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Major Carbohydrate, Polyol, and Oligosaccharide Profiles of Agave Syrup. Application of this Data to Authenticity Analysis

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ABSTRACT: Nineteen pure agave syrups representing the three major production regions and four processing facilities in Mexico were analyzed for their major carbohydrate, polyol, and oligosaccharide profiles, as well as their physicochemical properties (pH, °Brix, total acidity, percent total titratable acidity, and color). Additionally, the detection of intentional debasing of agave syrup with four commercial nutritive sweeteners (HFCS 55 and 90, DE 42 and sucrose) was afforded by oligosaccharide profiling employing both high performance anion exchange liquid chromatography with pulsed amperometric detection (HPAE-PAD) and capillary gas chromatography with flame ionization detection (CGC-FID). Results showed that the major carbohydrate and polyol in agave syrups were fructose and inositol with mean concentrations of 84.29% and 0.38%, respectively. Oligosaccharide profiling was extremely successful for adulteration detection with detection limits ranging from 0.5 to 2.0% for the aforementioned debasing agents. Also, all four of these possible adulterants could be detected within a single chromatographic analysis.

KEYWORDS: *agave syrup, carbohydrates, polyols, oligosaccharides, adulteration*

INTRODUCTION

Agave syrup, also known as agave nectar, is a recent (post 1990) food product from the Western region of Mexico which is produced from the sap of the agave plant, specifically, *Agave tequilana* and *Agave salmiana*, or blue and salmiana agave, respectively. This product has gained popularity as an alternative to traditional sweeteners, such as table sugar (sucrose) and honey, partially due to its low glycemic index (17−27) when compared to honey (55) and sucrose (68), and its status as vegan.[1−3]

To produce agave syrup the plant must grow for a minimum of six years to reach appropriate maturity so that it can be harvested by hand.[3] The major steps in agave syrup production are shown in Figure 1. Briefly, the heart or piña (weighing up to 68 kg) is isolated from the leaves and is then crushed into fibers via milling. The juice within the fibers is released by gravity in combination with hot water washing employing a diffuser, and is then filtered to remove particulates. The filtered juice is then subjected to natural hydrolysis where, over a period of hours, the temperature is increased to approximately 80 °C. During natural hydrolysis, the glycosidic enzymes in the juice (i.e., inulinase and β-fructosidase) convert inulin and fructans[4−6] to free monosaccharides, primarily fructose. Following hydrolysis, the juice is filtered and is then subjected to vacuum evaporation at approximately 90 °C to remove water and denature glycosidic activities, resulting in the finished syrup.[3] Approximately 10% of the agave harvest goes toward the production of agave syrup, with the remainder being used for the production of fermented beverages such as tequila and mescal, made from *A. tequilana* and *A. salmiana*, respectively.[3]

Agave syrup contains a very high carbohydrate content (>95% of the total soluble solids), with the major component being fructose, which has been reported to range from 55.6%[7] to 90%.[8] It is this high fructose content that gives agave syrup its low glycemic index and also makes it sweeter than syrups containing appreciable levels of glucose (e.g., honey) or sucrose (e.g., maple syrup) so that less agave syrup can be used to achieve the same level of sweetness, thus decreasing calorie intake. Minimal published work exists on the chemical composition of agave syrup, and this is most likely due to its relative newness to the market and the attention paid to the fermented beverage uses of this product.

Knowledge of the chemical composition of agave syrup is important not only for human health reasons but also for authenticity matters. Due to the increasing popularity of agave syrup's use as a table top sweetener and as a food ingredient, it has become a target for adulteration. The fact that agave syrup is primarily composed of carbohydrates results in the relatively simple and economically viable adulteration (i.e., debasing) of this material with less expensive nutritive sweeteners such as high fructose corn syrup (HFCS). Of particular concern is HFCS 90 due to its high fructose content which closely mimics the monosaccharide profile of pure agave syrup. However, other commercial nutritive sweeteners, such as beet/cane sucrose, dextrose syrups (DE), and other HFCS products (HFCS 42 and 55), are also of concern due to their large difference in pricing when compared to pure agave syrup.

One method to detect the possible adulteration of agave syrup with less expensive nutritive sweeteners is by looking for changes in its major (i.e., fructose and glucose) carbohydrate profile. However, this method may not be effective if the major carbohydrate profile of the adulterant is similar to that found naturally in agave syrup, as would be the case with HFCS 90. In this case another method is needed. One way to detect this type

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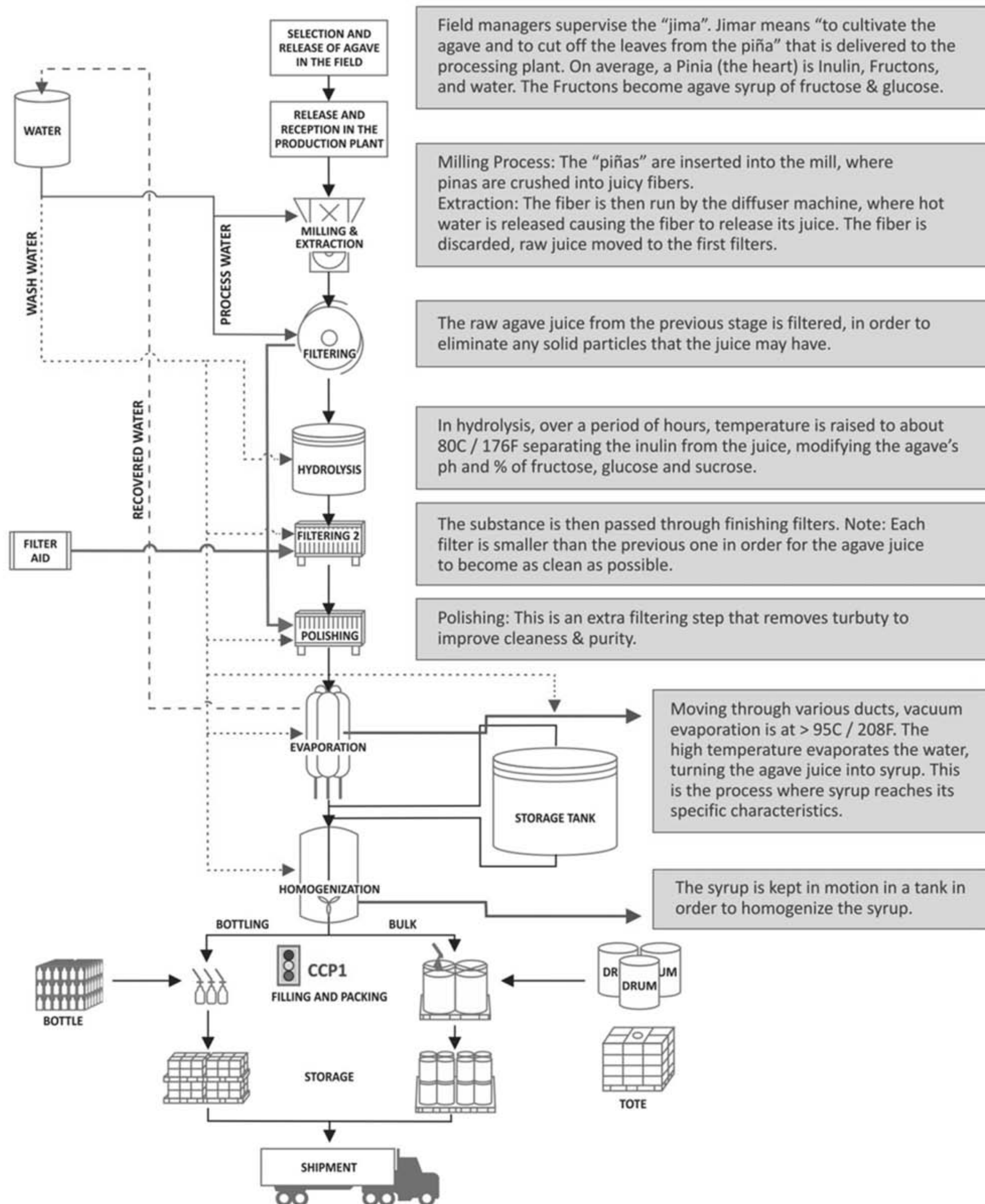

