

# Determination of Pectic Substances in the Presence of Dextrins

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The determination of pectins in the presence of dextrins in canned fruit tissue and in cut-out sirups was investigated. In cut-out sirups, dextrins present in the alcohol precipitate interfere with determination of pectins by the colorimetric carbazole method, and the pectins interfere with the chromatographic separation and determination of separated maltodextrins by copper reducing methods. The addition of

dextrins to pectins results in a 10% increase in carbazole values when added at equal concentration, and interference increases linearly with further increase in dextrin content. The Somogyi micromethod was even more sensitive to interference by pectins. A combination of precipitation with calcium and colorimetric carbazole determination of galacturonic acid in the precipitate was developed and found applicable.

During investigation of the effect of replacement of sucrose by corn sirup solids on the changes occurring in canned peaches during processing and storage, the authors observed that the alcohol precipitate obtained from cut-out sirups in the presence of added corn sirup solids differed in appearance and composition from that obtained from sucrose sirups (6). The precipitate was opaque, chalky in appearance, and sticky rather than the gelatinous translucent precipitate characteristic of pectins. The alcohol precipitates obtained from cut-out sirups containing added corn sirup solids were heavier and more voluminous. In the colorimetric carbazole determination, they develop a brownish red color on heating with concentrated sulfuric acid. Alcohol precipitates from sucrose sirups, however, were colorless before addition of carbazole.

Dische (7-9), in developing his carbazole procedure for uronic acids, observed that hexoses produce a brownish red color instead of the purple color characteristic of uronic acids. This was ascribed to the combination of brown color produced by hexoses heated with sulfuric acid with the red color produced with carbazole. Dische's colorimetric procedure was modified for the determination of pectic substances in cotton by Stark (21) and for pectic substances in fruits and vegetables by McComb and McCready (18, 19). While the carbazole method has been widely used for the determination of galacturonic acid and pectic substances (10), its limitations were recognized by Bitter and Muir (4). They proposed addition of borate to increase sensitivity of the procedure and increase stability of the pigment formed. McComb and McCready (18) reported hexoses not to interfere until their concentration relative to that of galacturonic acid was threefold or above. They did not investigate the possible interference due to dextrins or starches.

Appreciable interference of dextrins was observed with the carbazole colorimetric determination of pectins in alcohol precipitates obtained from sirups and in the alcohol-insoluble solids of fruit tissues. The extent of this interference was determined in cut-out sirups and in solutions of corn sirups and pectin. Quantita-

tive determination of pectic substances in the presence of dextrins by separation of dextrins by paper chromatography or charcoal column chromatography was not applicable (20, 24-27). Determination of pectic substances by direct titration (2, 10, 12, 13) or by ion exchange titration (1, 16, 17) also was not applicable. Precipitation of pectic substances as calcium pectate and gravimetric determination as such was not applicable—Carré and Haynes (5); Emmett (11)—because of the known entrapment of nonuronides in the calcium pectate gel (10). A combination of separation as calcium pectate with carbazole assay was satisfactory.

## Methods and Materials

**Corn Sirup.** In investigations on effect of corn sirup solids on yield and pectin content of alcohol precipitate, refined cane sugar (sucrose), commercial dextrose (Cerelese), and four different corn sirups were used. The corn sirups were prepared under the supervision of Corn Industries Research Foundation and used in canning tests carried out in 1955. Their description and composition as obtained from CIRF are given in Table I.

**Corn Sirup Dextrins.** Corn sirup dextrins were prepared from 43.2° Bé. 55 DE (dextrose equivalent) acid-converted, carbon-refined corn sirup obtained in 1962. This sirup was diluted with water to 40° Brix. Ninety grams of the diluted sirup were weighed into a predried, tared polyethylene centrifuge bottle, 151 ml. of 95% ethanol were added, and the sticky gelatinous sediment was separated by centrifuging, washed with 70% ethanol, then 95% ethanol, and finally acetone. The precipitate obtained, after drying in vacuo at 70° C. for 24 hours, weighed 4.85 grams. The corn sirup dextrins so prepared had a dextrose equivalent value of 5.50, as determined by the procedure of Bailey, Whelan, and Peat (3).

