No sulfur dioxide losses were observed during the recommended blending time. This was determined by gravimetric Monier-Williams analyses of several blended and unblended dried fruits. The error introduced by the transfer of a 10-gram aliquot with a calibrated pipet from the blender was found to be small, with a coefficient of variation of 1% in 10 trials of different dried fruits.

It was found that blank samples from a filtrate gave fairly constant values of about 0.02 in absorbance against water. Somewhat higher and inconstant values were obtained when the supernatant liquid was used. It is, therefore, possible for the estimation of sulfur dioxide content of dried fruits to use water for the 100% transmittance setting, whereupon a correction is applied to the absorbance readings.

The colorimetric method was compared with the methods of Nichols and Reed (*8*) and Monier-Williams (*1*). The results (Table I) indicate good agreement between the colorimetric and gravimetric Monier-Williams methods. The Nichols and Reed values were in every case higher than the values obtained by the other procedures. This may be due to the fact that no correction was made for blank samples, as unsulfured dried fruits were not available. It has been reported (*9*) that blank determinations by the Nichols and Reed procedure, may run to about 200 p.p.m. in some dried fruits.

Table I also presents replicate sulfur dioxide values on analyses of various dried fruits. The results indicate the adequate reproducibility of the colorimetric procedure.

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SUGAR IMPURITIES

Composition of "Floc" Formed in Acidified Sirups from Refined Granulated Cane Sugars

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Refined granulated cane sugar occasionally contains trace amounts of material which gradually precipitates from acidified sirups. This "floc" is undesirable in bottled beverages. Its major components are starch, lipides (wax), protein, ash constituents, and decolorizing carbon from the refining operation. Carbon and the organic components predominate. Silica is the major ash constituent. Possible test procedures for estimating floc in refined cane sugars were investigated. An improved procedure adapted from a floc test currently used in the beverage industry appears to offer the most promise.

ALTHOUGH REFINED SUGAR from both sugar cane and sugar beets is one of the purest industrial chemicals available, it occasionally contains a few parts per million of material which gradually precipitates from acidified sugar sirups. The precipitate, "floc," is undesirable in bottlers' concentrates, bottled drinks, and acidic pharmaceutical sirups.

Some progress has been made in alleviating the floc problem of refined beet sugar by modification and improvement of the processing methods, based on the findings (*9, 26*) that a saponin and its derivatives are chiefly responsible for this type of floc. The present investigation was undertaken to obtain information on the nature and composition of cane sugar floc and to explore possible methods for its estimation.

Sugars Used in Investigation

Commercial samples, including both flocculating and nonflocculating cane sugars, were obtained through the cooperation of members of the bottling and sugar refining industries. The five refined granulated sugars used for the isolation of floc were selected because they produced moderate to heavy floc in low pH sirups and were representative of different commercial refining processes. The processing histories and some compositional characteristics of the five sugars are given in Table I. The data reported are for the same components found in cane sugar floc.

Ash was determined by charring and incinerating the sugar in the presence of sulfuric acid at 550° C., following essentially the procedure of Valdez and Camps-Campins (*24*). Starch was determined colorimetrically as the starch-iodine complex in dilute perchloric acid solution by a slightly modified Balch procedure (*2-4*), adapted from Pucher *et al.* (*13, 19, 20*). Sweet potato starch of known purity was used as a standard. Soxhlet-extraction of 200 grams with chloroform for 42 hours, followed by processing the extract according to Browne and Zerban (*5*), was employed for determining wax. Protein content was calculated from total nitrogen values obtained by Kjeldahl digestion and by colorimetric evaluation using a special Nessler reagent (*25*). The relative amounts of decolorizing carbon (free carbon) were estimated by dissolving 25 grams of sugar in 100

ml. of aqueous phosphoric acid (pH 1.6), allowing to stand one-half hour, and filtering through a Millipore filter (Type HA, pore size 0.5 micron, Millipore Filter Corp., Watertown, Mass.). The filter disk was examined for carbon particles with a wide-field binocular microscope at 12× magnification.

