chromatographic and other systems. Factors common to a reasonable number of these systems are not immediately apparent. The authors have observed the conversion in spinach heated at temperatures in excess of 100° C. in plain tinplate cans, enameled tinplate cans, and glass test tubes. Aqueous solutions of *p,p'*-DDT in glass gave gas chromatographic evidence for DDE but not *p,p'*-TDE under similar conditions.

A suitable mechanism for the dechlorination has not yet been suggested. It is a matter of some importance, however, for pesticide residue analysts to be aware of the possibility of conversion in a variety of food products.

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SUGAR CANE STARCH

Comparison of Methods for Determination of Starch in Sugar Cane Juice

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Two colorimetric methods for determination of sugar cane starch are compared—iodometric and anthrone. A referee method is also described and applied to verify the results obtained by the other methods. Six commercial varieties of sugar cane currently being grown in Louisiana were taken at different stages of growth for starch determination. Because of the changing chemical nature of the starch during cane growth, the anthrone method, which facilitates total starch determinations independent of such changes, was used in analyzing a series of samples representing sugar cane at different degrees of maturity. Amylose was determined iodometrically in each sample. Data collected, as a result of a systematic investigation representative of a Louisiana harvest season, indicate that starch composition (amylose-amylopectin) varies during different stages of cane development in response to the metabolic processes of the plant. When plant tissues representing different sugar cane varieties are analyzed for starch, variations in composition are observed.

THE work reported was undertaken as part of a broader program based on the investigation of the physicochemical properties of sugar cane starch in relation to filtration problems encountered by the processors of refined sugar.

Wood (*10*), upon using the iodometric method for the determination of sugar cane starch, observed that equally concentrated solutions of starch from different plant sources give different color intensities with iodine. He established a factor for the conversion of what he termed relative values to absolute values, since he was using as a standard a starch from another parentage. However, he, like other investigators [Nielson and coworkers (*7*)], did not take into consideration that the method is valid for the quantitative determination of starch only after it is established that the ratio of amylose to amylopectin remains constant at different degrees of plant maturity, under various growing conditions,

and from one variety to another. Carter (*2*) showed that the iodometric method finds practical application while working with apples of maturities ranging from earliest commercial harvests to maturities slightly more advanced than is commercially desirable. This is understandable because he was working with fruit which for all practical purposes had reached full maturity. In the case of sugar cane, which is a tropical plant, domestic or subtropical climatic conditions necessitate commercial harvesting well before full maturity is reached. During the Louisiana sugar cane harvest period it is reasonable to suspect that the starch composition will vary considerably.

Although the iodometric method as proposed by Balch (*1*) for sugar cane starch determination has been accepted as a standard, to date no sugar cane research relating to starch composition during the harvest season has been reported.

Experimental

Sampling. The samples used represent screened (100-mesh) fresh sugar cane raw juice, milled from a number of varieties of sugar cane harvested during the 1963 and 1964 seasons in accordance with commercial practices—i.e., stalks were topped at the first hard joint. The samples which were used to show composition changes during maturation were taken periodically from replicated plots by variety.

Sample Preparation. Three methods of starch preparation were employed. In all cases a sufficient quantity of raw juice was weighed, being predetermined on the basis of the refractometer Brix (per cent soluble solids), and a knowledge of the approximate starch range for sugar cane, in combination with a consideration of the sensitivities of the analytical methods being studied.

Procedure 1. Sugar cane starch to be studied by the iodometric method was prepared in accordance with a technique which is attributed to Balch (*1*). The raw juice was centrifuged in stainless steel test tubes at 17,000 r.p.m. (r.c.f. = 34,800 × G) for 30 minutes. After supernatant was decanted, the precipitates from each were quantitatively transferred to 250-ml. borosilicate glass beakers with 40 ml. of calcium chloride (sp. gr. = 1.30). These were then boiled for 18 minutes in order to disperse the starch, after which time cooling was allowed and the dispersed starch transferred to 100-ml. volumetric flasks and brought to volume. After being centrifuged at 2000 r.p.m. to remove suspended solids, aliquots of the supernatant solution were taken for analysis.

For the preparation of starch extracts to be determined colorimetrically with anthrone, two techniques were used.

Procedure 2. A procedure described by McCready *et al.* (*6*) was employed, with minor modifications. The raw juice was centrifuged at 17,000 r.p.m. (r.c.f. = 34,800 × G) in stainless steel test tubes for 30 minutes. The precipitates were then extracted with hot 80% ethanol until negative tests of the supernatant were obtained with anthrone. This consistently required five extractions. The test tubes containing the residue were immersed in an ice water bath, and 6.5 ml. of 52% perchloric acid was added to each with constant stirring. Stirring was continued for about 5 minutes, and thereafter occasionally during 15 minutes; 20 ml. of water were then added, and the mixture was centrifuged. The aqueous starch solutions were decanted into 100-ml. volumetric flasks, and the extractions of the residue with perchloric acid repeated. The combined extracts and washes of each were then brought to volume with water. The solubilized starch was filtered with suction through S & S No. 589 paper to remove any remaining suspended solids. Appropriate aliquots of the dispersed starch were then taken for analysis.