Figure 1. Schematic of the major processing steps in the production of agave syrup. Reprinted with permission from ref 3. Copyright 2012 IOAA.

of adulteration is by oligosaccharide profiling using chromatographic techniques such as high performance anion exchange liquid chromatography with pulsed amperometric detection (HPAE-PAD) and capillary gas chromatography with flame ionization detection (CGC-FID). Previous work has been done using these techniques to detect the adulteration of other high carbohydrate foods, such as maple syrup,[9] honey,[10] and fruit juices,[11,12] with less expensive carbohydrate syrups. While some work has been done on the authenticity of tequila,[13,14] no work has been published to date on the detection of the adulteration of agave syrup. Therefore, the aim of this study was to develop a database on the chemical composition of pure agave syrup from the three major growing/production regions in Mexico, and to use this database to develop analytical methods to detect

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the adulteration of agave syrup with commercial HFCS (55 and 90) and a dextrose syrup (DE 42).

■ MATERIALS AND METHODS

Samples. Nineteen pure agave syrup samples representing two production years (2010 and 2011), four processors/processing facilities (Bioagaves; IMAG; Milpillas; PSA), and three major production regions (Tepic, Nayarit; Capilla de Guadalupe and Tepatitalán DeMorelos, Jalisco; Jilquipan de Juárez, Michoacán) in Mexico were analyzed in this study. Four of the syrup samples were produced from *Agave salmiana* (salmiana agave), and fifteen were produced from *Agave tequilana* (blue agave). In addition, a raw agave juice sample (*A. tequilana*) was also analyzed. Nutritive sweeteners used in this study as debasing agents (i.e., adulterants) were dextrose syrup (DE 42; CASCO, Etobicoke, ON, Canada), two high fructose corn syrups (HFCS 55 and HFCS 90; CASCO), and sucrose (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada).

Chemicals. Acetic acid, caffeic acid, catechin, chlorgenic acid, D-fructose (fructose), D-glucose (glucose), erythorbic acid, gallic acid, glucuronic acid, 4-hydroxybenzoic acid, 5-hydroxymethyl-2-furaldehyde (HMF), isocitric acid, maleic acid, malic acid, malonic acid, naringenin, phloridzin, quercetin, quinic acid, rutin, shikimic acid, D-sucrose (sucrose), sylon TP (TMSI + pyridine, 1:4), tartaric acid, and vanillic acid were purchased from Sigma-Aldrich Canada Ltd. Ascorbic acid, citric acid, formic acid, fumaric acid, hydrochloric acid, mannitol, oxalic acid, sodium acetate (NaOAc), sodium hydroxide (NaOH) solution (50% w/w), and succinic acid were obtained from VWR Canada (Mississauga, ON, Canada). *meso*-Inositol was obtained from Nutritional Biochemicals Corporation (Cleveland, OH, USA). Phosphoric acid, potassium phosphate dibasic (K_2HPO_4), and potassium phosphate monobasic (KH_2PO_4) were purchased from J. T. Baker (Phillipsburg, NJ, USA). The water used in this study was obtained from a Millipore Milli-Q water system (Millipore Corporation, Milford, MA, USA).

°Brix. Measurements were obtained using an Auto Abbe refractometer (Leica Inc., Buffalo, NY, USA) with temperature compensation. All samples were analyzed in duplicate.

Color. Sample color was determined using a ColorFlex EZ spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA) standardized with both black and white standards with values reported using L^*, a^*, b^* parameters. All samples were analyzed in triplicate.

pH. pH measurements were obtained using an Orion 3 pH meter (Thermo Fischer Scientific, Ottawa, ON, Canada) calibrated at pH 4.0 and 7.0. All syrup samples were diluted to 10.0% (w/v) in water prior to pH determination; the agave juice sample was analyzed as received. All samples were analyzed in triplicate.

Total Acidity (TA). Total acidity was determined using AOAC method 962.19[15] on 50.0 mL of a 10.0% (w/v) aqueous solution. All samples were analyzed in duplicate.

Percent Total Titratable Acidity (% TTA). To 50.0 mL of a 10.0% (w/v) aqueous solution was added three to four drops of 1.0% (w/v) phenolphthalein in ethanol indicator solution. The resulting solution was titrated to a faint pink end-point using 0.05 M NaOH. The % TTA was calculated as citric acid. All samples were analyzed in duplicate.

Sample Adulteration. Select agave samples and the three adulterants (DE 42, HFCS 55, and 90) were individually diluted to 5.5 ± 0.2 °Brix, and sample adulteration was done at the 1.0, 5.0, and 10.0% (v/v) levels. The oligosaccharide profiles of intentionally adulterated samples were analyzed by HPAE-PAD and CGC-FID as described below.

Monosaccharide and Polyol Analysis by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). Carbohydrate analysis by HPAE-PAD was carried out using a Dionex ICS 5000 HPLC system (Thermo Fischer Scientific) equipped with a Dionex AS autosampler, ICS 5000 electrochemical cell with a disposable gold electrode and a 25 μL injection loop. The potentials and durations of the gold electrode were as follows: E_1 = 0.10 V, t_1 = 0.00 s; E_2 = −2.00 V, t_2 = 0.41 s; E_3 = 0.60 V, t_3 = 0.43 s; E_4 = −0.10 V, t_4 = 0.44 s; E_5 = −0.10 V, t_5 = 0.50 s. Data acquisition was afforded with Dionex Chromeleon 7.0 software. All separations were carried out at room temperature (20−22 °C).

