JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

A Novel Approach for the Quantitation of Carbohydrates in Mash, Wort, and Beer with RP-HPLC Using 1-Naphthylamine for Precolumn Derivatization

Stefan Rakete and Marcus A. Glomb*

Institute of Chemistry, Food Chemistry, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Straße 2, 06120 Halle/Saale, Germany

ABSTRACT: A novel universal method for the determination of reducing mono-, di-, and oligosaccharides in complex matrices on RP-HPLC using 1-naphthylamine for precolumn derivatization with sodium cyanoborhydride was established to study changes in the carbohydrate profile during beer brewing. Fluorescence and mass spectrometric detection enabled very sensitive analyses of beer-relevant carbohydrates. Mass spectrometry additionally allowed the identification of the molecular weight and thereby the degree of polymerization of unknown carbohydrates. Thus, carbohydrates with up to 16 glucose units were detected. Comparison demonstrated that the novel method was superior to fluorophore-assisted carbohydrate electrophoresis (FACE). The results proved the HPLC method clearly to be more powerful in regard to sensitivity and resolution. Analogous to FACE, this method was designated fluorophore-assisted carbohydrate HPLC (FAC-HPLC).

KEYWORDS: *carbohydrate analysis, beer, FACE, HPLC-FLD, reductive amination, precolumn derivatization*

INTRODUCTION

The composition of beer is very complex. Carbohydrates represent a major part of the substances occurring in bottled beer. They are associated with the nutrition, taste, and physical properties of beer. The degradation of barley malt starch and of other carbohydrate polymers by inherent enzymes during mashing leads to an enormous diversity of carbohydrates in beer. The resulting spectrum strongly depends on enzyme activity and process control.[1] Major carbohydrates are glucose, maltose, and maltotriose. In addition, noticeable amounts of higher molecular carbohydrates are found. Further insights into the qualitative and quantitative nature of nonfermentable carbohydrates during beer brewing are highly desirable, for example, due to their influence on the generation of off-flavors derived from Maillard reactions. Up to now numerous methods for the determination of carbohydrates in diverse matrices have been published. In general, the analytical approach can be divided in two groups without and with derivatization.

The analyses of carbohydrates are related to several problems due to their polar and nonchromophoric character.[2] To overcome these difficulties high-performance anion exchange chromatography (HPAEC) coupled to pulsed amperometric detection (PAD) is one of the most frequently used methods.[3] HPAEC uses high-pH eluents (e.g., 0.5 M sodium hydroxide solution) to achieve a good resolution of mono- and oligosaccharides. By definition, HPAEC-PAD provides no structural information and, thus, is limited to the availability of reference standards. Coupling to mass spectrometry is complicated due to heavy salt loads in eluents and requires, for example, online desalting.[4] Moreover, high pH can trigger artifacts by epimerization or degradation.[3] In addition, carbohydrates are not efficiently ionized by electron spray ionization.[2] Alternatively, native carbohydrates can be determined by using alkali metal adducts for direct flow injection mass spectrometry.[5] Besides the problem of potential ion source fragmentation, especially for oligomers, this method lacks chromatographic separation and cannot distinguish carbohydrate isomers, such as maltose and isomaltose.

In contrast, derivatization is a convenient alternative to overcome the above problems. Different derivatization agents are known for liquid chromatography, electrophoresis, or mass spectrometry.[6] A special form of gel electrophoresis is fluorophore-assisted carbohydrate electrophoresis (FACE).[7] A charged fluorophore tag is introduced to the carbohydrate and enables separation on polyacrylamide gels. An additional method of choice is the modification of carbohydrates with nonpolar molecules. In this case derivatization diminishes the very polar character of native carbohydrates for better separation of especially large carbohydrates in reversed phase HPLC and often improves the efficiency of UV/fluorescence detection and mass spectrometric ionization.[8,9] In the literature only a very few such methods have been published, limited to the analyses of selected mono- or disaccharides.[10−13]

In this paper, we present a novel method for the simultaneous determination of reducing mono-, di-, and oligosaccharides in mash, wort, and beer on common RP-HPLC systems coupled to fluorescence detection and mass spectrometry. 1-Naphthylamine as the labeling agent provided a sufficient separation but also a tremendous increase in fluorescence activity and sensitivity for mass spectrometry. The method was then used to monitor the change of carbohydrate profiles at different stages of the brewing process. The performance of the new method was compared to that of FACE.

