Identification of Hydrolyzed Inulin Syrup and High-Fructose Corn Syrup in Apple Juice by Capillary Gas Chromatography

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METHOD AUTHOR:
NICHOLAS H. LOW
University of Saskatchewan, Department of Applied Microbiology and Food Science, 51 Campus Dr, Saskatoon, Saskatchewan, Canada S7N 5A8, Tel.: +1-306-966-8898; Fax: +1-306-966-5024

SUBMITTING LABORATORY:
MICHAEL A. MCLAUGHLIN and SAMUEL W. PAGE
U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Natural Products, Washington, DC 20204

PEER LABORATORIES:
BENJAMIN J. CANAS
U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Natural Products, Washington, DC 20204

ALLAN R. BRAUSE
Analytical Chemical Services of Columbia, Inc., Columbia, MD 21045

NICHOLAS H. LOW
University of Saskatchewan, Department of Applied Microbiology and Food Science, Saskatoon, Saskatchewan, Canada S7N 5A8

Abstract

A peer-verified, gas chromatographic (GC) method is presented for the identification of hydrolyzed inulin syrup (HIS) and high-fructose corn syrup (HFCS) in apple juice. The procedure involves determining the Brix value of the apple juice or apple juice concentrate and preparing a dilution of the test sample to 5.5° Brix. A 100 μL aliquot of the 5.5° Brix test solution is then freeze-dried in a GC autosampler vial. The sugars in the freeze-dried residue are converted to trimethylsilyl derivatives, by the addition of an appropriate silylation reagent, and the vial is heated at 75°C for 30 min. After derivatization, the solution is introduced into a gas chromatograph where the analytes are separated on a 30 m, 0.25 mm id DB-5 column. The method can use hydrogen, helium, or nitrogen as the carrier gas. The analytes and marker compounds are measured by use of a flame ionization detector. Commercial apple juice concentrates were diluted with one of the 2 syrups at 2 levels. Dilution was ascertained by the presence of retrograde sugar markers found in the 2 sugar syrups. All 3 laboratories involved in the study were able to identify the correct diluent in the blind, randomly coded, apple juice test portions. The levels of dilution in the test portions were 0, 6.9% (HIS), 16.0% (HIS), 8.1% (HFCS), and 17.0% (HFCS). No false positive results were reported. Quantitative conclusions can be drawn when the same syrup is used for dilution and as a reference standard.

1 Summary of Results of Verification Study

1.1 Matrices

This paper addresses the identification of the presence of 2 types of sugar syrups, high-fructose corn syrup (HFCS) and hydrolyzed inulin syrup (HIS), in apple juice concentrate.

1.2 Number of Samples

The submitting and peer laboratories independently performed analyses of 25 blind test samples and 3 controls, using the calibration curves prepared from 6 spiked standards.

1.3 Precision

1.3.1 Repeatability.—The level of addition of HIS or HFCS in each test sample was determined to evaluate the precision of the method. The results obtained with this procedure should not be interpreted as quantitative except in cases in which the actual diluent is used as the reference standard. The results were consistent among the 3 laboratories.

1.3.2 Reproducibility.—Reproducibility was evaluated by the analysis of 15 blind duplicate test samples. The reproducibility standard deviations, sR, were as follows: test sample HIS 1, 4.1%; test sample HIS 2, 7.8%; test sample HFCS 1, 7.2%; and test sample HFCS 2, 5.2%.

2 Safety Precautions

The primary source of hazardous material in this procedure is the trimethylsilyl (TMS) derivatizing agent trimethylsilylimidazole–pyridine (1 + 4). Trimethylsilylimidazole is a combustible liquid that is irritating to the skin, eyes, and mucous membranes. The toxicological properties have not been fully investigated. Pyridine is rapidly absorbed through the skin and can cause irritation. Pyridine vapor irritates the eyes and respiratory system and may cause headache, nausea, giddiness, conjunctivitis, and vomiting. It may also cause kidney, liver, and central nervous system damage as well as gas-
Figure 1. Chromatogram of HIS.

Figure 2. Chromatogram of HFCS.
trointestinal upset. Methanol is toxic as well as flammable. These materials should be handled in an exhaust hood with appropriate gloves. The hydrogen used by the gas chromatograph is extremely flammable. If hydrogen gas is used as the carrier gas, the gas chromatograph must be equipped with an automatic cutoff sensor. The gas chromatograph and the heating block can produce burns. Safety glasses should be worn at all times.

3 Scope

This procedure uses a TMS derivatization and gas chromatographic (GC) analysis for the identification of marker retrograde sugars from HIS and HFCS in apple juices and apple juice concentrates. This test covered a concentration range of approximately 5–20% added syrup (w/w).