Procedure 3. The referee procedure employed for the preparation of the starch extract, as described by Hassid and Neufeld (*4*), is based partly on that developed by McCready and coworkers, and partly on the method of Pucher and coworkers (*8*), and affords a means of quantitatively isolating the starch in a highly purified state. The first stage in preparing the starch extract was as recommended by McCready, described in Procedure 1. After centrifugation and perchloric acid extraction of the starch it was necessary to have prepared an aqueous solution of 20% sodium chloride, and an iodine–potassium iodide reagent. The iodine reagent was prepared by grinding 7.5 grams of iodine and 7.5 grams of potassium iodide with 150 ml. of water; the resulting solution was diluted to 250 ml. and filtered through a Whatman No. 3 paper with suction. From each 100 ml. of starch preparation, 25 ml. were transferred to a 40-ml. conical centrifuge tube; 5 ml. of sodium chloride solution and 2 ml. of the iodine–potassium iodide reagent were added, and the solution was mixed. After standing at least 20 minutes, the starch-iodine complex was centrifuged, and the supernatant liquid removed with extreme care to avoid loss of precipitate. The complex was then suspended in 5 ml. of ethanolic sodium chloride wash solution and centrifuged. With care, the supernatant fluid was drawn off and discarded, and the washing repeated. To the precipitate, 2 ml. of 0.25*N* ethanolic sodium hydroxide was added, and the tube gently shaken and tapped until all the blue color was discharged. Sufficient time for decomposition of the complex must be allowed. The liberated starch, representing each sample, was then centrifuged and washed twice with 5-ml. portions of ethanolic sodium chloride. Complete dispersion of the starch with hot water, as recommended by Hassid, was very difficult, and in some instances impossible. To disperse the starch completely, 5 ml. of 52% perchloric acid were added to the purified starch in accordance with the technique employed by Hoffpauir (*5*). The starch solutions were then transferred quantitatively to 25-ml. volumetric flasks, and diluted to volume with water. Appropriate aliquots were taken from these starch preparations for analysis.

Methods of Total Starch and Amylose Determination. Iodometric. Ten milliliters of starch preparation containing between 0.3 and 2.5 mg. of starch were transferred to a 100-ml. volumetric flask and diluted to approximately 50 ml. with distilled water. Added in sequence were 5 ml. of 10% acetic acid, 5 ml. of 10% KI, and 5 ml. of 0.02*N* KIO_3. This solution was made to volume and allowed 15 minutes for color development. The light transmittance was measured on a Coleman Junior spectrophotometer with a matched set of round cuvettes (24 × 110 mm.), at 7000 A. against a blank solution prepared from the same chemicals and amounts, excluding starch. The transmittance reading was translated in terms of milligrams of starch from a standard curve which was prepared from known amounts of a commercial grade of corn starch of known purity.

The amylose determinations were made iodometrically by the method of McCready (*6*) with 10-ml. portions of the starch extract. The transmittance readings of the Coleman spectrophotometer were made at a wavelength setting of 6600 A., and were translated in terms of milligrams of amylose from a standard curve prepared with known amounts of a commercial grade of amylose of known purity.

Anthrone. Aliquots of starch preparation containing between 0.023 and 0.135 mg. of starch (0.5 ml.) were transferred to 25 × 150 mm. borosilicate glass tubes, and each was diluted to 5 ml. with distilled water. The test tubes containing the starch samples, and one prepared as a blank containing the same chemicals and amounts, starch excluded, were immersed in an ice water bath. Ten milliliters of freshly prepared anthrone–sulfuric acid solution, prepared by dissolving 2 grams of anthrone in 1 liter of cold 95% sulfuric acid, were added slowly to each sample and well mixed in the tubes. The test tubes were then placed in a boiling water bath and heated for 7.5 minutes, after which time they were rapidly cooled. With the use of a matched set of square cuvettes (15 × 100 mm.), the Coleman Junior spectrophotometer was set for 100% transmittance at 6200 A. with the blank, and transmittance of the starch samples was measured. In calculating the amount of starch from a standard curve of D-glucose, the readings were multiplied by 0.90.

Results and Discussions

The results of the analyses by the three methods are shown in Table I. Raw juice samples were chosen randomly, and the starch results listed are not intended to reflect degree of maturity or variety.

