Maple Syrup Authenticity Analysis by Anion-Exchange Liquid Chromatography with Pulsed Amperometric Detection

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Methodology employing anion-exchange high-performance liquid chromatography coupled with pulsed amperometric detection was developed to determine the addition of high-fructose corn syrup and beet medium invert syrup to maple syrup. Fingerprint oligosaccharides were shown to be present in these inexpensive sweeteners, which were not detectable (area <30 000) in pure maple syrup. Eighty pure maple syrup samples representing the major geographical regions of production in North America were analyzed in this study. Thirty of these samples were intentionally adulterated with each individual inexpensive sweetener at levels of 5, 10, and 20%. The detection limit for each of these inexpensive sweeteners was 5%.

Keywords: *Maple syrup; authenticity; HPLC-PAD*

INTRODUCTION

Maple syrup adulteration is perceived to be a serious problem because it deceives the consumer and affects the economic stability of the maple syrup industry.

The major chemical constituent of maple syrup is carbohydrate, which accounts for ~98% of the total soluble solids (Stuckel and Low, 1995; Morselli, 1975). Sucrose is the major carbohydrate present in pure maple syrup, and its concentration ranges from 88 to 100% depending on syrup age and other natural, processing, and storage factors (Laing and Howard, 1990; Whalen and Morselli, 1985; King and Morselli, 1983; Morselli, 1975). Because of the high level of carbohydrates in pure maple syrup, adulteration of this product can be readily accomplished by addition of inexpensive sweeteners. This adulteration is economically advantageous because of the large cost differential that exists between sweeteners and maple syrup. Commercially available inexpensive sweeteners that may be employed as adulterants for maple syrup include high-fructose syrups (HFCS), beet/cane medium invert syrups (BMIS), and beet/cane sucrose.

Several methods to detect adulteration of maple syrup have been or are currently being examined/developed. These include (1) carbohydrate profiling, (2) matrix analysis, (3) carbon stable isotope ratio analysis (CSIRA), (4) conductivity measurements, and (5) site-specific natural isotope fractionation nuclear magnetic resonance spectroscopy (SNIF-NMR).

Carbohydrate profiling is based on the qualitative and quantitative measurement of glucose, fructose, and sucrose in maple syrup. Nonaged, pure maple syrup has been shown to have a combined glucose plus fructose concentration of <11−12% (Stuckel and Low, 1995; Morselli, 1975). Therefore, maple syrup samples with monosaccharide levels greater than 12% may be indicative of adulteration with inexpensive sweeteners which contain high levels of glucose and fructose, such as HFCS and BMIS. Disadvantages of this method include an inability to detect beet/cane sucrose at any level, poor adulterant detection limits, and the fact that stored or partially fermented pure maple syrups may contain much higher levels of monosaccharide (up to 25%; Low, 1993).

The matrix approach is based on the qualitative and quantitative analysis of the major chemical components present in maple syrup. These chemical components include carbohydrates, minerals, organic acids, and amino acids. Individual sample results are compared to mean/range values for authentic samples. Large deviations from the mean or values that are outside the analytically determined range for one or a combination of chemical components indicate possible adulteration. This technique is both laborious and expensive, and because a wide natural range exists for each chemical component, adulterated samples may go undetected by this method. In addition, carbohydrates, mineral salts, and organic acids (i.e. malic) are relatively inexpensive and can be used to "extend" maple syrup while maintaining a normal chemical profile.

An important breakthrough in the detection of cane sucrose and HFCS addition to maple syrup resulted from the discovery by Smith and Epstein (1971) and Bender (1971) that the $^{13}C/^{12}C$ ratio in organic compounds varied among certain types of plants. These studies revealed that plants such as corn and sugar cane, which use the Hatch-Slack (C_4) photosynthetic cycle, had lower amounts of ^{13}C than plants such as maple trees, sugar beets and potatoes, which use the Calvin (C_3) photosynthetic cycle. Carro et al. (1980) and Morselli and Baggett (1984), using this technique, established a CSIRA range of −22.31 to −26.63 for authentic maple syrup samples. Because of this wide natural CSIRA range for authentic samples, the limit of detection for this technique is approximately 15%. A more recent development (Paquin, 1994), based on the work of Doner (1985) for lemon juice, involved the isolation of malic acid from maple syrup followed by CSIRA analysis. According to the authors, this modification results in a detection limit of ~7.5% for cane sucrose and corn syrup in maple syrup.

Although no peer-reviewed publication exists on the use of conductivity for maple syrup adulteration detection, this technique is widely used in eastern Canada as a screening tool (Gouvernement du Quebec, personal communication). The method is based on the ability of a diluted maple syrup sample (approximately 25 °Brix) to conduct a current. The users of this technique report that a conductivity reading <1200 μS suggests the

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sample has glucose or granulated sugar added and that samples with these low readings require further analysis by CSIRA methodology.

