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Fast Analysis of Sugars, Fruit Acids, and Vitamin C in Sea Buckthorn (*Hippophaë rhamnoides* L.) Varieties

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A fast, one-step gas chromatographic method was developed to analyze trimethylsilyl (TMS) derivatives of sugars, fruit acids, and ascorbic acid in sea buckthorn (*Hippohaë rhamnoides* L.) berries. The method was applied to berry press juice of sea buckthorn of different origins grown in Finland during the 2003 and 2004 seasons. The method gave reliable results for D-fructose, D-glucose, ethyl-D-glucose, and malic, quinic, and ascorbic acids, which are the major sugars and acids in sea buckthorn juice. For the first time in sea buckthorn and evidently in any berry, the presence of ethyl β-D-glucopyranoside is reported. The structure of ethyl glucose was verified by high-performance liquid chromatography (HPLC), gas chromatography (GC), MS, and NMR analyses of both the isolated and the synthesized compounds. In the GC method, vitamin C was analyzed as ascorbic acid only, and dehydroascorbic acid was thus not taken into account.

KEYWORDS: Sea buckthorn (*Hippophaë rhamnoides* L.); fruit acids; gas chromatography; sugars; ethyl β-D-glucopyranoside; vitamin C

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) is a berry of high nutritive value with growing commercial importance in western countries. It has been used since ancient times in traditional Chinese medicine and nutrition to reduce the risk of numerous diseases and maintain good health. Especially, the high contents of vitamin C, carotenoids, tocopherols, sterols, and flavonoids have increased the interest of western consumers in the fruit (*1*−*5*). Sugars and fruit acids display an important contribution to the sensory properties of the berry. The ratio of sugars and organic acids in sea buckthorn is close to 1:1, which is exceptionally low as compared to other edible fruits and berries and which gives the products their characteristic sourness. On the other hand, the vitamin C concentration is in some cases only 1/10 less than that of sugars or acids in the berry. Fructose and glucose contribute typically over 90% of sugars and malic and quinic acids over 98% of all acids in sea buckthorn (*6*−*8*). In addition, the presence of an unknown sugar compound has been reported (*7*).

Among the different methods used for the analysis of sugars and organic acids in fruits and berries, mostly high-performance liquid chromatography (HPLC) is applied (*9*−*11*). HPLC is also commonly used for the estimation of vitamin C (*11*−*13*). However, we did not find any examples of applications in which sugars, fruits acids, and vitamin C would have been analyzed in a single chromatographic run. We have previously reported gas chromatographic (GC) analysis of isolated fractions of sugars and acids and HPLC analysis of vitamin C in the sea buckthorn fruit (*1*, *7*, *14*). The sample preparation for both of these methods is time- and resource-consuming and requires a large size of sample.

As the concentration of ascorbic acid (AA) in sea buckthorn is high, in some samples even close to that of sugars or acids, it is possible to use one and the same dilution to analyze all of the compounds in a single procedure. Our goal was to develop and test a reliable, fast, and easy-to-use GC method in which sugars, fruit acids, and AA can be simultaneously analyzed as trimethylsilyl (TMS) derivatives. The method was applied in the analysis of sea buckthorn berries of different origins grown in Finland. Also, a sugar compound previously unknown was identified.

MATERIALS AND METHODS

Samples. A professional sea buckthorn farmer in Riihimäki, southern Finland, cultivated sea buckthorn (*Hippophaë rhamnoides* L.) of cvs. Prevoshodnaya (PRE), Oranzhevaya (ORA), Chuiskaya (CHU), and Raisa (RAI) in 2003. The varieties Avgustinka (AVG), Botanicheskaya (BOT), and Trofimovskaya (TRO) were grown in 2003 and 2004, and the varieties Prozcharachnaya (PRO), Pertsik (PER), and RAI were grown in 2004 by an association on sea buckthorn in Turku, southwest Finland. All of the varieties were of the Russian origin, i.e., subspecies (ssp.) *mongolica*, except RAI, which is a Finnish variety of the ssp.

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rhamnoides. Berries were hand-picked when optimally ripe, frozen immediately at −20 °C, pooled after freezing, and stored for the analyses.

Reference Compounds. D-Glucose and L-malic acid were purchased from Fluka (Buchs, Switzerland), D-fructose and AA were purchased from Sigma, quinic acid was purchased from Chem Service (West Chester, PA), and citric acid was purchased from J. T.Baker (Devanter, Holland). Internal standards L-tartaric acid and D-sorbitol were obtained from Merck (Darmstadt, Germany). Ethyl β-D-glucopyranoside was synthesized in this project.

