FOOD
RESEARCH

Determination of simple carbohydrates in beer using SPME, on fiber derivatization and gas chromatography mass spectrometry

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Abstract

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. GC/MS has not been widely used for the analysis of carbohydrate contents in beer. The analysis of beer by using L-SPME, on fiber derivatization with bis(trimethylsilyl) trifluoroacetamide (BSTFA) and GC-MS showed the presence of some carbohydrates (D-arabinose, β-D-arabinopyranose, D-galactose, D-xylose, and D-mannose) never detected in beer. The analysis of three different types of beer showed that the presence of these carbohydrates can represent a significant part of the total amount of carbohydrates present in the beer.

1. Introduction

Beer is a fermented beverage made from malted grains, hops, yeast, and water (De Keukeleire, 2000; Duarte *et al.*, 2003). Major beer components are water, ethanol, and carbohydrates comprising fermentable sugars (i.e., fructose, maltose, and maltotriose), as well as glucose oligosaccharides (Brandolini *et al.*, 1995). 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 (Corradini *et al.*, 1997).

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 (Constant *et al.*, 1995; Wei and Ding, 2000). The carbohydrate content in beer is approximately 3-5%, while in some strong beers or malt beers it may be considerably higher (Belitz *et al.*, 2004). The carbohydrate content in beer can be an important element to estimate the impact of this beverage on human feeding (Bamforth, 2005).

The main chromatographic systems used for underivatized carbohydrates can be generalized as anion-exchange column with water containing bases or salts as the eluent (Torto *et al.*, 1995); cation-exchange column with water as the eluent (Clement *et al.*, 1992); alkyl-bonded silica gel column with water as the eluent (Rajakyla, 1986); and amine-bonded silica gel column with water-acetonitrile as the eluent (Macrae and Dick, 1981; Macrae *et al.*, 1982; Nikolov *et al.*, 1985; Clement *et al.*, 1992; Ferreira and Ferreira, 1997; Ferreira *et al.*, 1998).

In the brewing industry, specific chromatographic methods have been developed in order to evaluate these compounds (Coquet *et al.*, 1994; Lehtonen and Hurme, 1994; McLinn *et al.,* 1994; Madigan *et al.*, 1996; Castellari *et al.*, 2001; Cortacero-Ramirez *et al.*, 2004; Nogueira *et al.*, 2005).

Solid phase microextraction (SPME) has shown to be a powerful instrument for the determination of volatile organic compounds (VOCs) (Pawliszyn, 1997). SPME is an extraction method involving adsorption of analytes on a solid phase deposited on a silica fiber. The extraction of the components is achieved either by immersing the fiber into the liquid to be analyzed (L-SPME), or by simple contact with its headspace under static conditions (SHS-SPME). In the past, we reported several applications of SPME in the analysis of VOCs from truffles (Mauriello *et al.*, 2001; Mauriello *et al.*, 2004), olive oil (Bentivenga *et al.*, 2001; Bentivenga *et al.*, 2002; Bentivenga, D'Auria, Mauriello *et al.,* (2004), saffron (D'Auria, Mauriello and Rana, 2004; D'Auria *et*

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al., 2006), horseradish (D'Auria, Mauriello and Racioppi, 2004), honey (Bentivenga, D'Auria, Fedeli *et al.*, 2004), thymus (D'Auria *et al.*, 2005), oregano (D'Auria *et al.*, 2005), lavender (D'Auria *et al.*, 2005), and cheese (D'Auria *et al.*, 2006).

In this paper, we reported our results obtained using L-SPME on beer coupled with GC/MS after on-fiber derivatization of the analytes.

2. Materials and methods

SPME extractions were performed using a 60 μm carbowax-divinylbenzene fiber obtained from Supelco (Milan, Italy). The 10 mL samples were places in 20 mL vials with septa and agitation was performed using magnetic stirring. All the extractions were conducted at ambient temperature for 30 mins and were agitated using magnetic stirring. Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was obtained by Aldrich (Milan, Italy). To a 20 ml vial sealed with a septum, 100 μL of BSTFA were added. Derivatization was performed by piercing the septum with the SPME needle and exposing the fiber to the contained vapor for 60 mins at 70°C. The fiber was then introduced into the injection port of a HP6890 plus gas-chromatograph equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness). As for detector, a HP5973 mass selective detector (mass range: 15-800 amu; scan rate: 1.9 scans/s; EM voltage: 1435) was used while helium at 0.8 ml/min was used as the carrier gas. The injection port, equipped with glass insert (internal diameter 0.75 mm) was splitless at 250°C. The desorption time of 1.0 min was used. The detector was maintained at 230°C. The oven was maintained at 100°C for 5 mins, then the temperature increased until 250°C (30°C/min); then, the temperature increased until 300°C (3°C/min); finally, this temperature was maintained for 5 mins. All the analyses were performed in triplicate. The chromatograms obtained from the total ion current (TIC) were integrated without any correction for coelutions and the results were expressed in arbitrary surface unites (asu). All the peaks were identified from their mass spectra by comparison with spectra in Wiley6N and NIST98 libraries.

