CARBOHYDRATE CONTENT OF *LAGER* AND *ALE* BEERS

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Resumo

*Um método de HPLC com detecção por light scattering foi usado na quantificação da glucose, fructose, maltose, maltoriose e maltotetraose em amostras de cerveja de diferentes tipos (*lager, ale *e sem álcool,) produzidas em 10 países diferentes. Observou-se perfil semelhante de glúcidos dentro de cada tipo de cerveja de diferentes países. As cervejas* lager *eram, em geral, mais fermentadas que as ale e continham teores mais baixos de glúcidos. As cervejas sem álcool eram pouco fermentadas e continham teores superiors de hidratos de carbono fermentáveis.*

Abstract

A HPLC method with an evaporative light scattering detector was used for quantification of glucose, fructose, maltose, maltotriose and maltotetraose in beer samples of different types (lagers, ales and alcohol free) produced in 10 different countries. Within each type of beer similar carbohydrate patterns were observed for beers from different countries. Lager beers were in general more fully fermented (attenuated) than ales, and contain less residual carbohydrate. Low alcohol beers were poorly fermented and contained higher amounts of fermentable carbohydrate.

INTRODUCTION

Monitoring of beer carbohydrate composition may therefore be an important tool for modern brewing technology. Carbohydrates have been determined in some beers by chemical and enzymatic analysis. However, liquid chromatographic methods play an important role in determining carbohydrates, especially fermentable carbohydrates. HPLC methods can provide not only the qualitative analysis but also the quantitative determination[1-5].

The main chromatographic systems used for the separation of beer carbohydrates can be generalized as anion-exchange column with water containing bases or salts as the eluent and amine-bonded silica gel column with water-acetonitrile as the eluent[6-11]. Of these systems, an amine-bonded silica gel column is the one mostly used.

Underivatized carbohydrates lacks a chromophore and in combination with an isocratic separation refractive index detection can be performed while evaporative light scattering can be used following isocratic and gradient separations. Refractive Index (RI)[12]. 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)[10-11] is widely used as a semi-universal mass detector for HPLC. No chemical manipulation was involved and no derivatization was needed.

A HPLC method with an evaporative light scattering detector, previously validated for quantification of glucose, fructose, maltose, maltotriose and maltotetraose in beer[11] was used to study quantify monosaccharides and malto-oligosaccharides in beers of different types (lagers, ales and alcohol free,) produced in 10 different countries.

EXPERIMENTAL

Sampling

Twenty-nine beers from ten different countries were analysed in quadruplicate. Beers were grouped according to alcohol content and fermentation type, sampling is summarised in Table 1.

Table 1 - Samples were grouped according to beer type and ingredients mentioned in labels.

Beer type	Number of samples	Ingredients mentioned on the labels	Countries of origin
Alcohol free	4	Water, malt, hop, unmalted cereals	Portugal Spain
	3	Water, malt, unmalted cereals, sugars, hop, antioxidant (E224)	Portugal Brazil
Lager	6	Water, malt, hop	Portugal Denmark Germany Holland Czech Republic Brazil
	12	Water, malt, unmalted cereals or glucose syrup, hop, antioxidant (E224)	Portugal Denmark Holland Belgium Spain Brazil
Ale	4	Water, malt, hop	UK Germany Belgium Irland

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 10ºC.

Reagents and carbohydrates 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 Chemicals Co. (St Louis, MO, USA), glucose was supplied by Merck (Darmstradt, Germany) and maltose was supplied by Fluka. Standard solutions were prepared in a mixture of 50% water and 50% acetonitrile (v/v).

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 x 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 1ml/min. The temperature of the heated drift tube was 45ºC, the gas pressure was 3.0 bar, and gain 5.

