residues of alanine and phenylalanine possess tastes; the phenylalanine residue, phenylpyruvic acid, can be detected at very low concentrations.

Hypochlorous acid, monochloramine, or chlorine dioxide react with proline to yield a compound with a very low threshold taste.

Monochloramine deaminates alanine and phenylalanine at a slower rate than hypochlorous acid. Chlorine dioxide apparently deaminates phenylalanine to form the phenylpyruvic acid, although the mechanism of the reaction is not understood, as chlorine dioxide does not react with alanine.

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PLANT STARCH ANALYSIS

Rapid Determination of Starch in Apples

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A rapid colorimetric procedure was developed for determining starch in apples in the maturity range of commercial harvest and storage. The method was found to be accurate for the Jonathan, Golden Delicious, standard Delicious, and Winesap varieties over this maturity range. One fourth of the apple starch in these varieties appears to be amylose.

THE QUANTITATIVE DETERMINATION OF starch in plant material is usually a tedious and time-consuming procedure. Purification by extraction or precipitation and acid or enzymic hydrolysis are frequently required. The determination of starch in apples requires purification to remove sugars and also (when acid hydrolysis is used) polysaccharides such as pectic substances, which are partially hydrolyzed to reducing compounds (*14*).

Rapid colorimetric methods depending on the formation of colored starch-iodine complexes have been proposed by several workers (*6*, *11*, *12*, *15*). Most starch is composed of two fractions, a straight-chained amylose component and a branched-chain amylopectin fraction (*7*). Iodine reacts with amylose to give an intense blue complex with about six times as much color as the less intense blue-violet complex formed with amylopectin (*1*). Methods depending on the formation of these complexes therefore require that the amylose-amylopectin ratio be constant, or that the proportions be determined, if results are to be accurate. Methods involving specific rotation or enzymic hydrolysis are also dependent on a constant ratio. This ratio may vary with plant species (*1*, *12*), variety (*4–6*, *8*, *12*), maturity (*2*, *5*, *6*, *11*, *12*), and in the case of sweet corn with growing conditions (*2*). Nielsen and Gleason (*12*) have applied correction factors for iodine color values of starches from different plant sources. The literature thus indicates that the starch-iodine color complex can be used for the quantitative determination of starch in a given plant material only after it is established that the ratio of amylose to amylopectin is constant within the species and at different maturities, and under various growing conditions.

The time-saving possibilities of the colorimetric method justified an investigation of its applicability to several varieties of apples for which numerous starch analyses were required. This report describes the method developed and presents a comparison of results with those obtained by the method of Pucher, Leavenworth, and Vickery (*14*) on four apple varieties of different maturities. The procedure is essentially a modification of the method developed by Pucher and Vickery (*15*) and involves the use of perchloric acid as a starch solvent as proposed by Nielsen (*11*). The method was used in studies of the juice quality of Delicious apples (*9*).

Apparatus and Reagents

Photoelectric colorimeter, 620 mμ filter.
Food disintegrator.
Perchloric acid, 7.8*N*.
Potassium iodide, 5% solution.
Potassium iodate, 0.01*N* solution.
Sodium thiosulfate, 0.16*N* solution.