SNIF-NMR was initially developed for the detection of wine adulteration with beet sucrose (Martin et al., 1982). More recently, this technique has been applied to the detection of beet sucrose and BMIS adulteration of citrus juices (Martin and Guillon, 1993). This method is based on the controlled fermentation of the carbohydrates in a sample and subsequent analysis of the deuterium content at the methyl and methylene positions of the resultant ethanol by nuclear magnetic resonance spectroscopy. Although no published information exists on the application of this methodology to maple syrup adulteration, it has been reported to be successful in the detection of beet sucrose adulteration of maple syrup (G. Martin, personal communication).

This paper presents methodology for the detection of high-fructose corn syrup and beet medium invert sugar in maple syrup by analysis of trace oligosaccharides via anion-exchange high-performance liquid chromatography with pulsed amperometric detection. This work is based on "fingerprint" oligosaccharide methods previously developed for orange and grapefruit juice (Swallow et al., 1991; Low and Wudrich, 1993) and honey (Swallow and Low, 1994).

MATERIALS AND METHODS

Samples. Eighty pure maple syrup samples (Stuckel and Low, 1995) produced during a three year period were analyzed in this study. All samples were received from primary producers throughout North America and came with signed certificates attesting to their authenticity. Maple syrup samples originated in Quebec (27 samples), Ontario (18 samples), Vermont (14 samples), Massachusetts (8 samples), Wisconsin (8 samples), New Hampshire (4 samples), and Michigan (1 sample). All samples were stored at 4 °C until required for analysis.

Inexpensive Sweeteners. Commercial inexpensive sweeteners employed as adulterants in this study were HFCS (CPC International Inc., Decatur, IL; 2655 Invertose) and BMIS (Michigan Sugar Co., Saginaw, MI).

Sample Preparation. Samples were prepared by diluting the pure maple syrup samples to the appropriate °Brix (15 for HFCS detection and 25 for BMIS detection). Samples were then passed through separate columns (10 mL syringe barrels) containing ~5.5 cm^3 AG 50W-X8 cation-exchange resin, 100–200 mesh (Bio-Rad Laboratories, Richmond, CA), and ~5.5 cm^3 AG1-X8 anion-exchange resin (formate form), 100–200 mesh (Bio-Rad) followed by passage through a C_{18} Sep-Pak cartridge (Waters Chromatography, Milford, MA) and a 0.20 μm syringe filter (Corning Glass Works, Corning, NY). After preparation, the samples were brought to their final °Brix (5.5 for HFCS detection and 18 for BMIS detection), adulterated at levels of 5, 10, and 20% (w/w) with the appropriate adulterant (at an equivalent °Brix), and analyzed. Samples either were analyzed immediately or were stored at −20 °C until analyzed.

HPLC Oligosaccharide Analysis. The resulting samples were analyzed on a Waters 625 metal free gradient HPLC (Waters Chromatography) equipped with a Waters 712 Wisp autosampler and a Waters Model 464 pulsed amperometric detector (PAD) with a dual gold electrode and triple-pulsed amperometry set at sensitivities of 50 μA for HFCS and 20 μA for BMIS detection. The potentials and durations of the working electrode were maintained at $E_1 = 0.05$ V, $t_1 = 0.299$ s; $E_2 = 0.60$ V, $t_2 = 0.299$ s; and $E_3 = -0.80$ V, $t_3 = 0.499$ s. Oligosaccharide separation was achieved using a gradient program of sodium hydroxide (NaOH) and sodium acetate (NaOAc). The gradient parameters and mobile phases employed for this separation are shown in Table 1. Carbohydrate separation was accomplished using a CarboPac PA1 column (Dionex, Sunnyvale, CA; 250 × 4 mm) coupled with a CarboPac PA1 guard column (50 × 4 mm). A 200 μL injection volume was used, and the mobile phase flow rate was 1 mL/min. To minimize baseline drift, 300 mM NaOH was delivered post-column at a flow rate of 0.8 mL/min (Self-Flush Model 350 Pump, Rose Scientific, Edmonton, AB). The carbohydrate profile of the sample was plotted employing Millennium 2010 Chromatography Manager software (Waters Chromatography). Fingerprint oligosaccharide peaks were identified by comparing intentionally adulterated samples to pure maple syrup samples.

Table 1. HPLC-PAD Elution Program for Oligosaccharide Separation

time (min)	composition[a] %A	%B	%C
0.00	100	0	0
8.00	100	0	0
40.00	0	100	0
41.00	0	0	100
70.00	0	0	100
71.00	100	0	0
116.00	100	0	0

[a] % A, 100 mM NaOH (sodium hydroxide); % B, 100 mM NaOH/250 mM NaOAc (sodium acetate); % C, 300 mM NaOH. All gradients were linear.

