Analytical Methods

Characterisation of brewpub beer carbohydrates using high performance anion exchange chromatography coupled with pulsed amperometric detection

Giuseppe Arfelli [a,*], Elisa Sartini [b]

[a] Università degli Studi di Teramo, Facoltà di Bioscienze e tecnologie agro-alimentari e ambientali Via C. R. Lerici 1, 64023 Mosciano S.Angelo, TE, Italy
[b] Alma Mater Studiorum – Università di Bologna, Facoltà di Agraria, Dipartimento di Scienze degli Alimenti, P.zza G. Goidanich, 60, 47023 Cesena, FC, Italy

ABSTRACT

High performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) was optimised in order to quantify mannose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose content of beer. The method allows the determination of above mentioned oligosaccharides, in a single chromatographic run, without any pre-treatment. Limit of detection and limit of quantification were suitable for beer. Accuracy and repeatability were good for the entire amount considered.

Once optimised HPAEC PAD for the specific matrix, the second goal of this research was to verify the possibility to discriminate beers, depending on their style. The carbohydrates content of brewpub commercial beers was very variable, ranging from 19.3 to 1469 mg/L (mannose), 34.5 to 2882 mg/L (maltose), 141.9 to 20731 mg/L (maltotriose), 168.5 to 7650 mg/L (maltotetraose), 20.1 to 2537 mg/L (maltopentaose), 22.9 to 3295 mg/L (maltohexaose), 8.5 to 2492 mg/L (maltoeptaose), even in the same style of beer. However, the carbohydrates content was useful, jointed with other compounds amount, to discriminate different styles of beer. As a matter of fact, principal component analysis put in evidence beer differences considering some fermentation conditions and colour.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Beer is a fermented drink and the fermentation of sugars forms alcohols and other compounds responsible for taste, flavour and nutritional quality of beer. The composition of beer includes 3.3–3.4% of carbohydrates (75–80% dextrin's (>G4), 20–30% monosaccharides and oligosaccharides (<G4) and 5–8% pentosans (Cortecero-Ramírez, Hernáinz-Bermúdez de Castro, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2003). So carbohydrates are the major non volatile compounds of beer.

The determination of various malt oligosaccharides provides a useful control of the complex enzymatic system in beer brewing.

Refractive index (RI) detector is widely used for the analysis of sugar by HPLC in different type of beverages (Calull, Mercé, & Borrull, 1992; Castellari, Sartini, Spinabelli, Riponi, & Galassi, 2001; Castellari, Versari, Spinabelli, Galassi, & Amati, 2000; López-Tamames, Puig-Deu, Teixeira, & Buxaderas, 1996) but it is not selective and it has a rather limited sensitivity, it does not permit gradient elution and it is very sensitive to changes in flow rate and temperature (Martínez Montero, Rodríguez Dodero, Guillén Sánchez, & Barroso, 2004).

HPAEC PAD was developed to separate carbohydrates and alditols in different complex matrices such as citrus juices (White & Widmer, 1990), instant coffees (Bernal, Del Nozal, Toribio, & del Alamo, 1996), wine (Bernal et al., 1996; Zhu, Zhang, & Niu, 1997; Cataldi & Nardiello, 2003), beer (Madigan, McMurrough, & Smyth, 1996; Zhu et al., 1997), tobacco (Tang, Liang, Cai, & Mou, 2007), lake water and soil extract (Jahnel, Ilieva, & Frimmel, 1998), different foods and beverages (Andersen, & Sørensen, 2000; Cataldi, Campa, & De Benedetto, 2000; Pan, Liang, Cai, & Mou, 2008). This technique allows quantifying directly non derivatized carbohydrates reaching picomole levels with minimal sample preparation and clean up (Madigan et al., 1996).

Works carried out on beer (Castellari et al., 2001) using RI detector reported a LOD for maltose 150 times higher than that obtained using HPAEC PAD (anion exchange chromatography coupled with pulsed amperometric detection) and reported by Martínez Montero et al. (2004).

For sugar determination some different electrodes can be used such as a cuprum oxide modified electrode (Huang, Pot, & Kok, 1995), a copper electrode (Luo, Luo, & Baldwin, 1993) and an Au/Ni electrode (Casella, Guascito, & Cataldi, 1999).

---

* Corresponding author. Tel.: +39 0861 266901; fax: +39 0858071509.
  E-mail addresses: garfelli@unite.it (G. Arfelli), elisa.sartini@unibo.it (E. Sartini).

Also the evaporative light scattering detectors (ELSD) is used for the detection of carbohydrates (Coquet, Veuthey, & Haerdi, 1992; Lehtonen & Hurme, 1994; Nogueira, Silva, Ferreira, & Trugo, 2005). Compared to RI detector ELSD provides better sensitivity (LOD 0.2 μg/mL) better stability of chromatographic baseline and it isn't influenced by temperature (Clement, Yong, & Brechet, 1992).

Mass spectrometry (MS) was also used as a simple and sensitive method to determine carbohydrates without chromatographic separation or derivatization. For this purpose, different beer samples were analysed by means of flow injection into electrospray ionisation MS (Mauri, Minoggio, Simonetti, Gardana, & Pietta, 2002).

