determine the color intensities, using light of wave lengths near 6300 A. (*12, 18*). Prepare a daily standard curve, using 0, 50, and 100 micrograms of glucose containing the same amount of perchloric acid as that in the starch aliquots, and use this calibration curve to obtain the yield of glucose from starch. Using the Klett-Summerson colorimeter, the K-64 filter, and the 12.5-mm. tube, scale readings of color intensities from the anthrone-sugar reaction, with 0 to 100 micrograms or more of glucose, fall on a line passing through the origin. Multiply glucose found by 0.90 to convert to starch.

Determination of Amylose. Dilute 5 ml. of the sugar-free starch solution containing about 5 mg. of starch with about 400 ml. of water. Add 5 ml. of iodine–potassium iodide reagent, mix, and dilute to 500 ml. After at least 15 minutes, determine the intensity of the blue colors with light of wave lengths near 6600 A. Use an iodine blank containing the same amount of perchloric acid as that in the samples to be analyzed. The Klett-Summerson colorimeter scale reading with 5 mg. of pea amylopectin was 25 (iodine "sorption" 0.2%) and that with purified pea amylose was 330 (iodine "sorption" 18.4%), using the K-66 filter and 20-mm. glass cell. (The iodine sorptive values were determined by plotting the e.m.f. against milliliters of iodine solution. The end point was taken at the point of inflection.) Scale readings of mixtures of these components totaling 5 mg. fall on a straight line

connecting these points. Other starch fractions behave similarly (*7, 8, 11*). Correct the observed scale reading to 5 mg. of starch material and read the per cent amylose from the graph.

VARIABLES AND LIMITATIONS

In carrying out the procedure it is necessary to extract the sugars from the pea flour or slurry, inasmuch as they react with anthrone. Care must be exercised to prevent contamination of solutions with lint, saliva, or other carbohydrate-containing materials. Pea proteins in the concentrations encountered here do not interfere; they may contribute to turbidity but do not yield an appreciable color with anthrone.

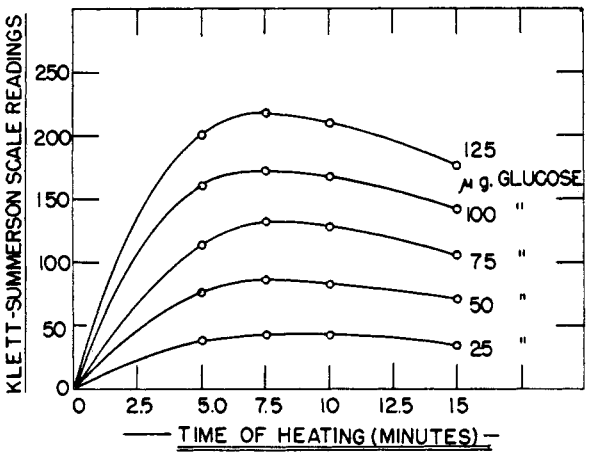

Figure 1. Effect of Time of Heating Glucose with Anthrone–Sulfuric Acid Reagent

Modification of the proposed method along the line of the procedure of Pucher *et al.* (*17*) will extend the scope of the method to other starchy vegetables or fruits.

It is necessary to carry out two extractions with dilute perchloric acid (*17*), because 5% of the starch is extracted in the second treatment. When amylose is to be determined, the starch extraction should be carried out in an ice bath. Significant decreases in intensity of the iodine-amylose blue solution results when amylose extracts are in contact with dilute perchloric acid (4.8 *M*) for even 30 minutes at 25° C. No measurable decreases occur at 0° C. for an hour or more. For the most precise measurements of the "blue value" it is advisable to discharge the iodine color with a crystal of thiosulfate and determine a possible turbidity blank. This blank, sometimes 2 divisions on the Klett-Summerson scale, should be subtracted from the gross reading of the blue values.

In the determination of starch with anthrone, the samples of sugar-free starch solution in test tubes are cooled in a water bath during the addition of the anthrone reagent. This precaution ensures a similar heating treatment for all samples in a given series and prevents erratic results from the heat developed by the water–sulfuric acid reaction. Figure 1 shows the results of heating various quantities of glucose in anthrone–sulfuric acid for different times. The heating period of 7.5 minutes produced the maximum color intensities measured with light of 6300 A. wave length.