Monosaccharide and polyol analysis was accomplished using a Dionex CarboPac PA1 column (4 × 250 mm) in series with a CarboPac PA1 guard column (4 × 50 mm). An isocratic mobile phase of 80 mM NaOH at a flow rate of 1.0 mL/min was employed for analyte separation. Quantification of fructose, glucose, inositol, and mannitol was afforded using external standard curves with R^2 values of 0.998 or greater. Standards for fructose, glucose, and inositol ranged from 5.0 to 100.0 ppm. Standards for mannitol ranged from 1.0 to 200.0 ppm. Agave syrup samples were prepared by dilution in water to 1.0% (w/v) for inositol and mannitol analysis and to 200.0 ppm for fructose and glucose analysis. The agave juice sample was prepared by dilution with water to 1.0% (w/v) for inositol and mannitol analysis and to 0.1% (w/v) for fructose and glucose analysis. All sample solutions were filtered using a 0.2 μm pore size syringe filter (13 mm diameter; Chromatographic Specialties, Brockville, ON, Canada) prior to HPAE-PAD analysis. All samples were analyzed in triplicate.

Oligosaccharide Analysis by HPAE-PAD. Oligosaccharide analysis was carried out by HPAE-PAD using a Dionex CarboPac PA100 column (4 × 250 mm) in series with a CabroPac PA100 guard column (4 × 50 mm). A gradient elution program (Table 1) was employed with the following mobile phases: 160 mM NaOH (solvent A); 160 mM NaOH/1.0 M NaOAc (solvent B); and 1.0 M NaOH (solvent C). The mobile phase flow rate was 1.0 mL/min. All samples were diluted to 5.5 ± 0.2 °Brix with water and were syringe filtered prior to HPAE-PAD analysis. All samples were analyzed in duplicate.

Table 1. HPAE-PAD Gradient Program for Oligosaccharide Separation[a]

time (min)	% A	% B	% C
0.0	100.0	0.0	0.0
1.5	100.0	0.0	0.0
2.0	99.2	0.8	0.0
25.0	76.0	24.0	0.0
25.1	0.0	100.0	0.0
28.0	0.0	100.0	0.0
28.1	0.0	0.0	100.0
32.0	0.0	0.0	100.0
32.1	100.0	0.0	0.0
40.0	100.0	0.0	0.0

[a]160 mM NaOH (A); 160 mM NaOH/1.0 M NaOAc (B); 1.0 M NaOH (C).

Oligosaccharide Analysis by Capillary Gas Chromatography with Flame Ionization Detection (CGC-FID). Oligosaccharide analysis by CGC-FID was carried out using an Agilent 6890 gas chromatograph equipped with an Agilent 6890 series injector autosampler (Agilent Technologies Canada Inc., Mississauga, ON, Canada). Oligosaccharide separation was afforded using an Agilent J&W DB-5 (95% dimethyl−5% diphenyl polysiloxane; 30 m × 0.25 mm, 0.25 μm film thickness) open tubular fused-silica capillary column (Agilent Technologies Canada Inc.). Samples were analyzed in the splitless mode with ultrapure hydrogen used as the carrier gas and was delivered at a constant flow rate of 1.2 mL/min and ultrapure nitrogen delivered at a flow rate of 30 mL/min as the makeup gas. The injection port temperature was maintained at 250 °C and the detector at 300 °C. Data acquisition and processing were carried out using Agilent ChemStation Rev. A.06.03 software (Agilent Technologies Canada Inc.).

The following temperature gradient program was used for sucrose quantification in pure agave samples: initial temperature of 215 °C for 0 min; 0.5 °C/min from 215 to 229 °C; 30 °C/min from 229 to 295 °C; hold at 295 °C for 10 min. The total run time was 40.20 min. Pure agave syrup samples were prepared by freeze-drying (Heto Lab

Table 2. Physicochemical Properties, Variety, and Province of Origin of the Analyzed Agave Syrup and Juice Samples

sample	variety[a]	province[b]	°Brix	pH	% TTA[c]	TA[d] (mequiv/kg)	color		
							L^*	a^*	b^*
1	B	N	75.17	4.73	0.02	5.36	6.39	−0.75	0.42
2	B	N	76.21	4.97	0.02	4.33	6.06	−1.23	1.54
3	B	N	75.74	4.89	0.03	4.55	6.07	−0.96	1.06
4	B	N	75.40	4.64	0.03	5.28	6.24	−0.91	0.33
5	S	N	75.11	5.00	0.02	4.30	5.73	−0.74	1.00
6	S	N	75.09	4.85	0.03	5.14	6.55	−0.64	1.04
7	S	N	74.69	4.80	0.03	6.38	5.46	−0.90	1.52
8	S	N	75.75	4.86	0.03	5.45	5.87	−0.47	1.44
9	B	J	77.42	4.62	0.03	4.50	5.85	−0.76	0.39
10	B	J	75.88	4.93	0.03	4.46	5.53	−0.69	0.55
11	B	J	76.22	4.91	0.03	4.64	5.83	−0.41	−0.05
12	B	J	74.61	5.38	0.03	4.39	6.07	−0.26	−0.47
13	B	J	77.47	4.44	0.05	10.85	7.16	−1.07	1.09
14	B	J	75.72	4.59	0.03	6.99	6.81	−0.82	1.09
15	B	J	76.66	5.50	0.04	7.14	7.30	−1.28	2.32
16	B	J	75.69	5.11	0.03	6.96	6.42	−0.95	0.44
17	B	M	75.65	4.18	0.05	8.57	5.67	−0.27	−0.32
18	B	M	76.20	4.41	0.02	4.28	5.32	−0.16	−0.51
19	B	M	74.94	4.64	0.02	3.82	5.64	−0.08	−0.79
20	B	N	21.41	4.17	0.49	76.97	6.20	−0.58	−1.51
mean[e]			75.77	4.81	0.03	5.65	6.10	−0.70	0.64
SD[f]			0.80	0.32	0.01	1.79	0.55	0.35	0.82

[a]B: Blue agave syrup. S: Salmiana agave syrup. [b]N: Nayarit. J: Jalisco. M: Michoacán. [c]Percent total titratable acidity (as citric acid). [d]Total acidity. [e]Excluding sample 20 (agave juice). [f]Standard deviation. Excluding sample 20 (agave juice).

Equipment, Allerod, Denmark) 100 μL of a 1.0% (w/v) aqueous solution of agave syrup in a 12 × 32 mm glass vial (Chromatographic Specialties) containing a 6 × 29 mm glass microinsert. To the resulting dried foam was added 100 μL of sylon TP, and the vials were capped and heated in a block heater (Denville Scientific Inc., Metuchen, NJ, USA) at 70 °C for one hour with shaking every 10−15 min. Quantification was carried out using a standard curve ($R^2 > 0.999$) employing sucrose concentrations ranging from 20.0 to 200.0 ppm.

