Acetic Acid, a Major Volatile Constituent of Brown Sugar: Its Origin and Measurement

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Acetic acid was identified as the major volatile constituent of commercial brown sugars. These sugars contained levels ranging from 31 to 827 ppm. The source of the acetic acid was found to be bacterial action in recycled sweet waters containing low levels of sucrose. Sugars manufactured without these waters had low to no detectable acetic acid levels. The method of analysis used—direct gas chromatography of sugars on the Dupuy inlet—provided a rapid, semiquantitative result. A high level of variability in some of the sugars was attributed to uneven distribution of acetic acid in these sugars. Other volatile constituents due to bacterial action were methanol, ethanol, acetaldehyde, and diacetyl.

Commercial brown sugar, a product of sugar refining that is known as "soft" sugar in the industry, is characterized by a unique odor and flavor. During an investigation of the volatiles responsible for this odor and flavor, it was noted that acetic acid was found in all samples and was present in a much higher concentration than any other volatile constituent (Godshall and Roberts, 1980).

Since the sugars being analyzed were from different sources around the world, representing various processing methods, it was of interest to determine acetic acid levels and sources.

One possibility for the origin of acetic acid was thermal or chemical degradation of sucrose during the manufacture of brown sugar. This seemed unlikely in view of the relatively mild conditions that normally exist in refining. A second obvious possibility was microbiological degradation of sucrose. This required that some processing parameter exist in common in all the refineries producing the brown sugar so that refineries in such diverse places as Canada, Austrialia, England, and Louisiana could produce brown sugars with high acetic acid levels.

Traditional brown sugars fall into two basic categories, "boiled" and "coated", which represent fundamentally distinct methods of production. A boiled soft sugar is crystallized from a dark refined syrup. The color of the sugar reflects the syrup from which it was crystallized. A coated sugar is a refined sugar that has been sprayed with a thin film of highly colored syrup or molasses to give it the characteristic color.

A survey of processes in use during the manufacture of brown sugar showed one process in common: all of the producers made use of bone char to decolorize various process liquors, and the water used to wash residual sugar off bone char prior to its regeneration was recycled for the manufacture of brown sugar. Other clarification and decolorization processes varied, but this procedure was used by all manufacturers of these sugars. This "sweet water" or "char washing" contains from 5 to 20% sucrose as well as other nutrients and seemed to be a possible source for the microbiological activity that could lead to acetic acid production. In addition, water used to wash sugar out of some vessels was also recycled.

A third type of colored sugar, known as "turbinado" sugar, is also available in American markets. This type of sugar is essentially a raw sugar that has been subjected to a cleanup procedure consisting of centrifugation in the presence of syrup or water, resulting in pale brown to golden crystals.

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Table I. Description of Sugars Used in Acetic Acid Determination

type of sugar	no. of samples	code
light or medium brown, boiled	8	A
dark brown, boiled	6	B
light brown, coated	3	C
dark brown, coated	3	D
turbinado	3	E
specialty sugars		
amorphous, processed with sweet water	2	F
amorphous, processed without sweet water	1	G
light brown, boiled, processed without sweet water	1	H
demerara	1	I

The analysis of underivatized acetic acid by gas chromatography presents many problems due to the acid's highly polar nature, which can lead to peak tailing and ghosting (Trombella and Ribeiro, 1980). A method was needed that would require minimum sample preparation and avoid the necessity of any type of solvent extraction. We decided to investigate the Dupuy external inlet (Legendre et al., 1979) for rapid quantitation of acetic acid. This inlet was developed for direct gas chromatography of volatile constituents in foods and had already been successfully used to identify volatile constituents in molasses (Godshall et al., 1980).

EXPERIMENTAL SECTION

Source of Brown Sugars. Twenty-five brown sugars and three turbinado sugars were contributed by refineries from the United States, Canada, England, and Australia. The sugars analyzed are categorized in Table I. All were of cane sugar origin. Low-purity (i.e., low in sucrose) sweet waters were contributed by a U.S. refinery. These consisted of water from char washing and water from vessel washing. Samples were preserved and chilled after sampling so that no further deterioration or microbiological action would occur.