Received: January 30, 2013
Revised: March 28, 2013
Accepted: March 30, 2013
Published: April 11, 2013

ACS Publications © 2013 American Chemical Society

dx.doi.org/10.1021/jf400463r | *J. Agric. Food Chem.* 2013, 61, 3828−3833

MATERIAL AND METHODS

Materials. Carbohydrate reference compounds had the highest available grade. 1-Naphthylamine and sodium cyanoborohydride were purum, and 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt monohydrate was for fluorescence analyses. Methanol was of HPLC grade. All chemicals were provided by Sigma-Aldrich (Munich, Germany), except maltotetraose (Boehringer, Mannheim, Germany).

Brewery Samples. Samples were drawn at different stages in the beer brewing process at the Hasseröder Brewery/Wernigerode, Germany. The samples were frozen immediately on site.

Sample Preparation. Frozen mash samples were defrosted and transferred to a centrifuge tube. Tubes were centrifuged for 5 min at room temperature. The supernatant was filtered through a folded paper filter. Wort and beer samples were defrosted and filtered directly. Mash and wort filtrates were diluted 10-fold to ensure excess of derivatization agent. One hundred microliters of the samples was transferred in a threaded culture tube and dried in a Speed Vac concentrator (Savant SPD121P, Thermo Scientific, Waltham, MA, USA) prior to derivatization.

Preparation of Carbohydrate Standards. The used standards are listed in Table 3. For identification, 0.1 mM solutions were prepared for each carbohydrate. One hundred microliters was transferred in a threaded culture tube and Speed Vac dried. For quantitation, a 0.1 mM mixed standard solution containing 10 carbohydrates in equimolar concentrations was prepared. For calibration, 10−250 μL of this stock solution was transferred in a threaded culture tube and Speed Vac dried, representing 0.01−0.25 mM in relation to the samples.

Derivatization. For carbohydrate labeling prior to HPLC analyses, a 0.2 M solution of 1-naphthylamine in 15% acetic acid and DMSO (50:50, v/v) was prepared. For electrophoreses, a 0.2 M solution of 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt monohydrate in 15% acetic acid was used. Fifty microliters of the respective solution and 50 μL of a 1 M sodium cyanoborhydride solution in DMSO were added to the dried samples. The tubes were capped, vortexed vigorously, and incubated for 24 h at 40 °C.

HPLC-FLD (Fluorescence Detection) Analyses. The derivatized samples were diluted with eluent B to concentrations appropriate for detection (Table 2). Eluents were water (A) and a mixture of methanol and demineralized water (70:30, v/v; B). Heptafluoric butyric acid (0.6 mL/L) was added to both eluents as ion pair reagent. Analyses were carried out on a Waters HPLC system consisting of a Waters 600-MS pump, a Waters 470 fluorescence detector, and a Waters 712 WISP autosampler (injection volume = 10 μL; Waters Corp., Milford, MA, USA). Chromatographic separations were performed on a stainless steel column (Knauer, Eurospher 100-5 C18, 250 × 4.0 mm, Berlin, Germany) using a flow rate of 1 mL min^{-1} at 20 °C (Jetstream 2 column oven, Jasco, Groß-Umstadt, Germany). The excitation was adjusted to 318 nm and the emission to 440 nm. The separation started at 65:35 (A:B). After 75 min, B was raised to 100% in 5 min to flush the column for 15 min. Within 5 min the composition of the eluents was changed to starting conditions and equilibrated for 15 min prior to injection of the next analysis. All samples were prepared at least three times. The limit of detection (LOD) and the limit of quantification (LOQ) were determined according to DIN 32645. Recovery rates for beer samples were generated by standard addition, precisely by increasing concentrations of the reference compounds by factors of 0.5, 1, and 2 compared to original values. The recovery rates for individual derivatized carbohydrates were as follows: 1, 91%; 2, 97%; 3, 93%; 4, 97%; 5, 109%; 6, 91%; 7, 91%; 8, 94%; 9, 92%; 10, 99%.