The oligosaccharide profiles of the adulterants and the pure and intentionally adulterated agave syrup samples were determined using CGC-FID employing the following temperature program: initial temperature of 210 °C for 10.00 min; 1 °C/min to 248 °C; 248 °C for 1.00 min; 30 °C/min to 295 °C; 295 °C for 12.00 min. The total run time was 62.57 min. Samples were prepared by freeze-drying 100 μL of a 5.5 ± 0.2 °Brix solution in a glass vial followed by the addition of 500 μL of sylon TP. Samples were heated at 70 °C for one hour with shaking every 10−15 min.

Organic Acid Analysis by High Performance Liquid Chromatography with Photodiode Array Detection (HPLC-PDA). Organic acid analysis by HPLC-PDA was accomplished on an Agilent 1100 series HPLC system (Agilent Technologies Canada Inc.) with a photodiode array detector controlled by ChemStation LC-3D software. Analyte separation was afforded employing a Restek Allure organic acids column (300 × 4.6 mm, 5 mm, 60 Å; Chromatographic Specialties Inc.) in conjunction with an isocratic mobile phase of 100 mM K_2HPO_4 adjusted to pH 2.5 with phosphoric acid. Analyte detection was at 226 nm. The mobile phase flow rate was 0.7 mL/min, and the sample injection volume was 20 μL. Agave syrup samples were prepared by dilution with water to produce a 5.0% (w/v) solution; samples were syringe filtered prior to analysis. Standards run in conjunction with the samples included acetic, ascorbic, benzoic, citric, erythorbic, fumaric, galacturonic, glucuronic, isocitric, maleic, malic, malonic, oxalic, quinic, shikimic, succinic, and tartaric acid at a concentration of 200 mg/100 mL. A standard containing glucose, fructose, and sucrose was also analyzed under the sample HPLC-PDA conditions to ensure the lack of interference by these compounds. All samples were run in duplicate.

Polyphenol Analysis by HPLC-PDA. Polyphenol analysis was conducted by HPLC-PDA employing a Prodigy ODS-3 (250 × 4.6 mm; 5 μm C_{18}, 100 Å) column (Phenomenex, Torrance, CA, USA) in series with a C_{18} guard column (Phenomenex). A gradient program was used for analyte separation and consisted of 50 mM KH_2PO_4 adjusted to pH 3.0 using phosphoric acid (solvent A) and 70% acetonitrile:30% solvent A (v:v) (solvent B). The linear gradient program was as follows: 100% A for 3 min, to 4% B at 6 min, to 10% B at 15 min, to 15% B at 30 min, to 20% B at 35 min, to 23% B at 50 min, to 25% B at 60 min, to 30% B at 66 min, to 50% B at 80 min, to 80% B at 85 min, which was held at 80% B for 5 min. Analyte detection was monitored at 254, 280, 360, and 520 nm. The mobile phase flow rate was 0.8 mL/min, and the sample injection volume was 20 μL.

Agave syrup samples were prepared by dilution with water to produce a 2.0% (w/v) solution; samples were syringe filtered prior to analysis. Standards run in conjunction with the samples included caffeic acid, catechin, chlorogenic acid, gallic acid, 4-hydroxybenzoic acid, naringenin, phloridzin, quercetin, rutin, and vanillic acid. All samples were run in duplicate.

RESULTS AND DISCUSSION

Physicochemical Properties. Results for color (L^*, a^*, b^*), pH, total soluble solids (°Brix), total acidity (TA), and percent total titratable acidity (% TTA) for the 19 agave syrup samples and the agave juice sample are shown in Table 2. Also identified in this table are the sample variety (i.e., blue [B] or salmiana [S]) and geographical location (i.e., Mexican Province) of agave syrup sample production.

The total soluble solids content of the agave syrup samples as measured by refractometry and reported as °Brix ranged from 74.61 to 77.47 with a mean value of 75.77. When compared to other nutritive sweeteners, this mean value was lower than that reported for honey of 80−83[16] and was higher than that for maple syrup of 68.0 °Brix.[17] Each of the agave syrup samples analyzed in this study had total soluble solids values that were

above the minimum Mexican standard value of 74.0 °Brix for authentic samples.[18] The agave juice sample had a total soluble solids content of 21.41 °Brix, which was much higher than those reported for maple sap (the starting material for maple syrup) of 1.6–3.7[19] and within the broad range of 7–76 °Brix reported for floral nectar (the starting material for honey).[20] As water is added during the initial stage of juice isolation from the agave plant fibers, the actual °Brix value of agave nectar (i.e., the undiluted juice from the agave plant) could be markedly higher. During agave syrup production, the water content of the juice is reduced via an evaporative process step (Figure 1), which results in a significant increase in the °Brix of the final syrup.

Agave syrup color measurements were observed both visually and as HunterLab L^*, a^*, b^* tristimulus values. In the HunterLab system, the L^* measures the lightness of the sample with a value of zero being black and 100 being white; the a^* value indicates red when positive and green when negative; and the b^* value indicates yellow when positive and blue when negative. The observed color of the agave syrup samples ranged from light amber (i.e., yellow-orange) to dark amber (i.e., orange-black). The mean tristimulus values for agave syrup samples showed a low L^* value of 6.10, and a^* and b^* values of −0.70 and 0.64, respectively. These instrumental values agree with those obtained visually of a dark syrup with a yellow-orange hue/chroma. Interestingly, the L^* value of agave juice was 6.20, which matched the mean for the agave syrup samples, indicating that the remaining processing steps of heating and evaporation do not significantly alter the lightness of the finished product. This is in contrast to that of maple syrup production, where the maple sap is colorless and becomes dark amber during the heating and evaporative process. Based on the color of the agave juice sample, which was taken before heating, the most likely explanation for the dark color of the juice would be due to enzymatic browning via polyphenol oxidase activity. Further coloring during agave syrup production would be due to a combination of caramelization and Maillard reactions.

Polyphenol analysis of the agave syrup samples showed only trace levels (<5 ppm) of compounds with absorbance values at 280 (with the exception of HMF), 360, and 520 nm and did not match the retention times of any of the standards analyzed in this study.

Agave syrup pH values ranged from 4.18 to 5.50 with a mean value of 4.81. Each of the agave syrup samples analyzed in this study had pH values that were within the Mexican standard range of 4.0–6.0 for authentic samples.[18] Based on the mean value of the samples in this study, agave syrup can be considered to be an alkaline food (i.e., pH >4.5)[21] which lies between the more basic maple syrup (pH of 6.66)[17] and the more acidic honey (pH of 3.91).[16] The pH of the agave juice sample was 4.17, and based on the mean pH result of 4.81 for the 19 syrup samples analyzed, an increase in pH is observed during water removal. This increase in pH during water removal has also been observed during the conversion of maple sap (pH range of 3.4–6.7) to syrup, which has been attributed to organic acid conversion to flavor compounds and/or microbial contamination.[19] Given the extremely low total acidity and total titratable acidities of the agave syrups studied, a more likely explanation for an increase in pH could be based on an increased concentration of mineral carbonates in the finished syrup.