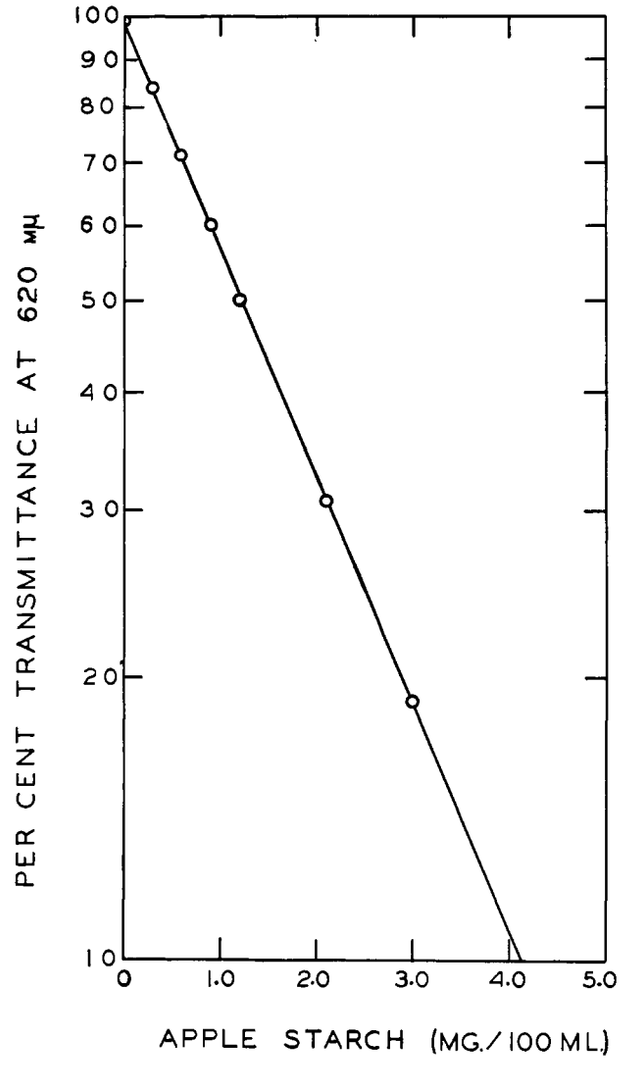
Analytical Procedure

Wedge-shaped slices cut from stem to calyx on opposite sides of each apple were ground 3 minutes in a food disintegrator to obtain a fine pulp. A 5.0-gram sample of this slurry was weighed into a 50-ml. beaker, and 20 ml. of 7.8*N* perchloric were added with rapid initial stirring to avoid high acid concentrations. Digestion was allowed to proceed for 5 to 10 minutes at room temperature and the mixture was then diluted with distilled water and transferred to a volumetric flask of a size depending on the approximate amount of starch in the sample.

In this study 500 ml. was found suitable for 1.5 to 3.0% starch, 250 ml. for 0.7 to 1.5% starch, and 100 ml. for a trace to 0.7% starch. The solution was mixed and filtered through a plug of borosilicate glass wool. To obtain fractional transmittance values between 20 and 80%, an aliquot containing 0.4 to 2.9 mg. of starch was transferred to a 100-ml. volumetric flask. One milliliter each of 5% potassium iodide and 0.01N potassium iodate was added and after a 5-minute delay to permit the iodine and starch to react, the solutions were made to volume and mixed, and the fractional transmittance was read at 620 mμ (red filter) in an Evelyn photoelectric colorimeter with standard macro absorption tubes. The instrument was set at 100% transmittance with a blank prepared from the same aliquot of starch and reagents as the sample, and also 2 drops of 0.16N sodium thiosulfate to remove starch-iodine color.

The percentage of starch in fresh apples was calculated from a standard curve (Figure 1), which was prepared from a dried, ground, alcohol-extracted residue of standard Delicious apples which had been analyzed by the method of Pucher, Leavenworth, and Vickery (*14*). To make the standard curve, the fractional transmittance values were determined from a digest of 0.5 gram of this material with 4.0 ml. of water and 20 ml. of 7.8N perchloric acid for 10 minutes. The digest was diluted and transferred to a 250-ml. volumetric flask. Different aliquots of known starch content were transferred to 100-ml. volumetric flasks, 1 ml. of potassium iodide and potassium iodate reagents added as in the above procedure, and transmittance readings taken. Jonathan, Golden Delicious, and Winesap apples gave standard curves nearly identical to that of standard Delicious shown in Figure 1.