4 References

5 Definitions

HIS = hydrolyzed inulin syrup; HFCS = high-fructose corn syrup; GC = gas chromatographic; LC = liquid chromatographic; and TMS = trimethylsilyl.

6 Principle

This method (1–4) uses the approach developed by Low and coworkers (5–8). The hydrolysis of complex carbohydrates such as starch and inulin produces, in addition to the desired mono- and disaccharides, minor amounts of retrograde sugars. These retrograde sugars can be used as markers of the addition of these sugar syrups to certain fruit juices and natural sweeteners. The method involves diluting an apple juice with liquid chromatographic (LC) grade water to 5.5° Brix, removing the water by lyophilization, and converting the sugars in the sample to TMS derivatives. The derivatized sugars are then analyzed by gas chromatography on a 30 m DB-5 (or equivalent) capillary column. The trace sugar profile of the analyze is examined and marker peaks (see Figures 1–4) from HIS and HFCS (these peaks have been identified as the anomers of isomaltose; 1, 3) are noted if present. No authentic apple juices analyzed to date exhibited the marker peaks when subjected to the same analytical procedure. The presence of HIS or HFCS marker peaks on analysis provides presumptive evidence for the addition of HIS or HFCS to the apple juice.

7 Standards

(7.1.1) HIS standards.—These adulterated juice concentrate standards were provided by the submitting laboratory. Authentic apple juice concentrates at 70° Brix had HIS of the same Brix value added at the following levels: 25% HIS standard, 25.0% w/w; 15% HIS standard, 15.0% w/w; and 5% HIS standard, 5.0% w/w.

(7.1.2) HFCS standards.—These adulterated juice concentrate standards were provided by the submitting laboratory. Authentic apple juice concentrates provided at 70° Brix had HFCS of the same Brix value added at the following levels: 25% HFCS standard, 24.9% w/w; 15% HFCS standard, 15.0% w/w; and 5% HFCS standard, 5.0% w/w.

8 Reagents and Supplies

(8.1.1) Derivatization reagent.—Reagent-grade Tri-Sil Z (Pierce Chemical Co., Rockford, IL), Sylon TP (Supelco, Inc., Bellefonte, PA), or Hydroxsil AQ (Regis Technologies, Inc., Morton Grove, IL). This reagent is trimethylsilylimidazole–pyridine (1 + 4). The reagent should be stored as directed by the manufacturer.

(8.1.2) Water.—LC grade.

(8.1.3) Acetone or methanol, for freezing test samples (optional).—Technical grade.

(8.1.4) Dry ice, for freezing test samples (optional).

(8.1.5) Gas for gas chromatograph.—High-purity helium, hydrogen, or nitrogen carrier gas; hydrogen; and compressed air.

9 Apparatus and Equipment

(9.1.1) Analytical balance.—Accurate to ± 0.01 g.

(9.1.2) Aluminum seal GC autosampler vials.—1.8 mL.

(9.1.3) Autosampler vial crimper.

(9.1.4) Adjustible-volume pipetter.—100 μL; for transfer of juice, juice concentrates, or dilutions of juice.

(9.1.5) Disposable pipet tips.—100 μL; for transfer of juice, juice concentrates, or dilutions of juice.

(9.1.6) Polypropylene scintillation vials (or equivalent).—20 mL.

(9.1.7) Pasteur pipets.—9 in.

(9.1.8) Pasteur pipet bulbs.

(9.1.9) Lyophilizer.—Shelf-type

(9.1.10) Freezer.—Capable of maintaining –20°C.

(9.1.11) Brix refractometer.

(9.1.12) Block heater.—Digital incubator block heater (Denville Scientific, Metuchen, NJ), or equivalent.

(9.1.13) Teflon-capped glass syringe.—For transfer of derivatization reagent.

(9.1.14) Computerized gas chromatograph with autosampler.—Hewlett-Packard (Palo Alto, CA) Model 6890, or equivalent. Injector should be packed with silanized glass wool.

(9.1.15) Capillary GC column.—Bonded poly(5% diphenyl/95% dimethylsiloxane) such as the DB-5 (J&W Scientific, Folsom, CA) or the Hewlett-Packard HP-5; 30 m length, 0.25 or 0.32 mm id, 0.25 μm film thickness.

10 Sample Preparation

The Brix value of the test sample was determined according to AOAC Method 932.14. A 5.5° Brix dilution of the apple juice or apple juice concentrate was prepared as the test portion by diluting the concentrate with LC grade water.

11 Controls Preparation

(11.1.1) LC-grade water control.—Used as a negative blank. This is LC-grade water that the collaborator used to dilute the concentrates and syrups in this study.