Statistical analysis

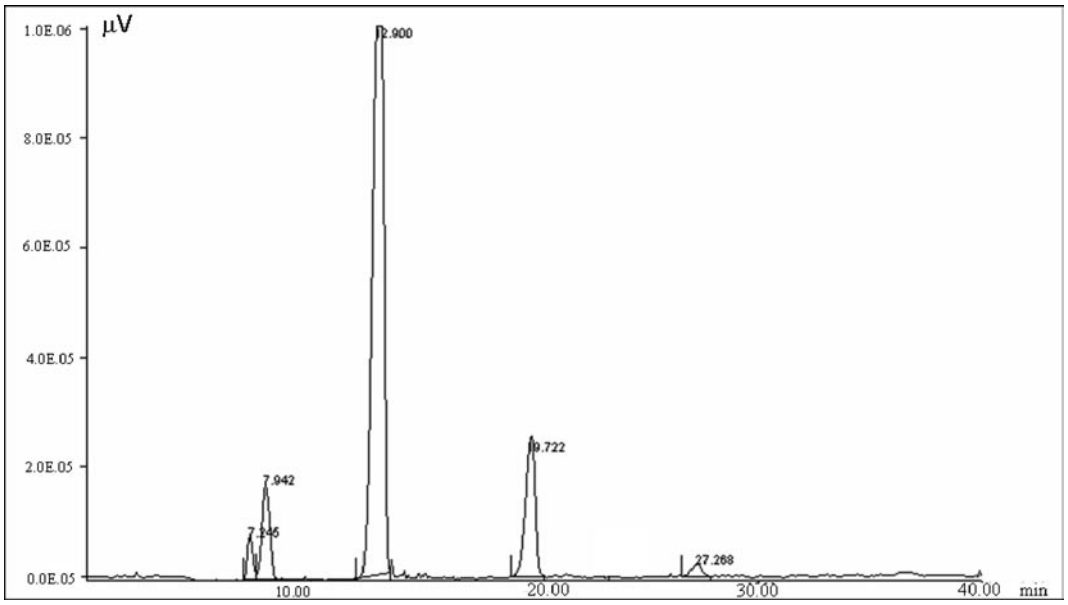
Data are presented as the mean±standard deviation. The results were statistically analysed by analysis of variance (ANOVA). Differences were considered significant for $p<0.05$. Statistical analyses were all performed with SPSS for Windows version 14 (SPSS Inc, Chicago, IL).

RESULTS AND DISCUSSION

Carbohydrates separation and quantification. 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 analysed in triplicate.

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 and were 0.005 g/L for fructose, 0.008 g/L for glucose and 0.01 g/L for maltose, maltotriose and maltotetraose. Figure 1 shows a typical chromatogram for a free alcohol beer sample.

Figure 1 - Typical chromatogram for separation of five carbohydrates in an alcohol free beer sample (chromatographic conditions described in the text): Fructose (t_R 7.245 min), Glucose (t_R 7.942 min), Maltose (t_R 12.900 min), Maltotriose (t_R 19.722min), Maltotetraose (t_R 27.268 min).



The reliability of the method in terms of precision and accuracy was verified previously in three different beer matrices, including free alcohol beer, beer 100% malt and beer with adjuncts[11].

Beer carbohydrate content. General inspection of the data was carried out for each type of beer (alcohol free, lager and ale) and each carbohydrate individually, using the minimum and maximum values observed, as well as the first quartile, the median and the third quartile. This procedure, which is suitable for skewed data, enabled the construction of Figures 2, 3 and 4, where results are displayed to favour mutual comparisons.

Figure 2 - Box and Whisker plots, obtained via non-metric univariate statistics, for monosaccharides and malto-oligosaccharides contents expressed in g L^{-1} for free alcohol beers.