Identification of Acetic Acid. The identification of acetic acid in brown sugar was confirmed by combined GC/MS using the Dupuy inlet. The conditions of mass spectrometry have been described previously (Godshall et al., 1980). The identity of acetic acid was confirmed by MS with some initial difficulty. The Dupuy inlet was equipped with a Na_2SO_4 condenser through which the

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volatiles passed prior to separation on the GC column, to prevent entrance of H_2O into the MS. We found that acetic acid was completely removed by the Na_2SO_4 trap at room temperature. However, it would pass through the condenser if the condenser was heated to 125 °C, and after this was done, acetic acid could be detected by GC/MS, with a purity correlation of 97.7%.

Measurement of Acetic Acid. The volatile constituents of the brown sugars and the sweet water were determined on a Hewlett-Packard 5750 gas chromatograph with the injection port modified to contain the Dupuy inlet for direct examination of volatiles.

For sugar analysis, 100–300 mg of brown sugar was firmly and uniformly packed between glass wool in a sample holder that was placed in the inlet. For sweet water analysis, approximately 0.1 mL of sweet water was injected onto glass wool prior to insertion of the sample holder into the Dupuy inlet. Samples were heated at 120 °C for 16 min, during which time the volatiles were swept from the sample with a flow of 40 mL/min helium and deposited at the head of the column. At this time, the column oven was switched on to 80 °C for 4 min and the temperature increased at 4 °C/min until acetic acid eluted, around 11 min.

The column used was a 7 ft × $^1/_8$ in. nickel column filled with Tenax-GC, 60–80 mesh, coated with 7% poly-MPE. The temperature of the flame ionization detector (FID) was 275 °C.

A calibration curve was prepared for acetic acid by injecting dilute aqueous solutions of acetic acid via the Dupuy inlet.

Isolation of Acetic Acid Bacteria. In order to characterize the microbiology associated with brown sugar production, three typical refinery streams were assayed. These were a dark syrup (about 65% sucrose content at about 50 °C), char sweet water (about 10% sucrose content at about 85 °C), and vessel washings (less than 5% sucrose at room temperature). Total plate counts were obtained on nutrient agar. Gram-positive organisms were selectively assayed on nutrient agar amended with 4 ppm of polymyxin sulfate to retard Gram-negative organisms. Counts were obtained from quadruplicate plates after 48-h incubation at 30 °C. Isolates representing the various colonial morphologies were picked and stored on nutrient agar slants.

The ability of each isolate to produce acetic acid in sweet water was determined by incubation in 20 mL of sterile 10% raw sugar solution for 5 days at room temperature. Aliquots of each culture were filtered through a 0.45-μm Millipore filter and assayed for acetic acid on a Hewlett-Packard 5880 gas chromatograph. The filtrates were made up to 0.2% formic acid to increase the sensitivity of detection, and 3 μL was injected directly into the gas chromatograph. The injection port temperature was 250 °C; the column temperature was held at 110 °C for 4 min and programmed to 146 °C at 6 °C/min. We determined that no acetic acid was produced by degradation of sucrose in the injection port. Contamination of the column with high molecular weight sucrose degradation compounds did occur. The column was periodically regenerated by multiple injections of water followed by a bake-out period of 1 h at 200 °C.

Cultures that produced acetic acid were regrown under the same conditions and sampled at 3, 5, and 7 days. Acetic acid producing bacteria were identified by standard biochemical and morphological characteristics.

RESULTS AND DISCUSSION

Acetaldehyde, ethanol, and diacetyl were the major volatile constituents identified in the char washings. Ethanol and acetic acid were identified in the vessel washings. Since the FID detector is not very sensitive to acetic acid and since there is some loss of acetic acid on the Tenax column due to binding and tailing at lower concentrations, the presence of acetic acid in the sweet waters was confirmed by diethyl ether extraction of acidified sweet waters. This showed the presence of 43 ppm of acetic acid in the vessel washings and its absence in the char washings. The char wash water was quite hot (80 °C) when used and could thus inhibit microbiological growth. The vessel washings, however, were at a lower temperature, allowing growth of acetic acid organisms. This pattern was found on two subsequent occasions when sweet waters were monitored for acetic acid content. Some refineries also use cool water to wash char, and this would be another source for acetic acid development.