(11.1.2) Sucrose control, 1% (by weight).—Prepared with LC-grade water and sucrose provided by the submitting laboratory. This was used as a marker for the pertinent region of the chromatogram.

(11.1.3) Authentic apple juice concentrate control.—Prepared by determining the Brix value and diluting to 5.5° Brix
with LC-grade water. The identity of this material was known to the analyst and was used as a negative control.

Controls were prepared for analysis according to the procedure used for sample preparation (see section 10).

12 Procedure

12.1 TMS Derivatization of Standards, Test Portions, and Controls

A small volume (100 μL) of the 5.5° Brix dilution of the test portion, control, or standard was transferred to an autosampler vial and frozen in a freezer or cooled in a dry ice-acetone or dry ice–methanol bath. The frozen test portion was then lyophilized. When the test portion was dry, 0.5 mL silylation reagent was added with a glass syringe, and the vial was capped and heated at 75°C for 0.5 h. The test solution was then ready for analysis. All standards and controls were run in triplicate. Test solutions were injected once because there were 5 blind duplicates of each test sample.

12.2 GC Parameters

(12.2.1) Helium or nitrogen carrier gas.—Injection volume: 2 μL; injector mode: splitless; injector temperature: 250°C; injector purge: for 1.5 min with helium at 60 mL/min; flame ionization detector temperature: 300°C; oven temperature program: 210°C for 15 min, from 210 to 290°C at 1°C/min, hold at 290°C for 20 min; carrier gas: helium or nitrogen at a constant velocity of 27 cm/s; detector hydrogen flow: 40 mL/min; detector air flow: 450 mL/min; detector makeup flow (nitrogen or helium): 30 mL/min; integration on detector set at zero.

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16.0% HIS in apple juice concentrate

| 1     | 16.0| 17.0| 16.0| 18.0| 16.0| 16.6 | 0.8  |
| 2     | 18.0| 17.8| 17.7| 17.5| 18.4| 17.9 | 0.3  |
| 3     | 15.8| 16.5| 13.3| 16.2| 16.0| 15.6 | 1.2  |
| Combined | |    |    |    |    | 16.7 | 1.3  |

8.1% HFCS in apple juice concentrate

| 1     | 8.0 | 10.0| 8.0 | 8.0 | 8.0 | 8.4  | 0.8  |
| 2     | 7.6 | 8.2 | 8.4 | 8.9 | 9.0 | 8.4  | 0.5  |
| 3     | 8.0 | 8.0 | 8.3 | 7.8 | 8.0 | 8.0  | 0.2  |
| Combined | |    |    |    |    | 8.3  | 0.6  |

17.0% HFCS in apple juice concentrate

| 1     | 18.0| 19.0| 18.0| 17.0| 16.0| 17.6 | 1.0  |
| 2     | 18.4| 18.8| 17.4| 16.9| 16.9| 17.7 | 0.8  |
| 3     | 16.4| 17.1| 16.2| 16.9| 16.9| 16.6 | 0.4  |
| Combined | |    |    |    |    | 17.3 | 0.9  |

Authentic apple juice concentrate (no adulterant)

| 1     | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | —    | —    |
| 2     | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | —    | —    |
| 3     | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | —    | —    |

a Lab 1 = Benjamin J. Canas, U.S. Food and Drug Administration; Lab 2 = Nicholas H. Low, University of Saskatchewan; and Lab 3 = Allan R. Brause, Analytical Chemical Services of Columbia, Inc.

b SD = Standard deviation.

c n = 15 for combined results.
(12.2.2) Hydrogen carrier gas.—Injection volume: 2 μL; injector mode: splitless; injector temperature: 250°C; injector purge: for 1.5 min with helium at 60 mL/min; flame ionization detector temperature: 300°C; oven temperature program: 210°C for 10 min, from 210 to 248°C at 1°C/min, hold at 248°C for 30 s, from 248 to 295°C at 30°C/min, hold at 295°C for 12 min; run time: approximately 62 min; carrier gas: hydrogen at a constant flow of 1.4 mL/min, with nitrogen as the makeup gas; total linear velocity: approximately 38 cm/s; detector hydrogen flow: 40 mL/min; detector air flow: 450 mL/min; detector makeup flow (nitrogen or helium): 30 mL/min; integration on detector set at zero.