**Pectin.** In the 1957-58 investigations Sunkist Growers Exchange citrus pectin was used. This was purified by dissolving the pectin in distilled water, precipitating with 70% ethanol, filtering, and thoroughly washing with 70% ethanol. The alcohol-precipitated pectin was then redissolved in water, demineralized by passing over cation exchange resin in the hydrogen form, reprecipitated, and dried.

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**Table I. Composition of Corn Sirups Used in Comparative Tests**

	Corn Sirup			
	55-1	55-2	55-3	55-4
Dextrose equivalent (DE)	52.4	51.6	59.7	61.5
Bé.	43.0	43.0	43.0	43.0
Conversion	Acid	Acid	Acid	Acid-enzyme
Refining	Vegetable carbon	Vegetable carbon and ion exchange resin	Vegetable carbon and ion exchange resin	Bone-char and ion exchange resin
pH	4.7	5.3	5.2	5.2
Ash (sulfated, %)	0.29	0.024	0.027	0.036
Saccharides (as % sugars present)				
Mono-	25.7	25.6	34.0	32.5
Di-	16.6	17.5	18.4	29.4
Tri-	12.8	13.1	13.0	16.4
Tetra-	9.9	9.9	8.1	6.4
Penta-	7.9	7.3	6.4	4.2
Hexa-	6.0	5.6	4.6	2.9
Hepta- and higher	21.0	20.8	15.4	8.0

In the 1964-65 investigations, citrus pectin, N.F., obtained from the Exchange Lemon Products Co., was purified by suspension in water and precipitation by addition of 2.8 volumes of ethanol. The precipitate was filtered, washed with ethanol, dried in vacuo overnight at 70° C., and then ground in a Wiley mill to pass a 60-mesh sieve. The pectin so prepared had an anhydrouronic acid content of 67% as determined by the carbazole method (18). Galacturonic acid monohydrate (Nutritional Biochemicals Corp., Cleveland, Ohio), purified by suspension in water, decolorized by passing through charcoal column, concentrated by evaporation, and finally crystallized at 2° C., was used as standard. This standard analyzed as 90.8% galacturonic acid monohydrate.

**Analyses.** Approximately 40% sirups were prepared by mixing 625-gram aliquots of corn sirups and 625 grams of distilled water or by dissolving 500 grams of sucrose or cerelese in 750 grams of water. To 250-gram aliquots of distilled water and the above sirups, 0, 0.25, and 1.0 gram, respectively, of purified citrus pectin were added and dispersed in a Waring Blendor. The alcohol-precipitable matter in these sirups was determined by gradually adding 300 ml. of 95% ethanol to 100-gram aliquots with stirring, allowing the precipitate to stand overnight, filtering through Whatman No. 5 filter paper, washing with 200 to 300 ml. of 70% ethanol, and drying to constant weight in vacuo at 70° C. Colorimetric carbazole determinations were made by the procedure described by McComb and McCready (18) with the Klett-Summerson filter photometer with a No. 54 filter. The free and total pectin carboxyl groups were determined by the modified Hinton titration procedure described by Gee, McComb, and McCready (12). The absorption spectra of the pigments formed by corn sirup dextrans with and without added pectin were obtained in a recording Cary spectrophotometer Model 15. The microvolumetric copper reduction procedure of Somogyi (14) was applied to determine