Figure 1. Relation of transmittance to starch content



Effect of Varying Conditions

Perchloric Acid Concentration For colorimetric analysis it is essential to disperse the starch in a fine, stable, colloidal suspension with minimum hydrolysis to sugar. Nielsen and Gleason showed that the concentration of perchloric acid best suited for extracting starch was 4.8N. High perchloric acid concentration, high temperature, and increased time of digestion tend to favor starch hydrolysis. Diluting 72% perchloric acid to about 7.8N avoids to a large extent the hydrolytic effect and the temperature rise due to heat of dilution (*12*).

To determine the effect of normality of perchloric acid on apple starch, varying concentrations of perchloric acid were studied. Twenty milliliters of 6.0, 7.2, 7.5, 7.8, 8.2, and 8.7N perchloric acid were added to duplicate 5.0-gram samples of apple pulp and mixed to obtain approximate normalities in the digest of 4.8, 6.0, 6.3, 6.5, 6.8, and 7.0N, respectively. One half of the samples were permitted to digest at room temperature 5 minutes and the remaining half 10 minutes. When 6.0N perchloric acid was added, the starch was not dispersed sufficiently in either the 5- or 10-minute digestion. Addition of 7.2N perchloric acid also failed to disperse the starch in 5 minutes, but dispersion was complete in 10 minutes. The most effective perchloric acid concentration for maximum color in all varieties, with 5 or 10 minutes of digestion, was in the range of 7.5 to 8.2N. However, in the case of Winesap, 5-minute digestion, with 7.8N added perchloric showed slightly less hydrolysis than 10-minute digestion. Use of 8.7N perchloric acid resulted in appreciably less color. As a result of this study, 7.8N perchloric acid was selected as most effective in solubilizing apple starch with a 5- to 10-minute digestion. The final normality of 6.5 in the resulting digest is considerably higher than that used by Nielsen (*11*).

After digestion of 5.0 grams of apple pulp with 20 ml. of 7.8N perchloric acid, the digest was diluted sufficiently to retard hydrolysis and thus eliminate the neutralization step of Nielsen (*11*). It was also unnecessary to add 2N acetic acid, because there was sufficient hydrogen ion in an aliquot of the digestion mixture to release all the iodine required. The 1.0-ml. aliquot of 0.01N potassium iodate, which was considerably less than that used by Pucher and Vickery (*15*) and Nielsen (*11*), was found adequate.

Time of Digestion A study was made to determine the effect of digestion time in the range from 3 to 30 minutes using 20.0 ml. of 7.8N perchloric acid and 5.0 grams of apple pulp. Five to 10 minutes' digestion completely dispersed the starch in all samples with maximum color development. Three minutes' digestion was inadequate for dispersing the starch in some samples, while less color, presumably due to hydrolysis, was obtained in samples digested 15 and 30 minutes.

In the case of dried, alcohol-extracted residues used in preparing standard curves, 5-minute digestion with 4.0 ml. of water and 20 ml. of 7.8N perchloric acid was preferred for Jonathan, Winesap, and Golden Delicious and 10-minute digestion for standard Delicious.

Stability of Starch. Ground apple pulp, when analyzed 2 to 3 hours after grinding, gave results comparable to those obtained immediately after grinding. This stability may be due to enzyme inhibition in ruptured apple tissue (*3*) or resistance of raw starch granules to enzyme attack. Color of colloidal suspensions of starch-iodine was also observed to be stable over considerable periods of time as reported by Nielsen (*11*).

Maturity and Variety In determining the suitability of the colorimetric method for apples, it was essential that samples from several varieties, representative of the range of maturity encountered in com-

mercial harvest, be studied. It was also desirable to study the method over several seasons and to include stored fruit as well as fruit ripened after harvest. The varieties selected for this study were Jonathan, Golden Delicious, Winesap, and standard Delicious. Samples included fruit harvested at maturities ranging from earliest commercial harvests to maturities more advanced than is commercially desirable. Fruit held in cold storage and fruit ripened at room temperature for periods up to 6 weeks after harvest were also studied. This range of maturities and conditions in the apple varieties studied was similar to that used in studies of the quality of apple juice (*9, 10*).