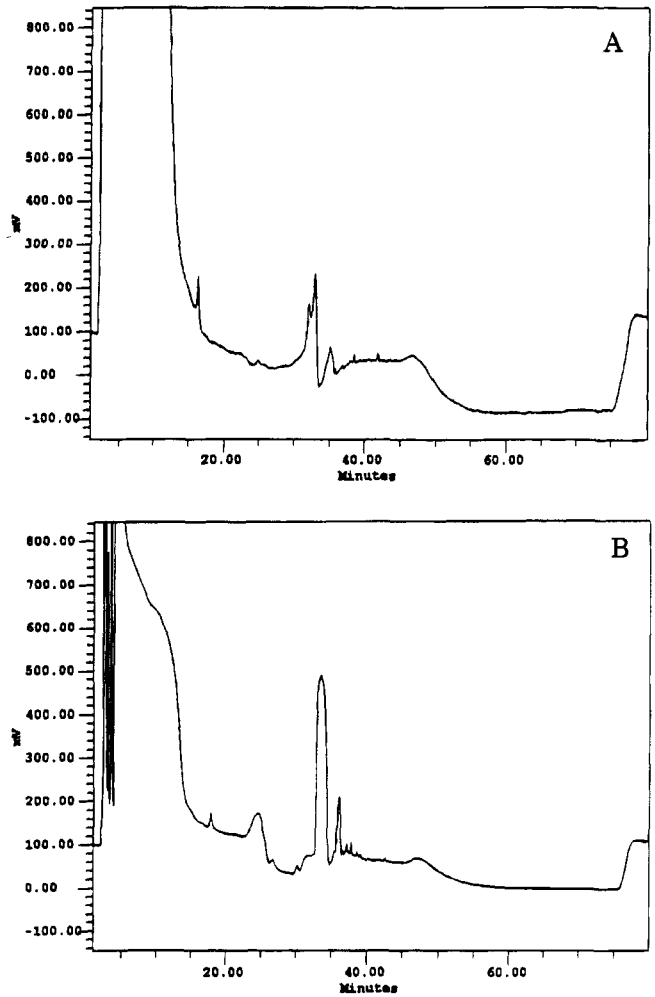

Figure 1. (A) HPLC-PAD chromatogram of a pure maple syrup sample (64; 5.5 °Brix) with low oligosaccharide content. (B) HPLC-PAD chromatogram of a pure maple syrup sample (30; 5.5 °Brix) with high oligosaccharide content.

RESULTS AND DISCUSSION

High performance liquid chromatography with pulsed amperometric detection (HPLC-PAD) was used to de-

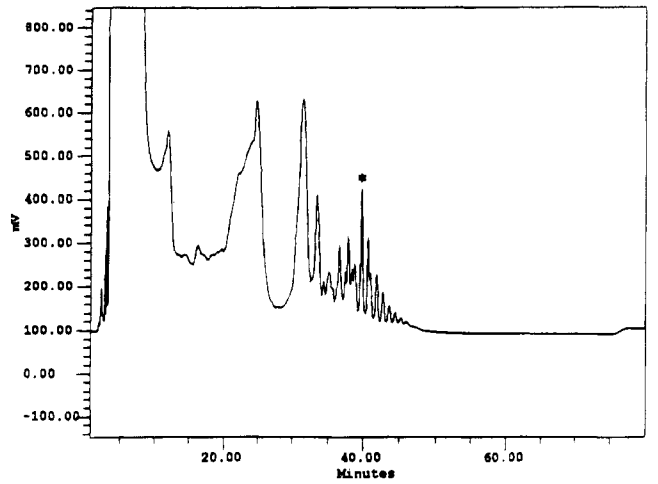

Figure 2. PLC-PAD chromatogram of HFCS 55 (5.5 °Brix; * indicates the fingerprint oligosaccharide used as a marker).

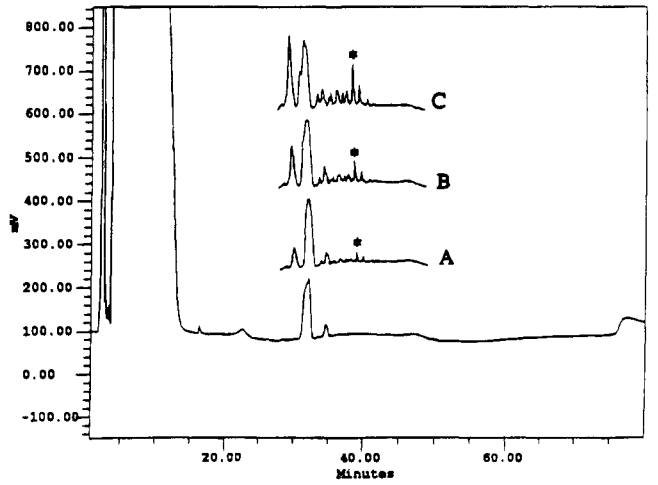

Figure 3. HPLC-PAD chromatogram of a maple syrup sample (64; 5.5 °Brix) and the same sample intentionally adulterated with HFCS 55 at levels of 5 (A), 10 (B), and 20% (C) (* indicates the fingerprint oligosaccharide used as a marker).

