Determination of Dextran and Starch in Cane Juices and Sugar Products

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Sugar cane, cane juice, and various products obtained therefrom frequently contain significant quantities of dextran and starch. Existing methods were not suitable for the rapid assay of these impurities in the presence of sucrose. A method is described for the rapid assay of mixtures of dextran and starch. Starch is determined colorimetrically and dextran turbidimetrically after enzymatic removal of the starch. Both of the impurities can affect technologic processes in cane sugar manufacture. Starch in raw sugar is associated with problems of filtration and in cane juice is related to the phosphorus content. The presence of dextran can indicate delay in harvesting cane or spoilage in juice.

A METHOD FOR THE RAPID ASSAY of mixtures of dextran and starch is described. Starch is determined colorimetrically and dextran turbidimetrically after the enzymatic removal of starch.

Sugar cane, cane juice, and various products derived therefrom frequently contain significant quantities of dextran and starch which interfere with technologic processes in sugar manufacture. Therefore it is desirable to devise rapid methods for their estimation. Existing methods are not suited for rapid assay, where dextran and starch occur together and in the presence of sucrose.

Methods considered for the determination of dextran included isolation and subsequent determination using anthrone (*9*), specific rotation (*12*), and refractive index (*10*), precipitation and direct weighing (*11*), and serological methods involving the reaction of dextran with pneumococcus antiserum (*5*). A turbidimetric method (*4*) used in clinical analysis was finally adopted for investigation.

In unheated raw cane juice the starch is in a granular form and may be separated from dextran by filtration or centrifugation. However, when the starch has been gelatinized by heat or retrograded by drying, its separation becomes more difficult. In such cases, it is usually isolated by extraction and estimated either colorimetrically as the iodine complex or as a reducing sugar after hydrolysis. A variety of extracting agents have been proposed; they include chloral hydrate, potassium hydroxide, formamide, dimethylformamide, and concentrated solutions of salts such as calcium chloride (*2, 3*).

Perchloric acid, proposed by Nielsen (*7*) appears to be the preferred extractant at the present time. Formamide has been used in the present investigation for a number of reasons. Early in this research it was anticipated that mixtures of starch and dextran could be determined by haze measurement, the starch then removed from an aliquot by enzymes, and the residual dextran determined by haze. Some extractants, particularly salts and to a lesser extent perchloric acid, caused a flocculation of the haze.

When this method for determining starch was abandoned and the flocculation of starch hazes by certain extractants ceased to be a factor, there still remained reasons for preferring formamide. Formamide does not require careful neutralization before addition of enzymes and no doubts can arise as to possible toxicity toward enzymes or hydrolytic attack on dextran. These points would have had to be investigated before the adoption of perchloric acid.

The accurate method of Pucher, Leavenworth, and Vickery (*8*) for the determination of starch was rejected as being time-consuming and was replaced by a colorimetric method which although more subject to error was considered suitable for the purpose.

Principle of Methods

When the sample of cane juice contains starch in a form easily separable from dextran by filtration, the filter cake containing the starch is extracted with boiling water and starch is determined colorimetrically in an aliquot. Standard curves have been prepared by similarly extracting cane starch and determining the relationship between the absorbance of the starch-iodine blue and the weight of starch determined as reducing sugar after acid hydrolysis. Dextran is estimated in the juice filtrate by adjusting an aliquot to 50% ethyl alcohol concentration and measuring the haze under standard conditions.

The concentration of dextran is read off a standard curve plotted by measuring the absorbance of hazes produced by known amounts of dextran.

This method of separation of starch and dextran is possible only in raw, unheated cane juice. In heated juices and in all other cases where starch and dextran occur in solution as mixtures they must be coprecipitated by alcohol in the presence of salts (*8*). The authors have dissolved the centrifuged alcohol precipitate of mixed polysaccharides by digestion with formamide. Starch is determined colorimetrically in an aliquot (the presence of dextran does not interfere) and the dextran is measured in another aliquot by haze measurement after the enzymatic removal of starch. The quantity of formamide present in the final solution in which the haze is read is insufficient to affect the reading.

Experimental

Preparation of Cane Starch. The method of Balch (*1*) was used. Raw cane juice, 100 ml., is mixed with 2 grams of diatomaceous filter aid and filtered and the cake is washed with cold water (3 × 10 ml.) followed by cold 70% alcohol (2 × 10 ml.), and boiling absolute alcohol (2 × 10 ml.). The alcohol is removed by sucking air through the cake for 15 minutes.