The total acidity (TA) of the agave syrup samples ranged from 3.82 to 10.85 mequiv/kg with a mean of 5.65 mequiv/kg. The TA of honey has been found to have range and mean values of 8.68–59.49 and 29.12 mequiv/kg, respectively.[22] This variance in TA has been attributed to both the nectar source and geographical origin of the honey.[23,24] The average TA of agave syrup was found to fall well below this range, which may be explained by the fact that agave syrup has been shown to have a higher average pH and is therefore less acidic than honey.

The percent total titratable acidity (% TTA) of the agave syrup samples ranged from 0.02 to 0.05% with a mean value of 0.03%. This range and mean value were much lower than those reported for honey of 0.17–1.17% and 0.57%,[25] respectively, and are supported by the higher pH and lower TA of agave syrup when compared to those of honey.

The TA and % TTA in agave juice sample were 76.97 mequiv/kg and 0.49%, respectively, and these values were much higher in the juice than the finished syrups. The significant drop in both TA and % TTA as a function of concentration also supports the hypothesis of increased production of mineral carbonates in the finished syrup.

Organic acid analysis of the agave syrup samples showed only trace levels of individual compounds with citric acid predominating at a concentration of <0.01% (w:w).

Major Carbohydrates and Polyols. The major carbohydrates and polyols identified in the agave syrup samples analyzed in this study were fructose and glucose, and inositol and mannitol, respectively. Their mean values and standard deviations are shown in Table 3.

The major carbohydrate found in the 19 agave syrup samples based on retention time comparison with standards was fructose with a concentration range of 71.86 to 92.13% and a mean of 84.29%. The other major carbohydrate identified was glucose with a concentration range of 4.73 to 15.06%, and a

Table 3. Major Carbohydrate and Polyol Concentrations in Pure Agave Syrup and Juice as Determined by HPAE-PAD and CGC-FID

sample	fructose (%)	glucose (%)	sucrose (%)	mannitol (%)	inositol (%)
1	88.90	8.40	ND[a]	1.20	0.43
2	88.84	7.71	ND	0.35	0.43
3	86.49	7.44	ND	0.83	0.35
4	86.80	6.80	ND	0.17	0.40
5	84.29	7.73	ND	1.06	0.38
6	82.37	11.28	ND	0.13	0.33
7	83.55	7.30	ND	0.51	0.38
8	91.09	7.15	ND	0.73	0.41
9	90.53	6.92	ND	0.04	0.31
10	92.13	5.27	ND	0.04	0.35
11	86.81	8.10	ND	0.09	0.32
12	86.36	7.80	0.15	0.02	0.36
13	75.20	12.80	0.20	1.31	0.39
14	81.15	9.95	0.16	2.54	0.42
15	74.51	13.13	0.04	0.48	0.42
16	71.86	15.06	ND	0.45	0.40
17	84.59	5.51	0.72	1.39	0.38
18	83.35	4.73	0.74	0.98	0.39
19	82.71	5.23	0.93	0.93	0.36
20	28.30	0.02	ND	0.17	0.27
Mean[b]	84.29	8.33	0.16[c]	0.70	0.38
SD[d]	5.58	2.87	0.28[c]	0.64	0.04

[a]ND: <0.01%. [b]Excluding sample 20 (agave juice). [c]Calculated using ND as 0.01%. [d]Standard deviation. Excluding sample 20 (agave juice).

mean of 8.33%. These results show that these two monosaccharides accounted for ∼93% of the total soluble solids content of agave syrup, and produce a mean fructose to glucose ratio of 10.1. The mean values for fructose + glucose for blue and salmiana agave syrups were 92.34 and 93.69%, respectively, and the minor differences in these values were shown to be insignificant ($p > 0.05$). The Mexican standard for agave syrup indicates that blue agave syrup should contain a minimum of 80.0% fructose and a maximum of 15.0% glucose, while salmiana agave syrup should contain a minimum of 70.0% fructose and a maximum of 25.0% glucose.[18] Based on these major carbohydrate results, 100% (4/4) of the salmiana agave samples and 87% (13/15) of the blue agave samples analyzed in this study met these standards.

The fructose and glucose concentrations in the agave juice sample were 28.30% and 0.02%, respectively, and accounted for ∼28% of the total soluble solids. These results show that the natural hydrolysis processing step converts inulin (a polysaccharide composed of linear fructose units β-(1→2) linked with a terminal glucose unit) to lower molecular weight fructans and ultimately to fructose and glucose.

During major carbohydrate analysis by HPAE-PAD it was assumed that the peak eluting at ∼10.4 min was sucrose, which was supported by previous reports that showed levels of this compound in agave syrup of up to 4%.[3,18] However, CGC-FID analysis of the oligosaccharide patterns of agave syrup did not support the HPAE-PAD findings for sucrose concentration and/or content, indicating that the major disaccharide in agave syrup, based on retention time comparison with a standard, is 1-*O*-β-D-fructofuranosyl-D-fructose; inulobiose. Analytical results by CGC-FID showed that 63% of the agave syrup samples analyzed in this study had sucrose concentrations <0.01% (quantitative detection limit) and that the maximum concentration observed was <1% (Table 3).

Inositol and mannitol (i.e., polyols) were identified in agave syrup based on retention time comparisons to standards and spiking experiments by both HPAE-PAD (Figure 2) and CGC-FID analytical protocols. The mannitol concentration in the agave syrup samples ranged from 0.02 to 2.54% with a mean of 0.70%. The inositol concentration range of 0.31 to 0.43% for these samples was quite narrow and yielded a mean value of 0.38%.

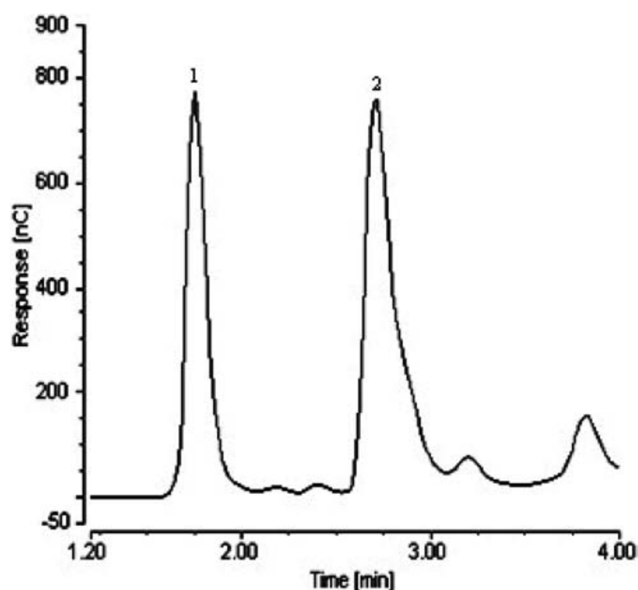

Figure 2. HPAE-PAD chromatogram of the major polyols in agave syrup (sample 3). Peaks: 1 = inositol; 2 = mannitol.