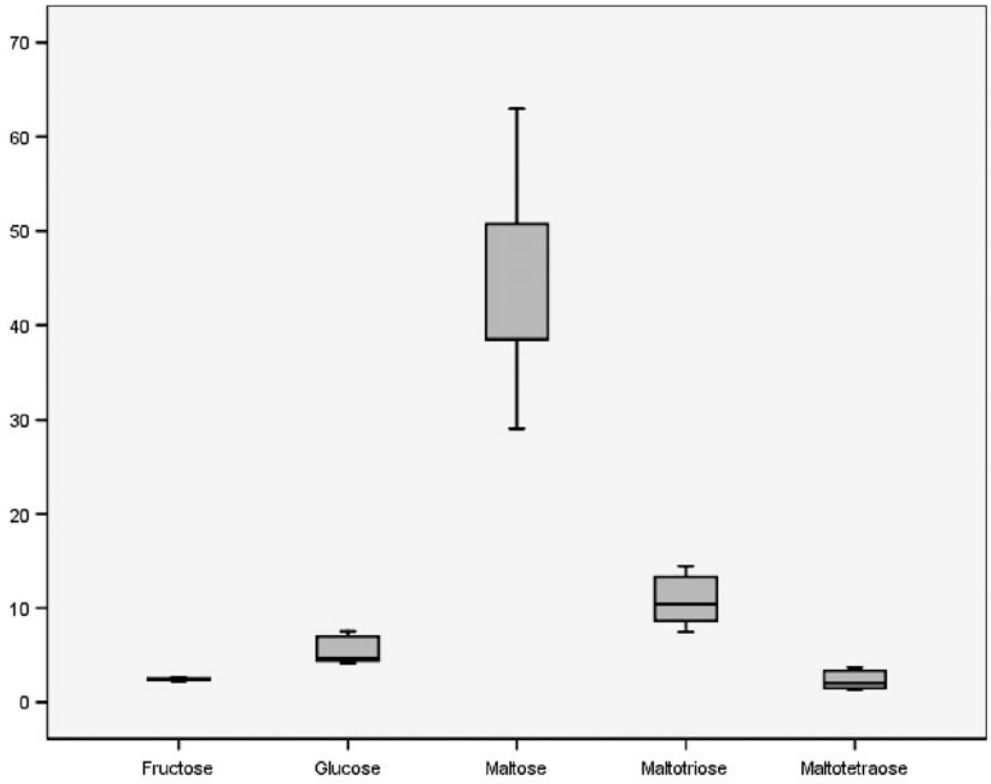


Figure 3 - Box and Whisker plots, obtained via non-metric univariate statistics, for monosaccharides and malto-oligosaccharides contents expressed in g L^{-1} for lager beers.

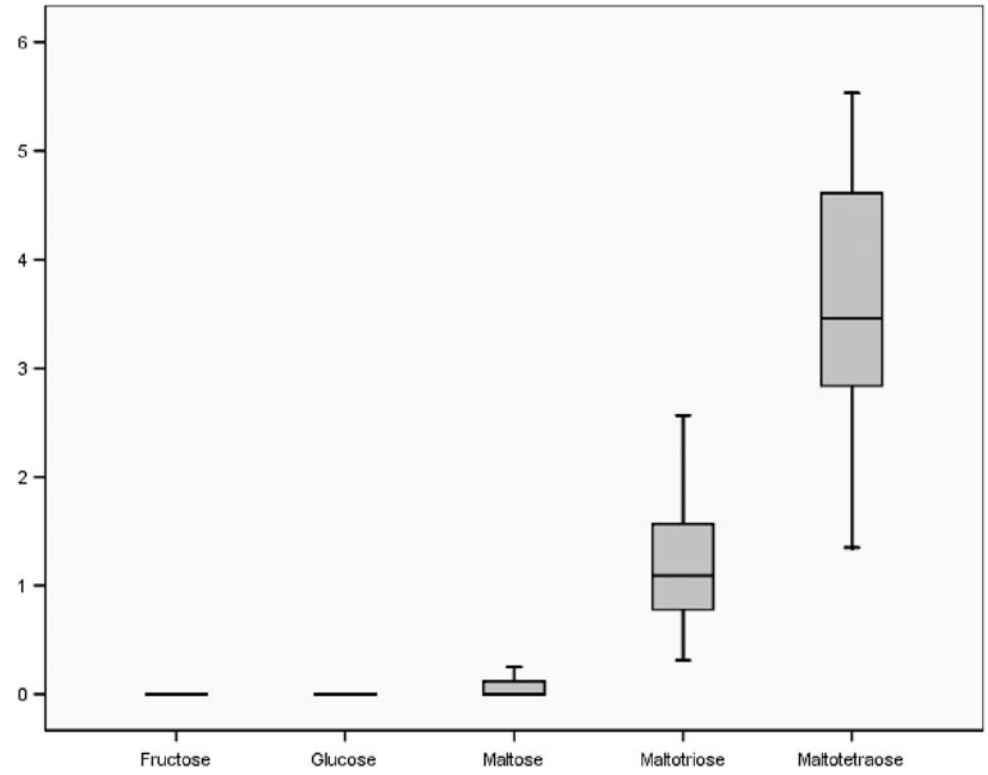


Figure 4 - Box and Whisker plots, obtained via non-metric univariate statistics, for monosaccharides and malto-oligosaccharides contents expressed in g L^{-1} for ale beers.