Sample Preparation. Fifty grams of sea buckthorn berries was thawed in the microwave oven with 225 W twice over 15 s and shaken out between each thaw and homogenized over 30 s by a Bamix mixer (Bamix, Metlen, Switzerland). The homogenate was filtered through a cheesecloth under vacuum conditions. The one and same juice subsample was used for the fractionation of sugars, acids, and vitamin C analysis by HPLC and for simultaneous GC analysis of sugars, acids, and vitamin C. All of the analyses were carried out in triplicate, except for the variety AVG in 2004, which was analyzed in six replicates.

Chemical Analysis. *Vitamin C by HPLC.* Vitamin C was analyzed as AA by HPLC after converting dehydroascorbic acid (DHAA) to AA with dithiothreitol (DTT, Promega Co., Madison, WI) (*1*, *12*, *14*). The berry juice was diluted 1:40 with DTT in water solution (final concentration, 80 mg/1 mL juice), and each juice sample was analyzed in duplicate. The dilution was kept for 2 h in the dark at room temperature and filtered (0,45 μm) for HPLC analysis. A sample of 20 μL was injected into a Shimadzu SLC-10A system (Shimadzu, Japan) with a UV detector. The column used was LiChrocharts 250-4 LiChrosphar RP-18, 5 μm (Merck). A buffer of 0.5% KH_2PO_4, containing 0.1% DTT with flow rate of 0.4 mL/min, was used as the mobile phase. Quantification was carried out with an external standard method.

Sugar and Acid Fractionations. Sugars and acids were analyzed according to the method applied earlier in our laboratory (*7*, *14*). The juice was diluted 1:20 in water, and the internal standards sorbitol and tartaric acid (Merck) and 2 mL of 0.1 N NaOH were added in the total volume of 20 mL. One milliliter of the dilution was fractionated by dual solid-phase extraction, where the lipophilic colors were adsorbed in the upper nonpolar cyclohexyl Isolute CH (EC) column (100 mg/mL) (International Sorbent Technology, Hengoed, United Kingdom) and the acids were trapped in the second, lower anion exchanger Isolute SAX column (International Sorbent Technology). From the SAX column, sugars were washed by water and the organic acids were eluted by 15 N formic acid. Both fractions were diluted, and the sample was evaporated to dryness and dried in a desiccator overnight. TMS derivatives of sugars and acids were prepared by adding Tri-Sil (Pierce, Rockford, IL) reagent for each fraction. The vials were closed with butyl Teflon septa, shaken vigorously by a Vortex (Vortex-Genie, Springfield, MA) for 3 min, and incubated at 60 °C for 30 min and at room temperature overnight.

Juice Preparation for Simultaneous Analysis of Sugars, Acids, and AA. The juice was diluted 1:20 in water and the internal standards sorbitol (0.1 g/100 mL in dilution) for sugars and vitamin C and tartaric acid (Merck) (0.05 g/100 mL) for acids were added in the dilution in the total volume of 20 mL and filtered (0.45 μm). A 100 μL amount of filtrate was evaporated to dryness, dried in a desiccator over P_2O_5 overnight, and silylated as described for fractionated samples.

GC. The TMS derivatives of sugar and acid fractions as well as of the nonfractionated juice samples were analyzed with Varian 3300 GC equipped with a flame ionization detector (FID) (Varian, Limerick, Ireland). The analyses were carried out with methyl silicone Supelco Simplicity-1 fused silica column (30 m, i.d. 0.25 mm; film thickness, 0.25 μm) (Bellefonte, PA). One microliter of sample was injected manually into the split injector (1:20). The temperature of the injector was 210 °C, and the detector temperature was 290 °C. The column temperature was programmed as 2 min at 90 °C, raised to 180 °C at rate of 8 °C/min, to 215 °C at rate of 4 °C/min and to final temperature 275 °C at rate of 12 °C/min, and held at 275 °C for 6 min.

Identification of the Unknown Sugar. *Isolation and Purification of the Compound.* The sugar fraction of sea buckthorn was isolated as described for the analysis of sugars. The sugars were separated, and the unknown compound was isolated with HPLC by Luna NH_2 phase column (Phenomenex, Torrance, CA) over acetonitrile:water (80:20) with a flow rate of 1.4 mL/min.

