Capillary Gas Chromatographic Detection of Invert Sugar in Heated, Adulterated, and Adulterated and Heated Apple Juice Concentrates Employing the Equilibrium Method

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The equilibrium method is introduced for the detection of invert sugar addition to apple juice. The method consists of a pre-equilibration of the sample with dry pyridine at 50 °C for 20 min followed by the addition of trimethylsilylimidazole and heating at 75 °C for 40 min. The resulting derivatized carbohydrates are then analyzed by capillary gas chromatography. This method was successfully used by independent laboratories to distinguish heated pure, intentionally adulterated (with invert sugar), and intentionally adulterated and then heated apple juice concentrates. The equilibrium method was shown to give significantly lower coefficients of variation for this sample set when compared to the original capillary gas chromatographic method. In addition, these results indicate that it may also be an effective method for the detection of medium invert sugar, depending on the level of the fingerprint oligosaccharides in this sweetener.

Keywords: *Authenticity; apple juice; invert sugar*

INTRODUCTION

The introduction of oligosaccharide fingerprinting (Swallow et al., 1991; Wudrich et al., 1993) employing high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) provided an important method to detect the undeclared addition of a variety of inexpensive sweeteners to citrus juices.

The direct application of this procedure to the detection of inexpensive sweetener addition to apple juice was more difficult because of the enzymes used during the extraction and clarification of this juice (N. H. Low, unpublished results). To extend this method to apple juice authenticity, a new procedure based on capillary gas chromatography (CGC) (Low, 1995, 1996) was developed. The CGC method was originally published (Low et al., 1994) to detect the undeclared addition of high-fructose syrups to pineapple juice and was later extended as a procedure for both total invert sugar and high-fructose syrup detection in apple and orange juices based on the presence of fingerprint oligosaccharides. More recently (Low and Hammond, 1996) it has been shown that this method can also detect the presence of inulin-derived syrups in apple juice. The method is based on the presence of fingerprint oligosaccharides in each of the aforementioned sweeteners that are either not present or are present at very low concentrations in the pure foods.

Due to the sensitivity of the CGC method and its ability to detect the addition of a wide variety of inexpensive sweeteners in a single chromatographic run, there has been considerable discussion about the methodology. To investigate both the applicability and robustness of the methodology, a number of collaborative/ring tests have been organized under the auspices of AOAC International and the International Fruit Juice Union (IFU). The method has recently been peer validated (McLaughlin et al., 1999) for the detection of high-fructose syrups from starch and inulin, and a full collaborative trial of the method for these two adulterants is nearing completion. Because the oligosaccharide fingerprints for all of the aforementioned sweeteners are either of extremely low concentration or not present in apple juice concentrate that has not been excessively heated, there is general consensus that this method has the ability to detect these types of adulterants in apple juice at low levels, typically in the region of 5%.

One of the difficulties observed in the application of the GCG method to the detection of invert sugar in apple juice has been the formation of these fingerprint oligosaccharides in concentrates that have been excessively heated (Low, 1996). An AOAC International collaborative study (Hammond et al., 1999) involved the analysis of 24 apple juice concentrate samples that were either pure, adulterated with total invert sugar (at levels of 10−30%), pure heat treated (105−120 °C for time periods of 5 and 10 min), or intentionally adulterated with total invert sugar at a level of 10% followed by heating at the aforementioned temperatures and times. The purpose of the collaborative study was to test the ability of the CGC oligosaccharide fingerprint method to distinguish pure, heated pure, intentionally adulterated, and heated intentionally adulterated samples. Of the 14 laboratories that participated in the study, 12 laboratories were able to distinguish each of these different groups of samples on the basis of the peak height ratio of the two invert sugar oligosaccharide

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10.1021/jf990457w CCC: $18.00
Published on Web 09/16/1999

peaks (IS_2/IS_1), and *no* false positive results were observed for the unadulterated samples in this collaborative study.

Although 12 of the 14 laboratories were able to use the method effectively to identify "pure", "heated", and "adulterated" samples, the ratio (IS_2/IS_1) of the invert sugar fingerprint oligosaccharides from laboratory to laboratory was quite variable. Following exclusion of two laboratories on statistical grounds, the coefficient of variation (CV) for the ratio of IS_2/IS_1 was typically 25%.