dextrans in the presence and absence of pectins. The corn sirup dextrin and citrus pectin solutions were adsorbed on a 1 to 1 mixture of Darco G-60 (Atlas Powder Co., activated carbon) and analytical grade Celite 535 (Johns Manville, diatomaceous earth preparation) column (22-mm. i.d., 180 mm. long) prepared by packing dry, washing with water then 50% ethanol, and used at a flow rate of 2 to 7 ml. per minute. In the modified calcium pectate method, the pectin in the aliquot selected was first precipitated as calcium pectate. The calcium pectate precipitate was then heated to boiling, centrifuged hot, washed with boiling water in glass centrifuge bottles, and separated by centrifuging at 8000 r.p.m. for 10 minutes. The washed precipitate was suspended in 0.5% Versene [(ethylenedinitrilo)-tetraacetic acid tetrasodium salt] solution at pH 6. The solution of calcium pectate in Versene was slow but was facilitated by heating and shaking. One hundred milliliters of Versene were required for solution of each 10 mg. of calcium pectate. The pectin content of the Versene extract was determined as described by McCready and McComb (19).

#### Results and Discussion

**Effect of Corn Sirup Solids on Yield of Alcohol Precipitate.** The weight of alcohol precipitate obtained from 100 grams of sirup containing 0, 0.1, 0.2, and 0.4% of added purified citrus pectin varied with type of sirup and concentration of added pectin. The weight of alcohol precipitate obtained was 0.10 gram for cerelese, 0.60 gram for sucrose, 10 to 12 grams for corn sirups 55-1 to 55-3, and 4.8 grams for corn sirup 55-4. The increase in weight of alcohol precipitates with increase in added pectin was variable and inconsistent. Apparently incomplete precipitation of pectins and dextrans by alcohol and loss after precipitation during washing occurred. More consistent results were obtained by precipitation with alcohol acidified with hydrochloric acid. This procedure resulted in decreased

weight of alcohol precipitate. It did not avoid irregularities in changes in weight with increase in added pectin. Chen and Joslyn (6) reported higher weights of alcohol precipitate from cut-out sirups obtained from canned clingstone peaches when sucrose was replaced with corn sirup. The alcohol precipitates obtained in sirups varied from 10 times as high initially to 3 times after storage for one year.

In water, finely suspended particles precipitated at 0.2% pectin, and a translucent gelatinous precipitate formed at 0.4%. With sucrose or cerelose, finely suspended particles formed at all levels of added pectin. In corn sirups the precipitate was white, sticky, and opaque.

**Recovery of Pectin by Carbazole Assay on Alcohol Precipitates.** Alcohol precipitates from pectin solutions in water, sucrose, or cerelose gave the usual pink to red color in the range of 10 to 90  $\mu\text{g}$ . per 2 ml. Those from corn sirups were tan, dark orange, brown, or dark brown in color depending on the relative concentration of dextrans and pectins present. The recovery of added pectin from carbazole assay of alcohol precipitates from water varied from 90 to 95%; 90 to 100% for cerelose and sucrose; and 110 to 180% for corn sirup. The higher the level of added pectin and the lower the dextrose equivalent of the corn sirup the higher were the results. This, as shown below, is due to interference from the dextrans present.

**Recovery of Pectin by Titration of Alcohol Precipitates.** While the titration of carboxyl groups, liberated after saponification and corrected for acetyl content of aliquots used, compared closely with results obtained by carbazole assay of Versene-pectinase extracts of fruit and sugar beet marcs (12), the recovery of added citrus pectin from sirups was low and variable. The citrus pectin used contained pectic substances that had an esterification degree of 75% and an average of 60% anhydrogalacturonic acid. The recovery of added citrus pectin in alcohol precipitates obtained from various sirups was variable and low. It varied from 30 to 80% at low levels of added pectin, to 35 to 90% at higher levels.