Table II. Bacterial Counts in Refinery Streams

stream	nutrient agar, mL^{-1}	amended nutrient agar,[a] mL^{-1}
dark syrup	35 600	16 500
char sweet water	ND[b]	ND
vessel washings	21 900 000	8 400 000

[a] Nutrient agar with 4 ppm of polymyxin sulfate. [b] ND = none detected.

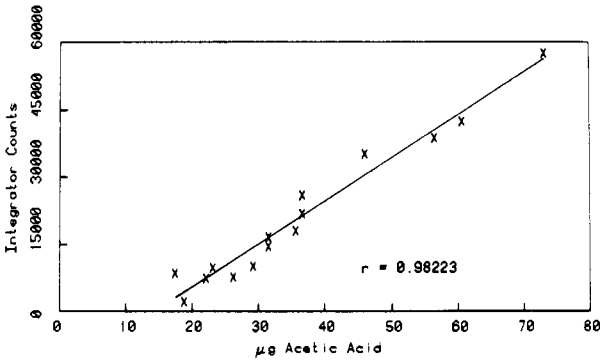

Figure 1. Calibration curve used for determination of acetic acid.

Plate counts are listed in Table II. As expected, the char sweet water was sterile while the vessel washings were heavily populated.

Of the 57 bacterial isolates obtained, 3 were found to produce acetic acid. We found it necesary to assay acetic acid in the filtrates by conventional GC injection because of low acid levels that could not be detected by using the Dupuy inlet, which can detect quantities of acetic acid in excess of 10 μg only. Injection of the filtrates in the presence of 0.2% formic acid allowed detection of as little at 0.1 μg of acetic acid.

Two of the three isolates were identified as *Enterobacter aerogenes*, which each produced 500 ppm of acetic acid. The other isolate was identified as *Serratia marcescens*; it produced about 40 ppm of acetic acid.

Other microbiologically produced volatiles identified in various cultures included methanol, ethanol, acetaldehyde, and diacetyl, as well as several other unidentified components.

The calibration curve for acetic acid on the Dupuy inlet is shown in Figure 1. The results showed a low level of sensitivity to acetic acid, with the minimum detectable amount being 14.4 μg of acetic acid. The correlation coefficient was 0.98223.

Recovery was tested by injecting acetic acid into refined sugar that was inside the sample holder and treating it in

Table III. Acetic Acid Levels in Brown Sugars

sugar[a]	origin	ppm of HAc
A-1	USA	325
A-2	USA	353
A-3	USA	418
A-4	USA	827
A-5	Canada	688
A-6	Canada	419
A-7	Australia	308
A-8	England	148
B-1	USA	346
B-2	USA	270
B-3	USA	387
B-4	USA	341
B-5	Canada	242
B-6	Canada	579
C-9	USA	163
C-10	USA	224
C-11	Canada	146
D-9	USA	439
D-10	USA	227
D-11	Canada	390
E-12	USA	72
E-13	USA	31
E-14	USA	37
F-8a	England	1598
F-8b	England	365
G-8	England	ND[b]
H-15	USA	ND
I-8	England	102

[a] The code letters used to identify the sugars are explained in Table I. Sugars with the same numbers came from the same refinery. [b] ND = not detected.

the same way as the brown sugars. The results, listed below, showed satisfactory recovery.

μg of added HAc	μg of recovered HAc	% error
60.2	63.4	+5.32
83.9	82.8	−1.31
134.2	137.2	+2.21

Blank runs with refined sugar alone indicated that no detectable levels of acetic acid or other volatiles were produced by degradation of sucrose in the inlet. Degradation undoubtedly occurs due to the high temperature employed, but the amounts of volatile degradation products were very low because of the small sample size and did not interfere with the analysis.