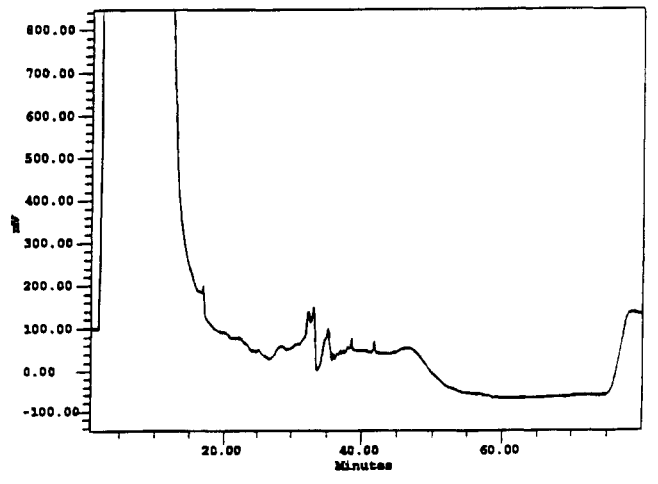

Figure 4. HPLC-PAD chromatogram of a pure maple syrup sample (17; 18 °Brix).

termine the oligosaccharide profile of pure and intentionally adulterated maple syrup samples. HPLC-PAD chromatograms which are representative of the pure maple syrup samples analyzed in this study are shown in Figure 1 (A, low oligosaccharide content; B, high oligosaccharide content). These chromatograms show the wide natural range in oligosaccharide content of

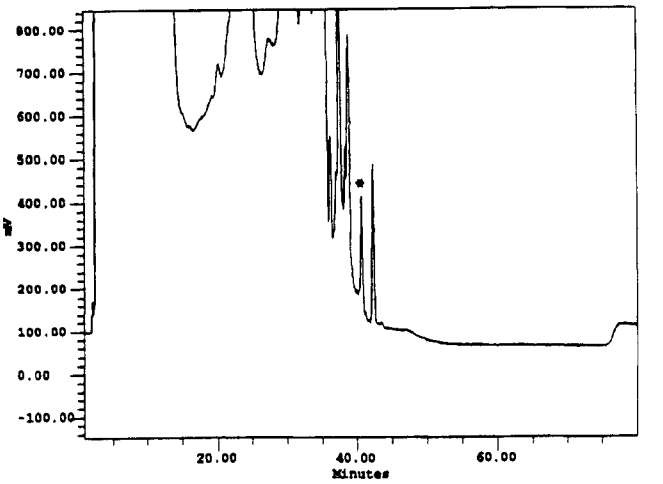

Figure 5. HPLC-PAD chromatogram of BMIS (18 °Brix; * indicates the fingerprint oligosaccharide used as a marker).