A similar CV for the ratio of the two invert sugar fingerprint oligosaccharides was observed in a more complex ring study organized by the IFU (Anonymous, 1999). This study consisted of apple juice concentrates adulterated with four sweetener syrups at levels of 2.5, 5.0, and 12.5% for medium invert sugar; 5.0, 7.5, and 15.0% for total invert sugar; 5.0 and 10.0% for high-fructose corn syrup (HFCS); and 5.0% for high-fructose inulin syrup (HFSI). In addition, pure apple juice concentrates and selected pure and intentionally adulterated samples were subjected to heating to a temperature of 127 °C. Seventeen of the 18 laboratories that participated in the ring test were able to identify the apple juice concentrate samples adulterated with HFCS and HFSI and 16 were able to identify the sample adulterated with 7.5% total invert sugar. However, ~37% of the laboratories classified the heated concentrate samples as adulterated with invert sugar, a false positive result, and seven identified the sample adulterated with 5% total invert sugar as pure, a false negative result. In addition, significant numbers (94 and 80%, respectively) of the laboratories were unable to detect the addition of 2.5 and 5.0% medium invert sugar, a false negative result. However, as the oligosaccharide method was not developed for this particular inexpensive sweetener (due to the low level of the fingerprint oligosaccharides present), these results were not surprising.

It was our hypothesis that the high CV observed in these studies was due to nonequilibrium of the IS_1 and IS_2 tautomers prior to derivatization. It has been shown that a reducing carbohydrate can exist in solution in up to six tautomeric forms (α,β-pyranose, α,β-furanose, acyclic carbonyl, and its hydrate) (Angyal, 1984). Therefore, we belived that a pre-equilibration step with base would afford mutarotation to a compound specific stable tautomeric ratio prior to derivatization.

This paper discusses recent developments in oligosaccharide fingerprinting for the detection of medium invert sugar in commercially prepared apple juice concentrate. We introduce the equilibrium method, which significantly reduces the interlaboratory variation in the ratio of IS_2/IS_1.

This method can be used to distinguish a heated apple juice concentrate from one that has been intentionally adulterated with medium invert sugar and then heated. Subsequently, this method may afford a lower CV for interlaboratory results for both this inexpensive sweetener and total invert sugar and may also reduce the number of false positive and negative results.

MATERIALS AND METHODS

Samples. Pure, Heated, and Intentionally Adulterated and Heated Apple Juice Concentrate Samples. A commercially produced (manufactured in the United States from a combination of Granny Smith and Red and Golden Delicious apples) apple juice concentrate (70 °Brix) with a Brix/acid ratio of 26 was used as the standard (test sample 1; pure apple juice concentrate) in this study.

A portion (95 kg) of this pure apple juice concentrate was diluted with distilled water (263 kg) to yield a 20 °Brix solution, which was in turn subjected to commercial evaporation (vacuum of 12−13 in.) at 85 °C to a final solid content of 70 °Brix (test sample 2; heated pure apple juice concentrate).

A portion (95 kg) of test sample 2 was then diluted with distilled water (263 kg) to yield a solid content of ~20 °Brix. This solution was then subjected to commercial evaporation at 85 °C to a final solid content of 70 °Brix (test sample 3; excessively heated pure apple juice concentrate).

A portion (85 kg) of test sample 3 was then diluted with distilled water (213 kg) to yield a solid content of ~20 °Brix. This solution was then subjected to commercial evaporation at 85 °C to a final solid content of 70 °Brix (test sample 4; excessively heated pure apple juice concentrate).

A portion (38 kg) of test sample 2 was mixed with 38 kg of a 50% medium invert sample (prepared by taking 19 kg of test sample 1 plus 19 kg of medium invert sugar) followed by the addition of 187 kg of distilled water (final total solid content of ~20%). Following mixing, this solution was commercially evaporated (85 °C) to 70 °Brix (test sample 5; heated and 25% adulterated sample).

A portion (43 kg) of test sample 2 was mixed with an equal weight (43 kg) of a 20% medium invert sugar sample (prepared by taking 80% of test sample 1 and 20% of medium invert sugar) followed by the addition of 214 kg of distilled water (final total solid content of ~20%). Following mixing, this solution was commercially evaporated (85 °C) to 70 °Brix (test sample 6; heated and 10% adulterated sample).