NMR Analysis of the Sugar Derivative. ^1H and ^{13}C NMR spectra of the isolated and synthesized sugar derivative were recorded on a Bruker Avance 400 spectrometer in deuterated water as a solvent at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm), and the coupling constants (*J*) are in Hertz (Hz). The following abbreviations are used to describe the multiplicity: d, doublet; t, triplet; q, quartet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; and dq, doublet of quartets. Two-dimensional (2D) spectra were run as a HHCOSY90 standard 2D experiment for HH homonuclear correlation and a HCCOW standard 2D experiment for CH correlation. ^1H NMR (D_2O): δ 4.49 (d, $J_{1,2}$ = 8.0 Hz, 1H, H_1), 3.99 (dq, ^2J = 10.0 Hz, ^3J = 7.1 Hz, 1H, $-CH_2-CH_3$), 3.93 (dd, $^2J_{6'6}$ = 12.3 Hz, $^3J_{6'5}$ = 2.2 Hz, 1H, $H_{6'}$), 3.75 (dq, ^2J = 10.0 Hz, ^3J = 7.1 Hz, 1H, $-CH_2-CH_3$), 3.73 (dd, $^2J_{66'}$ = 12.3 Hz, $^3J_{65}$ = 6.0 Hz, 1H, H_6), 3.50 (t, $J_{32} = J_{34}$ = 9.1 Hz, 1H, H_3), 3.47 (ddd, J_{54} = 9.6 Hz, J_{56} = 6.0 Hz, $J_{56'}$ = 2.2 Hz, 1H, H_5), 3.39 (dd, J_{43} = 9.1 Hz, J_{45} = 9.6 Hz, 1H, H_4), 3.27 (dd, J_{23} = 9.1 Hz, J_{21} = 8.0 Hz, 1H, H_2), and 1.25 ppm (t, J = 7.1 Hz, 3H, $-CH_3$). ^{13}C NMR (D_2O): δ 101.8 (C_1), 75.9 (C_3), 75.8 (C_5), 73.1 (C_2), 69.6 (C_4), 66.2 (-CH_2-CH_3), 60.8 (C_6), and 14.2 ppm ($-CH_3$).

Synthesis of the Ethyl β-D-Glucopyranoside. After NMR analysis of the unknown sugar, ethyl β-D-glucopyranoside was synthesized by dissolving glucose to ethanol:water (9:1) at 50 °C over 10 min with continuous stirring. The β-glycosidase from almonds was added to the mixture, and the reaction was performed over 72 h. The mixture was chromatographed over Celite and washed with ethanol:water (9:1). The filtrate was evaporated to dryness yielding a yellow crude suspension (*15*). Ethyl β-D-glucopyranoside was isolated and purified by HPLC as described above and identified by ^1H and ^{13}C NMR analysis and also as a TMS derivative with GC-MS (electron impact, EI) and with GC-FID coinjection with sea buckthorn sample.

Statistical Analyses. The statistical analyses were performed using SPSS (SPSS 12.0.1, SPSS Inc., Chicago, IL) and Unscrambler 9.2 (Camo Process AS, Oslo, Norway). To compare different methods, principal component analysis (PCA) was used to profile samples based on the chemical parameters studied.

RESULTS AND DISCUSSION

GC Analysis. A gas chromatogram of TMS derivatives of diluted sea buckthorn juice compounds is shown in **Figure 1**. Malic and quinic acids contributed over 98% of the acids in the juice. Trace amounts of citric acid were also found. Fructose and glucose are known to be the main sugars in the berry (*6*−*8*). The three isomeric forms of fructose (α- and β-D-furanose and β-D-pyranose) and two of glucose (α- and β-D-pyranose) were the dominating sugar peaks in the chromatogram. Previous reports indicated the presence of an unknown sugarlike compound (*7*, *10*, *14*), which was identified to be ethyl β-D-glucopyranoside (**Figures 1**−**3**). The retention time of ethyl glucose in GC analysis, 19.2 min, was somewhat longer than that of AA (**Figure 1**). In HPLC analysis, the retention time of 4.3 min was noticeably shorter than that of other sugars (**Figure 2**). The EI-MS spectrum of the TMS derivative of ethyl glucose is typical for the TMS derivative of a sugar (**Figure 3**). Interpretation of the structure was not possible based on the mass spectrum only. After identification of the compound by NMR, the mass spectrum is, however, specific enough for fingerprint identification, when used together with retention time behavior. The relative amount of ethyl glucose among sugars varied greatly from 2 to 5% in most varieties to 45% in RAI.