LC-MS/MS Analyses. Sample preparation was according to FLD analysis. Analyses were performed on a Jasco HPLC system (AS2057plus and PU-2080plus). The LC system was connected directly to the probe of the mass spectrometer. The mass analyses were performed using an Applied Biosystems API 4000 quadrupole instrument (Applied Biosystems, Foster City, CA, USA) equipped with an API source using an electrospray ionization (ESI) interface. The LC system was connected directly to the probe of the mass spectrometer. Nitrogen was used as sheath and auxiliary gas. For electrospray ionization in positive mode the following specifications are used: sprayer capillary voltage, 4.5 kV; nebulizing gas flow, 60 mL/min; heating gas, 50 mL/min at 500 °C; and curtain gas, 50 mL/min. For multiple reaction monitoring (MRM), declustering potential (DP), collision energy (CE), and cell exit potential (CXP) were optimized by syringe injection of available reference standards (Table 1). For identification of unknown carbohydrates Q1MI (multiple ions) mass spectra were performed by calculating the nominal masses for the derivatized high polymeric dextrins (DP > 8).

Table 1. Mass Spectrometric Parameters for Derivatized Carbohydrates (MRM Mode)

	mass (amu)				
	Q1	Q3	DP	CE	CXP
ribose/arabinose/xylose	278.3	156.2	31	30	27
glucose	308.3	156.2	75	32	27
maltose/isomaltose	470.4	308.3	71	27	20
maltotriose	632.5	308.3	72	35	18
maltotetraose	794.5	308.3	100	45	17
maltopentaose	956.6	308.4	108	56	17
maltohexaose	1118.7	308.4	125	70	16
maltoheptaose	1280.9	308.5	93	81	17

FACE. Derivatized samples were adjusted to 1 mL with an aqueous solution of 62.5 mM tris(hydroxymethyl)aminomethane hydrochloride and 20% glycerol. Separation was carried out on an acrylamide gel (20%) with 3.3% cross-linkage. Two microliters of the sample solution was transferred to the gel. The gel buffer consisted of an aqueous solution (pH 8.5) of 89 mM tris(hydroxymethyl)-aminomethane, 89 mM boric acid, 2 mM EDTA, and 8 mM urea. Prior to analyses, 0.75 mm thick gels were prepared with the Mini-Protean Tetra Cell system (Bio-Rad, Munich, Germany). Electrophoreses were performed in buffer solution (25 mM TRIS, 129 mM glycine). Evaluation of the gels was done on a photodocumentary station (Bio-Rad) by fluorescence detection. All samples were prepared at least two times. The LOD and LOQ were determined according to DIN 32645 (Table 2).

Table 2. Validation Data of HPLC-FLD versus FACE Analysis

	FA-HPLC-FLD	FACE
LOD (μM)	1.2	110
LOQ (μM)	3.8	313
calibration (μM)	5−250	100−1500
linearity (R^2)	0.995−0.999	0.893−0.995
recovery rates (%)	91−109	nd
CV (%)	<5	up to 50

Mass Spectra of Derivatized Carbohydrate References. Mass spectra were generated from HPLC runs in full scan mode (m/z 100−2000) by injection of individual derivatized reference compounds.

Ribose: t_R 64.8 min; m/z 300.4 ([M + Na]$^+$, 27%), 278.5 (45), 260.4 (12), 242.4 (13), 170.5 (61), 168.6 (26), 156.4 (100), 144.3 (91), 143.3 (86), 129.3 (54).

Xylose: t_R 60.8 min; m/z 300.4 ([M + Na]$^+$, 25%), 278.5 (28), 260.4 (16), 242.4 (11), 170.5 (44), 168.6 (21), 156.4 (100), 144.3 (69), 143.3 (71), 129.3 (47).

Glucose: t_R 54.5 min; m/z 330.5 ([M + Na]$^+$, 28%), 308.4 (51), 290.5 (31), 272.5 (7), 170.6 (36), 168.4 (13), 156.3 (100), 144.3 (49), 143.3 (37), 129.3 (30).

Maltose: t_R 49.1 min; m/z 492.6 ([M + Na]$^+$, 19%), 470.6 (35), 452.6 (2), 308.5 (100), 290.6 (13), 156.4 (6), 144.3 (4).

Isomaltose: t_R 43.2 min; m/z 492.6 ([M + Na]$^+$, 23%), 470.6 (36), 452.6 (1), 308.5 (100), 290.6 (11), 156.4 (4), 144.3 (2).