The cake containing the starch is dispersed in about 25 ml. of water, boiled for several minutes, and filtered. The cake is re-extracted with boiling water. The starch in the combined extracts is precipitated by adding alcohol up to 70% concentration, allowing it to stand,

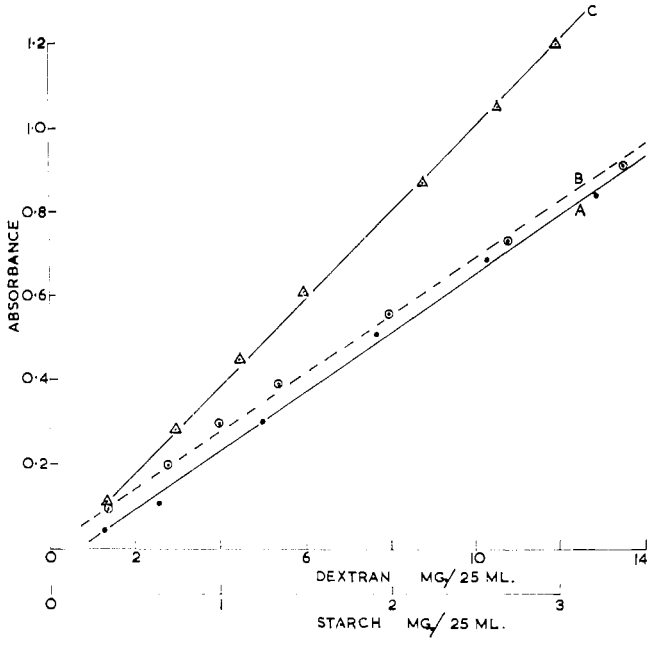

Figure 1. Standard curves

A. Dextran B. Cane starch C. Potato starch

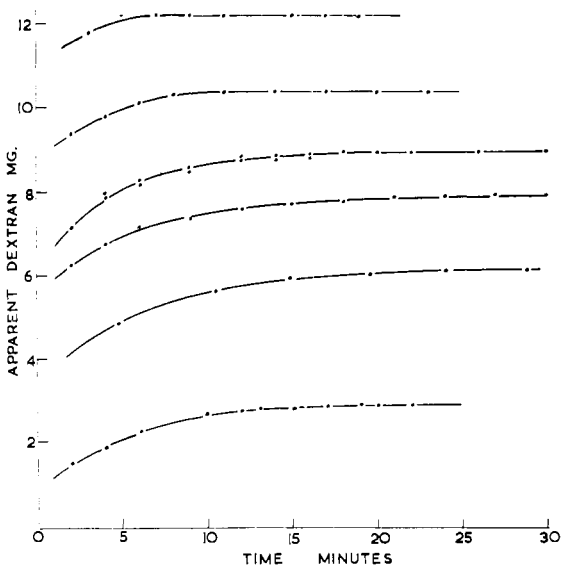

Figure 2. Rate of haze formation for dextran in 50% ethyl alcohol

and collecting it by centrifugation. The starch is again dissolved and reprecipitated before taking it up in the final solution.

This solution is used for determining the relationship between the absorbance of the iodine color and the amount of starch as described under "Preparation of Standard Curves."

Preparation of Dextran. A solution of purified dextran is prepared either from a culture of a strain of *Leuconostoc mesenteroides* or from cane juice that has undergone spontaneous dextranous fermentation. The culture is mixed with 0.1 volume of 10% trichloroacetic acid and filtered using a filter aid. Dextran is prepared by adding alcohol up to a concentration of 50 volume %, settling the precipitated dextran, and redissolving it in water using a high-speed stirrer to break up gels.

The dissolving and precipitation is repeated twice more, filtering (filter aid) between the second and third precipitations. The final aqueous solution is evaporated under reduced pressure to approximately 2% concentration. Dextran solids are determined by drying and corrected for a small ash content. This solution, which may be stored under refrigeration, is used for the preparation of a standard curve.