NMR Analyses. The structure of the unknown sugar derivative was unambiguously determined as ethyl β-D-glucopyranoside by high resolution ^1H and ^{13}C NMR spectroscopy and confirmed by mass spectrometry. All peaks of the NMR spectra

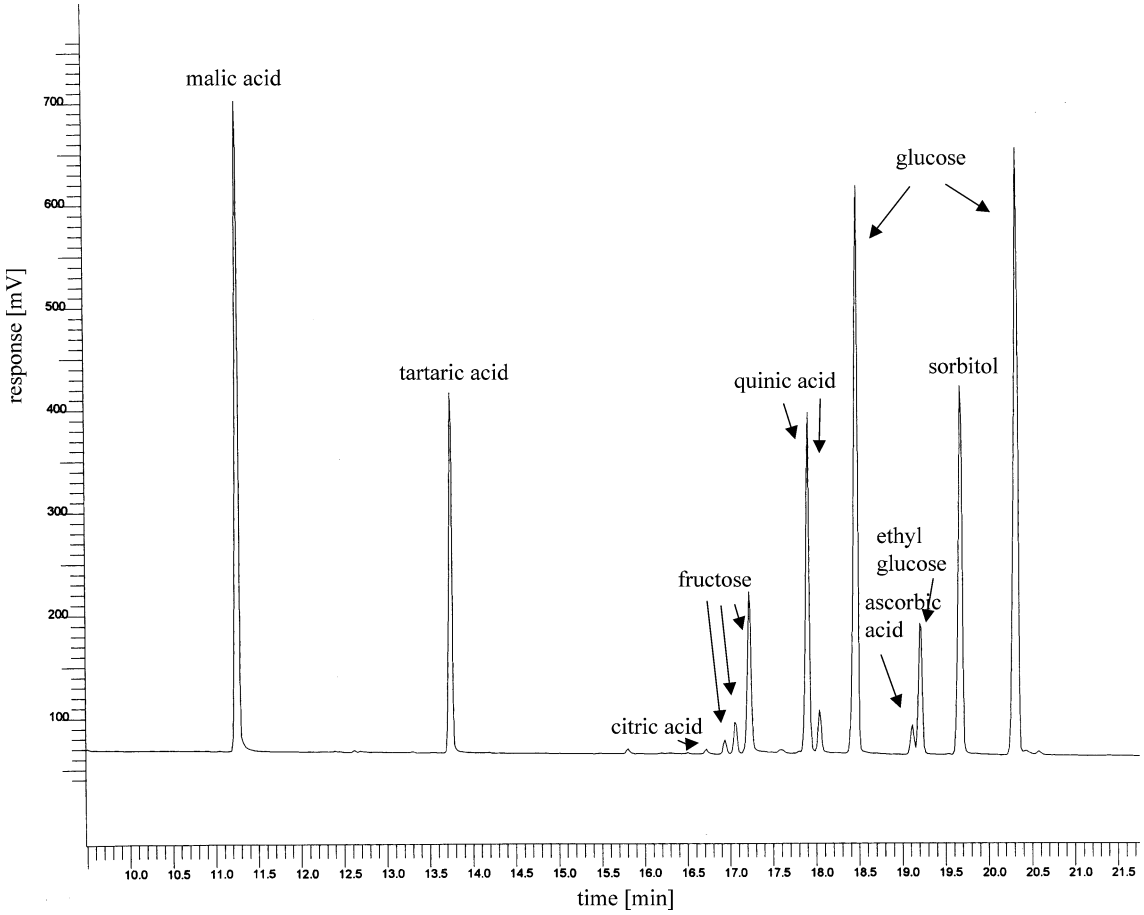

Figure 1. GC-FID chromatogram of sugars, organic acids, and AA as their TMS derivatives from sea buckthorn juice of variety TRO.

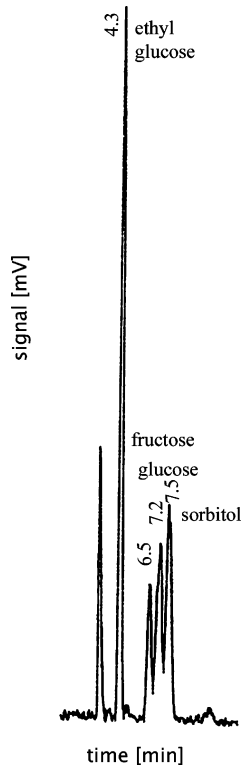

Figure 2. HPLC-ELSD chromatogram of sugar fraction in sea buckthorn variety RAI.

were explicitly assigned by the aid of 2D HHCOSY90 and HCCOW standard 2D experiments for the HH homonuclear and CH correlations, respectively. The spectra were identical to those obtained for purified ethyl glucose synthesized by the method of Goncalves and co-workers (*15*).

Reliability of the GC Analyses. The quantitative GC analyses of sugars and acids, including AA, were calculated using correction factors based on internal standards and the analysis of AA by HPLC using an external standard curve (*14*, *16*). The detector response of ethyl glucose was not defined, and it was estimated to have the same response with glucose. Reliability of the methods was tested by coefficients of variation with six subsamples of the variety AVG (**Table 1**). Both methods applied showed good repeatability for the acids and sugars in the berry. The variation in vitamin C content measured with GC method was slightly high, but it was still in the limits for reliable results.

