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Separation and quantification of beer carbohydrates by high-performance liquid chromatography with evaporative light scattering detection

Luciana C. Nogueira[a], Filipe Silva[b], Isabel M.P.L.V.O. Ferreira[b,*], L.C. Trugo[a]

[a] *Laboratório de Bioquímica Nutricional e de Alimentos, Instituto de Química, Universidade Federal do Rio de Janeiro, Brazil*
[b] *REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 4050-047 Porto, Portugal*

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Abstract

An HPLC method with an evaporative light scattering detector was optimized and validated for quantification of carbohydrates in beer. The chromatographic separation was achieved using a Spherisorb NH_2, 5 μm chromatographic column and gradient elution with acetonitrile/water. The determinations were performed in the linear range of 0.05–5.0 g/L for fructose, 0.05–5.0 g/L for glucose, 0.05–15.0 g/L for maltose, 0.05–10.0 g/L for maltotriose, and 0.05–5.0 g/L for maltotetraose. The detection limits were 0.005 g/L for fructose, 0.008 g/L for glucose, and 0.01 g/L for maltose, maltotriose, and maltotetraose. The reliability of the method in terms of precision and accuracy was evaluated in three beer matrices, low alcohol beer, 6% alcohol beer, and beer made with part of adjuncts (4.5% alcohol). Relative standard deviations (RSDs) ranged between 1.59 and 5.95% ($n = 10$), and recoveries ranged between 94 and 98.4%.

Keywords: Beer; Carbohydrates

1. Introduction

Beer is a fermented beverage made from malted grains (usually barley), hops, yeast, and water [1,2]. It has a complex composition, containing a vast number of compounds. Major beer components are water, ethanol and carbohydrates comprising fermentable sugars (i.e., fructose, glucose, maltose, and maltotriose) as well as glucose oligosaccharides [3]. Fermentable sugars directly contribute to the sweetness of beer, whereas carbohydrates with more than four glycosyl units can be beneficial to the perception of beer in that they contribute to body or mouthfeel [4].

Quantitative evaluation of malto-oligosaccharides provides a useful control of the complex enzymatic system in beer brewing, particularly when changes in procedure are contemplated [5]. In this respect it is important to control not only the total amount of fermentable carbohydrates formed but also the relative amounts of the different sugars.

The analysis of carbohydrates is generally carried out by high-performance liquid chromatography (HPLC), which can provide not only the qualitative analysis but also the quantitative determination [6]. The main chromatographic systems used for the separation of underivatized carbohydrates can be generalized as anion-exchange column with water containing bases or salts as the eluent [7]; cation-exchange column with water as the eluent [8]; alkyl-bonded silica gel column with water as the eluent [9] and amine-bonded silica gel column with water–acetonitrile as the eluent [8,10–14]. Of these systems, an amine-bonded silica gel column is the one mostly used.

Refractive index (RI) [8,9,12–15] measurement is the most popular detection method for carbohydrates. However, it has many disadvantages, such as lacking sensitivity, temperature and flow-rate dependent, and incompatibility with gradient elution. Evaporative light scattering detection (ELSD) [16] is widely used as a semi-universal mass detector

* Corresponding author. Tel.: +351 222078929; fax: +351 222003977.
E-mail addresses: luciana.nogueira@ig.com.br (L.C. Nogueira), isabel.ferreira@ff.up.pt (I.M.P.L.V.O. Ferreira).

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for HPLC. It is based on the detection of solute molecules by light scattering after nebulization and evaporation of the mobile phase, so it is suitable to detect the nonvolatile compounds such as lipids [17,18] and carbohydrates [8,10,11]. However, some disadvantages of this detector are described, because its response is proportional to the compound mass not being limited by their spectral feature, some authors find that quantification by ELSD may represent a problem, since it is described that response factor is linear at very low or high levels of analytes [19].

In this work, a HPLC method with an evaporative light scattering detector was optimized and validated for quantification of carbohydrates in beer.

2. Experimental

2.1. Sampling

Three different samples of pilsner type, were analyzed. The first two samples were prepared from the same wort, 100% malt, and fermented during 24 h (sample 1: low alcohol beer, sample 2: 6% alcohol beer, added with alcohol of cereals). The third sample was added with part of adjuncts (sample 3: standard pilsner, added with high maltose syrup, 4.5% alcohol).