**Interference of Dextrin in Photometric Carbazole Determination of Pectin.** The effect of addition of corn sirup dextrin to citrus pectin on Klett values for carbazole pigment absorption is shown in Table II and Figure 1. The data in Table II show that, as the amount of dextrin added is increased, the absorbance increases. The interference of dextrin is small when the dextrin-pectin ratio is 0.5 or less, but is appreciable at a ratio of 1.0, and increases linearly thereafter. The data in Figure 1 indicate that the interference due to dextrin is the same at all levels of pectin concentration tested, and this is true of the effect of pectin on absorbance of pigment formed at various levels of dextrin. While data on the absorption spectrum of the pigments produced in the carbazole test for various galacturonides, hexoses, and pentoses have been reported (7-9, 15, 18, 21-23) no data on starch or dextrans were reported. The absorbance spectra of the pigment formed by corn sirup dextrans, alone and in presence of pectin, are shown in Figure 2. This shows that the maxi-

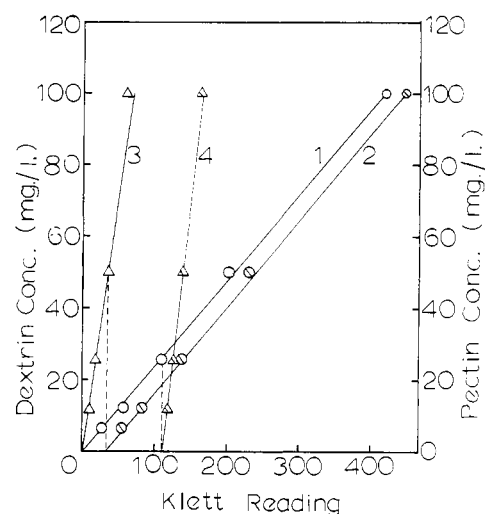


Figure 1. Effect of dextrin on determination of pectin by carbazole

1. Pectin
2. Pectin + 50 mg./liter of dextrin
3. Dextrin
4. Dextrin + 25 mg./liter of pectin

Table II. Effect of Dextrin on Carbazole Method

Dextrin Added to 25 Mg. of Pectin, Mg.	Dextrin-Pectin Ratio	Klett Value	AGA <sup>a</sup> Eq. Wt. Calculated, Mg.	Interference, %
0	0	111	16.5	0
12.5	0.5	115.5	17.4	5.5
25	1.0	122	18.3	10.9
50	2.0	137	20.5	24.2
100	4.0	165	24.5	48.5

<sup>a</sup> Anhydrogalacturonic acid.

imum for pectin is 532  $m\mu$ —McComb and McCready (18) reported a maximum of 525, Dische (7) and Stutz (22, 23), 527—while that for dextrin is 542  $m\mu$ . Holzman, MacAllister, and Neimann (15) and others have reported values near 545  $m\mu$  for hexoses. Pectins show a secondary maximum at 405  $m\mu$ , but this is lower, and shifted to 415  $m\mu$  in the case of dextrin.

**Interference of Pectins in Somogyi Volumetric Micro-method for Sugars Applied to Dextrans.** Somogyi's reagent was applied to determination of maltose and maltodextrans by Bailey, Whelan, and Peat (3), but because of interference with this reagent when dextrans were hydrolyzed to glucose with acid, a modified procedure was used by Whelan, Bailey, and Roberts (24). Whistler and Duffy (25) determined the reducing power of the isolated maltodextrans by an iodine method. The authors used Somogyi's reagent in the volumetric determination of corn sirup dextrans present in alcohol precipitates. The modification used was that described by Hodge and Hofreiter (14).

The effect of added pectin on the copper reduction by dextrin method for reducing sugars is shown in Table