From these six test samples, six other test samples were prepared as follows: (a) 1:1 (v/v) mixing of test sample 4 with medium invert sugar (test sample 7; heated and 50% adulterated sample); (b) 1:1 (v/v) mixing of test sample 4 with test sample 7 (test sample 8; excessively heated and 25% adulterated sample); (c) 4:1 (v/v) mixing of test sample 4 with test sample 7 (test sample 9; excessively heated and 10% adulterated sample); (d) 1:1 (v/v) mixing of test sample 3 with 100% medium invert sugar (test sample 10; excessively heated and 50% adulterated sample); (e) 1:1 (v/v) mixing of test sample 3 with test sample 7 (test sample 11; excessively heated and 25% adulterated sample); and (f) 4:1 (v/v) mixing of test sample 3 with test sample 7 (test sample 12; excessively heated and 10% adulterated sample);

Sweetener Syrup/Adulterant. The sweetener syrup used in this study was a beet medium invert sugar (75 °Brix; National Food Processors Association, Washington, DC).

Sample Preparation. Samples were diluted to 5.5 ± 0.1 °Brix in HPLC grade water (e.g., Milli-Q Water System, Milford, MA) and were filtered through a 0.45 μm syringe filter (e.g., Corning Glass Works, Corning, NY).

An aliquot of this solution (100 μL) was transferred to a gas chromatographic autosampler vial (12 × 32 mm; 1.5 mL); frozen in either acetone/dry ice or a freezer (−5 °C), prior to lyophilization for between 1.5 and 16 h depending on the efficiency of the vacuum concentrator (e.g., Heto Model VR-1; Heto Lab Equipment, Allerod, Denmark).

In selected experiments, 10 μL aliquots of gentiobiose (300 mg/L) and phenyl-β-D-glucopyranoside (200 mg/L; both from Sigma-Aldrich Co. Ltd., Mississauga, ON) were added as internal standards prior to lyophilization.

Sample Derivatization. The following sample derivatization protocols were used for this study.

(a) Original Method. To the lyophilized sample was added 500 μL of *N*-trimethysilylimidazole in pyridine (1:4, v/v; Sylon-TP, Supelco Inc., Bellefonte, PA). The sample was then capped and heated at 75 ± 2 °C for 1 h (heating block; Denville Scientific Inc., Metuchen, NJ).

(b) MSHFBA Method. Samples were treated as in (a) with the exception that the derivatizing agent employed was 500 μL of *N*-methyl-*N*-trimethylsilylheptafluorobutyramide (MSHFBA) in 1-methylimidazole (95:5, v/v; both from Aldrich Chemi-

cal Co., Milwaukee, WI). Solubilization of the sample was gradual using this derivatization procedure.

(c) Temperature Variation Method. Selected samples were treated as in (a) or (b) with the exception that the derivatization temperature employed was either room temperature (~22 °C) for 12 h or 50 ± 2 °C for 1 h.

(d) Equilibrium Method. To the lyophilized sample was added 400 μL of dry pyridine (Sigma-Aldrich Chemical Co. Ltd.). The sample was then capped and heated for 20 min at 50 ± 2 °C. To this solution was added 100 μL (through the vial septum) of *N*-trimethylsilylimidazole (Sigma-Aldrich Chemical Co. Ltd.), and the reaction mixture was heated at 75 ± 2 °C for 40 min.

Sample Analysis. Oligosaccharide fingerprint analysis was performed on a capillary gas chromatographic system (HP 6890 or HP 5890; Hewlett-Packard, Mississauga, ON) employing either a J&W DB-5 (0.25 mm × 30 m; 0.25 μm film thickness; J&W Scientific, Folsom, CA) or an HP-5 (0.25 mm × 30 m; 0.25 μm film thickness; Hewlett-Packard) capillary column. The carrier gas was either UHP hydrogen (HP 6890) at a flow rate of 1.4 mL/min (employing the constant flow mode) or UHP helium (HP 5890) at a total linear velocity of 27 cm/s. Analysis was carried out in the splitless mode with either a 1.0 or a 2.0 μL injection volume (depending on the sensitivity of the instrument). Sample carbohydrates were eluted by employing one of the following temperature programs (based on carrier gas): (a) hydrogen at 210 °C for 10 min followed by a 1 °C/min gradient to a final temperature of 248 °C; this temperature was maintained for 30 s and was followed by a 30 °C/min gradient to a final temperature of 290 °C, which was held for 12 min (approximate run time of 62 min); (b) helium at 210 °C for 15 min followed by a 1 °C/min gradient to a final temperature of 290 °C, which was held for 20 min (approximate run time of 115 min).