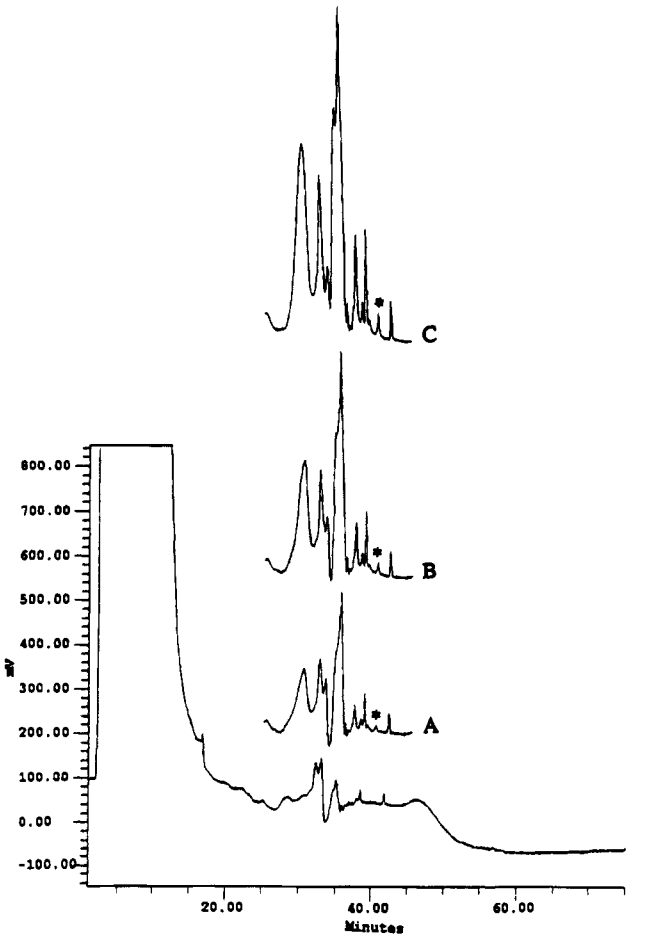

Figure 6. HPLC-PAD chromatogram of a pure maple syrup sample (17; 18 °Brix) and the same sample intentionally adulterated with BMIS at levels of 5 (A), 10 (B), and 20% (C) (* indicates the fingerprint oligosaccharide used as a marker).

pure maple syrup. The HPLC-PAD carbohydrate profile of HFCS 55 is shown in Figure 2. The gradient mobile phase program used to generate these chromatograms separates carbohydrates on the basis of size/molecular weight. Therefore, as carbohydrate molecular weight increases, retention time increases. Each of the 80 pure maple syrup samples analyzed in this study had a relatively simple oligosaccharide (peaks with relative retention times >20 min) profile when compared to that of HFCS. Major differences in oligosaccharide profiles of pure maple syrup and HFCS were observed in the

Table 2. Peak Areas and Retention Times of 30 Pure Maple Syrup Samples Intentionally Adulterated with High-Fructose Corn Syrup (HFCS) at Levels of 5, 10, and 20%

sample[a]	origin[b]	pure sample peak area[c]	RT[d]	peak area 5% HFCS	peak area 10% HFCS	peak area 20% HFCS
1	VT	ND[e]	38.82 ± 0.07[f]	0.28 ± 0.01	0.58 ± 0.01	1.17 ± 0.02
3	WI	ND	38.66 ± 0.08	0.28 ± 0.01	0.57 ± 0.01	1.14 ± 0.00
7	NH	ND	38.62 ± 0.11	0.27 ± 0.00	0.54 ± 0.00	1.10 ± 0.02
8	NH	ND	39.01 ± 0.20	0.28 ± 0.00	0.59 ± 0.01	1.10 ± 0.02
13	VT	ND	39.14 ± 0.10	0.28 ± 0.00	0.59 ± 0.00	1.18 ± 0.01
14	VT	ND	38.79 ± 0.04	0.28 ± 0.01	0.58 ± 0.01	1.16 ± 0.02
20	PQ	ND	38.58 ± 0.08	0.28 ± 0.00	0.56 ± 0.01	1.14 ± 0.02
21	PQ	ND	39.07 ± 0.31	0.28 ± 0.00	0.58 ± 0.01	1.16 ± 0.01
23	PQ	ND	38.55 ± 0.15	0.28 ± 0.00	0.57 ± 0.00	1.07 ± 0.02
28	PQ	ND	39.07 ± 0.22	0.28 ± 0.01	0.56 ± 0.01	1.11 ± 0.02
29	PQ	ND	39.11 ± 0.35	0.28 ± 0.00	0.55 ± 0.01	1.12 ± 0.02
30	PQ	ND	39.06 ± 0.40	0.29 ± 0.01	0.57 ± 0.01	1.16 ± 0.02
31	VT	ND	39.56 ± 0.31	0.29 ± 0.01	0.57 ± 0.01	1.17 ± 0.03
32	VT	ND	39.44 ± 0.12	0.27 ± 0.01	0.56 ± 0.00	1.17 ± 0.03
35	MA	ND	38.77 ± 0.32	0.28 ± 0.01	0.58 ± 0.01	1.17 ± 0.02
38	VT	ND	38.44 ± 0.29	0.28 ± 0.01	0.58 ± 0.02	1.16 ± 0.02
40	VT	ND	39.05 ± 0.38	0.27 ± 0.01	0.57 ± 0.01	1.15 ± 0.02
43	WI	ND	39.01 ± 0.07	0.28 ± 0.01	0.58 ± 0.02	1.16 ± 0.03
45	WI	ND	39.41 ± 0.46	0.27 ± 0.00	0.56 ± 0.01	1.18 ± 0.04
47	WI	ND	38.75 ± 0.33	0.28 ± 0.00	0.58 ± 0.02	1.11 ± 0.02
51	ON	ND	39.13 ± 0.40	0.28 ± 0.01	0.59 ± 0.02	1.16 ± 0.03
55	ON	ND	38.87 ± 0.29	0.27 ± 0.00	0.57 ± 0.01	1.15 ± 0.03
56	ON	ND	38.71 ± 0.47	0.29 ± 0.02	0.59 ± 0.03	1.09 ± 0.03
59	ON	ND	39.58 ± 0.20	0.28 ± 0.01	0.57 ± 0.01	1.14 ± 0.02
60	ON	ND	38.57 ± 0.11	0.28 ± 0.01	0.56 ± 0.02	1.09 ± 0.04
61	ON	ND	39.01 ± 0.53	0.27 ± 0.01	0.55 ± 0.02	1.18 ± 0.03
63	ON	ND	39.06 ± 0.35	0.29 ± 0.01	0.56 ± 0.02	1.08 ± 0.04
64	ON	ND	38.77 ± 0.41	0.29 ± 0.02	0.57 ± 0.02	1.12 ± 0.03
71	PQ	ND	39.56 ± 0.34	0.28 ± 0.01	0.57 ± 0.01	1.15 ± 0.04
76	PQ	ND	39.00 ± 0.17	0.29 ± 0.01	0.58 ± 0.01	1.16 ± 0.03
X^g			38.97	0.28	0.57	1.14
δ^f			0.31	0.01	0.01	0.03
CV[h]			0.01	0.02	0.02	0.03