As reported above, the technique HPAEC PAD is more precise, accurate and sensitive compared to other methods for the determination of sugars such as HPLC-RI. In addition, the possibility to a gradient elution increases the chance of a better resolution of chromatographic peaks in a short time, because of the matrix analysed.

The aim of this work was to improve the utilisation of anion exchange chromatography coupled with pulsed amperometric detection to determine maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose and other carbohydrates (mannose and maltose) useful to control beer production process using a technique characterised by high sensibility. Furthermore, we want to check the possibility to use oligosaccharides data to discriminate different kinds of brewpub beer.

2. Experimental

2.1. Beer Samples

To develop the analytical method, a commercial pale lager beer, purchased in a market, was used.

The developed method was used to analyse twenty six samples of brewpub beer. Eighteen bottled samples were purchased and eight samples were bottled directly in the brewpub.

Sixteen samples of pale beer, seven samples of amber beer and three samples of dark beer were analysed. Dilutions were selected for each sample analysed and ranged from 10 to 2000 times. Samples were, also, filtered through a 0.20 μm nylon filter (Millipore, Milan, Italy) and then injected.

2.2. Chemical and reagents

Sodium hydroxide 50% solution for HPLC (ACS reagent), water (ACS reagent) and carbohydrate standards (Mannose, Maltose, Maltotriose, Maltotetraose, Maltopentaose, Maltohexaose and Maltoheptaose) were purchased from Sigma–Aldrich (Milan, Italy).

2.3. Analysis of carbohydrates

2.3.1. Instrumentation

Analyses were carried out using an HPAEC–PAD Dionex (Dionex Spa Milan, Italy) equipped with a pump GP 50 Gradient pump and with an ED 50A electrochemical detector (pulsed amperometry) including a detection cell with a gold electrode and a pH-Ag/AgCl reference electrode. Sample was injected through a 7515 rheodyne injection valve (Rheodyne, Cotati, CA, US) (loop 25 μL) and column temperature was controlled at 30 °C using a LC25 chromatography oven (Dionex Spa Milan, Italy). Data were analysed using a personal computer equipped with a software Chromeleon 6.6 (Dionex S.p.A. Milan, Italy). Eluents were used under helium splashing.

A CarboPac PA 10 Analytical Column (4 × 250 mm) and a CarboPac PA 10 Guard Column (4 × 50 mm) were used (Dionex Spa Milan, Italy).

2.3.2. Compounds quantification

Standard calibration curves were obtained injecting standard solutions, as external standard, with a concentration ranging from about 0.2 mg/L to 13 mg/L.

The instrumental limits of detection (LOD S/N = 3) and quantification (LOQ S/N = 10), for every single compound, were calculated on the basis of baseline noise. Baseline noise determination was carried out considering a peak to peak measurement within 3 min selected in three different parts of the chromatograms of carbohydrate standard injection. We prefer to utilise this computation method for the determination of LOD to can compare our findings with the ones of other authors, which utilised this computation method for the determination of LOD in sugars analysis (Cai, Liu, Shi, Liang, & Mou, 2005; Panagiotopoulos, Sempéré, Lafont, & Kerhervé, 2001; Tang et al., 2007; Wei & Ding, 2000; Zhu et al., 1997).

2.3.3. Accuracy and precision

To determine the accuracy, recovery performances were calculated injecting a commercial pale beer spiked with 4 increasing amounts of pure compounds. Original amounts were evaluated analysing the commercial samples using the proposed chromatographic method. All samples were analysed in triplicate. Recovery was calculated for each compound as the percent ratio between the observed and the expected values. Intraday and interday repeatability were checked both for the retention times and peak areas of each compound analysing the same beer sample by the same operator, for five times a day and for three consecutive days.

2.4. Further analyses

Other parameters were determined on the beer samples: dry extract, pH, optical density 420 nm and ethanol according to EC (Commission Regulation (EEC) 2676/90, 1990); bitterness units, haze after chilling and turbidity according to AOAC methods (1990).

2.5. Statistical analysis

Analysis of variance (two-way ANOVA), Tukey test, and principal component analysis (PCA) were carried out using Minitab Release 13.13 software (Minitab Inc., Pine Hall, PA, USA).

Asymmetry was calculated as follows:

$$\frac{n}{(n-1)(n-2)} \sum \left(\frac{xi - \bar{x}}{s} \right)^3$$

3. Results and discussion

3.1. Method optimisation

Peak identification was made comparing retention time with those of pure standards and spiking beer samples with standard solutions.

In order to optimize the elution, several tests were carried out modifying condition previously experimented by Moreno, Olano, Santa-María, & Corzo, 1999 or by the Dionex laboratories and published in the application form number 46 (Dionex Corp. Application Note n 46). These methods did not have a good separation of the firsts chromatographic peaks and also the recoveries were not good for beer. Beer is a more complex matrix than those analysed in the previous reported methods. So, starting from the latter methods, numerous elution gradients were tried, both isocratic or not in the first elution minutes, able to resolve the initial peaks. In