Injector and detector temperatures (independent of carrier gas) were maintained at 250 and 300 °C, respectively. Detector makeup gas was UHP nitrogen at a flow rate of 30 mL/min.

Statistical Analysis. Experimental data were processed using StatView 4.01 (Abacus Concepts, Berkeley, CA) at the 5% significance level.

RESULTS AND DISCUSSION

Carbohydrate analysis of the pure apple juice (at 11.5 °Brix) used in this study showed sorbitol, glucose, fructose, and sucrose concentrations of 0.41, 1.10, 5.95, and 2.44%, respectively. The Brix-to-acid ratio of this juice was 26.

Analysis Employing the Original CGC Method. The original CGC method developed by Low (1995, 1996) was based on the detection of total invert sugar addition to apple juice via the presence of two fingerprint oligosaccharides with retention times (based on a 0.25 mm × 30 m, 0.25 μm film thickness column) of ~39.3 (IS_1) and ~39.9 min (IS_2), respectively. In this study we chose medium invert sugar as the adulterant. Major carbohydrate analysis of this sweetener (at 12.0 °Brix) showed glucose, fructose, and sucrose concentrations of 3.30, 3.03, and 5.40%, respectively. Medium invert sugar also contains the same two fingerprint oligosaccharides that are present in total invert sugar; however, the concentrations of these oligosaccharides are significantly lower. In most commercial total invert sugar (at 5.5 °Brix) samples (Low, 1996) the peak heights for IS_1 and IS_2 are ~53 and ~184 pA, respectively, for a peak height ratio (IS_2/IS_1) of 3.5. In this commercial sample of medium invert sugar the peak heights (at 5.5 °Brix) for IS_1 and IS_2 were ~27 and ~88 pA, respectively, for a peak height ratio of 3.3. Therefore, medium invert sugar as an adulterant provides an analytically challenging (with respect to detection limits) problem for the oligosaccharide fingerprint method.

Table 1. Peak Height Ratio (IS_2/IS_1) for Invert Sugar Fingerprint Oligosaccharides Employing the Original CGC Method

sample	lab 1	lab 2	lab 3	lab 4	mean	CV[a]	SD[b]
1	nd[c]	1.50	1.00	nd	nc[d]	nc	nc
2	1.20	1.60	1.10	1.40	1.33	0.17	0.22
3	1.30	1.40	1.00	1.40	1.27	0.15	0.19
4	1.30	1.60	1.10	1.40	1.35	0.15	0.21
5	2.30	2.60	1.90	2.40	2.30	0.13	0.29
6	1.70	2.50	1.40	1.90	1.88	0.25	0.46
7	3.50	4.70	2.50	3.10	3.45	0.27	0.93
8	1.50	2.30	1.30	2.00	1.77	0.26	0.46
9	1.40	1.60	1.10	1.60	1.42	0.17	0.24
10	2.90	3.60	2.10	2.00	2.65	0.28	0.75
11	2.10	2.70	1.70	2.20	2.17	0.19	0.41
12	1.60	2.10	1.30	1.70	1.68	0.20	0.33

[a] Coefficient of variation. [b] Standard deviation. [c] Not detectected (peak height < 4 pA). [d] Not calculated.

In the first set of experiments, the 12 samples were analyzed by four independent laboratories employing the original CGC fingerprint oligosaccharide method using either helium (laboratories 3 and 4) or hydrogen (laboratories 1 and 2) as the carrier gas. The results from this analysis are reported in Table 1.

These results yielded CVs that were similar to those found in previous ring/collaborative studies employing this methodology (Anonymous, 1999). The results obtained for each specific laboratory indicated that pure, heated, and intentionally adulterated (with medium invert sugar) and then heated apple juice concentrate samples could be distinguished on the basis of their IS_2/IS_1 ratio. However, the interlaboratory results obtained for the same samples showed a high CV. Because of this high variation it would be impossible for these four laboratories to adopt a "standard" peak height ratio for pure, heated, and intentionally adulterated and heated apple juice concentrate samples. For example, a comparison of laboratories 1 and 3 for samples 3 (heated pure concentrate) and 8 (25% adulterated and heated concentrate) gave the following results: 1.30 and 1.50 (laboratory 1) and 1.00 and 1.30 (laboratory 3), respectively. Therefore, a standard peak height ratio of 1.30 that may be adopted by laboratory 3 for an adulterated sample could not be accepted by laboratory 1 as this value would give a false positive result. In addition, a standard peak height ratio of 1.30 for a pure heated concentrate that may be adopted by laboratory 1 could not be accepted by laboratory 3 as this value would give a false negative result. A positive result from this experiment was the fact that the choice of carrier gas did not have a significant effect ($p < 0.05$) on the peak height ratio of the fingerprint oligosaccharides.