The amount of acetic acid determined in each of the sugars is listed in Table III. Figure 2 shows a typical chromatogram of a brown sugar.

The results showed high levels of acetic acid in most of the sugars. The turbinado sugars (code E) were much lower, less than 100 ppm. This reflected the different production method used to make turbinado sugars. Turbinado sugars are raw sugars that have been centrifuged in the presence of a fine spray of water to wash off some of the molasses film on the crystals, resulting in a light golden sugar with large crystals. These sugars are quite different in odor and flavor from commercial refined brown sugars.

Sugar H was a brown sugar manufactured to a buyer's specifications made without sweet water but rather with tap water. It contained no detectable levels of acetic acid, and although it had the appearance of a normal brown sugar, it possessed a quite different aroma. This is further confirmation of the role of sweet water in the production of acetic acid in brown sugars.

Sugars F and G were made by an experimental method that involved high temperature processing without vacuum crystallization. The F sugars were processed in the usual manner with recycled sweet waters; F-8a contained a very high level of acetic acid while F-8b contained a more normal amount. Sugar G, on the other hand, which was similar to a turbinado in that it was produced directly from a raw sugar, had no detectable levels of acetic acid. It had very few other volatiles in its profile as well. The coated sugars (codes C and D) had acetic acid levels that were within the range exhibited by the boiled sugars (code A and B).

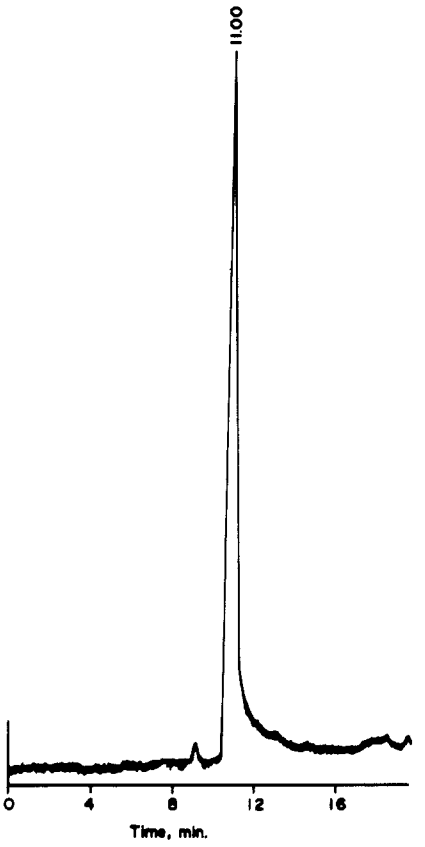

Figure 2. Chromatogram of acetic acid in a light brown sugar.

The retention of volatile compounds in hot, crystallizing solutions has not received much study. The study of volatile retention in freeze-dried and spray-dried systems has received more attention (Massaldi and King, 1974; Reineccius and Coulter, 1969). Acetaldehyde was retained in hot crystallizing sucrose after vacuum-drying to a level of 0.05–0.2% (U.S. Patent 3-767-430). Flink and Karel (1970) found retention of volatiles occurred in freeze-dried sucrose up to 3.27% by weight. They postulated that volatiles were trapped in amorphous microregions between hydrogen-bonded carbohydrate molecules. Niediek and Babernics (1979) found that sucrose in the amorphous state sorbed aroma substances at a rate several orders larger than in the crystalline state. It is possible that in the manufacture of amorphous brown sugars, there are two competing effects that contribute to the volatile profile: (1) greater absorption of volatiles in the amorphous state and (2) loss of volatiles due to high temperature.

The acetic acid values obtained with the Dupuy inlet showed a high coefficient of variation (CV), ranging from 2.42% (sugar C-11) to 49.2% (sugar C-10). The mean CV was 16.9%. This was attributed to uneven distribution of acetic acid in the sugars, which was magnified by the small sample size used in the analysis. However, this is an adequate method for rapid, semiquantitative determination of acetic acid levels when many sugar samples need to be assayed.