The starch contents of these samples, determined by the McCready and Hassid

Table I. Comparison of Colorimetric Methods for Determination of Starch in Sugar Cane Raw Juice and of Amylose Fraction Representative of Each Sample Acquired during 1963 Louisiana Harvest Season

Sample[a]	Starch Content (Soluble Solids, Basis), % McCready et al.[b] (6) (anthrone)	Hassid et al.[c] (4) (anthrone)	Balch (1) (iodomet.)	Amylose Content [Total Starch Basis, McCready (6)]
1a	0.166	0.166	0.132	53.1
1b	0.161	0.160		54.9
2a	0.093	0.100	0.072	47.7
2b	0.101	0.098		46.7
3a	0.106	0.094	0.025	26.1
3b	0.108	0.085		25.8
4a	0.090	0.081	0.063	44.4
4b	0.088	0.087		46.2
5	0.186	...	0.124	48.4
6	0.092	...	0.042	30.3
7	0.102	...	0.047	30.8

[a] a and b designate duplicates.
[b] Solvent extraction of sugars.
[c] Extraction of sugars and iodine precipitation of starch (referee).

methods, are in good agreement; however, the results obtained by the iodometric method, in all instances, are lower than those of the other methods. When the amylose quantitative values decrease, there is a corresponding increase in the difference between iodine starch values and those determined by the anthrone method.

Variation in composition of sugar cane starch from that of the corn starch standard is primarily responsible for the error introduced in the results obtained iodometrically. The corn starch used as a standard for the iodometric method was found to contain 55.3% amylose. However, the per cent deviation exhibited between the amylose present in the standard starch and that of the sugar cane starch does not coincide with the per cent difference between the methods employed. This may be explained in terms of the ratio of the extinction coefficient of the components (amylose, amylopectin) contained in a mixture at a particular wave length, as demonstrated by Richter (*9*). Other factors which have not yet been interpreted may partly account for the error: differences in the iodine-absorption capacities between cornstarch amylose and that of sugar cane starch amylose which is related to a difference in starch species, or to a difference in the physical state of the starch granules, as shown by Cotton and coworkers (*3*).

Samples of sugar cane (25-stalk bundles) representing six commercial varieties were chosen periodically for an assay of starch composition variations representative of a full period of harvest. Duplicate samples of each variety were taken; however, individual samples of each pair harvested on the same date were selected from two different field plots. Results of this study are shown in Table II.

Since this particular phase of the study was not made with the intent of promulgating the reasons for the observed changes in starch composition, but to show that such changes do occur, only generalizations relating to the reasons for composition changes are made. In any event, a possible single factor, or combination of factors contributing to the variations, such as agronomy, variety, or climate, would be difficult to ascertain from the data shown since no pronounced trends are manifested in relationship to sampling date or variety. However, there are considerable sample-to-sample and varietal variations in starch composition. An early October hurricane may have promoted abnormal growth during the harvest season, and a combination of this and the late season mild temperature which prevailed could produce extensive lateral budding, thus affecting the metabolic processes of the cane.

There is a drop in total starch shown by all varieties on October 9 (Table II), which represents the first sampling after the hurricane of October 3. In terms of relative values, this observation would

Table II. Determination of Total Starch and Amylose Representative of Sugar Cane Samples Taken Periodically during 1964 Louisiana Harvest Season

	Sugar Cane Variety											
	CP 44-101		NCO-310		CP 52-68		CP 48-103		CP 55-30		L 60-1	
	Starch Content[a] and Amylose,[b] %											
Harvest Date	Total	Amylose	Total	Amylose	Total	Amylose	Total	Amylose	Total	Amylose	Total	Amylose
9-9-64	0.047	41.07	0.039	38.46	0.070	35.80	...	...	...	...	...	...
	0.053	35.66	0.048	28.43	0.070	30.86	...	...	...	...	...	...
9-16-64	0.032	48.24	0.047	24.57	0.068	44.44	...	...	...	...	...	...
	0.033	48.61	0.051	31.05	0.090	44.44	...	...	...	...	...	...
9-23-64	0.038	45.94	0.045	32.73	0.105	46.05	...	...	...		...	...
	0.046	41.51	0.044	33.90	0.102	40.66	...	...	...	...	...	...
9-24-64	0.046	39.06	0.042	32.05	0.092	40.78	0.039	47.86	0.012	49.38	0.020	46.59
	0.041	35.35	0.042	27.77	0.074	32.71	0.035	52.26	0.009	51.28	0.022	38.58
10-1-64	0.051	33.33	0.040	23.06	0.087	44.18	0.064	37.73	0.025	26.39	0.033	21.15
	0.047	37.04	0.053	24.44	0.106	29.77	0.040	42.72	0.031	21.49	0.042	23.80
10-9-64	0.031	41.97	0.043	24.90	0.052	48.61	0.056	37.24	0.014	27.08	0.022	30.86
	0.028	36.55	0.033	28.88	0.054	34.29	0.032	38.46	0.018	24.00	0.023	30.03
10-16-64	0.023	46.29	0.032	37.03	0.053	43.20	0.040	39.80	0.014	33.33	0.024	33.33
	0.030	40.74	0.043	33.51	0.054	44.97	0.034	44.64	0.016	33.33	0.034	26.26
11-13-64	0.098	41.76	0.117	43.17	0.152	45.15	0.104	38.74	0.040	24.88	0.082	23.08
	0.116	41.99	0.160	38.96	0.133	43.92	0.134	41.37	0.059	31.43	0.078	20.51
12-1-64	0.056	40.12	0.053	37.88	0.169	54.26	0.040	31.24	0.017	51.89	0.021	39.68
	0.094	44.51	0.036	40.07	0.176	53.37	0.047	52.84	0.012	55.55	0.023	36.11