dx.doi.org/10.1021/jf400463r | J. Agric. Food Chem. 2013, 61, 3828−3833

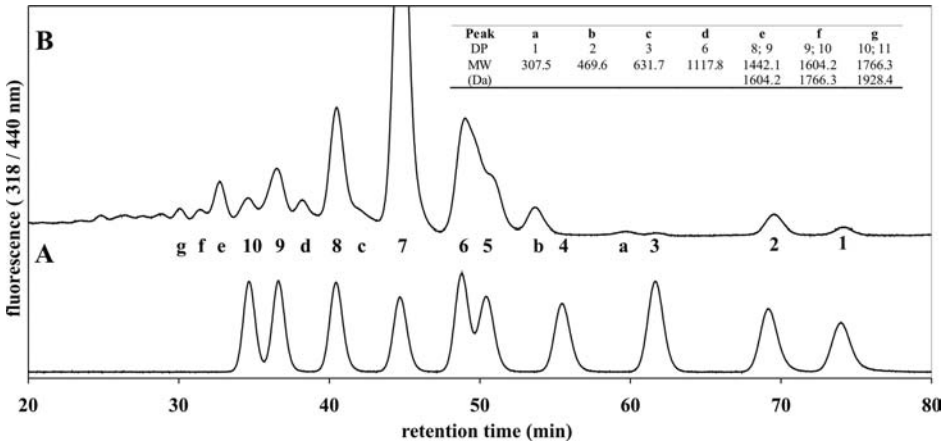

Figure 1. HPLC-FLD chromatogram of beer (B) and a 0.1 mM carbohydrate mix standard solution (A). The numbering is according to Table 3. Molecular weight (MW) and the degree of polymerization (DP) of unknown peaks (a–g) are given in the inset and were verified by mass spectrometry.

Maltotriose: t_R 44.7 min; *m/z* 670.8 ($[M + K]^+$, 1%), 654.7 (21), 632.7 (100), 614.8 (1), 470.5 (2), 308.5 (75), 290.6 (3).
Maltotetraose: t_R 39.7 min; *m/z* 832.8 ($[M + K]^+$, 2%), 816.9 (21), 794.8 (100), 470.5 (2), 308.5 (9), 144.4 (2).
Maltopentaose: t_R 36.0 min; *m/z* 994.8 ($[M + K]^+$, 3%), 978.8 (41), 956.9 (100), 632.5 (9), 470.7 (4), 308.6 (17), 290.3 (2).
Maltohexaose: t_R 32.7 min; *m/z* 1141.0 ($[M + Na]^+$, 60%), 1119.0 (100), 794.9 (51), 632.7 (43), 470.5 (26), 308.5 (89).
Maltoheptaose: t_R 30.9 min; *m/z* 1303.0 ($[M + Na]^+$, 31%), 1281.1 (73), 995.9 (14), 956.8 (64), 794.8 (100), 65246 (39), 632.7 (87), 470.6 (27), 308.5 (99).

RESULTS AND DISCUSSION

HPLC Separation. 1-Naphthylamine (NA) was chosen as derivatization agent due to its fluorescence activity and nonpolar character. Nevertheless, NA is still soluble in DMSO/acetic acid mixtures and therefore suitable for derivatization of carbohydrates in aqueous matrices. For separation, several chromatographic parameters were optimized. Due to the ion pair reagent heptafluoric butyric acid (HFBA) NA was strongly retained (after 80 min) and did not interfere with target analytes. Ion pair reagents were recently used to increase retention of basic amines in reversed phase chromatography, for example, amino acids.[14] Surprisingly, tagged carbohydrates apparently did not interact with HFBA. This was verified in the absence of HFBA using formic acid as eluent additive, which resulted in no significant changes of retention times, except for NA (around 30 min). It can be assumed that the polar carbohydrate residue prevents interaction with HFBA. This is in contrast to Roberts et al., who described a significant effect of ion pair reagents on retention times of both primary and secondary amines.[15]

The optimized parameters provided a satisfying separation for all derivatized reference compounds used (Figure 1). The size of the carbohydrate residue and thus increasing polarity correlated negatively with retention time, clearly shown by the elution order of linear dextrins (**4**, **5**–**10**). In addition, with increasing polarity HPLC resolution decreased. Nevertheless, peaks at early retention times of beer sample chromatograms indicated the occurrence of several carbohydrates (Figure 1, a–g). They remained unknown due to the absence of reference compounds. Next to molecule size, stereoconfiguration had an important impact on separation. For example, ribose (**1**) was separated from xylose and arabinose. Unfortunately, it was not possible to chromatographically separate the latter (**2**). Isomaltose (**6**) eluted earlier than maltose (**4**), indicating that the glycosidic bond position influences retention properties. Constitutional isomers can also be separated by HPAEC.[16] Thus, maltotriose and isomaltose were not completely separated. The method was validated for all available reference substances using HPLC-FLD (Table 2).