The range of results obtained using this method were also quite broad with the maximum of 2.50−4.70 observed for sample 7 and the lowest of 1.00−1.40 observed for sample 2.

In a selection of samples two internal standards, gentiobiose (reducing disaccharide) and phenyl-β-D-glucopyranoside (nonreducing glucoside), were used in an attempt to reduce interlaboratory variation in the peak height ratio. Results (not reported) from these experiments yielded CVs similar to those observed without the use of internal standards.

Analysis Employing the MSHFBA Reagent. In the second set of experiments, the 12 samples were analyzed by the four independent laboratories employing the MSHFBA derivatizing agent using the same gas chromatographic conditions as in the original CGC

Table 2. Peak Height Ratio (IS_2/IS_1) for Invert Sugar Fingerprint Oligosaccharides Employing the MSHFBA Reagent

sample	lab 1	lab 2	lab 3	lab 4	mean	CV[a]	SD[b]
1	nd[c]	1.00	0.60	1.00	nc[d]	nc	nc
2	1.22	0.84	0.65	0.94	0.91	0.26	0.21
3	1.21	0.89	0.68	0.94	0.93	0.23	0.22
4	1.28	0.89	0.70	0.99	0.97	0.25	0.24
5	1.95	1.41	1.08	1.61	1.51	0.24	0.36
6	1.54	1.22	0.86	1.29	1.23	0.23	0.28
7	2.00	1.75	1.29	2.08	1.78	0.20	0.36
8	1.91	1.43	1.03	1.58	1.49	0.25	0.36
9	1.54	1.14	0.83	1.11	1.15	0.25	0.29
10	2.41	1.74	1.29	2.13	1.89	0.26	0.49
11	1.95	1.59	1.07	1.58	1.55	0.23	0.36
12	1.56	1.13	0.80	1.24	1.16	0.33	0.38

[a] Coefficient of variation. [b] Standard deviation. [c] Not detectected (peak height < 4 pA). [d] Not calculated.

method. It was noted by all participants that the initial solubility of the freeze-dried samples in the MSHFBA reagent was poor and that solubilization occurred slowly during the derivatization procedure. The results from this analysis are reported in Table 2.

The MSHFBA reagent resulted in a significant ($p < 0.05$; based on mean results) lowering of the peak height ratio for all samples (with the exception of sample 1, for which statistical analysis was not performed). The peak height ratio decrease for the MSHFBA derivatized samples was ~30% when compared to those derivatized using trimethylsilylimidazole in pyridine and was due to the increase in peak height for IS_1 with virtually no change in peak height for IS*2*.

The CV for the samples analyzed using the MSHFBA reagent was significantly higher ($p < 0.05$) for six of the samples, was the same for three (samples 6, 8, and 10), and was significantly ($p < 0.05$) lower for two (samples 7 and 9).

As was observed for the 12 samples analyzed using the original CGC methodology, laboratories were able to distinguish pure, heated, intentionally adulterated, and subsequently heated apple juice concentrates using the MSHFBA reagent. However, the adoption of a standard peak height ratio for pure, heated, and intentionally adulterated and subsequently heated apple juice concentrates employing this reagent would lead to a larger percentage of false negative and false positive results. For example, if peak height ratios of <1.00 for pure juice and 1.10 as a minimum for 10% adulterated were adopted as the standard, then laboratory 1 would label all (with the exception of sample 1, for which no ratio was determined) samples as adulterated (three false positives), whereas laboratory 3 would have six false negative results (samples 5, 6, 8, 9, 10, and 11) employing the same standard ratios.

Analysis Employing Temperature Variation. Three of the samples (4, 7, and 9) representative of excessively heated pure concentrate (sample 4), intentionally adulterated (50%) with medium invert sugar (sample 7), and intentionally adulterated (10%) with medium invert and then subsequently heated (sample 9) apple juice concentrates were subjected to two different heating and derivatization time treatments. The sample treatments were either 50 °C/1 h or room temperature/12 h. The results from the analysis of these samples by the four laboratories are shown in Table 3.