Contents of Fruit Acids, Sugars, and Vitamin C. The contents of fruit acids, sugars, and vitamin C are presented in **Figures 4**−**6**, respectively. Data of acids (**Figure 4**) and sugars (**Figure 5**) are both from the isolated respective fractions and from the dried, nonfractionated juice. HPLC analysis of vitamin C covered both AA and DHAA, whereas the GC analysis as TMS derivatives gave the content of AA only (**Figure 6**).

The content of malic acid varied from 1.9 to 3.6 g/100 mL, and that of quinic acid varied from 0.9 to 2.5 g/100 mL juice. The citric acid content was less than 0.05 g/100 mL in all of the samples. The fructose content ranged from 0.2 to 3.5 g/100 mL, and that of glucose ranged from 1.5 to 4.2 g/100 mL. Typically, only glucose and fructose are indicated to be the sugars of sea buckthorn; the compound often reported as unknown was identified as ethyl β-D-glucopyranoside. The content of this sugar varied from 0.3 to 1.0 g/100 mL. Even

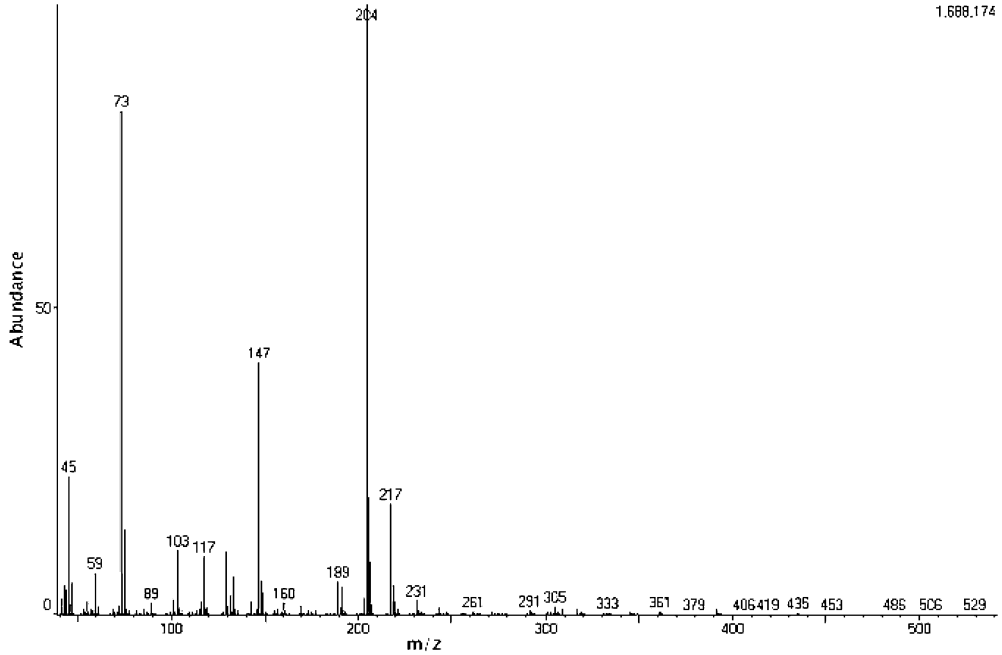

Figure 3. EI-MS spectrum of the TMS derivative of ethyl β-D-glucopyranoside.

Table 1. Coefficients of Variation (CV%) for the Methods Used in the Analysis of Sea Buckthorn Juice Variety AVG

method used	malic acid	quinic acid	fructose	glucose	ethyl β-D-glucopyranoside	vitamin C
our previously used methods ($n = 6$) (*1*, *14*)	7.2	10.4	10.8	8.0	11.7	10.4
fast method ($n = 6$)	5.7	7.4	6.9	3.6	7.8	16.3

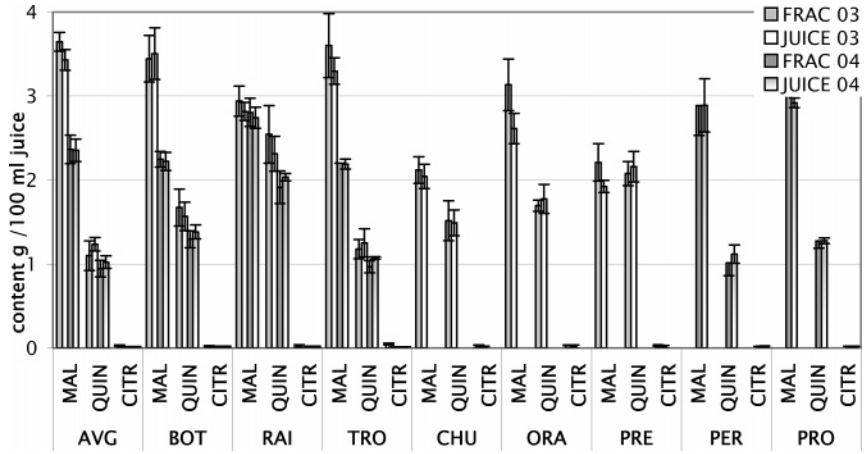

Figure 4. Fruit acids in sea buckthorn samples analyzed as TMS derivatives of fractionated juice samples (frac) and as straight juice sample (juice). Abbreviations: mal, malic acid; quin, quinic acid; and citr, citric acid. Sea buckthorn varieties are abbreviated as indicated in the Materials and Methods.