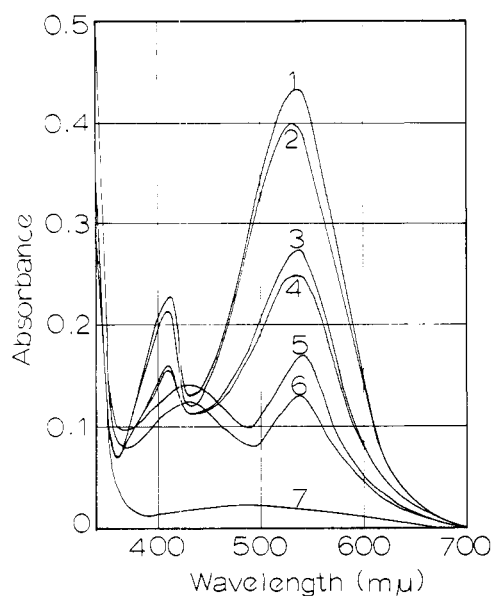


Figure 2. Absorption spectra of pectin-dextrin-carbazole pigment

1. Pectin, 50 mg./liter, + dextrin, 50 mg./liter
2. Pectin, 50 mg./liter
3. Pectin, 25 mg./liter, + dextrin, 50 mg./liter
4. Pectin, 25 mg./liter
5. Dextrin, 100 mg./liter
6. Dextrin, 50 mg./liter
7. Dextrin, 50 mg./liter

III. Apparently, the method cannot be used when pectins are present. An error of 50% is introduced when the ratio of dextrans to pectin is 5, and the error is increased more than linearly with increasing pectin content.

**Carbon-Celite Chromatography for Separation of Dextrans from Pectins.** In the separation of dextrans by carbon column chromatography, oligosaccharides with different molecular weight were expected to be eluted out of the column at increasing concentrations of ethanol. Since purified samples of higher maltosaccharides—i.e., tri-, tetra-, penta-, etc.—were not available, standard curves for each saccharide were not established. The amount of sugars eluted was calculated as its dextrose equivalent.

The recovery of dextrans, when 30% ethanol was used as the sole solvent, was only 71%. Dextrans were reported to be washed out completely by 30% ethanol

Table III. Effect of Pectin on the Somogyi Copper Micromethod for Determination of Dextrans

Sample, Mg.		Dextrin-Pectin Ratio	0.005N Thiosulfate, Net Ml.	Interference, %
Dextrin	Pectin			
2.5	0		3.50	0
2.5	0.5	5 to 1	5.20	49
2.5	1.0	5 to 2	8.00	129
2.5	1.25	5 to 2.5	9.75	180

(20, 26). When the column was washed by increasing strength of ethanol—i.e., H<sub>2</sub>O, 5% EtOH, 10% EtOH, 15% EtOH, and 30% EtOH—the total recovery of dextrans was increased to 92.0%. It was surprising, however, that reducing sugar was detected in the 5% EtOH effluent which should contain only maltose. If this fraction is regarded as the impurity or attributed to error, then the total recovery would be reduced to only 84%. The 10% EtOH effluents could contain maltotriose as reported by Whistler and Durso (26) and Whistler and Hickson (27), and 15 and 30% EtOH contain higher maltosaccharides.

On the other hand, the total recovery of pectin from the carbon column was very poor, amounting to only 36%. Pectin, as expected, was found only in the water and low EtOH fractions, not in the higher EtOH effluents.

In view of the low recovery of corn sirup dextrans from the carbon column, and the elution of appreciable amounts of pectin in alcohol solutions of 10 to 20% at which appreciable amounts of dextrin saccharides are eluted, carbon column chromatography was abandoned for separation of dextrans from pectin-dextrin mixtures.

**Combination of Precipitation as Calcium Pectate with Carbazole Color Development.** The recovery of pectin in the absence and presence of dextrin by precipitation as calcium pectate and then resolution in versenate and assay by carbazole reagent, shown in Table IV, was satisfactory.

The per cent recovery of pectin was high, averaging 96%. This indicates there was very little loss during the precipitation and washing with hot water. Samples containing both pectin and dextrin gave the same high recovery of pectin as samples with pectin alone, indicating that dextrans do not interfere.

The interference of Versene in the carbazole method was also investigated and found to be very small (less than 1%) as reported also by McComb and McCready (18).

Table IV. Recovery of Pectin by Precipitation as Calcium Pectate and Determination by Carbazole Method

Amount of Sample, Mg.		Pectin Found, Mg.	Recovery, %
Pectin	Dextrin		
10	0	10.5	105
20	0	18.4	92
30	0	29.4	98
40	0	38.4	96
10	10	8.6	86
20	20	18.6	93
30	30	29.6	99
40	40	39.2	98
0	20	0	0
0	40	0	0

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