2.2. Sample preparation

The beer samples were degassed for 15 min in an ultrasonic bath model Bandelin Sonorex RK (Bandelin, Berlin, Germany), diluted (1:2) in acetonitrile and filtered through a 25 mm organic syringe filter (0.2 μm pore size). All the samples were stored at 10 °C.

2.3. Reagents and carbohydrate standards

All reagents used were of analytical grade purity. Solvents for HPLC were filtered trough 0.22 μm NL 17 filters and degassed under vacuum for at least 15 min before use. Maltotriose, maltotetraose and fructose were supplied by Sigma (St. Louis, MO, USA), glucose was supplied by Merck (Darmstadt, Germany) and maltose was supplied by Fluka. Standard solutions were prepared in a mixture of water–acetonitrile (50:50, v/v).

2.4. Apparatus

The chromatographic analysis was carried out in an analytical HPLC unit (Jasco, Japan), equipped with a low pressure quaternary pump (PU-1580), an evaporative light scattering detector (LSD, Sedex 75, France) and a type 7125 Rheodyne injector with a 10 μL loop. A Borwin Controller Software (JMBS Developments) was also used. The column was a Spherisorb NH_2, 5 μm, 250 mm × 4.6 mm i.d.

Gradient elution was carried out with a mixture of two solvents. Solvent A consisted of acetonitrile and solvent B consisted of water. Carbohydrates were eluted increasing the proportion of solvent B from 19 to 25% over 40 min: 0–19 min, 19% B; 20–40 min, 25% B. The flow-rate was 1 mL/min. The temperature of the heated drift tube was 45 °C, the gas pressure was 3.0 bar, and gain 5.

2.5. Statistical analysis

Data are presented as the mean ± SD. The results were statistically analyzed by analysis of variance (ANOVA). Differences were considered significant for $p < 0.05$. Statistical analyses were all performed with SPSS for Windows version 11.5 (SPSS, Chicago, IL).

3. Results and discussion

3.1. Separation and quantification of carbohydrates

The optimization of the HPLC procedure was focused on the chromatographic separation as well as on the detector operational parameters. The stationary phase selected required the use of acetonitrile–water mixture for peak separation. Different gradient conditions were assayed at a flow rate of 1 mL/min, with the aim of obtain a well-resolved chromatogram.

The three instrumental parameters affecting sensitivity were: temperature of the detector, the nebulizing gas pressure and the gain. The temperature of the detector was studied in the interval 40–60 °C. A temperature of 45 °C was enough to allow complete solvent evaporation and therefore giving negligible noise. A flow-rate (pressure) of air set at 3 bar and a gain of 5 provided a good sensitivity and adequate signal-to-noise ratio. Fig. 1 shows a typical chromatogram for sep-

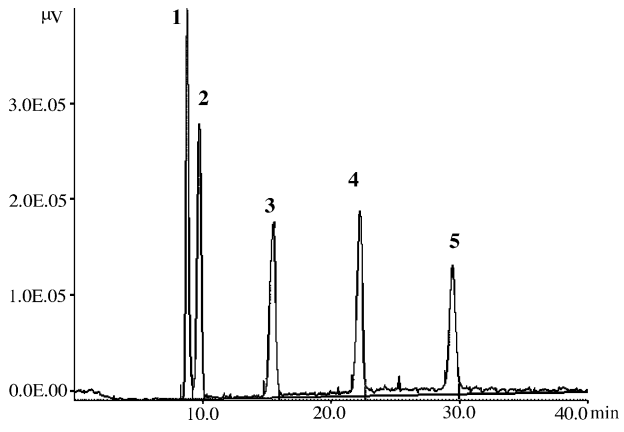

Fig. 1. Typical chromatogram for separation of five carbohydrates (chromatographic conditions described in the text): (1) fructose (t_R 8.76 min), (2) glucose (t_R 9.71 min), (3) maltose (t_R 15.45 min), (4) maltotriose (t_R 22.21 min), (5) maltotetraose (t_R 29.41 min). The concentration of standards in the mixture was 2 g/L.

Table 1
Calibration curves determined by the external standard method

Carbohydrates	Concentration range (g/L)	n[a]	Slope[b] (area counts/g)	Intercept[b] (area counts)	r[c]
Fructose	0.50–2.5	5	2728.9	−339.39	0.9988
Glucose	0.50–5.0	5	2514.9	−111.31	0.9932
Maltose	0.50–15.0	5	2324.2	−200.2	0.9997
Maltotriose	0.50–10.0	5	2827.4	−800.09	0.9967
Maltotetraose	0.50–5.0	4	1979.6	−294.95	0.9943

[a] Number of points considered for the regression. Each point represents the average of three injections of each standard solution.
[b] Standard deviation in the slope.
[c] Correlation coefficient.

aration of the five carbohydrates used in the optimization process.