Comparison of Methods

Preparation Of Samples The Pucher, Leavenworth, and Vickery method (*14*) was selected for comparison with the colorimetric method because it provided for the complete purification of apple starch through quantitative precipitation as a starch-iodine complex. Twenty-five grams of pulp from the same sample used for the colorimetric method were treated with 100 ml. of 95% ethyl alcohol, heated to boiling, and then filtered and washed with 95% alcohol to remove sugars. The residue was dried and ground for analysis.

Table I. Determination of Starch in Apples at Three Stages of Ripeness

Apple Variety	Sample	Starch Content[a] (Fresh Basis), % Pucher et al.	Rapid colorimetric
Jonathan	a[b]	1.04	1.02
	b	0.582	0.611
	c	0.334	0.332
Standard Delicious	a	2.57	2.57
	b	1.16	1.19
	c	0.458	0.499
Winesap	a	2.44	2.25
	b	0.946	0.919
	c	0.501	0.500
Golden Delicious	a	0.939	1.11
	b	0.569	0.611
	c	0.429	0.486

[a] Mean value of duplicate samples.
[b] Stage of ripeness.

The results of duplicate analyses by the two methods are shown in Table I. The starch content of these samples serves as an index of the range of maturities involved. These results are typical of comparisons made during three seasons.

Analysis of variance (Table II) indicates that differences between these two methods were not significant (*P*, probability, not significant at 5% level > 0.05). Variance between duplicates was just as great, if not greater, than variation between the methods. The interactions of method with variety, method with maturity, and method with maturity and variety are not significant ($P > 0.05$). These results would indicate that the ratio of amylose to amylopectin is sufficiently constant between varieties and maturities to make a colorimetric procedure practical.

Table II. Analysis of Variance of Starch Values

By methods of Pucher, Vickery, and Leavenworth (*14*) and rapid colorimetric for 48 samples of standard Delicious, Golden Delicious, Jonathan, and Winesap apples in 1953[a]

Cause of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F[c]
Method	0.0015	1	0.0015	0.07
Variety	5.3825	3	1.7941	89.25[b]
Maturity	14.2434	2	7.1217	354.31[b]
Method × variety	0.0402	3	0.0134	0.67
Method × maturity	0.0025	2	0.0013	0.06
Maturity × variety	3.9148	6	0.6524	32.46[b]
Method × variety × maturity	0.0297	2	0.0050	0.25
Error	0.4830	24	0.0201	
Total	24.0976	47		

[a] Analysis of variance based on mean for duplicates of each sample.
[b] Significant at 1% level.
[c] Variance ratio, $\frac{\text{mean square of group mean}}{\text{mean square of individuals}}$.

Assuming iodine color developed by starches in the apple to be identical with that from purified cornstarch fractions, additional evidence was obtained on the proportion of amylose and amylopectin in apple starch. This determination, made by the method of McCready and Hassid (*7*), showed that standard Delicious and Golden Delicious starches contain approximately 26% amylose, whereas Jonathan and Winesap starches contain approximately 25% amylose. These values are in good agreement with the 24.8% amylose content found in Newtown Pippin (*13*).

The accuracy of this colorimetric method depends primarily on the constancy of the amylose-amylopectin ratio and the precision of the starch analysis used to make the standard curve. To obtain most reliable results, a representative apple sample must be taken and matched colorimetric tubes must be used for the sample and blank. The measurement of 1.0 ml. of 0.01*N* potassium iodate must be accurate. With the rapid starch method it was possible to obtain duplicate starch values in closer agreement than those found by the procedure of Pucher, Leavenworth, and Vickery (*14*). A single starch determination may be made in 15 minutes when a 5-minute digestion is used.

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