Only trace quantities of the five components were found in the refined sugars, ash being present in the greatest amount. The starch content of sugar 3 was exceptionally low. The granular (vegetable) carbon used in processing this sugar may have been responsible for removal of starch (*4*). The sugars containing the greatest quantities of decolorizing carbon are the ones which gave the largest amounts of floc in the isolation work described below. Unfortunately, no samples of sugars prepared without the use of decolorizing carbon were available.

Table I. Processing Histories and Amount of Floc-Forming Constituents of Selected Refined Granulated Cane Sugars

Sugar No.	Processing History	Ash, P.P.M.	Starch, P.P.M.	Lipides, Wax, P.P.M.	Protein, N × 6.25, P.P.M.	Carbon, Free[a]
1	Affination of raw sugar; washing in centrifugals; Jacobs clarification of melted sugar liquor; filtration through bone char; decolorized liquor boiled for granulated sugar	76	24	13	44	2
2	Affination of raw sugar; washing in centrifugals; Williamson clarification of melted sugar liquor; filtration through bone char; decolorized liquor boiled for granulated sugar	110	34	10	91	4
3	Affination of raw sugar; washing in centrifugals; Jacobs clarification of melted sugar liquor; filtration through bone char, then through granular (vegetable) carbon; decolorized liquor boiled for granulated sugar	78	6	20	47	5
4	Affination of raw sugar; washing in centrifugals; melting and mechanical filtration of sugar liquor through filters of Sweetland type, with filter aid added; filtration through bone char; boiling in vacuum pans to granulated sugar	104	28	12	25	1
5	Same as for 4	114	37	13	56	3
	Average	96	26	14	53	..

[a] Relative amounts: 1 = lowest; 5 = highest in carbon.

Isolation of Floc

Sirups prepared according to the Coca-Cola Co. floc test (*21*) were used for large-scale isolation of floc. The floc formed in such sirups is comparable to that formed in a typical sirup used in the beverage industry for beverage manufacture. In the isolation experiments, sterilization in conjunction with the use of fairly high sugar concentrations (50 to 55 Brix) and low pH (1.5 to 1.6) prevented the growth of mold and bacteria. Separation by filtration or high speed centrifugation of the high-Brix sirups was not feasible. However, after sedimentation, siphoning, and concentrating the floc to a minimum volume, the sirup could be diluted to 25 to 27 Brix with aqueous phosphoric acid (pH 1.6), and the floc isolated and washed by high speed centrifugation. This eliminated the use of filter paper through which some of the finer floc particles might pass, and also facilitated the analysis and determination of the composition of the isolated floc. The procedure used for isolation of floc in a typical large-scale experiment was as follows:

A 10-gallon batch of 54% sugar sirup was prepared, using sterile glassware, equipment, and distilled water. Fifty-six pounds of refined granulated cane sugar were dissolved in 21 liters of distilled water in a 12-gallon borosilicate glass solution bottle. The sirup was acidified to pH 1.5 to 1.6 by the addition of 155 grams of 85% phosphoric acid (ACS grade) dissolved in 500 ml. of water, and filtered batchwise with suction through a large, coarse-porosity fritted-glass funnel to remove gross foreign matter, mainly fibers. The large area and coarse porosity of the filter prevented significant loss of floc constituents. The filtered sirup was collected in a 12-gallon borosilicate glass bottle; the bottle was capped and the sirup was allowed to stand until the floc coagulated and settled. Floc usually formed in 6 or 7 days and settled sufficiently for further processing in about 30 days. As much of the sirup as possible was removed from above the floc by gentle, stepwise siphoning.