The external standard method was used to calibrate the chromatographic system for carbohydrates quantification. For this purpose, sugars standard solutions with different concentrations (0.05–5.0 g/L for fructose, 0.05–5.0 g/L for glucose, 0.05–15.0 g/L for maltose, 0.05–10.0 g/L for maltotriose, and 0.05–5.0 g/L for maltotetraose) were used, according to the quantity of these compounds in the beer matrix. Each solution was analyzed in triplicate.

Calibration curves between peak areas and the mass of analyte injected were linear for the five carbohydrates following the equation $Y = aX + b$. The values of the slope, intercept and correlation coefficient are given in Table 1. These results are in good agreement with those obtained by other authors [20].

Identification of the carbohydrates in beers was performed by comparison with the retention times of the standards. The detection limit values were estimated as the concentration providing a signal three times higher than the standard deviation of the background noise. Thus, successive dilutions were performed to find the smallest concentrations that could be measured without confusion with background noise. Detection limits were 0.005 g/L for fructose, 0.008 g/L for glucose, and 0.01 g/L for maltose, maltotriose, and maltotetraose, respectively.

3.2. Validity of the method

The reliability of the method in terms of precision and accuracy was evaluated in the three beer matrices, low alcohol beer, 6% alcohol beer, and beer made with part of adjuncts (4.5% alcohol).

The precision of this method was evaluated taking into account its relative standard deviation (RSD) for 10 analyses of each beer sample. RSDs ranged between 2.78–2.89%, 3.02–3.11%, 1.59–4.77%, 2.89–4.79%, and 4.12–5.95%, respectively, for fructose, glucose, maltose, maltotriose, and maltotetraose.

Recovery studies were carried out to determine the accuracy of the method. Samples were analyzed before and after the addition of known amounts of fructose, glucose, maltose, maltotriose, and maltotetraose it was found that recoveries ranged between 94 and 98.4%. These results confirmed that although the matrix composition is complex, it does not cause interference effects. The presence of alcohol and other compounds from fermentation does not affect the accuracy, because no significant differences were found between samples 1 and 2, with similar composition except alcohol content, and between sample 3 that suffered higher fermentation and addition of adjuncts. A statistical one-way ANOVA test was used, in order to verify whether the average recoveries obtained, for each variable (fructose, glucose, maltose, maltotriose, maltotetraose) could be considered different or not, for the three samples. This turned to be possible as recovery values, for each sample, had a normal distribution (Shapiro–Wilk Test) and homocedasticity of variances (Levene Test). Results obtained for ANOVA showed that, with 95% confidence, that there were no significant differences between recovery values obtained for the three samples analyzed concerning the carbohydrates in study.

3.3. Chromatograms and results for beer samples

Figs. 2 and 3 show the typical chromatograms for samples 1 and 3, respectively.

Table 2 presents the concentrations of fructose, glucose, maltose, maltotriose, and maltotetraose in beer samples. As expected samples 1 and 2 provided similar quantitative carbohydrate profile, these samples were prepared from the same wort and suffered short fermentation, thus, great part of fructose, glucose and maltose remained in beer. Sample 3 pre-

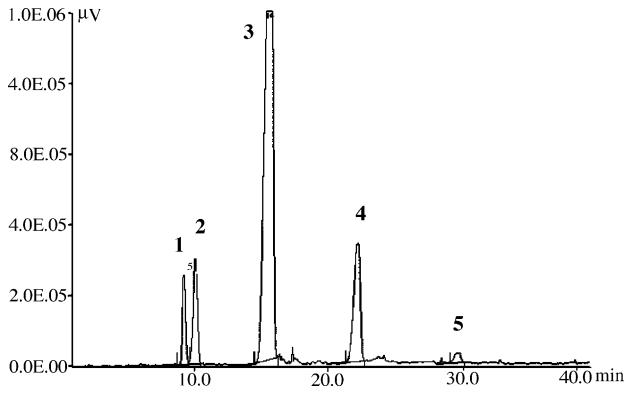

Fig. 2. Typical chromatogram for beer sample 1. The numbers correspond to the numbers in Fig. 1 with respect to peak identification.