Mass Spectrometry. All LC-MS/MS experiments were performed with electron spray ionization in positive mode (ESI^+-MS). The fragmentation pattern for derivatized maltotetraose is given in Figure 2. Besides the protonated molecule [M + H]$^+$ at 794.5 Da sodium (+23 Da) and potassium (+39 Da) adducts were found. These adducts were stable and showed only minor fragmentation and were not used for mass spectrometric analysis. The modification with NA enhanced the sensitivity by a factor of approximately 100 compared to native carbohydrates (ESI^+-MS). In contrast, the deprotonated

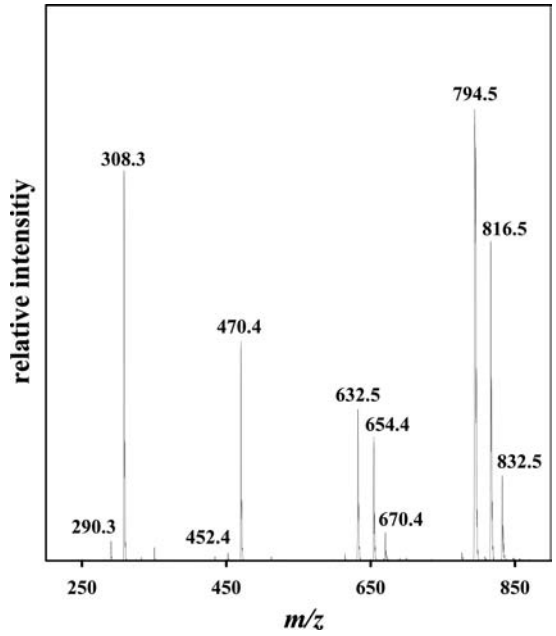

Figure 2. ESI^+ spectra of maltotetraose after derivatization with 1-naphthylamine ($[M + H]^+$ = *m/z* 794.5).

dx.doi.org/10.1021/jf400463r | J. Agric. Food Chem. 2013, 61, 3828–3833

Table 3. Concentrations (Grams per Liter) of Carbohydrates at Selected Stages of Brewing for HPLC-FLD and FACE

	start of mash		end of mash		wort		bottled beer	
carbohydrate	FA-HPLC	FACE	FA-HPLC	FACE	FA-HPLC	FACE	FA-HPLC	FACE
ribose (1)	0.03	nd	0.1	nd	0.1	nd	0.04	nd
arabinose/xylose (2)	0.08	nd	0.3	nd	0.2	nd	0.08	nd
glucose (3)	3.82	3.1	16.5	26	9.2	12.3	0.03	0.1
maltose (4)	4.45	2.8	80	40	41.3	47.1	0.07	0.2
maltotriose (5)	0.61	nd	9.3	20	7.8	32.7	0.7	1.0
isomaltose (6)	0.75	nd	0.6	nd	0.4	nd	0.9	1.4
maltotetraose (7)	0.41	nd	10.1	9.1	5.5	11.4	5.1	4.3
maltopentaose (8)	0.22	nd	3.8	5.0	2.6	6.9	2.6	6.0
maltohexaose (9)	0.16	nd	2.7	4.8	1.6	6.9	1.7	2.6
maltoheptaose (10)	0.16	nd	1.2	2.7	0.9	4.3	1.0	2.4

molecule $[M - H]^-$ (ESI^--MS) showed a thousandfold lower intensity than ESI^+-MS. Heptafluorobutyric acid adducts were also not found for ESI^--MS. Nonetheless, Saarnio et al. used ESI^--MS in combination with alkaline eluents for native carbohydrates.[17] $[M + H]^+$-MS/MS experiments revealed losses of glucose units (−162, −180, and −342 Da), which were prevalent and typical for all derivatized oligosaccharides. These fragmentations were used to develop a sensitive MRM method. However, with increasing carbohydrate residue, sensitivity decreased; for example, the LOD for maltotetraose was 10 μM compared with 1 μM for HPLC-FLD. Thus, for quantitation the HPLC-FLD was used.