[a] For complete information on samples see Stuckel and Low (1995). [b] Abbreviations: VT, Vermont; WI, Wisconsin; NH, New Hampshire; PQ, Quebec; MA, Massachusetts; ON, Ontario. [c] × 10^6 μV s. [d] Retention time. [e] Not detected (peak area <30 000). [f] Standard deviation. [g] Mean. [h] Coefficient of variation.

region of ~37−40 min. In the chromatograms of pure maple syrup (Figure 1A,B) few detectable peaks were observed after ~35 min, while several peaks were observed in HFCS (Figure 2). On the basis of the oligosaccharide profiles of all 80 pure maple syrup samples a fingerprint peak with a relative retention time of ~39 min was used to detect the adulteration of maple syrup with HFCS. When compared to standards, the relative retention time of this peak corresponded to a DP5 (maltopentose, retention time of ~38.7 min) or DP6 (maltohexose, retention time of ~39.5 min) oligosaccharide. This peak was determined to be unique because it was absent (i.e. had an area <30 000) in each of the 80 pure maple syrup samples analyzed. Figure 3 shows a pure maple syrup sample (sample 64) and the same sample (from ~29 to 49 min) intentionally adulterated with HFCS at levels of 5, 10, and 20%. The fingerprint oligosaccharide peak (*) was found to increase incrementally in both height and area as the amount of adulterant added increased. The peak eluting at ~30 min was not used as a fingerprint oligosaccharide as a similar peak was observed in several of the pure maple syrup samples analyzed in this study. Table 2 presents the relative retention times and peak areas of 30 pure maple syrup samples intentionally adulterated with HFCS at levels of 5, 10, and 20%. The mean peak areas for the fingerprint oligosaccharide in the 30 intentionally adulterated samples were 0.28 × 10^6, 0.57 × 10^6, and 1.14 × 10^6 for 5, 10, and 20%, respectively. The results obtained showed a narrow range for each of these adulteration levels and increased incrementally (r^2 = 1.000) as would be expected if maple syrup contained no detectable level of the fingerprint oligosaccharide and if the methodology was reproducible.

To detect BMIS addition to maple syrup, a more concentrated sample (18 °Brix) was required for HPLC-PAD analysis. A representative chromatogram of the carbohydrates present in pure maple syrup under these more concentrated conditions is shown in Figure 4. The HPLC-PAD oligosaccharide profile of BMIS under identical conditions is shown in Figure 5. These modifications resulted in some loss of baseline stability; however, differences in the oligosaccharide profiles were observed when pure maple syrup was compared to BMIS. Each of the 80 pure maple syrup samples had a relatively simple oligosaccharide profile in the 35−45 min region under these conditions, while BMIS contained a high level of oligosaccharides in this region. On the basis of the oligosaccharide profiles of BMIS and the 80 pure maple syrup samples, a fingerprint peak with a relative retention time of ~40 min was used to detect the presence of this inexpensive sweetener. The relative retention time of this peak corresponded to a DP6 (maltohexose) or DP7 (maltoheptose, retention time of ~40.5 min) oligosaccharide. This peak was determined to be unique because it was absent (i.e. had a peak area of <30 000) in each of the 80 pure maple syrup samples analyzed. Figure 6 shows a pure maple syrup sample (17) and the same sample (from ~25 to 45 min) intentionally adulterated with BMIS at levels of 5, 10, and 20%. As the amount of adulterant added to the pure maple syrup sample increased, there was an incremental increase in both peak height and area of the fingerprint oligosaccharide (*). The peaks eluting