Table 2
Results obtained in the monitoring of carbohydrates in beers[a]

Samples	Fructose	Glucose	Maltose	Maltotriose	Maltotetraose
1	2.6 ± 0.04 a	4.6 ± 0.5 a	38.5 ± 0.6 a	8.1 ± 0.2 a	1.4 ± 0.1 a
2	2.4 ± 0.07 a	4.2 ± 0.2 a	38.3 ± 0.4 a	7.4 ± 0.3 a	1.1 ± 0.1 a
3	nd b	nd b	0.35 ± 0.00 b	1.3 ± 0.1 b	2.9 ± 0.1 b

[a] Values are expressed as mean ± SD of two determinations (g of carbohydrate/L). (a, b) means columns without common superscripts are significantly different ($p < 0.05$).

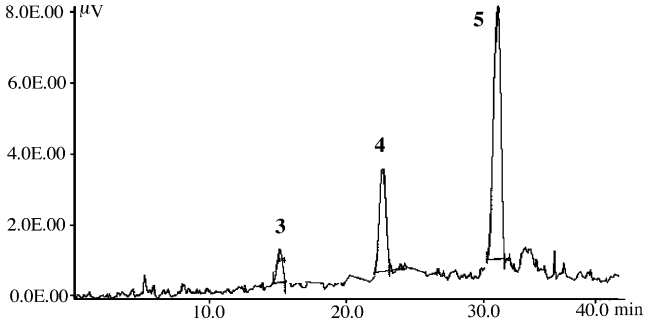

Fig. 3. Typical chromatogram for beer sample 3. The numbers correspond to the numbers in Fig. 1 with respect to peak identification.

sented significantly lower sugar content owing to an extended fermentation process.

4. Conclusions

Beer monosaccharides and malto-oligosaccharides can be separated easily using a simple gradient elution profile. No chemical manipulation is involved and no derivatization is needed. Sensitivity and stability of baseline is better than that achieved using RI detection frequently described in literature. Appropriate accuracy and precision was obtained in the analyses of beer samples with variable alcohol content and different composition.

References

[1] D. De Keukeleire, Quim. Nova 23 (2000) 108.
[2] I.F. Duarte, M. Godejohann, U. Braumann, M. Spraul, A.M. Gil, J. Agric. Food Chem. 51 (2003) 4847.
[3] V. Brandolini, E. Menziani, D. Mazzotta, P. Cabras, B. Tosi, G. Lodi, J. Food Composition Anal. 8 (1995) 336.
[4] C. Corradini, G. Canali, I. Nicoletti, Sem. Food Anal. 2 (1997) 99.
[5] A. Plaga, J. Stumpfel, H. Fiedler, Appl. Microbiol. Biotechnol. 32 (1989) 45.
[6] Y. Wei, M. Ding, J. Chromatogr. A 904 (2000) 113.
[7] N. Torto, T. Buttler, L. Gorton, G. Marko-Varga, H. Stalbrand, F. Tjerneld, Anal. Chim. Acta 313 (1995) 15.
[8] A. Clement, D. Young, C. Brechet, J. Liq. Chromatogr. 15 (1992) 805.
[9] E. Rajakyla, J. Chromatogr. 353 (1986) 1.
[10] R. Macrae, J. Dick, J. Chromatogr. 210 (1981) 138.
[11] R. Macrae, L.C. Trugo, J. Dick, Chromatographia 15 (1982) 476.
[12] Z.L. Nikolov, M.M. Meagher, P.J. Reilly, J. Chromatogr. 319 (1985) 51.
[13] I.M.P.L.V.O. Ferreira, M.A. Ferreira, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 3419.
[14] I.M.P.L.V.O. Ferreira, A.M.P. Gomes, M.A. Ferreira, Carbohydr. Polym. 37 (1998) 225.
[15] M. Boumahraz, V.Y. Davydov, A.V. Kiselev, Chromatographia 15 (1982) 751.
[16] Y. Mengerink, H.C.J.D. Man, S. van der Wal, J. Chromatogr. 552 (1991) 593.
[17] B. Marcato, G. Cecchin, J. Chromatogr. A 730 (1996) 83.
[18] C.D. Chang, D.J. Harris, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 1119.
[19] J.S. Perona, L.J.R. Barrón, V. Ruiz-Gutiérrez, J. Chromatogr. A 706 (2001) 173.
[20] Y. Wei, M.Y. Ding, J. Chromatogr. A 904 (2000) 113.