According to the literature the cleavage can occur on both sides of the *O*-glycosidic bond.[18] Still, ESI^+-MS analyses did not reveal the character of the glycosidic bond or the stereo configuration; for example, maltose and isomaltose showed the same fragmentation pattern. In contrast, Zhu et al. were able to distinguish structurally similar carbohydrates via ESI^--MS.[19] Nonetheless, the degree of polymerization (DP) could be assigned to unknown peaks in beer (Figure 1, a−g) on the basis of mass spectrometry. The occurrence of oligosaccharides with up to 16 glucose units was confirmed. In contrast to HPLC-FLD analyses, mass spectrometry showed two peaks for carbohydrates up to 11 glucose units. For carbohydrates with a DP > 11 only single peaks were detected, although the peak shape suggested two substances. It is likely that these two peaks for each DP represent linear dextrins and limit dextrins of the same molecular weight, respectively.

Apparently, oligosaccharides are prone to ion source fragmentation. MRM experiments of a mixture of reference compounds showed for maltotetraose the same mass transitions that were expected for maltose; that is, attempts to reduce ion source fragmentation did not lead to complete disappearance of this phenomenon. Thus, without chromatographic separation, mass spectrometry analyses could lead to false interpretation of DPs.

Carbohydrate Profiles of Mash, Wort, and Beer Samples. With the new method the carbohydrate profiles at different stages of the brewing process were examined. Table 3 gives the concentrations of carbohydrates at the main technological steps of lager type beer brewing. Ground malt contained predominantly glucose and maltose. It also contains sucrose in concentrations up to 2% in dry matter.[20] Sucrose could not be determined by the present method due to its nonreducing character. As expected, glucose, maltose, and maltotriose were the main starch degradation products in mash, representing almost 90% of the determined carbohydrates. The concentrations of dextrins maltotetraose (DP 4) to maltoheptaose (DP 7) were almost equal at the beginning of brewing. During mashing maltotetraose showed the strongest increase within the higher molecular dextrins. This must be explained by the weak activity of α- and β-amylases to cleave low molecular weight dextrins.[21] Therefore, an increasing amount of DP 4 in relation to DP 7 in lautered mash was observed. Amylopectin contains only minor amounts of α-1,6-glycosidic linkages (every 15−30 glucose units), and the degradation leads to limit dextrins. Therefore, isomaltose as a representative of limit dextrins was found only in low concentrations of 0.6 g/L. The occurrence of additional high molecular weight limit dextrins was not definitely proved due to the absence of reference compounds. It is likely that high molecular weight limit dextrins are formed and degraded simultaneously in the brewing process. Mass spectrometric analyses revealed carbohydrates of the same molecular weight like linear dextrins and therefore gave evidence for their occurrence.

During the passage of mash to wort all carbohydrate concentrations decreased due to washing out of the draff with water. During wort boiling only minor decreases of carbohydrates were found (data not shown). After fermentation, maltotetraose was confirmed as the dominating dextrin in bottled beer. In contrast, glucose, maltose, and maltotriose were almost completely metabolized by yeast.[22] Whereas maltotriose concentrations in bottled beer are relatively high, only traces of maltose and glucose were detected. For maltose and maltotriose, transport proteins are recognized as the limiting factor for their absorption into yeast cells.[22] In contrast, dextrins with four or more glucose units are not utilized and remain in equal concentrations in bottled beer as in wort. Pentoses are also utilized by yeast, but in the presence of glucose, pentose degradation is significantly decreased.[23] Thus, pentose concentrations were only partially reduced during fermentation.

Comparison of HPLC-FLD versus FACE. Fluorophore-assisted carbohydrate electrophoresis (FACE) allowed simultaneous analyses of 10 samples in a short time. This is an advantage compared to long HPLC runs. An image of a FACE gel is given in Figure 3. For glucose to maltoheptaose sufficient resolution was achieved. Unfortunately, the pentoses could not be separated from the derivatization agent and, thus, were not quantitated. Despite the rapid separation times and the possibility of multiple samples on one gel, the preparative effort of FACE was unequally extensive compared to HPLC-FLD. Comparative quantitative data from brewing samples based on HPLC-FLD and FACE are given in Table 3. On the one hand, the results, especially for bottled beer or dextrins with DP > 3, were in comparable ranges. On the other hand,