The remaining sirup and floc were transferred to a 7-liter conical glass percolator. After the floc had again settled, the supernatant sirup was removed by siphoning. The sirup containing the concentration of floc was rinsed into a beaker with sirup, diluted with an equal volume of aqueous phosphoric acid (pH 1.6), transferred batchwise to a 100-ml. conical-tipped centrifuge tube, and centrifuged for 20 minutes at 6000 r.p.m. (10,000 × *G*). The clear supernatant solutions were discarded. The floc was resuspended and washed in the tube with aqueous phosphoric acid (pH 1.6) until free of sugar, and then washed with two 20-ml. portions of distilled water to remove phosphoric acid. The isolated floc was dried in vacuo at room temperature over phosphorus pentoxide. The water and any of the phosphoric acid washings which were not entirely clear were diluted to 70% alcohol concentration by the addition of 95% ethyl alcohol to precipitate a small amount of floc. This was isolated by high-speed centrifugation and washed with 70% alcohol until free of sugar. This floc was dried in vacuo, combined with the main portion, ground in a small Sillimanite mortar to assure homogeneity, and finally redried in vacuo prior to weighing to obtain the yield.

The above isolation procedure was employed for flocs 2 through 5 (Table II). In the case of floc 1, the procedure was basically the same except that the water washings from the floc were analyzed directly rather than by using alcohol to precipitate the floc from the washings.

Characterization of Floc

The scheme employed for characterization of the isolated floc was devised after preliminary exploratory tests and semiquantitative analyses on a small amount of floc isolated from 1 gallon of acidified sirup prepared from sugar 2. Microscopic examination of the floc showed the presence of tan or brownish material, black specks (presumably carbon particles), and other particles. Micro ashing indicated that the floc was about one-third inorganic material containing appreciable quantities of silica. Major combustible con-

Table II. Composition of Floc Isolated from Acidified Sirups Prepared from Refined Granulated Cane Sugars

Sample No.	Floc Isolated[a]		Composition of Floc, Moisture-Free Basis, %					
	Mg.	P.p.m.	Ash	Carbon, free	Starch	Lipides, wax	Protein, N × 6.25	Total
1	56.7	2.2	22.1	35.9	24.8	9.7	7.9	100.4
2	118.9	4.7	34.4	28.3	20.5	15.5	3.6	102.3
3	150.2	5.9	45.9	22.8	8.7	24.5	3.3	105.2
4	49.4	1.9	22.1	26.7	7.0	36.7	6.4	98.9
5	84.3	3.3	19.1	20.0	30.0	22.3	4.7	96.1
Average	91.9	3.6	28.7	26.7	18.2	21.7	5.2	100.5

[a] Per 56 lb. sugar.

stituents appeared to be free carbon, starch, and lipides. The material gave a slight Biuret test for protein, contained only trace amounts of phosphorus (probably phosphoric acid used in sirup preparation), and gave a negative test for pectin (*16*).

The following analytical and fractionation system was used to obtain the quantitative composition of the floc: Micro samples of the floc were ashed in platinum boats at 600° C. After the ash was weighed, sulfuric acid was added and the sample was reashed to determine sulfate ash. The essentially white ash was analyzed spectrographically for inorganic constituents by the line-width method (*17, 18*). The remaining floc was exhaustively extracted in a small centrifuge tube with hot, redistilled commercial pentane, using a centrifugation technique, to remove wax or lipides. After evaporation of solvent and vacuum drying, the weight of wax or lipides was obtained. The waxy, semisolid material was whitish to light yellow in color. This material was reserved for subsequent chromatographic investigation. The lipide-free floc residue was vacuum-dried to remove the last traces of solvent. Portions of this material were used for the remaining identification work. Starch was determined by extraction with (1 + 1) perchloric acid, followed by colorimetric evaluation of starch-iodine using a procedure adapted from Pucher *et al.* (*19, 20*). Native sweet potato starch of known purity was used as a reference standard and the absorbances were measured at 635 mμ. A micro Kjeldahl procedure was employed for determination of total nitrogen. Protein was calculated as N × 6.25. Carbon (free) was determined as follows: A sample of lipide-free floc was weighed into a micro platinum filter crucible. The material was extracted exhaustively at room temperature with (1 + 1) perchloric acid to remove starch and metallic impurities and help dehydrate silica, washed with water to remove the acid, and then extracted several times with hot 70% ethyl alcohol and hot absolute ethyl alcohol to remove saponins, phosphatides, etc., if present. The residue (which should consist predominantly of carbon, silica, and protein) was dried in a micro oven for 2 hours at 105° C., weighed, and then ashed for 1 hour at 600° to 650° C. The loss in weight on ignition was corrected for the amount of protein present. The balance of the weight loss is reported as free carbon, although it is possible that unidentified organic substances (*10*) are also present.