3. Results and discussion

The beer samples were analyzed by using L-SPME technique followed by on-fiber derivatization by using bis(trimethylsilyl) trifluoroacetamide. The adsorbed analytes were analyzed using a GS/MS apparatus (Okeyo and Snow, 1998). Figure 1 shows the typical chromatogram we obtained. We can see that we were able to identify only some components while most of the

compounds were not identified. We identified the presence of D-arabinose, β-D-arabinopyranose, D-galactose, D-xylose, and D-mannose. In Figure 2, the mass spectra registered for these compounds as trimethylsilyl derivatives was shown. The identification was obtained by comparison with both Wiley6 mass spectra library and pure samples. Furthermore, we were not able to identify the presence of fructose, maltose, maltotreose, and maltotetrose, the most important carbohydrates present in the beer (Coquet *et al.*, 1994; Lehtonen and Hurme, 1994; McLinn *et al.*, 1994; Madigan *et al.*, 1996; Castellari *et al.*, 2001; Cortacero-Ramirez *et al.*, 2004; Nogueira *et al.*, 2005). It is noteworthy that, in our knowledge, those compounds (D-arabinose, β-D-arabinopyranose, D-galactose, D-xylose, and D-mannose) were not identified in beer until now.
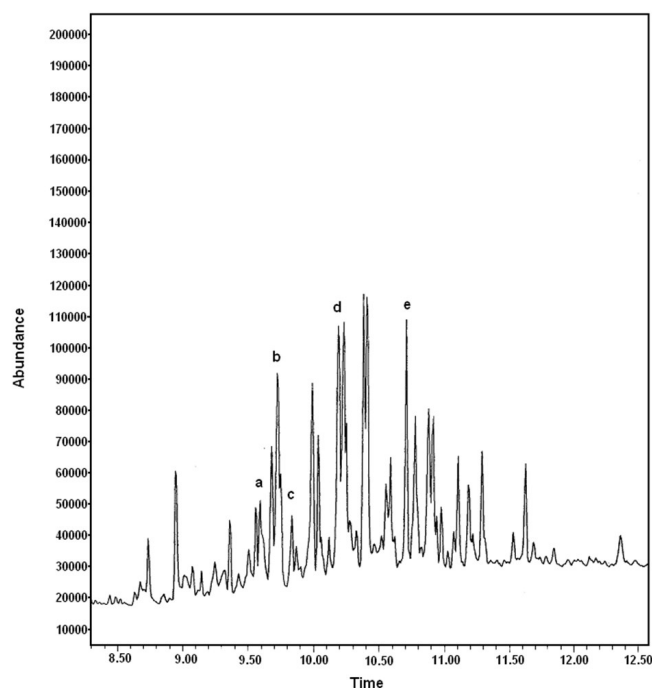

Figure 1. GC-MS analysis of a beer; a) β-D-arabinopyranose, b) D-arabinose, c) D-galactose, d) D-xylose, e) D-mannose.

Figure 3 reports the calibration curves for the five carbohydrates identified. A good calibration curve optimizing the absorption conditions was obtained. We observed that stirring of the samples needed for obtaining good results. Furthermore, we optimized also the post-derivatization procedure, where the time of exposition of the fiber and temperature were controlled.

On the basis of these results, we determined the concentration of these carbohydrates in three types of beer. We tested a lager, a pilsner, and a malt beer. The results are reported in Table 1.