Acetic acid, therefore, appears necessary for the perception of typical brown sugar aroma and flavor. Sugars

in which it was absent (G-8, H-15) or very low, as in the turbinado sugars, are not perceived by tasters as normal brown sugars.

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Registry No. Acetic acid, 64-19-7; sucrose, 57-50-1; acetaldehyde, 75-07-0; diacetyl, 431-03-8; methanol, 67-56-1; ethanol, 64-17-5.

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Kinetics of Hydrolysis of Dimeric and Trimeric Methionine Hydroxy Analogue Free Acid under Physiological Conditions of pH and Temperature

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Liquid feed supplements based on DL-methionine hydroxy analogue free acid (DL-MHA free acid, DL-2-hydroxy-4-(methylthio)butyric acid) contain considerable amounts of oligomeric MHA free acids. As a first approach to physiological decomposition the hydrolysis of dimeric and trimeric MHA free acid in dilute hydrochloric acid solution is investigated. By use of HPLC it is possible to follow the changes in concentration of all species. The hydrolysis reactions are found to be first order with respect to the oligomer concentration. The rate constant increases with HCl concentration and temperature (activation energy for dimeric MHA free acid: 72 kJ/mol). The rate constants obtained at 37 °C in 0.1 N aqueous HCl correspond to half-lives of 1.8 and 1.6 days for dimeric and trimeric MHA free acid, respectively. The results indicate that oligomeric MHA free acids show rather slow hydrolysis under physiological conditions of pH and temperature.

Essential amino acids such as DL-methionine are of general importance in the animal nutrition industry as supplements to broiler feed, hog feed, etc., in order to increase the protein value. Crystalline DL-methionine, purified to a minimum of 99% by weight, is the major commercial product used. A substitute product, which is used to a lesser extent, is the calcium salt of DL-2-hydroxy-4-(methylthio)butyric acid (MHA, a registered trademark of Monsanto Co.) with a purity of 93% by weight.

A liquid product containing 88% of DL-MHA free acid (DL-methionine hydroxy analogue free acid, DL-2-hydroxy-4-(methylthio)butyric acid) (Alimet, a registered trademark of Monsanto Co.) has recently been introduced as a feed supplement. Like other α-hydroxycarboxylic acids, DL-MHA free acid inevitably polymerizes in concentrated solution. This polymerization occurs via intermolecular esterification and results in a mixture of different stereoisomers. It was reported that an equilibrium solution of 85% DL-MHA free acid contains 64.5% by weight monomeric DL-MHA free acid, 20.1% by weight dimeric (linear and cyclic) MHA free acid, and 1.3% by weight trimeric MHA free acid (Cummins, 1973). A recent investigation on 88% DL-MHA free acid solution (Ivey, 1981) indicated that the equilibrium concentrations of polymers are even higher (17.6% by weight dimeric MHA free acid, 8.8% by weight trimeric MHA free acid, and 4.4% by weight higher oligomers). These oligomers are significant because it has been reported that the polymeric MHA free acids "are not useful as feed components" (Nufer, 1966). Boebel and Baker (1982) found that MHA free acid polymers had an efficacy only 54% that of DL-methionine when compared on a molar basis.

So with regard to the nutritional value one must assume that a hydrolysis of the polymers to the monomer is needed. As a first approach to physiological decomposition we have investigated the hydrolysis of dimeric and trimeric MHA free acid in dilute hydrochloric acid solution as a function of HCl concentration and temperature.

MATERIALS AND METHODS

Solutions were concentrated in a rotary evaporator. The existence of mono-, di- and trimeric MHA free acid was verified by ^{1}H NMR, IR, and mass spectra.

Monomeric DL-MHA free acid (I) was prepared by the following preparative route via ester saponification: DL-MHA-Ca (548 g) was dissolved in water (4.5 L) and activated charcoal and kieselgur were added. The mixture was then heated and filtered while still hot. To remove Ca, the filtrate was passed through an ion-exchange column (Lewatit S 100 from Bayer, Federal Republic of Germany). Concentrating the eluate gave DL-MHA free acid (451 g,

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