Oligosaccharide Analysis. The oligosaccharide profiles of the nineteen agave syrup samples were examined by HPAE-PAD, and a representative chromatogram is shown in Figure 3B. Shown in Figure 3A is a fructooligosaccharide standard.

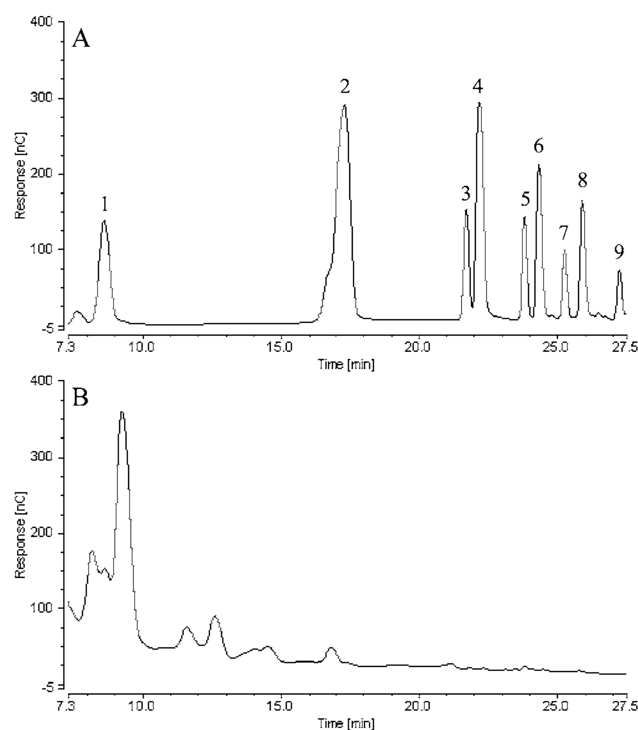

Figure 3. HPAE-PAD chromatograms of (A) fructooligosaccharide standard and (B) 2.75 °Brix agave syrup (sample 3). Peaks: 1 = F_2; 2 = F_3; 3 = GF_4; 4 = F_4; 5 = GF_5; 6 = F_5; 7 = GF_6; 8 = F_6; 9 = F_7 (G = glucose, F = fructose).

The major oligosaccharides present in agave syrup as determined by HPAE-PAD comparison to the standard were identified as degree of polymerization (DP) of 2−4 (retention time range of ∼8 to 20 min), with DP 2 (i.e., inulobiose) predominating. The major oligosaccharides present in agave syrup have been reported to be fructans with a DP ranging from 3 to 29.[5] The fructans present in *A. tequilana* have previously been shown to be composed of a complex mixture of fructooligosaccharides which contain linear β-(1→2) linkages as well as many β-(2→6) branch points.[5,26] Results from this study indicate that the natural hydrolysis conditions and time used in the production of these syrups afforded significant inulin/high molecular weight fructan hydrolysis as minimal levels of oligosaccharides greater than DP 2 were observed. These results also suggest that the oligosaccharide profile of agave syrup is dependent on how the juice is processed. For example, differences in natural hydrolysis processing time and temperature may lead to different oligosaccharide profiles in the finished product.

Authenticity. Adulteration detection can follow two major pathways. The first is called untargeted and is based on a knowledge of the natural chemical composition range of pure samples; when sample analysis shows results outside this range, it is considered to be adulterated, however the adulterant is

unknown. In the present study, this type of adulteration may be observed if an agave syrup sample showed chemical composition results outside the major carbohydrate profiles and/or the narrow range observed for inositol in the pure samples (Table 3). The second pathway is referred to as targeted, where an analytical method is developed and/or applied so as to identify the debasing of a product with a specific adulterant. This second approach to adulteration detection is extremely important as sophisticated product adulteration can be accomplished with debasing agents that are chosen to maintain the chemical composition of a sample within its natural range so that it would not indicate untargeted adulteration.

Because agave syrup is rich in carbohydrates (Table 3), a facile and economically viable method of adulteration of this product can be attained by debasing with commercially available caloric sweeteners. Through the appropriate choice of an adulterant, the major carbohydrate (i.e., fructose and glucose) profile of a debased agave syrup could be maintained within its natural range, which would make authenticity detection analytically difficult. For this section of the study four commercial caloric sweeteners were employed as adulterants that would meet this requirement; these included HFCS 55 and 90, sucrose, and a dextrose equivalent syrup (DE 42).

The most likely carbohydrate syrup to be used for agave syrup debasing would be high fructose corn syrup (HFCS), based on its monosaccharide composition. Two possible candidates for this purpose are HFCS 55 (55% fructose; F/G ratio of 1.2) and 90 (90% fructose; F/G ratio of 9.0). Based on the mean monosaccharide results obtained in this study and the HFCS F/G ratios, HFCS 55 and 90 adulteration could be done at appreciable (>30%) levels while still maintaining major carbohydrate values within their natural range.

Isotope ratio mass spectrometry (IRMS) has been used to detect the adulteration of fruit juices, honey, and maple syrup with HFCS.[27] The success of the IRMS procedure is based on the way in which plants fix carbon dioxide photosynthetically. As such, plants can be characterized into two main groups, those that follow the Calvin cycle (C3 pathway) and those that employ the Hatch−Slack pathway (C4 pathway). The success of this method has been due to the fact that most plants (fruits, vegetables, nectar used by bees for honey production) are C3, whereas two of the major caloric carbohydrate sweeteners, corn (used for HFCS) and cane sucrose, are C4. The difference between C3 and C4 materials can be measured by IRMS as an observed difference in $^{13}C/^{12}C$ ratios, with detection limits for these two adulterants in the 5−15% range.[28,29] A significant limitation of this method is in its application to plants such as agave[30,31] that fix carbon dioxide by the less common Crassulacean acid metabolism (CAM pathway) which obviates the use of $^{13}C/^{12}C$ ratios for HFCS adulteration detection in agave syrup.

An alternative method to detect the adulteration of foods is by oligosaccharide profiling employing HPAE-PAD and/or CGC-FID.[32] The application of CGC-FID oligosaccharide profiling to agave syrup adulteration with HFCS is shown in Figure 4A−C. Pure agave syrup (Figure 4A) showed a series of oligosaccharides with retention times ranging from ∼18 to ∼45 min which corresponded mainly to disaccharides based on retention time comparison with standards. The intentional debasing of this syrup with 5 and 10% HFCS 90 (Figure 4B,C) shows the presence of α- and β-isomaltose (O-α-D-glucophyranosyl-(1→6)-D-glucose) with retention times of ∼41.9 and ∼45.4 min, respectively. The HFCS 90 used as the adulterant in these experiments contained ∼184 ppm isomaltose and based on a detection limit of 3× the signal-to-noise ratio, the detection limit for this material would be ∼1% (v:v). Debasing experiments were also conducted with HFCS 55 (chromatograms not shown), which had an isomaltose content of ∼314 ppm, indicating that a significantly lower detection limit would be observed for this material. Detection limits for HFCS detection by oligosaccharide profiling could also be lowered by increasing the volume of sample injected, or by increasing the concentration and/or volume of the original sample prior to