dx.doi.org/10.1021/jf400463r | J. Agric. Food Chem. 2013, 61, 3828−3833

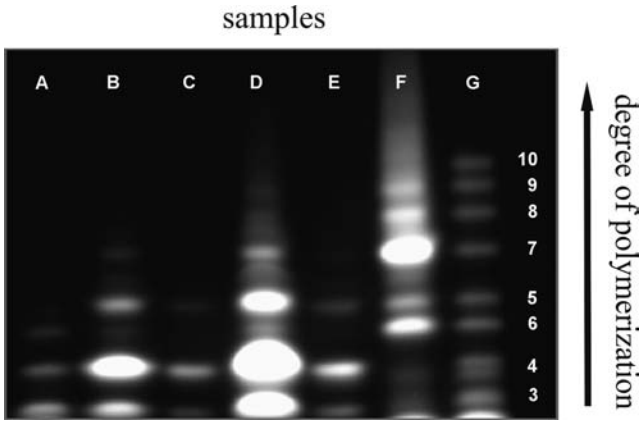

Figure 3. FACE electropherogram of selected brewing stages (A–G): (A) mash, start; (B) mash, end (1:100); (C) mash, end (1:1000); (D) wort (1:10); (E) wort (1:100); (F) beer; (G) 1 mM mixed standard solution. The numbers in parentheses indicate dilution. Carbohydrates (3–10) refer to Table 3.

clear differences in absolute values for certain carbohydrates were observed, for example, for maltose, at the end of mashing, showing twice the concentration for HPLC-FLD compared to FACE. Furthermore, the sensitivity of FACE was relatively poor. The lowest concentration detectable was 100 μM, which was 100-fold above the LOD of HPLC-FLD. Nonetheless, the sensitivity was sufficient for most samples (cf. mash/start). On the basis of the present results FACE must be evaluated as inferior to the novel HPLC-FLD method (Table 2). Variation coefficients up to 50% revealed a lack of precision of FACE, which must be explained by relative diffuse spot areas, but especially by a weak resolution, producing rather semi-quantitative results. This became most evident in the region of high polymeric carbohydrates. With HPLC-FLD, in particular with MS coupling, single peaks were detected regardless of retention time.

In conclusion, we developed a sensitive and simple method for the determination of several reducing carbohydrates during the brewing process based on HPLC separation and precolumn derivatization. Widely available reversed phase columns coupled to fluorescence detectors or mass spectrometers have clear advantages compared to previously used methods. The direct combination with mass spectrometry combines high sensitivity with information on the DP. We showed that this method provides a sufficient resolution for the present task. Furthermore, we verified the occurrence of high molecular weight carbohydrate isomers in beer. However, the novel method opens many opportunities for the determination of carbohydrates in complex matrices also found in other foods. The unique precolumn derivatization enables carbohydrate analyses without the necessity of special instrumentation. Due to the introduction of 1-naphthylamine, we call this method fluorophore-assisted carbohydrate HPLC (FAC-HPLC).

AUTHOR INFORMATION

Corresponding Author

*Phone: ++049-345-5525784. E-mail: marcus.glomb@chemie.uni-halle.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Hasseröder Brewery (Wernigerode/Germany) for the opportunity of direct access to the production facilities during sampling.

REFERENCES

(1) MacGregor, A. W.; Bazin, S. L.; Macri, L. J.; Babb, J. V. Modelling the contribution of α-amylase, β-amylase and limit dextrinase to starch degradation during mashing. *J. Cereal Sci.* **1999**, *29*, 161–169.

(2) Lamari, F. N.; Kuhn, R.; Karamanos, N. K. Derivatization of carbohydrates for chromatographic, electrophoretic and mass spectrometric structure analysis. *J. Chromatogr., B* **2003**, *793*, 15–36.

(3) Lee, Y. C. High-performance anion-exchange chromatography for carbohydrate analysis. *Anal. Biochem.* **1990**, *189*, 151–162.

(4) Bruggink, C.; Poorthuis, B. H. M.; Deelder, A. M.; Wuhrer, M. Analysis of urinary oligosaccharides in lysosomal storage disorders by capillary high-performance anion-exchange chromatography-mass spectrometry. *Anal. Bioanal. Chem.* **2012**, *403*, 1671–1683.

(5) Mauri, P.; Minoggio, M.; Simonetti, P.; Gardana, C.; Pietta, P. Analysis of saccharides in beer samples by flow injection with electrospray mass spectrometry. *Rapid Comm. Mass Spectrom.* **2002**, *16*, 743–748.

(6) Harvey, D. J. Derivatization of carbohydrates for analysis by chromatography; electrophoresis and mass spectrometry. *J. Chromatogr., B* **2011**, *879*, 1196–1225.

(7) O'Shea, M. G.; Samuel, M. S.; Konik, C. M.; Morell, M. K. Fluorophore-assisted carbohydrate electrophoresis (FACE) of oligosaccharides: efficiency of labelling and high-resolution separation. *Carbohydr. Res.* **1998**, *307*, 1–12.