Preparation of Dextran Standard Curve (Figure 1, *A*). Aliquots (containing 1 to 12 mg. of dextran) are pipetted from a suitable dilution of the dextran solution into 25-ml. volumetric flasks, containing 0.5 ml. of 10% trichloroacetic acid solution. The total volume of the aqueous solution is adjusted to 12.5 ml. If subsequent determinations are to be made on solutions containing sucrose, this should be included in the standard, because it slightly diminishes the absorbance. Ethyl alcohol is added from a buret to the 25-ml. mark with swirling and timing is started. The contents are gently mixed without violent shaking. The haze is read *vs.* the water dilutions of the aliquots at 720 mμ after 15 minutes (Figure 2, for effect of time) and a curve is drawn of the absorbance *vs.* the weight of dextran. The curve (Figure 1, *A*) is based on dextran prepared from cultures of *L. mesenteroides* ATCC No. 8357 (American Type Culture Collection, 211 M St. N.W., Washington, D. C.)

Preparation of Cane Starch Standard Curve (Figure 1, *B*). The starch content of the solution whose preparation has been described is first determined by acid hydrolysis (*8*). Then suitable aliquots containing up to 3 mg. of starch are pipetted into 25-ml. volumetric flasks and diluted, and 5 ml. of iodine solution are added (0.005*N* containing 20 ml. of hydrochloric acid per liter) and made up to volume. The solutions are read at 700 mμ *vs.* a water blank of the same aliquot.

Preparation of Potato Starch Standard Curve (Figure 1, *C*). A solution of undried potato starch of known moisture content was prepared by suspending an accurately weighed amount in water and gelatinizing it by swirling the container in boiling water. The container should be held in boiling water for about 15 minutes with occasional swirling and the solution carefully inspected for the presence of uneven dispersion before cooling and making up to volume. A concentration of about 2.0 mg. per ml. is convenient.

A series of 50-ml. centrifuge tubes containing 10 grams of pure sucrose in each is prepared, aliquots of the prepared starch solution containing up to 15 mg. are added, and a solution is prepared by stirring after the total water has been adjusted to 10 ml. in each tube.

Ethyl alcohol, 24 ml., is added to each tube to raise the alcohol concentration to 70% based on the 10 ml. of water present. A few drops of saturated potassium chloride are added. The tube is vigorously shaken to aid flocculation and allowed to stand overnight.

Each tube is centrifuged and decanted, and the residue is extracted with 4 ml. of 50% aqueous formamide, for 50 minutes, on a boiling water bath. The sides of the centrifuge tube are occasionally washed and rubbed down with water to return into the solution, material which has deposited on the sides. Each formamide extract is made up to 25 ml. A 5-ml. aliquot is pipetted into a 25-ml. volumetric flask and 5 ml. of iodine solution are added and made up to volume. The absorbance is read at 700 mμ against distilled water and a curve of absorbance *vs.* potato starch concentration is plotted (Figure 1, *C*).

Analytical Methods

Starch and Dextran in Raw Cane Juice. The starch is concentrated by filtering the juice with a 2% diatomaceous filter aid and washing the filter cake as described under "Preparation of Cane Starch." After the second extraction with boiling water, the two extracts totaling about 40 ml. are combined and mixed in a 50-ml. graduated cylinder. The volume is noted and the absorbance of an aliquot is measured after the addition of 5 ml. of the iodine

solution to the aliquot in a 25-ml. volumetric flask and making up to volume. The milligrams of cane starch in the aliquot are read from the standard curve (Figure 1, *B*).

The method of reading the absorbance should follow exactly the method employed in constructing the cane starch standard curve.

If dextran is to be determined in the juice, the procedure is varied by adding 20 ml. of 10% of trichloroacetic acid to 100 ml. of raw juice and then proceeding with the filtration. The juice filtrate is removed for the determination of dextran before any washings of the cake enter the Büchner flask.

An aliquot of the filtered juice containing up to 12 mg. of dextran is added to a 25-ml. volumetric flask and the total aqueous volume is made up to 12.5 ml. Ethyl alcohol is added and the procedure is then repeated as under "Preparation of Dextran Standard Curve." Milligrams of dextran per aliquot are read off (Figure 1, *A*) and allowance is made for the dilution of the juice with trichloroacetic acid when calculating the results.

Starch and Dextran in Raw Sugars. Variations of the procedure described for this analysis will be applicable whenever starch and dextran are both in solution and cannot be separated by simple filtration. Ten grams of raw sugar and 10 ml. of water are stirred until dissolved in a 50-ml. centrifuge tube.

Ethyl alcohol (24 ml.) and 4 drops of saturated potassium chloride solution are added and the tube is stoppered, shaken vigorously and allowed to stand, preferably overnight.