Statistical analysis of the results obtained by the four laboratories showed a general trend of lower CVs for each of the samples when compared to those analyzed

Table 3. Peak Height Ratio (IS_2/IS_1) for Invert Sugar Fingerprint Oligosaccharides Employing Trimethylsilylimidazole/Pyridine (1:4, v/v) at Different Temperatures and Times

sample	lab 1	lab 2	lab 3	lab 4	mean	CV[a]	SD[b]
4[c]	1.24	1.39	1.26	1.71	1.40	0.16	0.22
4[d]	1.18	1.51	1.20	1.36	1.31	0.12	0.15
7[c]	2.67	3.11	2.90	3.18	2.96	0.08	0.23
7[d]	2.52	3.21	2.65	3.19	2.89	0.12	0.36
9[c]	1.54	1.82	1.66	2.08	1.78	0.13	0.23
9[d]	1.70	1.77	1.59	1.59	1.69	0.05	0.09

[a] Coefficient of variation. [b] Standard deviation. [c] 50 °C/1 h. [d] Room temperature/12 h.

Table 4. Peak Height Ratio (IS_2/IS_1) for Invert Sugar Fingerprint Oligosaccharides Employing the Equilibrium Method

sample	lab 1	lab 2	lab 3	mean	CV[a]	SD[b]
1	0.76	0.69	nd[c]	nc[d]		
2	0.74	0.79	0.83	0.79	0.06	0.05
3	0.83	0.78	0.72	0.78	0.07	0.06
4	0.81	0.83	0.73	0.79	0.07	0.05
5	1.31	1.33	1.23	1.29	0.04	0.05
6	1.19	1.06	1.04	1.10	0.07	0.08
7	1.63	1.67	1.99	1.76	0.11	0.20
8	1.18	1.32	1.12	1.21	0.09	0.10
9	1.10	0.99	0.96	1.02	0.07	0.07
10	1.64	1.71	1.69	1.68	0.02	0.04
11	1.28	1.41	1.23	1.31	0.07	0.09
12	0.96	1.02	0.97	0.99	0.04	0.04

[a] Coefficient of variation. [b] Standard deviation. [c] Not detectected (peak height < 4 pA). [d] Not calculated.

using the original method. However, the range for these results was still quite broad with a maximum of 1.54−2.08 for sample 9 (50 °C/1 h) and a minimum of 1.59−1.77 for sample 9 (room temperature).

Analysis Employing the Equilibrium Method. The 12 samples were analyzed by three of the four independent laboratories employing the equilibrium method. One of the laboratories had difficulties applying the methodology due to suspected impurities in the pyridine used. The equilibrium method involves treatment of the freeze-dried sample with dry pyridine at 50 °C for 20 min, which is then followed by the addition of the derivatizing agent *N*-trimethylsilylimidazole. The resulting mixture is then heated at 75 °C for 40 min. The results from this analysis are shown in Table 4. The addition of a known volume of 2-hydroxypyridine, which has been shown to catalyze the mutarotation of nonreducing carbohydrates (Hudson, 1907), may more rapidly (<20 min) afford equilibration prior to derivatization.

The peak height ratios obtained (comparison of mean values) employing the equilibrium method were significantly lower ($p < 0.05$) than those obtained using the original CGC method. It was observed from the chromatograms for these samples that this peak height ratio difference was due to the peak height increase in IS_1 while IS_2 remained relatively constant. For example, in sample 2 the peak heights (laboratory 1) for IS_1 and IS_2 employing the original CGC method were 11.5 and 14.1 pA, respectively, for a peak height ratio of 1.2. When the equilibrium method was used for this sample, the peak heights for IS_1 and IS_2 were 18.8 and 13.9 pA, respectively, for a peak height ratio of 0.74. The lack of peak height change in IS_2 indicates that this peak corresponds to a nonreducing carbohydrate as no alteration in the anomeric (α and β) ratio was observed and supports our ongoing work on the isolation of these two