The proximate composition of the floc from each sugar is given in Table II. From 49.4 to 150.2 mg. of floc were actually isolated from the sirups prepared from each 56 pounds of sugar. The recoveries indicate the order of magnitude, but were not quantitative, because it was impractical to isolate the small amount of floc which remained suspended in the sirup. The trace quantities of floc material isolated represent from 1.9 to 5.9 p.p.m. of the sugars. As evident from the data in Table II, the composition of cane sugar floc varies over a considerable range. The recovery, averaging 100.5%, is considered well within the errors of sampling and analysis of materials of this kind.

Decolorizing carbon introduced in the sugar in the refining operations accounted for a considerable percentage of each of the flocs. Bone char was used in refining all of the sugars. Sugar 3 received an additional treatment with granular (vegetable) carbon subsequent to bone char filtration. This may be responsible in part for the fact that floc isolated from this sugar was low in starch content (8.7%). The other flocs contained appreciable amounts of starch, with the exception of sample 4, which had only 7% of this constituent. This low value cannot be reconciled with the high starch content (30%) for floc 5, isolated from a sugar which had a similar processing history. The starch in the isolated flocs undoubtedly had its origin as the starch normally found in small amounts in sugar cane (*2*), a small part of which still remains in refined cane sugars (*4*). Occasionally starch granules have also been found settled on the bottom of bottles of carbonated beverages (*12*). Although it is probable that the starch was modified somewhat during processing and refining of the sugar and isolation of the floc, it still gave a characteristic blue color with iodine, having only a slight reddish or purplish cast. The absorption spectrum of the starch-iodine complex in dilute perchloric acid solution in the presence of iodine-iodide, as determined with a Cary Model 14 recording spectrophotometer, was found to have a rather broad band with a maximum at approximately 600 mμ.

A specially prepared "native" sweet potato starch, containing approximately 21% of the blue-staining amylose fraction and 79% of the amylopectin fraction (*8*) was used in the present work as a reference standard for colorimetric evaluation of floc starch. Its iodine complex gave an absorption spectrum similar to that for floc starch with a maximum at 585 to 595 mμ. The slight shift to shorter wave lengths would indicate the presence of slightly more amylopectin in sweet potato starch than in floc starch. This is borne out by the work of Swanson (*23*), who observed a maximum at 650 mμ in the amylose-iodine spectrum and a maximum at 540 mμ for amylopectin-iodine, the effect of the two differently staining polysaccharides being additive. The slight difference in spectral characteristics of floc starch and sweet potato starch would not have a significant effect on the analysis of floc for starch because of the very broad absorption maximum.

The lipides (wax) contents of the flocs ranged from 9.7 to 36.7% and averaged 21.7%. Cuticle wax and lipides are normal constituents of sugar cane and are found in trace amounts in refined sugars (Table I). It has been suggested that the flocculation sometimes noted in carbonated beverages is attributable to wax present in the sugar from which they were prepared, and some refiners try to remove the wax as completely as possible and test the sugars for it (*5*). The present investigation shows that although wax is a contributor, several other components are involved in the formation of floc.

Because protein contributes to the turbidity of clarified cane juices (*10*) and would be expected to precipitate in the acidified sirups, the nitrogen in the floc is believed to be protein nitrogen. The material gave a positive Biuret test for protein and apparently did not contain phosphatides. Protein was the constituent present in the smallest amount in each of the flocs. The highest concentration of protein was 7.9% in floc 1.