The procedure is then the same as described for the "Preparation of the Potato Starch Standard Curve." After dispersion with formamide, aliquots are taken for the separate determination of starch and dextran. Starch is determined on a 5-ml. aliquot as described under "Potato Starch Standard Curve" using 5 ml. of iodine solution and a final volume of 25 ml. The absorbance is read *vs.* a blank prepared from 1 ml. of the original formamide solution plus 4 ml. of water. The results are calculated from Figure 1, *C*.

Dextran is determined on a 10-ml. aliquot pipetted into a 25-ml. volumetric flask, to which are added 1 ml. of phosphate buffer (pH 6.9) and 1 ml. of filtered diluted saliva (1 + 1). The contents are incubated for 30 to 45 minutes at 40° C. and samples originally high in starch are tested for completeness of removal by spotting them into iodine solution. The flask is cooled and 0.5 ml. of 10% trichloroacetic acid solution is added. The haze is developed by adding alcohol as described under "Preparation of Dextran Standard Curve." The dextran content of the aliquot is read off Figure 1, *A*.

Discussion

The proposed method for determining dextran is nonspecific, but it has been shown valid for the dextran produced by a number of strains of *L. mesenteroides*, which is the species of *Leuconostoc* commonly found in cane juice and sugar products. The presence of an unusual polysaccharide of the dextran type which can form in stale cane (*6*) and possesses similar solubilities, in aqueous alcohol, to mesenteroides dextran has introduced a problem. This material gives no color with iodine, but is attacked by salivary amylase to yield lower polymers soluble in 50% ethyl alcohol, whose presence would therefore not be detected in the dextran assay. If the presence of this material is suspected, the enzymatic removal of starch should be carried out with 10 mg. of "Bacterase" (Norman Evans and Rais, Ltd., England) instead of with saliva, as this enzyme does not attack the 1:4 linkages in the polysaccharide.

Determination of Dextran. The recovery of dextran by haze analysis from solutions containing known amounts is satisfactory (Table I), provided the standard curve has been based on dextran from a similar species of *Leuconostoc* organism. Different strains within the species, appear to yield dextran with characteristics sufficiently close to enable satisfactory analysis. Dextran from other species of *Leuconostoc* may have different solubility characteristics and determination would require a standard curve based on this material. It is shown in Table I that dextran from *L. dextranicum* gives less haze and is accordingly underestimated if the standard mesenteroides dextran curve is used.

Recoveries of dextran of 90 to 98% from mixtures of known composition containing sucrose and starch have been obtained. Salivary amylase makes a trace attack on normal dextrans, presumably, because a small proportion of the 1:4 linkages are available to the enzyme.

Rate of Haze Formation. The absorbance of the haze produced by the addition of alcohol increases with time and becomes reasonably constant after 15 minutes, at which point it should be read (Figure 2). Dextran hazes in 50% ethyl alcohol are, unlike starch, not prone to flocculation which upsets the absorbance reading.

Violent shaking of the suspension, particularly in the higher range of concentrations should however be avoided, and a visual inspection made of the contents in the cuvettes after reading to check for the absence of flocculation.

Determination of Starch. In earlier work, attempts were made to analyze starch-dextran mixtures by haze determination before and after the enzymatic removal of starch. These attempts were abandoned when it was found that the hazes developed by the mixtures were not additive for the two components. Starch by itself can be determined by haze measurement, but the suspension is more readily flocculated than dextran. Violent shaking causes flocculation and some starch-extracting agents such as perchloric acid and particularly calcium chloride also do so. Formamide is more satisfactory and this is one of the reasons which initially prompted its use. One disadvantage of formamide is that it must not be present in starch solutions which are to be acid-hydrolyzed, because it is itself hydrolyzed and will interfere. Starch can be satisfactorily separated for hydrolysis from aqueous formamide solutions by precipitation with alcohol.