Table 3. Peak Areas and Retention Times of 30 Pure Maple Syrup Samples Intentionally Adulterated with Beet Medium Invert Sugar (BMIS) at Levels of 5, 10, and 20%

sample[a]	origin[b]	pure sample peak area[c]	RT[d]	peak area 5% BMIS	peak area 10% BMIS	peak area 20% BMIS
1	VT	ND[e]	39.55 ± 0.15[f]	0.29 ± 0.00	0.58 ± 0.01	1.17 ± 0.03
3	WI	ND	39.46 ± 0.09	0.29 ± 0.01	0.59 ± 0.01	1.16 ± 0.03
7	NH	ND	39.72 ± 0.11	0.29 ± 0.00	0.60 ± 0.00	1.18 ± 0.02
8	NH	ND	40.01 ± 0.28	0.29 ± 0.01	0.59 ± 0.01	1.20 ± 0.02
13	VT	ND	39.94 ± 0.18	0.29 ± 0.00	0.59 ± 0.00	1.21 ± 0.03
14	VT	ND	39.79 ± 0.34	0.30 ± 0.01	0.61 ± 0.01	1.19 ± 0.02
20	PQ	ND	38.88 ± 0.21	0.30 ± 0.00	0.58 ± 0.01	1.18 ± 0.02
21	PQ	ND	40.07 ± 0.11	0.29 ± 0.01	0.58 ± 0.01	1.20 ± 0.02
23	PQ	ND	39.75 ± 0.35	0.29 ± 0.00	0.59 ± 0.00	1.18 ± 0.02
28	PQ	ND	39.77 ± 0.22	0.30 ± 0.01	0.60 ± 0.01	1.13 ± 0.02
29	PQ	ND	40.01 ± 0.30	0.29 ± 0.00	0.59 ± 0.03	1.15 ± 0.02
30	PQ	ND	39.66 ± 0.25	0.29 ± 0.01	0.57 ± 0.01	1.19 ± 0.03
31	VT	ND	39.76 ± 0.11	0.29 ± 0.01	0.58 ± 0.01	1.18 ± 0.03
32	VT	ND	39.94 ± 0.22	0.30 ± 0.01	0.59 ± 0.00	1.18 ± 0.02
35	MA	ND	39.77 ± 0.39	0.30 ± 0.02	0.58 ± 0.01	1.21 ± 0.04
38	VT	ND	39.44 ± 0.39	0.30 ± 0.01	0.61 ± 0.02	1.14 ± 0.02
40	VT	ND	40.05 ± 0.38	0.30 ± 0.01	0.59 ± 0.01	1.16 ± 0.02
43	WI	ND	40.01 ± 0.03	0.31 ± 0.01	0.58 ± 0.02	1.16 ± 0.03
45	WI	ND	40.21 ± 0.37	0.29 ± 0.00	0.59 ± 0.01	1.18 ± 0.04
47	WI	ND	39.75 ± 0.33	0.29 ± 0.00	0.60 ± 0.01	1.19 ± 0.02
51	ON	ND	40.13 ± 0.44	0.30 ± 0.01	0.59 ± 0.02	1.16 ± 0.03
55	ON	ND	39.87 ± 0.09	0.29 ± 0.00	0.61 ± 0.01	1.18 ± 0.03
56	ON	ND	39.71 ± 0.31	0.29 ± 0.00	0.59 ± 0.02	1.14 ± 0.03
59	ON	ND	39.58 ± 0.22	0.28 ± 0.01	0.59 ± 0.01	1.20 ± 0.02
60	ON	ND	39.57 ± 0.17	0.29 ± 0.01	0.62 ± 0.02	1.21 ± 0.03
61	ON	ND	40.21 ± 0.37	0.29 ± 0.01	0.58 ± 0.02	1.18 ± 0.03
63	ON	ND	40.06 ± 0.15	0.29 ± 0.02	0.57 ± 0.01	1.16 ± 0.04
64	ON	ND	39.77 ± 0.32	0.29 ± 0.01	0.57 ± 0.02	1.22 ± 0.03
71	PQ	ND	39.56 ± 0.24	0.30 ± 0.01	0.59 ± 0.01	1.24 ± 0.02
76	PQ	ND	39.90 ± 0.37	0.29 ± 0.00	0.60 ± 0.03	1.18 ± 0.04
X[g]			39.80	0.29	0.59	1.18
δ[f]			0.27	0.01	0.01	0.02
CV[h]			0.01	0.02	0.02	0.02

[a] For complete information on samples see Stuckel and Low (1995). [b] Abbreviations: VT, Vermont; WI, Wisconsin; NH, New Hampshire; PQ, Quebec; MA, Massachusetts; ON, Ontario. [c] × 10^6 μV s. [d] Retention time (minutes). [e] Not detected (peak area <30 000 μV s). [f] Standard deviation. [g] Mean. [h] Coefficient of variation.

at ~30, 36, 39, and 42 min were also examined as possible fingerprints for BMIS addition but were rejected because of the presence of similar peaks in some authentic samples. Table 3 presents the relative retention times and peak areas of 30 pure maple syrup samples intentionally adulterated with BMIS. The mean peak areas for the fingerprint peak in the 30 intentionally adulterated samples were 0.29 × 10^6, 0.59 × 10^6, and 1.18 × 10^6 for 5, 10, and 20%, respectively. As was the case for HFCS adulteration of maple syrup, the results obtained showed a narrow range for each of these adulteration levels and increased incrementally (r^2 = 1.000) as would be expected if maple syrup contained no detectable level of the fingerprint oligosaccharide.