The remaining major fraction of the floc consists of ash constituents. An average of 28.7% of ash was present. One sample (No. 3) contained 45.9%. Al-

Table III. Proportional Composition of Combustible and Ash Fractions of Floc

Floc No.	Floc: Combustible fraction[a], %	Floc: Ash fraction[b], %	Combustible Fraction: Carbon, free, %	Combustible Fraction: Starch, %	Combustible Fraction: Lipides, wax, %	Combustible Fraction: Protein, % N × 6.25	Ash Fraction: SiO_2, %	Ash Fraction: Other, %
1	77.9	22.1	45.8	31.7	12.4	10.1	71.0	29.0
2	65.6	34.4	41.7	30.2	22.8	5.3	77.6	22.4
3	54.1	45.9	38.4	14.7	41.3	5.6	82.9	17.1
4	77.9	22.1	34.8	9.1	47.8	8.3	52.8	47.2
5	80.9	19.1	26.0	38.9	29.0	6.1	69.8	30.2
Average	71.3	28.7	37.3	24.9	30.7	7.1	70.8	29.2

[a] 100 − % ash in floc.
[b] Analytically determined as % ash in floc.

though the sugars from which the sirups were prepared are not abnormally high in ash content (Table I), they contain a few parts per million of silica along with other ash constituents. Practically all of the silica present in cane sugars is in an insoluble form, according to Alexander and Parrish (*1*), being produced by the precipitation of the soluble silica as silicon dioxide (hydrated) during concentration of the juice and boiling of the sirup in sugar manufacture. It would be anticipated that this silica, as well as any silica present in the sugar in the form of diatomaceous filter aid from the refining process, would be insoluble in the acidified sirup and would concentrate in the floc. Other more soluble inorganic constituents of the sugar would remain in solution. That this is the case is borne out by the data in Table III. Most of the ash fraction of the floc consists of silica, which comprised from 52.8 to 82.9% of the ash from the five flocs. A further indication that the ash is primarily siliceous and does not contain appreciable amounts of metallic oxides and carbonates is afforded by the fact that sulfate ashing of the floc gave ash contents comparable to those obtained by dry ignition. The nonsilica fraction of the ash contained iron, aluminum, and copper, and in some instances trace amounts of manganese, tin, zinc, boron, and calcium. The iron contents of the ash ranged from 0.5 to 0.6% (as ferric oxide). Aluminum contents varied from 1 to 1.6% as aluminum sulfate, sulfate ash. The amount of copper was variable, ranging from 0.3 to 3.8% as copper sulfate (sulfate ash).

The proportional composition of the combustible fraction of the floc is given in Table III. Free carbon and the organic substances predominate in each of the five flocs analyzed. The flocs contained an average of 71.3% of these combustibles. Decolorizing carbon accounted for 37.3%, on the average, closely followed by wax and starch which averaged slightly lower. Protein represented a considerably smaller percentage. In a study of the chemical composition of materials causing turbidity in clarified sugar cane juices (*10*), it was found that organic substances predominate, averaging 72%. About two thirds of this organic material consisted of starch, protein, and lipides. The ash was primarily siliceous, as was observed for floc ash in the present study. The over-all composition of the cane sugar floc and the material causing turbidity are therefore similar in some respects. However, the former contains appreciable quantities of decolorizing carbon and usually has higher proportions of starch and lower amounts of lipides and proteins in its combustible fraction.

Paper Chromatographic Examination of Floc Lipides

A limited investigation of the nature of the lipides (wax) fraction extracted from the isolated flocs with petroleum ether was undertaken. Because of the small amounts of material available, it was necessary to resort to chromatographic techniques. The procedure of Dieckert and Reiser (*6*, *7*), which employs silicic acid–impregnated glass fiber paper, was used. The two solvent systems utilized were 2% (v./v.) ethyl ether in iso-octane, and pure iso-octane. Spots on the chromatograms were detected by spraying with concentrated sulfuric acid, followed by heating over an exposed-coil electric heater. In most cases, about 5 to 10 γ of floc lipides or of known compounds were chromatographed. The knowns consisted of saturated glycerides such as tristearin and distearin, palmitic acid, oleic acid, ceryl alcohol, cholesteryl palmitate, saponin from beet sugar, a cottonseed oil containing unsaturated glycerides, and an authentic sample of purified sugar cane cuticle wax (melting point 77–79° C.). These substances were selected on the basis that their chromatographic behavior covers in general the various classes of lipide and waxy materials which might be present in floc "lipides."