though in most samples it existed in trace amounts only, it is worth noticing its abundance in the variety RAI, even up to 45% of the total sugars. Both sugars and acids showed good repeatability within and between the two methods (**Table 1** and **Figures 4** and **5**). No statistical differences were seen between the two methods applied. The high malic acid content together with low sugars is commonly responsible for the known strong acidity of sea buckthorn berries (*14*, *17*).

The content of vitamin C in the juice samples studied was only moderate, ranging from as low as 29 to 128 mg/100 mL. In our earlier reports on wild berries of ssp. *sinensis* from high altitudes in China, concentrations even as high as 1300 mg/100 mL have been found. The lowest ones that we have recognized were in some unknown ssp. *mongolica* clones, 10 mg/100 mL. (*1*)

The principles of the two methods of vitamin C analysis applied differ significantly from each other. In the HPLC method, both AA and DHAA were quantified based on DTT treatment, whereas in the GC method the conversion was not done. In all of the samples from 2004, both procedures gave identical results concerning vitamin C, indicating the common absence of DHAA at harvesting time by this year. In samples from 2003, the GC method displayed vitamin C contents typically 10−20% less than those obtained from HPLC analysis. The two growing seasons were different. The temperature sum, i.e., the content of total sunny hours, was 1298 h during the

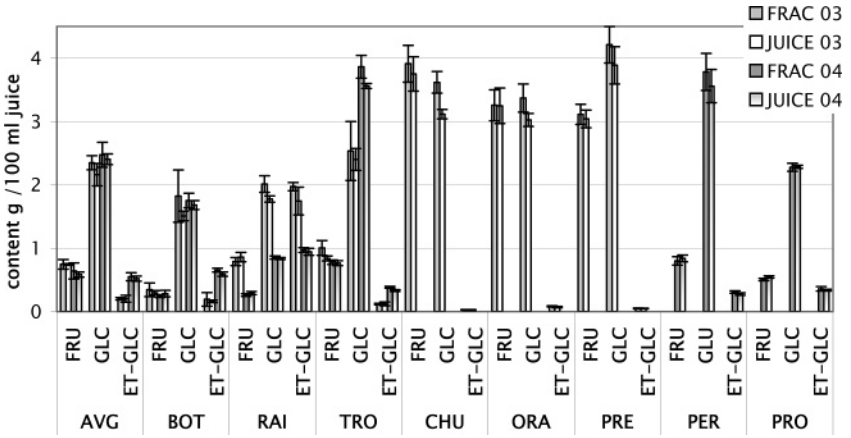

Figure 5. Sugars in sea buckthorn samples analyzed as TMS derivatives of fractionated juice samples (frac) and as straight juice sample (juice). Abbreviations: fru, fructose; glc, glucose; and et-glc, ethylglucose. Sea buckthorn varieties are abbreviated as indicated in the Materials and Methods.

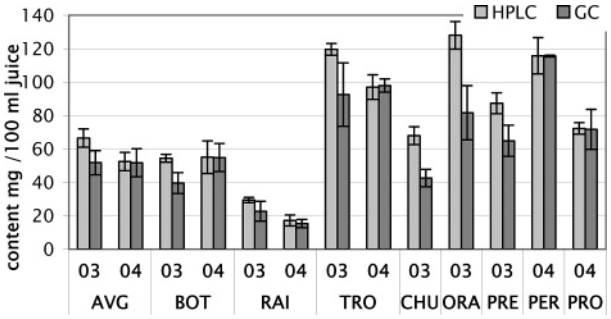

Figure 6. Vitamin C in sea buckthorn juice samples analyzed with HPLC (AA and DHAA) and as TMS derivatives (AA). Sea buckthorn varieties are abbreviated as shown in the Materials and Methods.

season 2003, whereas in 2004 it was 1065 h only, equaling on average two sunny hours more per day in 2003. This difference could increase the proportion of DHAA in 2003, because of oxidative stress induced by the abundant sunshine.