To assess the reproducibility of retention times and peak areas, six maple syrup samples were intentionally adulterated separately with 10% HFCS and 10% BMIS and were analyzed in duplicate according to the aforementioned HPLC-PAD methods. The results from these analyses are shown in Table 4. The mean retention time and peak area for HFCS adulteration with 10% HFCS were 38.83 min and 0.58 × 10^6, respectively, with standard deviations of 0.21 and 0.01 × 10^6, respectively. The BMIS fingerprint peak had a mean retention time and peak area of 40.03 min and 0.60 × 10^6, respectively, with standard deviations of 0.24 and 0.01 × 10^6, respectively.

In this study, adulteration was performed on finished syrups that were prepared following the standard industrial practice of ~104 °C for 1.5–2.0 h (Willits and Hills, 1976). To investigate the effect of an abusive heat treatment on adulteration detection, two maple syrup samples (3 and 59) were intentionally adulterated, one at a level of 10% with HFCS and the other at a level of 10% with BMIS. Each of these previously finished maple syrup sample was then subjected to a second heat treatment of 1.5 h at 104 °C. Samples were prepared and analyzed as previously presented for HFCS and BMIS adulteration detection. The results showed that the fingerprint oligosaccharide was present regardless of when the syrup was adulterated. The peak area of the fingerprint oligosaccharide for HFCS was 0.58 × 10^6, which compared favorably with the area of 0.57 × 10^6 previously found for this same sample (3). The peak

Table 4. Reproducibility of Retention Times and Peak Areas of Six Maple Syrup Samples Intentionally Adulterated with 10% HFCS and 10% BMIS

sample	HFCS fingerprint peak t_r[a]	HFCS fingerprint peak area[b]	BMIS fingerprint peak t_r	BMIS fingerprint peak area
3	39.04	0.58	39.66	0.60
3	39.18	0.58	39.51	0.51
31	38.91	0.57	40.01	0.60
31	38.79	0.58	40.12	0.59
45	38.77	0.57	40.06	0.59
45	38.60	0.57	40.31	0.59
59	38.92	0.58	40.03	0.60
59	38.87	0.57	40.17	0.62
71	39.00	0.58	40.09	0.60
71	38.61	0.57	40.02	0.61
79	38.86	0.58	40.37	0.60
79	38.43	0.59	40.07	0.60

[a] Retention time (minutes). [b] × 10^6 μV s.

area for the fingerprint oligosaccharide for BMIS was 0.60×10^6, which compared favorably with the area of 0.59×10^6 previously found for the same sample (59).

CONCLUSIONS

Maple syrup adulteration is considered to be one of the major problems currently encountered by the maple syrup industry. As the major soluble solids present in maple syrup are carbohydrate, adulteration of maple syrup can be readily accomplished by the simple addition of inexpensive sweeteners to the finished syrup. The cost differential between inexpensive sweeteners and pure maple syrup is considerable; therefore, there is an economic incentive to adulterate this product. In addition, glucose and fructose levels in maple syrup are variable (i.e. from 0 to 12%), and from analyses performed in our laboratory maple syrup experiences an increase in these carbohydrates as storage time increases (up to 25% in syrups stored at 4 °C for >8 months). On the basis of these factors, the adulteration of maple syrup with inexpensive sweeteners that contain high levels of glucose and fructose (i.e. HFCS or BMIS) may, but not necessarily, be limited to ~12%. However, even at this level, maple syrup adulteration would still be economically viable.

Prior to this research there was no published analytical method available for the detection of HFCS or BMIS addition to maple syrup at levels of 5–12%. Fingerprint oligosaccharide methodology was developed for the detection of both HFCS and BMIS addition to maple syrup. In each case an oligosaccharide that was either unique to the inexpensive sweetener or present at low concentrations in the pure samples was identified and used as a fingerprint for adulteration detection. The limit of detection for each of the developed methods was 5%.

ACKNOWLEDGMENT

We thank the North American Maple Syrup Council and the International Maple Syrup Council for providing authentic samples as well as financial support for this research.

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Received for review July 31, 1995. Accepted September 11, 1995.[⊗]

JF9500997

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1995.