The chromatographic characteristics of the floc lipides were similar in most respects to those of the sugar cane cuticle wax. The major component of each had an R_f of 0.94 to 0.96 in each of the two solvent systems. The cuticle wax appeared to contain at least two slower-moving minor components which were not completely resolved. One of these moved at approximately the same rate (R_f 0.60, 2% ether–iso-octane) as the minor component of the floc lipides. The other traveled slightly faster (R_f ca. 0.67). A small amount of material remained at the origin, for both the floc lipides and the cuticle wax. Although beet sugar saponin remains at the origin in the solvent systems employed, the trace component at the origin for floc lipides is probably a minor wax constituent rather than saponin. The latter should not be appreciably soluble in the petroleum ether used for isolation of the floc lipides. The major component of the floc lipides is believed to be a mixture of wax esters, similar to those present in sugar cane cuticle wax. Nonglyceryl esters of this type are known to move rapidly in the chromatographic system employed (*6*). It has been reported (*27*) that semirefined sugar cane wax contains 78 to 82% of wax esters. The minor component of the floc lipides (R_f 0.58 to 0.63) is probably a mixture of free fatty acids (oleic acid, R_f 0.59; palmitic acid, R_f 0.62) and/or glycerides (refined, winterized cotton seed oil, R_f 0.59). Warth (*27*) reports the presence of approximately 14% free fatty and wax acids and small amounts of glycerides in sugar cane wax. Some slight differences in the chromatographic behavior of the native cuticle wax and the floc lipides would be anticipated, inasmuch as the native cuticle wax is probably chemically modified or fractionated to some extent during sugar manufacturing operations.

Test Methods for Estimation of Floc

A fairly extensive study was made of possible test procedures for estimating floc in refined cane sugars. Improved floc tests are needed in the sugar and beverage industries for control purposes, and would also be useful in any studies concerned with the production of sugars of minimized floc forming tendencies.

Two approaches were investigated: (1) improving existing floc tests to make them faster or more applicable, and (2) devising entirely new test procedures based on chemical or physical measurements. Several floc tests now commonly used were applied to selected cane sugars of known flocculating characteristics. Three tests described by Sabine (*21*), known as the 7-Up test, the Holly Sugar Corp. test, and the Great Western (also Utah-Idaho) test, appear to be designed primarily for testing beet sugar. They failed to produce floc from selected cane sugars known to contain floc. The floc test recently proposed by Johnson and Diehl (*14*), as well as the Pepsi Cola Co. test

(*11*), based on the coagulation of negatively charged colloids with quaternary amines with determination of the resulting turbidity, was applied to cane sugars ranging from "no floc" to "heavy floc" types. Several different quaternary amines were tried. The procedures failed to differentiate the cane sugars tested.

The Coca-Cola test (*21*) was satisfactory for cane sugars, but the 10 days' observation time is longer than desired. Attempts were made to modify this test to make it faster and more practical for large numbers of sugar samples. The use of mineral acids other than phosphoric, or of other sirup concentrations, gave no improvement in the test procedure. High speed agitation or prolonged shaking of test sirups at various temperatures did not promote faster floc coagulation. It was observed that floc was somewhat heavier and, for most sugars tested, formed 1 to 2 days sooner if the test sirups were heated 15 to 20 minutes in a boiling water bath prior to standing. Prolonged heating was detrimental because color bodies began to separate from the sirups. The test procedure was also modified so that much smaller quantities of sirup could be prepared in containers better suited for the observation of floc and storage of large numbers of test sirups. This procedure is merely a modification of the Coca-Cola floc test; therefore the same qualitative assessment as to what constitutes a light, medium, or heavy floc applies.