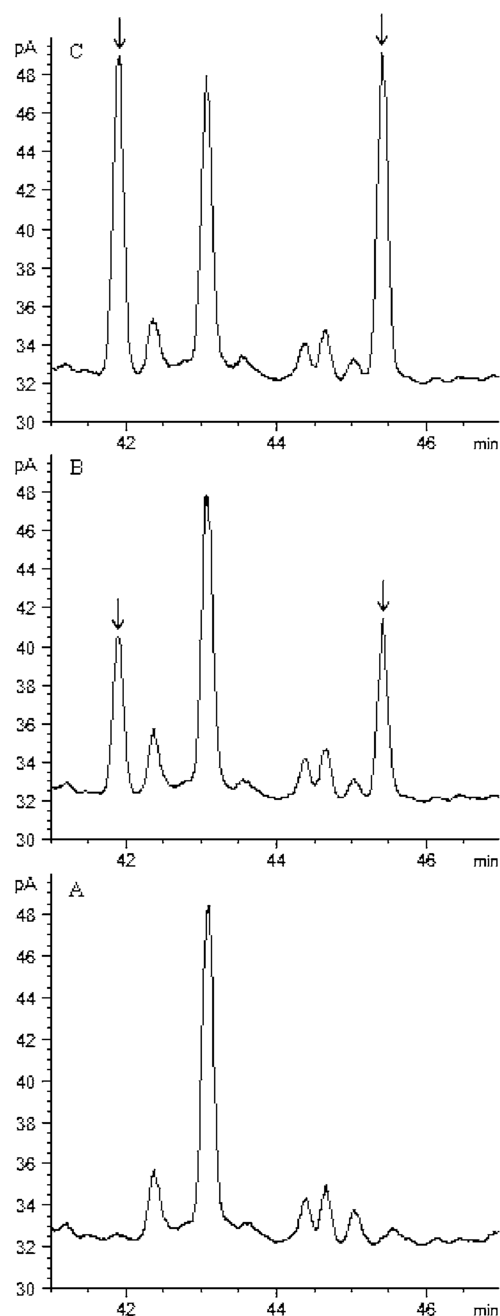

Figure 4. CGC-FID chromatograms from 41.0 to 47.0 min of (A) pure agave syrup (sample 19) and the same syrup intentionally adulterated with (B) 5% and (C) 10% (v/v) HFCS 90 at 5.5°Brix. Fingerprint oligosaccharides are indicated by arrows.

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freeze-drying. For example, in the present study 100 μL of a 5.5 °Brix sample was used for freeze-drying followed by derivatization, which results in a derivatization agent to active hydrogen (i.e., OH groups of the carbohydrate) ratio of ~5:1 (assuming that all of the carbohydrates were monosaccharides; that is, 5 OH groups per molecule). Based on these results, either the volume of sample or the original sample concentration could be doubled.

Dextrose equivalent syrups are produced commercially from plant starch sources (e.g., corn) employing a two-step enzymatic process with α-amylase (liquefaction step employing an endohydrolase; EC 3.2.1.1) followed by glucoamlyase (saccharification step; EC 3.2.1.3).[33] During the production of DE syrups, starch is hydrolyzed to produce lower molecular weight dextrose polymers and glucose. As the DE number increases, so does the concentration of glucose in the final product. The HPAE-PAD oligosaccharide profiles of a representative pure agave syrup and the same syrup intentionally debased with 10% DE 42 (v:v) are shown in Figure 5A and

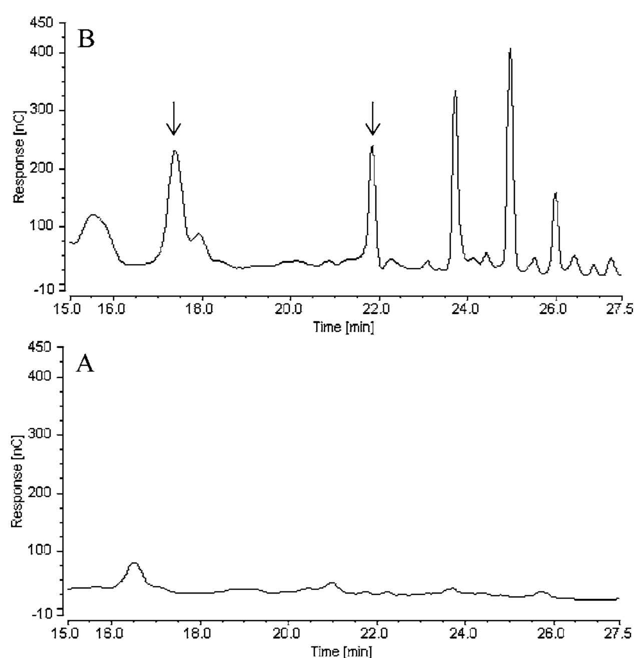

Figure 5. HPAE-PAD chromatograms from 15.0 to 27.5 min of (A) pure agave syrup (sample 2) at 5.5 °Brix and (B) agave syrup (sample 2) intentionally adulterated with 10% (v/v) DE 42 at 5.5 °Brix. Fingerprint oligosaccharides are indicated by arrows.

Figure 5B, respectively. The oligosaccharide profile of the intentionally debased sample shows significant levels of both maltose (O-α-D-glucopyranosyl-(1→4)-D-glucopyranose; retention time of ~17.4 min) and maltotriose (O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose; retention time of ~21.8 min) plus a number of other dextrose polymers. Under the experimental conditions used in this study, the detection of DE 42 at levels as low as 2.5% by HPAE-PAD was possible. Based on the polysaccharide structural differences between agave (inulin; polyfructose) and dextrose equivalent syrups (starch; polyglucose), the presence of dextrose polymers would clearly indicate adulteration. Employing a glucose concentration of 18% for DE 42[34] and the mean glucose result (8.33%) for the pure agave syrups from this study, debasing with DE 42 at levels of 5−10% would be readily possible without exceeding the natural range (4.73−15.06%) for this carbohydrate in agave syrup while still maintaining a fructose concentration >80% (assuming an initial fructose concentration of ~85% in the debased agave syrup). Debasing with a lower numbered DE syrup would be easier to detect by HPAE-PAD due to the presence of higher concentrations of low molecular weight (DP 2−25) glucose-oligosaccharides.