[a] Soluble solids basis (McCready-anthrone).
[b] Based on total starch.

not have been so obvious if the starch analyses had been performed iodometrically, since some of the samples show an increase in amylose. Also, on December 1, when the effects of lateral budding would be most pronounced because of mild temperatures, the drop in total starch as shown in Table II would not have been evident iodometrically for varieties CP 55-30 and L 60-1 since there was a substantial increase in the amylose fraction of the starch.

The iodometric method of starch determination, which has found broad acceptance and application in sugar cane research, is not reliable under the conditions previously cited, especially when analyzing raw juice from sugar cane which was grown domestically. The anthrone method has been found suitable for sugar cane starch work, producing results which are completely independent of any changes in starch composition. Limited preliminary experiments (unpublished) have shown this method to be adaptable to refinery liquors as well as raw juices, and in both applications reproducibility has been excellent.

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RESIDUE ANALYSIS

Spectrophotofluorometric Determination of Reserpine Residue in Poultry Tissues

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The fluorescence induced by the reaction of reserpine with nitrous acid has been used for its determination in poultry products. A cleanup procedure eliminates interfering compounds and other biological constituents. The method is sensitive to about 0.2 μg. of reserpine and will determine in the range of parts per billion, utilizing the spectrophotofluorometer. The accumulation of reserpine in poultry tissues and in eggs of birds on a medicated diet containing recommended levels is "relative zero" or nondetectable.

RESERPINE has proved effective as a stress - ameliorating agent for chickens, and is used to control and prevent aortic rupture in turkeys (*1*). It is available commercially as Serpasil Premix 0.08% in a carrier of confectioner's sugar and soybean feed (*2*). Reserpine is usually incorporated in feed at a level of 1 to 2 parts of reserpine per million for layers and broilers, and as little as 0.2 p.p.m. to aid in the prophylaxis and treatment of internal bleeding in turkeys. Considerations of human health made it necessary to determine whether significant amounts of reserpine residue were present in the edible parts and eggs of birds receiving a diet containing reserpine in recommended levels. For this reason, an investigation was undertaken to provide a method for the estimation of reserpine in poultry tissues and eggs in submicro quantities. Because of the low treatment levels involved, extremely sensitive, as well as specific, analytical procedures were required for its determination in biological material.

The extreme sensitivity of fluorescent measurements suggested an investigation of this technique. Sheppard, Wagle, and Plummer (*8*) noted that reserpine produced fluorescence in solutions of sulfuric acid and carboxylic acids, and in solutions of chlorinated hydrocarbons after exposure to ultraviolet light. Glazko and colleagues (*3*) found that solutions of reserpine in ethylene dichloride became strongly fluorescent in the presence of trichloroacetic acid and a small amount of nitroprusside. The latter was utilized by these authors to study the metabolism of reserpine in white rats, dogs, and monkeys. Poet and Kelly (*7*) described a fluorometric procedure suitable for determining reserpine in blood and urine, which is based on heating solutions of reserpine in sulfuric acid containing selenious acid. A modification of this method was utilized by Hess, Shore, and Brodie (*5*) to study the persistence of the drug administered in a large dose to rabbits. The current method (*9*) used by regulatory agencies for the analysis of medicated feeds is based on nitrous acid–induced fluorescence. This paper is a continuation and extension of the nitrite reaction to the determination of reserpine in parts per billion in biological material utilizing the spectrophotofluorometer.

Method Development

Although the nitrite technique was finally adopted for this work, a survey of the fluorescent response of reserpine in various reagents was first undertaken in an effort to ascertain the method of maximum sensitivity.

A stock solution was prepared containing 10 μg. per ml. of reserpine in methanol. Measurements were made at

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