Cane starches from fresh juices that have been separated by filtration and extracted with boiling water appear to vary slightly in absorbance with the variety of cane. The absorbance of cane starch per milligram appears equal to about 0.65 mg. of formamide extracted potato starch or 1.06 mg. of soluble starch (British Drug Houses). The procedure adopted in the extraction for preparation of the standard curve should be closely followed when analyzing cane juices. Starch which is present in raw sugar appears, both from color measurement and from its behavior toward amylases, to be considerably retrograded. Direct color measurement of the iodine complex in the sirup is unsatisfactory and preliminary precipitation of the starch, followed by redispersion with a solvent such as formamide, is necessary. The results are probably valid for normal raw sugars but the exact extent of the retrogradation and the resulting unavailability of the starch for color formation in sugars

Table I. Analysis of Dextran Solutions

Organism and Source	Dextran By weight, Mg.	Dextran By turbidity, Mg.
L. mesenteroides		
Culture	2.42	2.32
	2.42	2.42
Cane juice 1	2.05	1.98
Cane juice 2	1.07	1.12
Av. recovery 95%		
L. dextranicum		
Culture	4.06	3.27
	2.42	1.96
	2.42	2.01
	2.34	1.97
	4.20	3.35
	4.20	3.15
Av. recovery 80%		

that have been heated or stored for long periods remains to be investigated.

Because of the difficulty of obtaining a nonretrograded cane starch from a raw sugar to serve as a color standard, the authors have preferred to use an alcohol-precipitated formamide solubilized potato starch as a standard (Figure 1, *C*).

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BUTTERFAT OXIDATION

Evaluation of Lea's Aldehyde Determination Method

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Lea's method for determination of aldehyde in fats is excellent for *n*-heptanal. For normal aldehydes with more than seven carbon atoms, recovery decreased with increasing chain length and limiting values were reached with the C_9 and C_{10} aldehydes. For "aldehydes" from autoxidized milk fat the value found by Lea's method is arbitrary, because these carbonylic compounds do not behave like heptanal. Incomplete recovery can be caused by low solubility in water and low reactivity with bisulfite. Too high recovery may be obtained with unsaturated carbonyl compounds as a result of reaction of the double bonds. Reaction products and yields were examined by isolation of the aldehyde by solvent extraction after decomposition of the bisulfite complex. "Milk-fat aldehyde" was of ketonic character; the yield was about 1/3 to 1/10 as compared to synthetic aldehyde.

LEA'S METHOD for the determination of aldehyde involves an iodometric titration of bisulfite bound by the carbonyl group (*4*). A fat sample in benzene is shaken with a bisulfite solution. The excess of the bisulfite is removed from an aliquot of the aqueous layer by the reaction with iodide. The bound bisulfite is liberated with sodium bicarbonate and titrated with standard iodine. Lea claims quantitative recovery of *n*-heptanol.

As saturated aldehydes of medium molecular weight (heptanal) were at that time considered mainly responsible for the objectionable flavors and odor of oxidized fats, Lea proposed this method for estimating fat oxidation. Tamsma (*5*, *6*) and others have since shown that a wide variety of saturated and unsaturated carbonyl compounds, both aldehydes and ketones, are formed during fat oxidation. These may behave differently from heptanal, and recovery by Lea's bisulfite procedure is therefore not necessarily complete.

The present study was undertaken to investigate the extent and nature of aldehydes recovered by Lea's method from milk fat as compared with saturated normal aldehydes with 7 to 10 carbon atoms.

Since completion of this study, development of chromatographic methods has begun to make available more accurate knowledge of the nature of carbonylic compounds produced from autoxidized fats. Carbonylic oxidation products from butterfat appear to be largely ketonic rather than aldehydic, whereas in most fats they are very largely aldehydic. For this reason butterfat is hardly a fair choice of substrate on which to "evaluate" the Lea method, because ketones react more slowly and incompletely with bisulfite than do aldehydes. For this same reason, bisulfite extraction offers an interesting method for fractionation of the carbonyl compounds from butterfat.

Experimental Procedure

Synthetic Aldehydes. Commercial preparations (Givaudan-Delawanna, Inc., New York, N. Y.) of various aldehydes were purified by distillation under reduced pressure (about 12 mm.) to constant boiling point, density, and refractive index. The density was determined by the pycnometer method, and the refractive index (D line) with an Abbe refractometer, both at 20° C. Data for the purified aldehydes are presented in Table I.

Aldehydes were unstable at −15° C., as indicated by increases in density, refractive index, and melting point, and decrease in recovery by Lea's determination. In a dilute solution containing 1 to 6 μmoles per ml. of solution in a nitrogen atmosphere in the dark, purified aldehydes could be kept unchanged, at room temperature, for 2 months or more.

The ultraviolet absorption of the aldehydes in Skellysolve B was determined for solutions of approximately 4 μmoles of aldehyde per ml. Methods

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