Tri-Sil reagent used in the GC method does not silylate the oxo groups of DHAA, and the derivative will not be volatile enough for GC analysis. When DHAA in juice was converted to AA with DTT and the sample preparation was performed as previously described, only 60% of DHAA was recognized according to the GC results. Thus, analysis of DHAA by converting it to AA was questionable for accurate quantitative results and the method needs more thorough optimization.

Comparison of the Different Methods. PCA is applicable when all of the variables collectively characterize each, and all, of the samples. In this study, PCA is used to see if the samples are characterized differently due to the method of analysis (**Figure 7**) and in order to get an overview of the capability of different methods to detect the differences between the samples. For example, the closer the corresponding samples between the two methods lay on the plot, the more similarly the methods describe the samples. Respectively, the farther the samples lay from each other, the more different they are with respect to the measured parameters. The first two principal components (PCs) explained 74% of the variance of the data. The first PC explains the differences in sugar content. There is a strong positive correlation between fructose and glucose contents (Pearson's correlation = 0.650, $p < 0.01$), as well as between glucose and AA (Pearson's correlation = 0.710, $p < 0.01$). On the other hand, the content of ethyl glucose has a strong negative

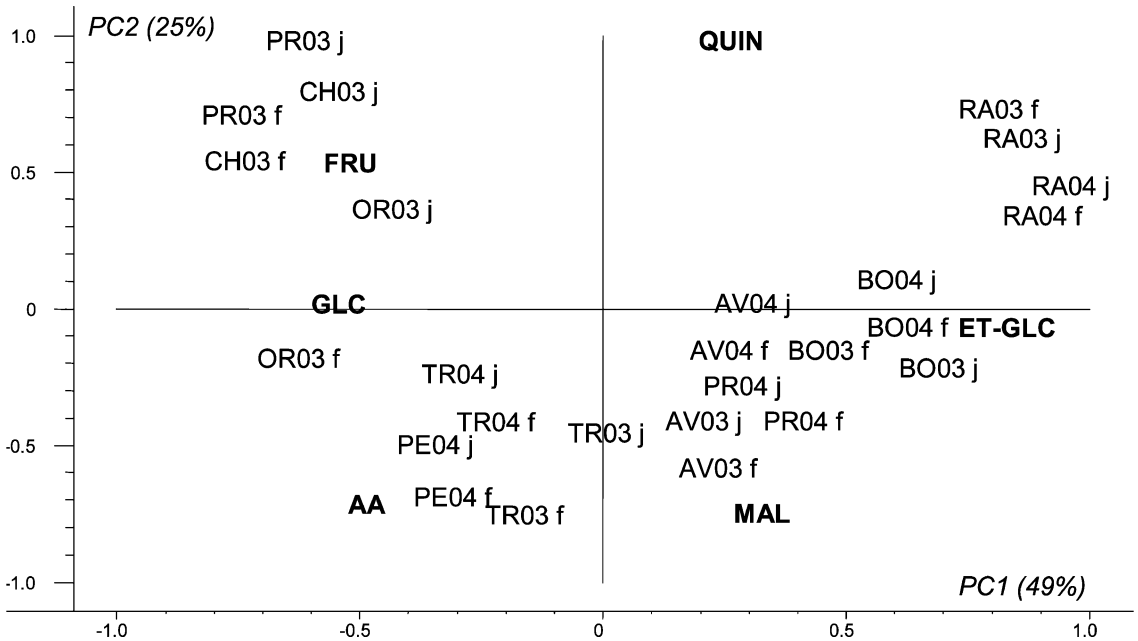

Figure 7. Differences in PCA between sea buckthorn varieties and methods used. MAL, malic acid; QUIN, quinic acid; FRU, fructose; GLC, glucose; f, fractionation or HPLC in vitamin C; and j, juice.

correlation with glucose (Pearson's correlation = −0.510, $p < 0.01$) and AA (Pearson's correlation = −0.647, $p < 0.01$) contents. The second PC explains the differences in acid content in the samples, the samples with high malic acid content laying in the upper part of the PCA biplot. The two methods studied profiled the samples identically in 2004, which are seen as fractionated (f) and direct GC measured (j) samples next to each other in the plot. On the other hand, different samples are grouped around the plot showing the overall difference between the samples. In 2003, the difference in vitamin C content moved the samples analyzed by HPLC (f) toward AA attributes as the contents measured by HPLC were higher.

On the basis of the results of this study, the direct GC analysis of sugars and acids of sea buckthorn juice as TMS esters and ethers is a reliable and repeatable method. It is easy to apply, and it is time-saving as there is no need for complex sample preparation steps. As the variation in sea buckthorn berries in a bush is limited, large sample amounts are not usually required to perform representative analysis. The direct GC method is suitable also in breeding operations, where often a large number of samples need to be screened and typically only small amounts of sample are available.