Only the pilsner beer did not show the carbohydrates we analyzed. The lager beer showed 471 mg/L of these carbohydrates while the malt beer showed 202 mg/L for four carbohydrates. It did not show D-mannose.
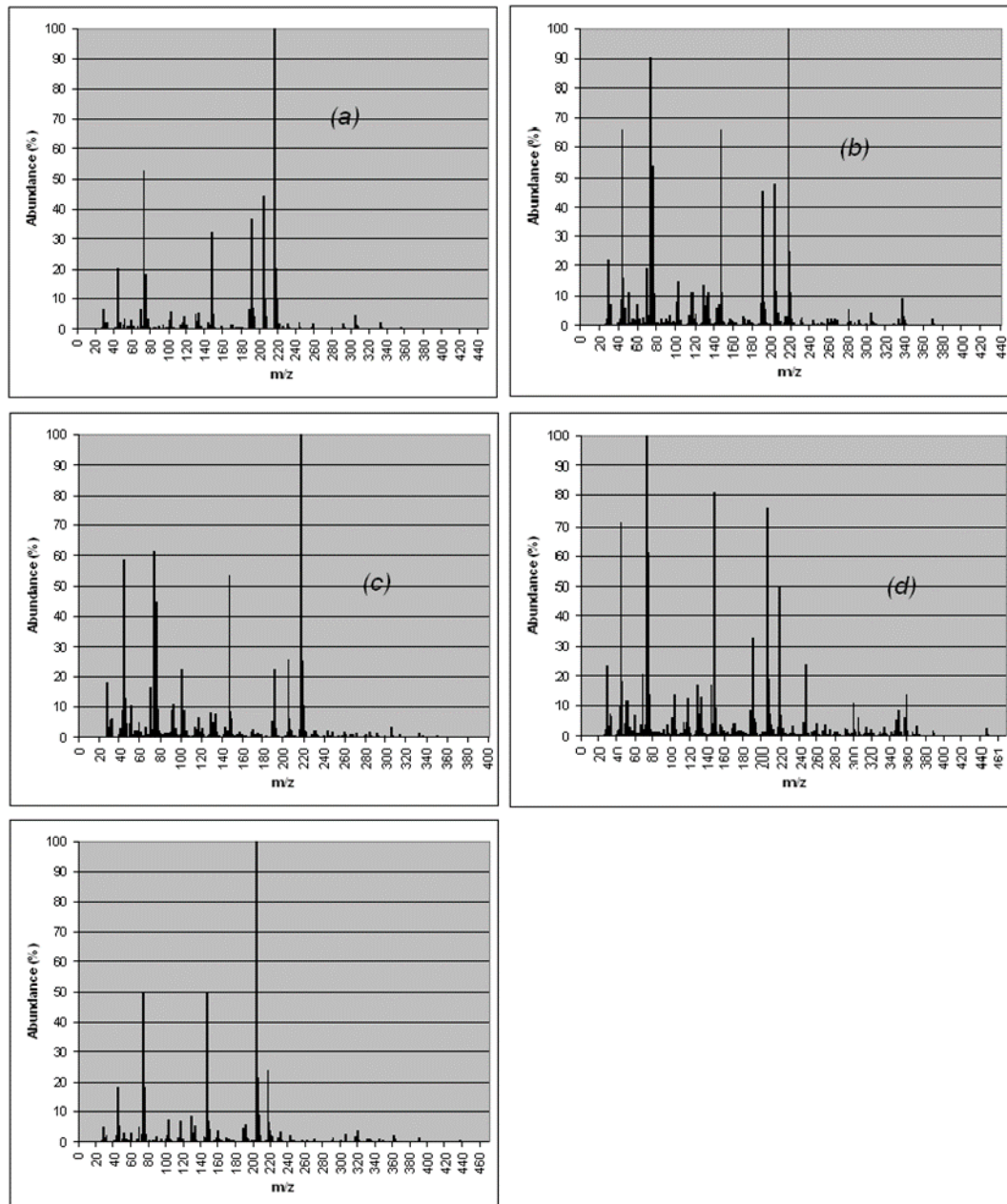
Figure 2. Mass spectra of tetrakis-O-trimethylsilyl derivatives of a) D-arabinose, b) b-D-arabinopyranose, c) D-galactose, d) D-xylose, and e) D-mannose found in beer samples.

Table 1. Carbohydrate amounts in beer.