13 Interpretation and Calculations

The suspect syrups contain characteristic marker compounds that can be located by careful examination of the chromatogram. The region of the chromatogram from 15 to 110 min (from 15 to 50 min when hydrogen is used as the carrier gas) contains the peaks of interest as well as the sucrose marker peak. The temperature program/carryer flow should be adjusted as necessary for the sucrose peak to appear in the chromatogram at approximately 44 min with nitrogen or helium carrier gas or at approximately 28 min with hydrogen carrier gas. The HIS have distinctive marker peaks (Figures 1 and 3) at approximately 32.6 and 45.4 min with nitrogen or helium carrier gas (20.3 and 34.0 min with hydrogen carrier gas). The marker peaks are found on either side of the sucrose peak (at approximately 44.3 min with helium and 29 min with hydrogen). Both marker peaks must be present to use as presumptive evidence for the presence of HIS.

The HFCS produce 2 marker peaks (Figures 2 and 4) at approximately 58.4 and 61.9 min with helium carrier gas and 41.0 and 45.0 min with hydrogen carrier gas. As in the case of the HIS, both peaks must be present to provide presumptive evidence of HFCS.

The areas and heights of these indicator peaks may vary with the source of HIS and HFCS; thus, quantitative conclusions should not be drawn unless samples of HIS or HFCS used in the dilution scheme are compared. For this interlaboratory study, the results were reported in terms of the proportion of diluent in the test portion as well as the presence or absence of the diluent. The level of diluent addition was determined by comparing the results from the analysis of the unknowns with a calibration curve calculated from external standards of HIS and HFCS (sections 7.1.1 and 7.1.2). The heights of the 2 marker peaks were measured and added together. The sum of these peak heights represented the variable that correlates directly with the percentage of diluent in the test sample. This process was repeated for all of the standards. The mean of the peak height sums was calculated for each set of standard triplicates. The calibration curve was plotted with the percent diluent on the x-axis and the mean of the peak height sum for that level of dilution on the y-axis. The collaborators were instructed not to force the curve through zero. The peak height sums from the chromatograms of the unknowns and the calibration curve were used to determine the percent diluent in the test portions.

14 Test Results Report

Table 1 displays the data obtained by the peer laboratories. Five test samples of apple juice concentrate were analyzed by this method. Blind, randomly coded test samples were provided to the peer laboratories. A qualitative standpoint, all 3 laboratories were able to correctly determine the identity of the diluent in the test portions. No false positives or false negatives were reported by any of the 3 laboratories. Quantitatively, the precision of the intralaboratory results were demonstrated by standard deviations of less than 1.5. An interlaboratory comparison of precision indicated that the largest standard deviation (1.3) was produced for the test samples containing the highest levels of the diluent HFCS. The results indicated that the method could be quite accurate in determining the level of dilution when the diluent syrup is available. All of the results from the individual analyses were within 2% of the actual level of the diluent present in the test portion.

15 Quality Assurance

15.1 Critical Control Points

A device impervious to solvent (such as a glass syringe) should be used to transfer the derivatizing agent, which degrade plastic syringes and pipet tips. Material from the plastic can contaminate the test portion and complicate the chromatogram. These contaminant peaks and the marker peaks can appear in the same time frame in the chromatogram and can lead to inaccurate conclusions. Exposure of the derivatized test solutions to any appreciable amount of water leads to rapid degradation of the TMS derivatives. Immediate derivatization of the freeze-dried residues is essential to the validity of the test because the lyophilized test portions will begin to absorb water from the atmosphere as soon as they are removed from the freeze-drier. The TMS derivatives are also susceptible to degradation at room temperature. The TMS derivatives should not be left at room temperature for >24 h. The derivatizing agent is also susceptible to degradation, and it should be stored as directed by the manufacturer. The correct column length and column diameter are critical to the method (see section 9.1.15). For example, megabore columns with the same film thickness do not afford the same resolution and elution pattern. They are, therefore, inappropriate for use in this method.

16 Comments and Discussion

Authentic apple juice and concentrates do not contain any substances known to interfere with this analysis. The 2 marker peaks used to determine the presence of HIS in apple juice are
unique compounds that are not present in any of the other commercially available syrups. The 2 peaks from the anomers of isomaltose, which were used in this method to identify HFCS, could also be found in other commercial syrups produced from starch because of the similarities in the production processes.

The sensitivity of the method to the presence of these diluents and their quantitation depends on the specific lot of syrup used in the adulteration scheme. The concentration of the marker peaks is known to vary from lot to lot and from manufacturer to manufacturer. This variability in the sugar syrups makes determining the sensitivity of the method problematic for unknowns. Low (1–3) indicates that the lower limit of measurement for this method is 5%. If the syrup used for diluting the apple juice is available, a lower limit measurement is possible.

Overall, the analysts were pleased with the method because it provided a relatively rapid, inexpensive procedure for screening apple juices for the presence of HIS and HFCS. No compounds normally found in apple juice were found to interfere with the proper interpretation of the results.