The application of CGC-FID oligosaccharide profiling to a pure and a DE 42 adulterated (at 1.0%) agave syrup sample is shown in Figure 6A,B. The chromatogram of the debased

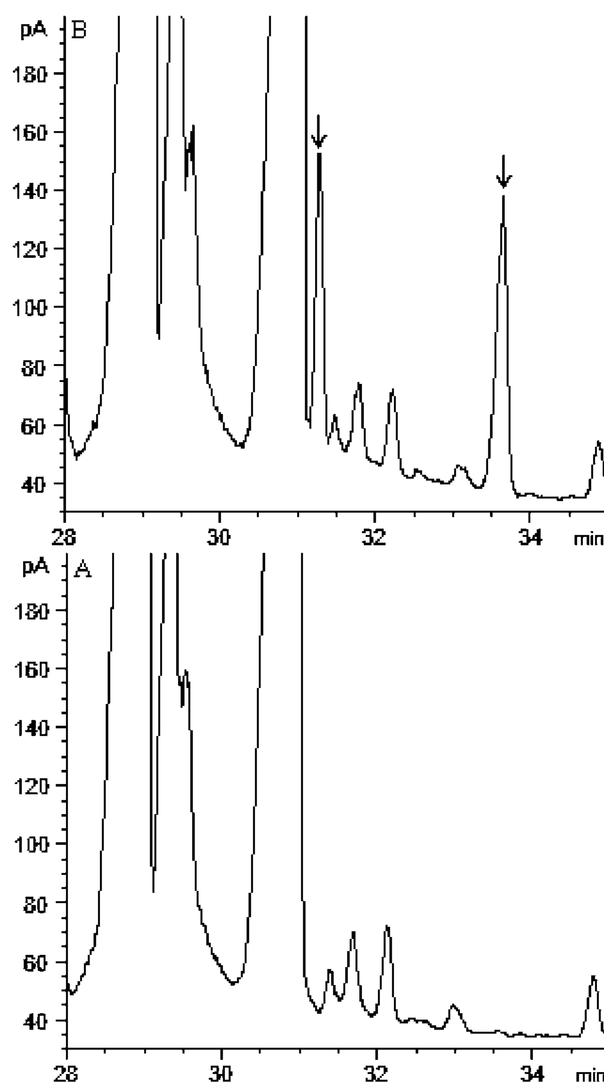

Figure 6. CGC-FID chromatograms from 28.0 to 35.0 min of (A) pure agave syrup (sample 19) and (B) agave syrup (sample 19) intentionally adulterated with 1.0% (v/v) DE 42. Fingerprint oligosaccharides are indicated by arrows.

sample shows the presence of α- and β-maltose with retention times of ~31.2 and ~33.6 min, respectively. The α-maltose peak overlaps with some of the naturally occurring oligosaccharides in agave syrup; however, the β-maltose peak elutes in a region of the chromatogram that is free from this issue. Under the experimental conditions employed in this study, the detection of DE 42 at levels below 0.5% is readily attained.

Sucrose is a common adulterant used in high carbohydrate foods, such as fruit juices, honey, and maple syrup,[35] due to both its price and its ubiquitousness in nature. Sucrose is derived from both sugar cane and beet, and its detection as an adulterant in foods has been accomplished employing a number of analytical methods including IRMS,[27] deuterium nuclear magnetic resonance spectroscopy,[30] pyrolysis mass spectrometry−chemometrics,[36] and Fourier transform infrared spectroscopy−chemometrics.[37] Both the success of the applied analytical technique for sucrose detection and its detection limits are highly dependent on the sample set tested, sucrose source (i.e., beet and/or cane), and the database of pure samples used for standard values/comparative purposes. In general, the detection limits for sucrose detection by these methods in select foods are in the range of 5−15%. In this study, oligosaccharide profiling employing CGC-FID was used to determine the oligosaccharide profiles of pure agave syrup and the same syrup intentionally debased with 1.0% beet sucrose (Figure 7A,B). The retention time for sucrose under these experimental conditions was ∼26 min, and this compound could be readily detected as a distinct peak in the intentionally debased sample. As presented previously, CGC-FID analysis of the 19 pure agave syrup samples showed a mean concentration of 0.16% and a maximum of 0.93% (Table 3). Based on these results, debasing of pure agave syrup with beet/cane sucrose at a level >1.0% would be indicative of adulteration as it would result in a final syrup with a concentration greater than the maximum observed in this study. The developed CGC-FID method for detecting the debasing of agave syrup with sucrose at this concentration would not be possible by any of the aforementioned analytical methods.

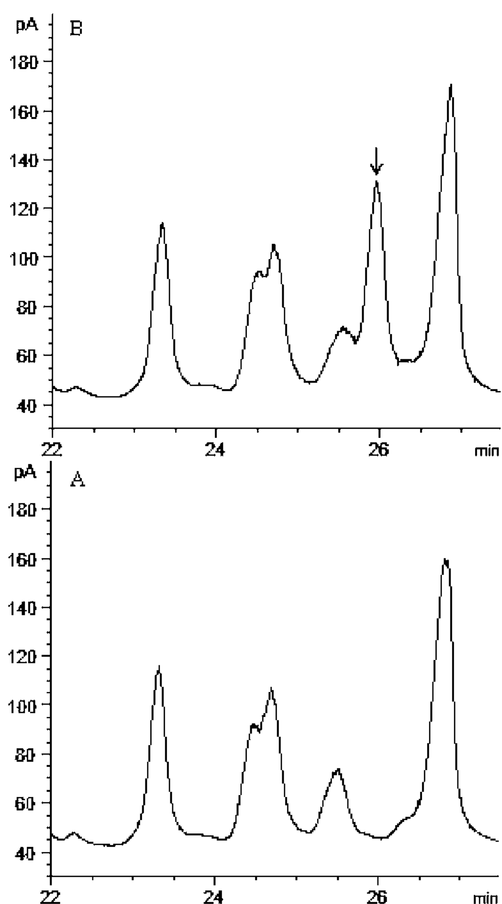

Figure 7. CGC-FID chromatograms from 22.0 to 27.5 min of (A) pure agave syrup (sample 1) and (B) agave syrup (sample 1) intentionally adulterated with 1.0% (v/v) sucrose. Sucrose is indicated using an arrow.

These results clearly show that oligosaccharide profiling employing HPAE-PAD and/or CGC-FID provides a rapid and facile method to detect the undeclared addition of low levels (0.5−2.0%) of DE syrups, HFCS, and beet/cane sucrose to agave syrup in a single chromatographic run (i.e., CGC-FID).

The economics of adulteration with any of the aforementioned caloric sweeteners is best illustrated by their price differential. As an example, the wholesale cost of high fructose corn syrup (55 or 90) is approximately \$0.53/kg,[38] whereas agave syrup pricing is about \$2.80/kg.[39] Therefore, the debasing of this material with as little as 5% would result in a significant profit margin based on metric tonne sales.

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Notes

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■ ABBREVIATIONS USED

CGC-FID, capillary gas chromatography with flame ionization detection; DE, dextrose equivalents; DP, degree of polymerization; HFCS, high fructose corn syrup; HMF, 5-hydroxymethyl-2-furaldehyde; HPAE-PAD, high performance anion exchange liquid chromatography with pulsed amperometric detection; IRMS, isotope ratio mass spectrometry; TA, total acidity; % TTA, percent total titratable acidity

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