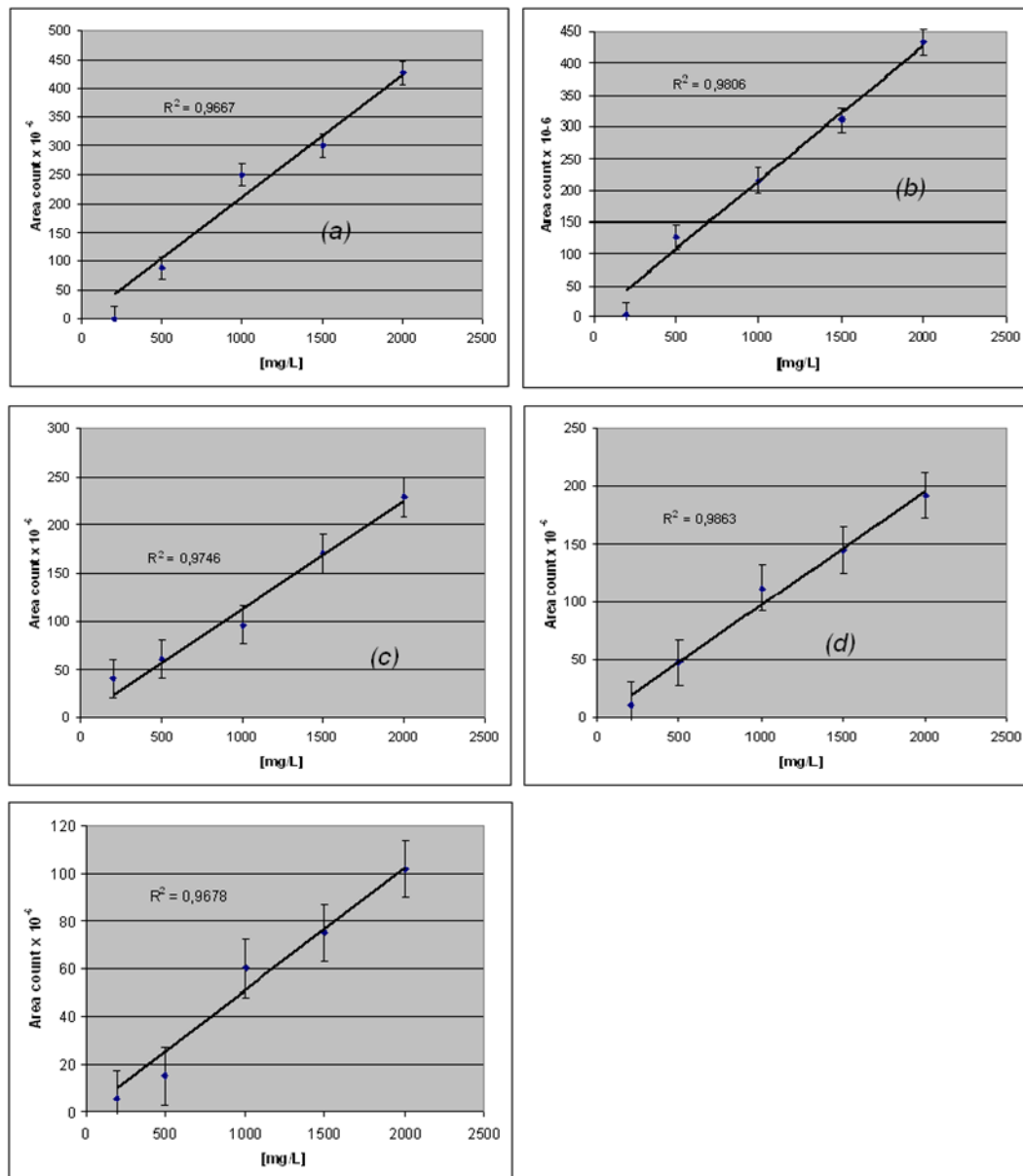| Carbohydrate	Lager (mg/L)	Malt (mg/L)	Pilsner (mg/L)
D-Arabinose	66±3	37±2	---
β-D-Arabinopyranose	31±2	13±2	---
D-Galactose	32±2	68±3	---
D-Xylose	142±4	84±3	---
D-Mannose	200±4	---	----

Figure 3. Calibration curves for a) D-arabinose, b) b-D-arabinopyranose, c) D-galactose, d) D-xylose, and e) D-mannose.

In conclusion, we have shown that SPME can be a useful method for the determination of some minor components of the beer. We identified the presence of five carbohydrates never detected before. The concentration of these carbohydrates is in the range 0.2-0.5 g/L and then they represent ca. 10% of the total carbohydrate amount in beer (Belitz *et al.*, 2004).

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