Modified Coca-Cola Floc Test. Transfer 63 grams of sugar to a sterile 4-ounce oil sample bottle. Dissolve in 44 ml. of sterile distilled water. Add 0.4 gram of 85% phosphoric acid in 10 ml. of distilled water. Mix thoroughly. Final pH of test sirup should be 1.5 to 1.6. Heat in a boiling water bath for 15 to 20 minutes. Remove from the bath, cap the bottle, and allow to stand to check floc development. Examine periodically with a strong light beam. If present, floc usually appears between the fourth and sixth days. Seven days' observation time is adequate.

Physical methods such as conductivity, surface tension, cataphoresis, and ultraviolet fluorescence were found to be unsatisfactory as the basis of a floc test for cane sugars. Negative colloids such as gold sol, or positive colloids such as iron sol, were ineffective for test purposes.

It was observed that a light floc can be produced in acidified nonflocculating cane sugar sirups by adding soluble starch, silica, sugar cane cuticle wax, and finely ground bone char in the amounts normally found in flocculating sugars. Results were not conclusive when single components or combinations of fewer of the components were added. Even more striking was the heavy floc produced in acidified light floc sugar sirups by the addition of normal amounts of the four floc components.

In view of the possibility that one of the floc components may be the key to floc formation, attempts were made to develop rapid floc tests based on the determination of each of the major floc constituents in refined sugars. Silica was determined in test sirups and sugars, after ashing and carbonate fusion, by a colorimetric procedure adapted from Schwartz (*22*). It was observed that most, if not all, of the total silica in refined cane sugars (approximately 4 to 12 p.p.m. for the flocculating and nonflocculating sugars tested) is insolubilized in floc test sirups acidified to pH 1.56 and can be removed by filtration through Millipore HA filters (0.5 micron pore size). However, the quantity of silica present is not a good index of the flocculating tendency of cane sugars. Some no floc sugars contained as much or more total silica than some of those which formed floc. Similarly, total nitrogen contents of the sugars determined by a colorimetric Nessler procedure were not indicative of their tendency to form floc. The sugars tested contained from about 8 to 15 p.p.m. of total nitrogen. Attempts to use oil-soluble dyes to stain lipides as the basis of a colorimetric floc test were unsuccessful. Furthermore, extraction of a floc-type sugar with chloroform to remove lipides or wax failed to prevent or reduce floc formation in sirups prepared from the extracted sugar.

A rapid, colorimetric procedure for determination of starch in cane sugars was developed and applied to sugars ranging from no floc to heavy floc types. This procedure, which should be useful for rapid testing of sugars for relative starch contents, is outlined briefly: Dissolve 25.0 grams of sugar in 100 ml. of aqueous phosphoric acid (pH 1.5 to 1.6). Allow to stand approximately 30 minutes, then filter with suction through a Millipore HA filter disk. Wash the filter twice with small amounts of aqueous phosphoric acid. Solubilize the starch by adding 20 ml. of (1+1) perchloric acid [1 volume of 60% perchloric acid + 1 volume of water] to the filter and let it stand for 10 minutes. Filter with suction into a 50-ml. volumetric flask and wash the filter with several small portions of water. Develop the starch-iodine color by adding 1 ml. of 5% potassium iodide and then 5 ml. of 0.01*N* potassium iodate, by pipet. Make to volume, mix well, and determine the absorbance of the solution against a reagent blank at 600 to 650 mμ in a colorimeter or spectrophotometer. Calculate starch content by means of a standard curve obtained using sweet potato starch of known purity.

Starch values obtained by this rapid procedure are approximately one half as large as those found using the more lengthy Balch method (*2–4*). Apparently some of the starch particles are smaller than 0.5 micron and are not retained on the Millipore filter. If desirable, it is probable that use of a layer of diatomaceous filter aid on the Millipore filter would aid in trapping more of the starch. At any rate, the rapid procedure should be adequate to obtain relative starch contents of sugars, because the sugars tested in the present investigation ranged from a liquid sugar containing only 0.5 mg. of starch per 100 grams to a heavy floc granulated sugar which contained 21 mg. of starch per 100 grams.