Starch, sucrose, pentoses, and pectic acid were heated with the anthrone reagent. The intensities of the colors compared to a glucose standard are presented in Table I. Starch and sucrose yield the calculated quantities of glucose as if they were first hydrolyzed to simple sugars. Pentoses and the uronic acids, both of which yield furfural among their dehydration and degradation products, did not interfere in the concentration encountered here.

Air-dried starches, starch fractions, and some fresh wrinkled peas were analyzed by the proposed method and by acid hydrolysis, as suggested by the association of Official Agricultural Chemists (*1*), followed by Hassid's method (*5, 6*) for determining reducing sugars. A factor of 0.9 was used, although Etheredge (*3*) and others (*10*) reported factors varying from 0.90 to 0.94 to convert glucose to starch. A summary of these results is presented in Table II. The results are averages of two or more determinations and variations in replicate analyses agree within ±1.5%. The precision of anthrone values is equal to the method using acid hydrolysis. Close agreement may be obtained between the two methods if a factor of 0.92 is used for the conversion of acid hydrolysis–glucose values to starch.

Table I. Direct Analyses by Anthrone-Sugar–Sulfuric Acid Reaction

Substance, 100 γ	Glucose Equivalent	
	Found, γ	Theory, γ
Glucose	100	100
Sucrose	106[b]	105.2
Starch[a]	110	111
Arabinose	2	...
Xylose	2	...
Pectic acid	3	...

[a] Soluble starch, reagent grade, according to Lintner.
[b] Fructose yields same quantitative color reaction as glucose.

RESULTS AND DISCUSSION

Single samples (about 20 grams of dried peas) of several varieties of smooth and wrinkled dried peas were analyzed for starch and amylose. Starch was also isolated from several samples of

Table II. Analyses of Starches, Starch Fractions, and Fresh Wrinkled Peas[a] by Direct Acid Hydrolysis (Factor 0.90) and Proposed Anthrone Reaction

Sample	Starch, %		Anthrone/Acid Hydrolysis Ratio
	Acid hydrolysis	Anthrone	
Wisconsin sweet pea amylopectin	87.2	90.0	1.03
Wisconsin sweet pea amylose	87.2	89.1	1.02
Alaska pea amylopectin	88.0	88.2	1.00
Alaska pea amylose	85.6	88.7	1.03
World Record pea starch	84.1	86.5	1.03
Thomas Laxton pea starch	74.8	75.6	1.01
Mammoth White pea amylopectin	84.4	88.2	1.045
Mammoth White pea amylose	87.8	90.0	1.02
Alah pea amylose	86.3	90.0	1.025
Alah pea amylopectin	85.0	87.5	1.03
Fresh wrinkled peas (sieve size 3)	15.3[b]	15.8	1.03
Fresh wrinkled peas (sieve size 8)	25.8[b]	26.1	1.01

[a] Ferry Morse No. 9, Thomas Laxton variety.
[b] Results presented on dry-solids basis.

Table III. Determination of Starch and Amylose in Dried Peas and Pea Starch

Variety and Sample	Seed Type	Granule Type	Starch, %	Amylose in Starch, %
Alderman peas	Wrinkled	Compound	33.9	68
Alderman starch	Wrinkled	Compound	..	65[a]
Stratagem peas, 1	Wrinkled	Compound	33.8	69
Stratagem peas, 2	Wrinkled	Compound	32.8	65
Stratagem starch, ?	Wrinkled	Compound	..	60[a]
Stratagem starch, 1	Wrinkled	Compound	92.8	72
Thomas Laxton peas, 1	Wrinkled	Compound	33.1	71
Thomas Laxton starch, 1	Wrinkled	Compound	94.0	72
Perfection peas, 1	Wrinkled	Compound	33.3	69
Perfection starch, 1	Wrinkled	Compound	92.2	70
Perfection starch, ?	Wrinkled	Compound	..	69[a]
Steadfast peas, 1	Wrinkled	Compound	36.8	70
Steadfast starch, 1	Wrinkled	Compound	..	98[b]
First and Best peas, 1	Smooth	Simple	42.8	35
First and Best starch, 1	Smooth	Simple	94.6	36
Alaska peas, 1	Smooth	Simple	45.1	37
Alaska starch, 1	Smooth	Simple	93.6	37
Early Bird starch	Smooth	Simple	..	29[b]

[a] Calculated from iodine "sorptive" capacity of starch (*9*).
[b] Calculated from iodine-starch "blue value" of starch (*16*).