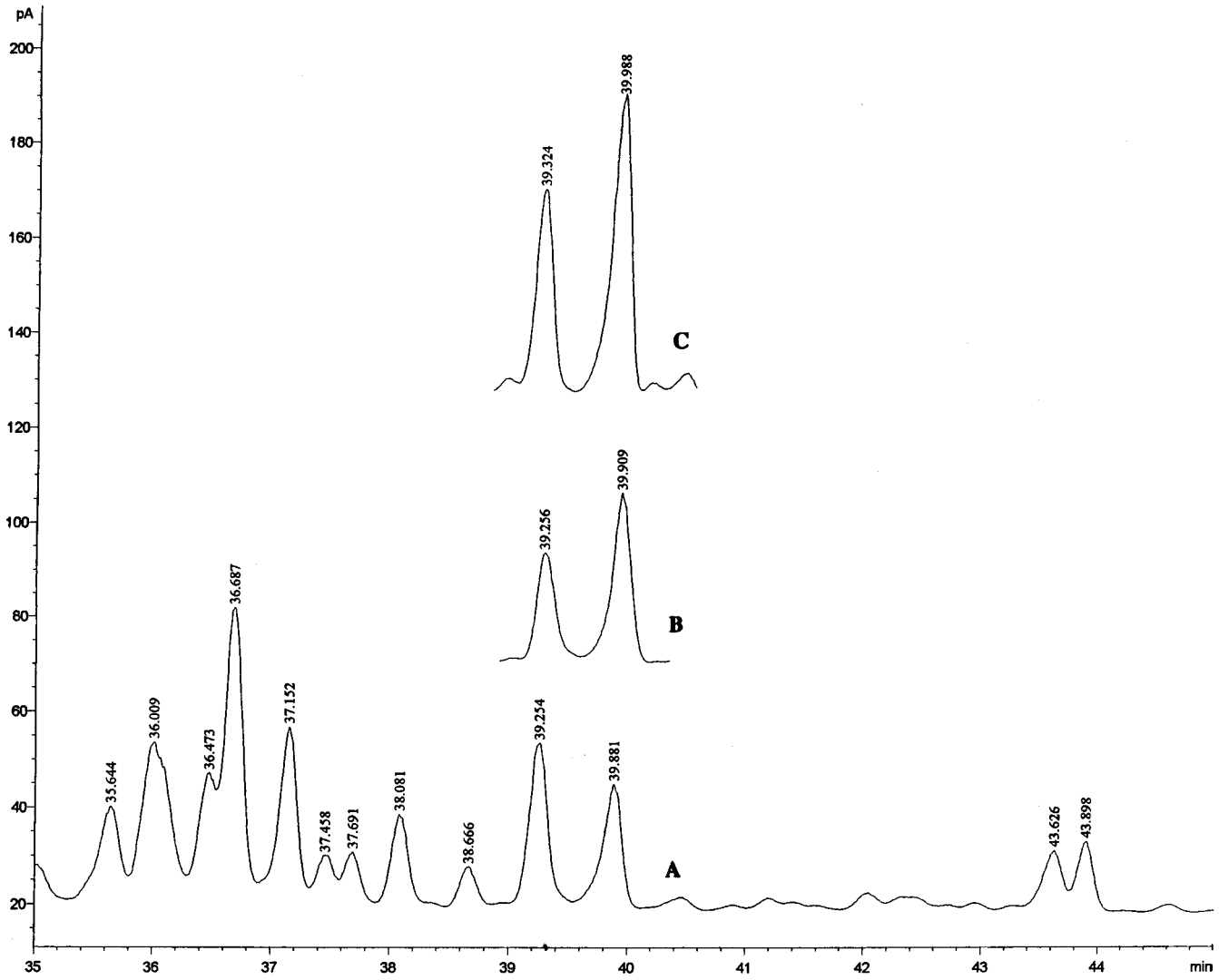

Figure 1. Expanded region (35−45 min) of the capillary gas chromatograms of a heated pure (A; test sample 4), an intentionally adulterated and heated (B; test sample 6), and an intentionally adulterated and excessively heated (C; test sample 9) apple juice concentrate. Fingerprint oligosaccharides IS_1 and IS_2 have approximate retention times of 39.3 and 39.9 min, respectively.