It was found that the content of starch in refined sugars is not a good index of the relative flocculating tendencies of the sugars. Although some no floc sugars were very low in starch content, others contained moderate amounts of starch. Most of the refined sugars tested contained from about 1 to 5 mg. of starch per 100 grams.

Some interesting observations were made in connection with an investigation of the feasibility of determining the quantities of decolorizing carbon in sugars as a means of rating sugars for floc. Carbon was isolated by filtering acidified sirups (pH 1.5 to 1.6) through Millipore HA filters. Microscopic examination of the filters showed that floc-type sugars generally contained slightly more carbon particles than the nonflocculating ones. However, the difference was not sufficiently great to make this practical as a floc test. Nevertheless, in a series of sugars, all of which produced floc, those containing the greatest quantities of decolorizing carbon produced the largest amounts of floc (Tables I and II). Perfect rank correlation between the relative amount of carbon in the sugar and quantity of floc isolated was obtained by the method of Spearman (*15*). Similarly, rank correlation coefficients of 0.90 indicated significant correlation between carbon in sugar and parts per million of carbon in floc, and between parts per million of carbon in floc and the quantity of floc isolated. None of the other individual constituents known to be present in sugar floc gave such significant correlations. This may be due to the fact that only a small percentage of the ash, starch, wax, and protein content of the sugars is present in the isolated floc. The average values were as follows: 1.3% of the total ash; 3.3% of the total starch; 5.5% of the total wax; and 0.35% of the total protein. Although absolute values for carbon (free) are not available, it is felt that a considerably higher percentage of the total decolorizing carbon in the sugar is found in the floc. It is postulated that the formation of floc may be due largely to the aggregation of colloidal materials such as starch, silica, protein, and wax on the nuclei of finely divided decolorizing carbon. The low pH of the acidified sugar sirups is optimum for coagulation of the floc colloids. It should be possible to minimize the floc-forming tendencies in cane sugars by removing most of the carbon and the other floc colloids prior to crystallization of the sugar.

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FOOD ANTIOXIDANTS

Biochemistry of Erythorbic Acid. Human Blood Levels and Urinary Excretion of Ascorbic and Erythorbic Acids

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Recent widespread use of erythorbic acid as an antioxidant in foods raises the question of masking of true L-ascorbic acid levels in assessment of nutriture. The usual methods for determining ascorbic acid in blood do not distinguish between these acids. Comparative blood level trials in five normal subjects showed that the ingestion of erythorbic acid which has less than 1% of the vitamin C activity of L-ascorbic in animals, can lead to overestimation of the state of vitamin C nutrition as judged by blood levels. A rapid, quantitative paper chromatographic technique for separation of the isomers showed no significant displacement of L-ascorbic by 300-mg. doses of erythorbic acid.

ERYTHORBIC ACID, also known as D-araboascorbic or D-isoascorbic acid, a stereoisomer of L-ascorbic acid (Figure 1), has been widely used in foods in recent years as the antioxidant properties of this more easily synthesized isomer are similar (*2*) to those of L-ascorbic acid. However, marked differences have been reported in the chemical, physical, and biological properties of the two isomers as summarized in Table I. In guinea pigs, erythorbic acid was claimed to be only about 5% as potent as L-ascorbic acid with respect to antiscorbutic activity (*14*) and to effects on serum phosphatase (*3*) and excretion of intermediary tyrosine metabolites (*11*). This 1 to 20 relationship does not hold for the marked differences in reactivity of the isomers with ascorbic acid oxidases (*7*) and reductases (*4, 13*).

Recent guinea pig tests (*9*) have shown the activity of erythorbic acid to be due to its sporing action on L-ascorbic acid. In severely depleted animals, erythorbic acid has less than 1% of the antiscorbutic activity of L-ascorbic acid. Attempts to demonstrate antagonism between the isomers were unsuccessful in serum phosphatase tests in guinea pigs (*3*), but complete inhibition by erythorbic acid of an ascorbic acid oxidation enzyme in a fungus has been demonstrated (*7*). Ikeuchi (*5*) reported urinary excretion of erythorbic and ascorbic acid after dosage to vitamin C-deficient humans,