Table IV. Starch and Amylose in Fresh Wrinkled-Seeded Peas[a]

Sample	Sieve Size	Solids, %	Starch, %	Amylose in Starch, %	Amylose per Pea, Mg.
Lot 1	3	20.0	15.8	44	22
	4	21.6	15.8	47	34
	5	22.0	17.1	59	56
	6	23.1	20.1	63	82
	8	25.7	26.1	67	150
Lot 2	3	19.8	15.1	44	21
	4	20.6	14.6	55	36
	5	21.6	15.4	65	55
	6	23.0	20.6	69	92
	8	26.2	26.4	69	157

[a] Ferry Morse No. 9, Thomas Laxton variety.

these peas and analyzed for amylose. The results, presented in Table III, are compared with amylose determinations conducted by others. The samples were not the same in all cases. The results, however, are in general agreement with those of Nielsen (*14*, *15*), Hilbert and MacMasters (*9*), and Peat, Bourne, and Nicholls (*16*) in the conclusion that starch of wrinkled peas is high in amylose. The highest amylose content encountered here is 72%; the authors' analyses of Steadfast peas indicate 70% of amylose in the starch rather than 98% as previously reported (*16*). It is concluded that wrinkled-seeded peas with compound starch granules contain about 34% starch of amylose content near 70%, and smooth-seeded peas 44% starch of amylose content near 36%.

Two lots (different harvest dates) of several sieve sizes of fresh peas (Ferry Morse No. 9, a wrinkled-seeded garden variety) were analyzed for starch and amylose (Table IV). The dry weight, total starch, and amylose in both the peas and the starch increase with sieve size. Peas are graded by size and there is a definite correlation between size and tenderness in any one variety grown in the same locality and harvested on the same date. There is nothing to refute the contention that a No. 4 size pea in one pod might be as mature as a No. 6 pea in a different pod. These data, however, seem to indicate that size did correlate with amylose content of the starch. This method of measuring both starch and amylose in peas may offer a means whereby maturity can be correlated with a chemical property.

CONCLUSION

A rapid method for the determination of starch and amylose in peas is described, by which samples as small as a single pea can easily be analyzed. Starch is estimated by the glucose-anthrone–sulfuric acid reaction. Amylose is determined by its iodine color reaction. The accuracy is at least equal to that of other methods and a great saving of time is realized.

Analyses of both dried and fresh peas are presented. Wrinkled peas contain about 34% starch of 70% amylose content. Smooth peas contain 44% starch of 36% amylose content.

The ratio of amylose to amylopectin in wrinkled pea starches increases as the peas mature.

ACKNOWLEDGMENT

The cooperation of F. E. Lindquist and W. C. Dietrich of this laboratory in supplying frozen peas, and of A. L. Potter in determining the iodine-sorptive capacities of pea amylose and amylopectin, is gratefully acknowledged.

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RECEIVED February 20, 1950.

Determination of Pectic Substances in Cotton

Colorimetric Reaction with Carbazole

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A colorimetric method for the estimation of pectic materials in cotton is described. It involves the reaction of the extracted pectic acid with carbazole to give a colored reaction product.

PECTIC substances account for an appreciable portion of the noncellulosic constituents in cotton fiber (*3*). In studying the distribution of pectic acid and its correlation with the growth and properties of the fibers, a number of investigators have developed methods for its determination in cotton. Several (*1*, *4*, *5*) are based on the preliminary extraction with ethyl alcohol (4 to 24 hours), followed by extraction of the pectic acid and its precipitation as calcium pectate. The precipitates are difficult to filter and wash, and require long periods of drying to attain constant weight (*1*). In addition, they must be purified (*5*). Other methods (*4–6*), based on decarboxylation with hydrochloric acid require special apparatus and close attention over extended periods of time, frequently longer than the normal working day. Calculations of the results obtained by all these methods are based on empirical factors.

A colorimetric method is now presented, which is based on the evaluation of the extracted pectic acid by the reaction of carbazole with hexuronic acids (*2*). The values obtained are expressed in terms of anhydrogalacturonic acid, which is the fundamental unit of the pectic acid chain. This method uses samples