ABBREVIATIONS USED

AA, ascorbic acid; DTT, dithiothreitol; DHAA, dehydroascorbic acid; ssp., subspecies; PC, principal component; PCA, principal component analysis; TMS, trimethylsilyl.

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LITERATURE CITED

(1) Kallio, H.; Yang, B. R.; Peippo, P. Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. *J. Agric. Food Chem.* **2002**, *50*, 6136−6142.

(2) Yang, B. Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries. Ph.D. Thesis, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland, 2001.

(3) Beveridge, T.; Li, T.; Oomah, B.; Smith, A. Sea buckthorn products: Manufacture and composition. *J. Agric. Food Chem.* **1999**, *47* (7), 3480−3488.

(4) Rösch, D.; Bergman, M.; Knorr, D.; Kroh, L. W. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agric. Food Chem.* **2003**, *51*, 4233−4239.

(5) Yang, B.; Kallio, H. Lipophilic components of sea buckthorn (*Hippophaë rhamnoides* L.) seeds and berries. In *Seabuckthorn (Hippophaë L.): A Multipurpose Wonder Plant*; Singh, V., Ed.; Daya Publishing House: New Delhi, India, 2005; Vol. 2., pp 70−97.

(6) Ma, Z.; Cui, Y.; Feng, G. Studies on the fruit character and biochemical compositions of some forms within Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis)* in Shanxi, China. *Proceedings of International Symposium on Sea Buckthorn (H. rhamnoides L.)*, Xian, China, Oct 19−23, 1989; The Secretariat of International Symposium on Sea Buckthorn: Xian, China, 1989; pp 106−112.

(7) Kallio, H.; Yang, B.; Tahvonen, R.; Hakala, M. Composition of sea buckthorn berries of various origins. *Proceedings of International Workshop on Sea Buckthorn IWS-99*, Beijing, China, Aug 29−Sept 2, 1999; International Centre for Research and Training on Sea Buckthorn: Beijing, China, 1999; pp 17−23.

(8) Zhang, W.; Yan, J.; Duo, J.; Ren, B.; Guo, J. Preliminary study of biochemical constitutions of berry of sea buckthorn growing in Shanxi Province and their changing trend. *Proceedings of International Symposium on Sea Buckthorn (H. rhamnoides L.)*, Xian, China, Oct 19−23, 1989; The Secreteriat of International Symposium on Sea Buckthorn: Xian, China, 1989; pp 96−105.

(9) Shui, G.; Leong, P. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. *J. Chromatogr. A* **2002**, *977*, 89−96.

(10) Beveridge, T.; Harrison, J.; Drover, J. Processing effects on the composition of sea buckthorn juice from *Hippophaë rhamnoides* L. cv. Indian Summer. *J. Agric. Food Chem.* **2002**, *50*, 113−116.

(11) Romero Rodriguez, M. A.; Vazquez Oderiz, M. L.; Lopez Hernandez, J.; Simal Lozano, J. Determination of vitamin C and organic acids in various fruits by HPLC. *J. Chromatogr. Sci.* **1992**, *30*, 433−437.

(12) Gökmen, V.; Acar, J. A simple HPLC method for the determination of total vitamin C in fruit juices and drinks. *Fruit Process.* **1996**, *5*, 198−201.

(13) Speek, A. J.; Schrijver, J.; Schreus, W. H. P. Fluorometric determination of total vitamin C and total isovitamin C in foodstuffs and beverages by high-performance liquid chromatography with precolumn derivatization. *J. Agric. Food Chem.* **1984**, *32*, 352−355.

(14) Tiitinen, K. M.; Hakala, M. A.; Kallio, H. P. Quality components of sea buckthorn (*Hippophaë rhamnoides*) varieties. *J. Agric. Food Chem.* **2005**, *53*, 1692−1699.

(15) Goncalves, P. M. L.; Roberts, S. M.; Wan, P. W. H. Regioselective acylation of carbohydrate derivatives using lipases leading to a facile two-step procedure for the separation of some alpha- and beta-glucopyranosides and galactopyranosides. *Tetrahedron* **2004**, *60*, 927−932.

(16) Kallio, H.; Hakala, M.; Pelkkikangas, A.-M.; Lapveteläinen, A. Sugars and acids of strawberry varieties. *Eur. Food Res. Technol.* **2000**, *212*, 81−85.

(17) Tang, X.; Kälviäinen, N.; Tuorila, H. Sensory and hedonic characteristics of juice of sea buckthorn (*Hippophaë rhamnoides* L.) origins and hybrids. *Lebensm.-Wiss. Technol.* **2001**, *34*, 102−110.

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