(8) Ding, C. X.; Wang, L. Y.; Tian, C. L.; Li, Y. L.; Sun, Z. W.; Wang, H. L.; Suo, Y. R.; You, J. M. LC-DAD-ESI-MS characterization of carbohydrates using a new labeling reagent. *Chromatographia* **2008**, *68*, 893–902.

(9) Unterieser, I.; Mischnick, P. Labeling of oligosaccharides for quantitative mass spectrometry. *Carbohydr. Res.* **2011**, *346*, 68–75.

(10) Fischer, K.; Wacht, M.; Meyer, A. Simultaneous and sensitive HPLC determination of mono- and disaccharides, uronic acids, and amino sugars after derivatization by reductive amination. *Acta Hydrochim. Hydrobiol.* **2003**, *31*, 134–144.

(11) Fu, D. T.; Oneill, R. A. Monosaccharide composition analysis of oligosaccharides and glycoproteins by high-performance liquid-chromatography. *Anal. Biochem.* **1995**, *227*, 377–384.

(12) Yodoshi, M.; Tani, A.; Ohta, Y.; Suzuki, S. Optimized conditions for high-performance liquid chromatography analysis of oligosaccharides using 7-amino-4-methylcoumarin as a reductive amination reagent. *J. Chromatogr., A* **2008**, *1203*, 137–145.

(13) Vinogradov, E.; Bock, K. Structural determination of some new oligosaccharides and analysis of the branching pattern of isomaltooligosaccharides from beer. *Carbohydr. Res.* **1998**, *309*, 57–64.

(14) Piraud, M.; Vianey-Saban, C.; Petritis, K.; Elfakir, C.; Steghens, J. P.; Bouchu, D. Ion-pairing reversed-phase liquid chromatography/electrospray ionization mass spectrometric analysis of 76 underivatized amino acids of biological interest: a new tool for the diagnosis of inherited disorders of amino acid metabolism. *Rapid Comm. Mass Spectrom.* **2005**, *19*, 1587–1602.

(15) Roberts, J. M.; Diaz, A. R.; Fortin, D. T.; Friedle, J. M.; Piper, S. D. Influence of the Hofmeister series on the retention of amines in reversed-phase liquid chromatography. *Anal. Chem.* **2002**, *74*, 4927–4932.

(16) Koizumi, K.; Kubota, Y.; Tanimoto, T.; Okada, Y. High-performance anion-exchange chromatography of homogeneous D-gluco-oligosaccharides and D-gluco-polysaccharides (polymerization degree-greater-than-or-equal-to-50) with pulsed amperometric detection. *J. Chromatogr.* **1989**, *464*, 365–373.

(17) Saarnio, K.; Teinila, K.; Aurela, M.; Timonen, H.; Hillamo, R. High-performance anion-exchange chromatography-mass spectrometry method for determination of levoglucosan, mannosan, and

galactosan in atmospheric fine particulate matter. *Anal. Bioanal. Chem.* **2010**, *398*, 2253−2264.
(18) Domon, B.; Costello, C. E. A systematic nomenclature for carbohydrate fragmentations in Fab-MS MS spectra of glycoconjugates. *Glycoconjugate J.* **1988**, *5*, 397−409.
(19) Zhu, A. P.; Romero, R.; Huang, J. B.; Clark, A.; Petty, H. R. Maltooligosaccharides from JEG-3 trophoblast-like cells exhibit immunoregulatory properties. *Am. J. Reprod. Immunol.* **2011**, *65*, 54−64.
(20) Allosio-Ouarnier, N.; Quemener, B.; Bertrand, D.; Boivin, P. Application of high performance anion exchange chromatography to the study of carbohydrate changes in barley during malting. *J. Inst. Brew.* **2000**, *106*, 45−52.
(21) Narziß, L. *Abriss der Bierbrauerei*, 7th ed.; Wiley-VCH: Weinheim, Germany, 2005.
(22) Zastrow, C. R.; Hollatz, C.; de Araujo, P. S.; Stambuk, B. U. Maltotriose fermentation by *Saccharomyces cerevisiae*. *J. Ind. Microbiol. Biotechnol.* **2001**, *27*, 34−38.
(23) Subtil, T.; Boles, E. Competition between pentoses and glucose during uptake and catabolism in recombinant Saccharomyces cerevisiae. *Biotechnol. Biofuels* **2012**, *5*.