fingerprint oligosaccharides (N. H. Low, unpublished results). The significant peak height change in IS_1 would appear to indicate that one of the anomers of a reducing carbohydrate is represented by this peak. This peak height change was not accompanied by a similar but opposite peak height change in any of the other peaks observed in the chromatogram; however, it is noted that the peak of interest could lie underneath the off-scale sucrose peak. Although the structures of IS_1 and IS_2 have not yet been completed, the relative retention times of these oligosaccharides when compared to those of standards indicate that they are most likely disaccharides. The range of results obtained for each of the 12 individual samples was much smaller than those obtained in any of the previous experiments run. This small range resulted in a CV which was less than half that observed in the original CGC method. By employing this methodology it may be possible to set a standard peak height ratio for pure, heated, and intentionally adulterated and subsequently heated apple juice that could be employed by all laboratories using the fingerprint oligosaccharide technique for the detection of invert sugar addition to apple juice. The results from these samples (see Table 4) would suggest that an IS_2/IS_1 peak height ratio <0.90 could be used to distinguish adulterated from nonadulterated samples; this ratio could be raised to <0.95 to increase the safety margin for false positive results. These results also show that it was possible to detect (on the basis of the level of fingerprint oligosaccharides in this commercial sample only) medium invert sugar at a level of ∼10% in apple juice concentrate. As total invert sugar has been shown to have a much greater concentration of these two fingerprint oligosaccharides (Low, 1995), the detection of this sweetener employing this methodology would be more facile. Expanded (35−45 min) capillary gas chromatograms for a heated pure sample (test sample 4), an intentionally adulterated (with medium invert sugar) and heated sample (test sample 6), and an intentionally adulterated (with 10% medium invert sugar) and then excessively heated apple juice concentrate (test sample 9) are shown in Figure 1.

When compared to the other experiments run, the range of results for each of the samples was the lowest observed. For example, the maximum range was observed for sample 7 of 1.63−1.99, whereas the lowest range of 0.96−1.02 was observed for sample 12.

Conclusions. The equilibrium method, which involves an initial reactant equilibration with dry pyridine prior to derivatization with *N*-trimethylsilylimidazole,

resulted in significantly lower CVs for the 12 samples analyzed in this study by independent laboratories. It was noted that all experimental procedures used in this study, including the original CGC method, could distinguish pure, heated pure, intentionally adulterated, and intentionally adulterated and then heated apple juice concentrate samples. However, the equilibrium method provides an improvement on interlaboratory results for these types of samples when compared to the original CGC method. In addition to improved reproducibility among laboratories, the equilibrium method may also provide a means of detection for medium invert sugar that has a much lower concentration of the two fingerprint oligosaccharides which are markers for invert (either total or medium) sugar addition to apple juice.

LITERATURE CITED

Angyal, S. J. The composition of reducing sugars in solution. *Adv. Carbohydr. Chem. Biochem*. **1984**, *42*, 15−68.

Anonymous. Detection of sugar syrup addition to juices by capillary gas chromatography. IFU Recommendation No. 4, 1999.

Hammond, D. A.; Brause, A.; Hofsommer, H.-J.; Low, N. H. The assessment of the capillary gas chromatographic method for the detection of invert syrup addition to apple juices; a collaborative study. *J. AOAC Int*. **1999**, submitted for publication.

Hudson, C. S. Catalysis by acids and bases of the mutarotation of glucose. *J. Am. Chem. Soc*. **1907**, *29*, 1571−1574.

Low, N. H. Apple and orange juice authenticity analysis by capillary gas chromatography with flame ionization detection. *Fruit Process*. **1995**, *11*, 362−367.

Low, N. H. Fruit juice authenticity analysis via capillary gas chromatography with flame ionization detection. *J. AOAC Int*. **1996**, *79*, 724−737.

Low, N. H.; Hammond, D. A. Detection of inulin in apple juice by capillary gas chromatography. *Fruit Process*. **1996**, *4*, 135−139.

Low, N. H.; Brause, A.; Wilhelmsen, E. Normative data for commercial pineapple juice from concentrate. *J. Assoc. Off. Anal. Chem*. **1994**, *77*, 965−975.

McLaughlin, M.; Hammond, D. A.; Low, N. H. Peer validation of the "Low" method for apple juice adulteration with HFSI and HFCS. *J. AOAC Int*. **1999**, in press.

Swallow, K. W.; Petrus, D.; Low, N. H. Detection of orange juice adulteration with beet medium invert sugar using anion exchange liquid chromatography with pulsed amperometric detection. *J. Assoc. Off. Anal. Chem*. **1991**, *74*, 341−345.

Wudrich, G. G.; McSheffrey, S.; Low, N. H. Detection of the addition of variety of inexpensive sweeteners to pure orange juice (adulteration) by high performance liquid chromatography. *J. Assoc. Off. Anal. Chem*. **1993**, *76*, 342−354.

Received for review May 5, 1999. Revised manuscript received July 29, 1999. Accepted August 3, 1999. This research was financially supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC; to N.H.L.), the Processed Apples Institute (to N.H.L.), and the National Food Processors Association (NFPA).

JF990457W