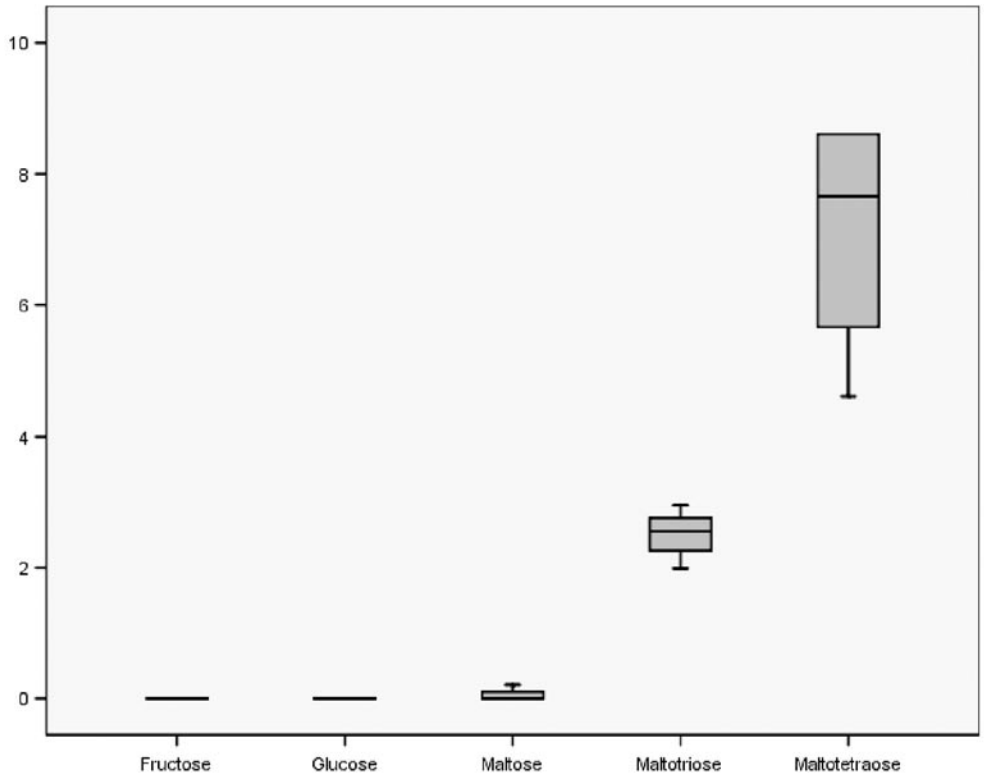


Figure 2 presents the concentration of fructose, glucose, maltose, maltotriose and maltotetraose in alcohol free beer samples. Similar qualitative profile was obtained for the six beer samples. However, within brands quantitative differences were observed for glucose, maltose and maltotriose contents. These samples suffered short fermentation, thus, great part of fructose, maltose, maltotriose and maltotetraose remained in beer.

As expected lager and ale beers presented significantly lower sugar content owing to an extended fermentation process (Figures 3 and 4). During fermentation the yeast absorbs and ferments first all the glucose and then maltose. Some yeasts, can also utilize maltotriose. Lagers were in general more fully fermented than ales and contained less residual carbohydrates.

In conclusion, strong differences between the compositions in terms of carbohydrates were observed; consequently, bivariate statistics were performed.

Bivariate statistics. The mean values of carbohydrate contents of free alcohol, lager, and ale beers are tabulated in Table 2. One-way ANOVA was used as to study whether there were significant differences between the mean scores of each carbohydrate across the different types of beer. Lager beers were grouped together because the carbohydrate spectra were similar, whether or not mash tun adjuncts were used. In general, carbohydrate contents within each type of beer were normally distributed, and homoscedaticity was also observed. The aforementioned ANOVA indicated that significant differences were noted for all carbohydrates – Tukey's post-hoc test was used to find out where such differences were located (Table 2).

Table 2 - Mean results obtained in the monitoring of carbohydrates in beers*.

Beer type	Fructose	Glucose	Maltose	Maltotriose	Maltotetraose
Alcohol free	2.46±0.13[a]	5.60±1.53[a]	44.13±12.14[a]	10.89±2.89[a]	2.39±1.03[a]
Lager	nd[b]	nd[b]	0.05 ± 0.10[b]	1.25 ± 0.71[b]	3.45 ±1.31[a]
Ale	nd[b]	nd[b]	nd[b]	2.51 ± 0.40[b]	7.11 ± 1.91[b]

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

CONCLUSIONS

Within each type of beer similar carbohydrate patterns were observed for beers from different countries. Low alcohol beers were poorly fermented and contained higher amounts of fermentable carbohydrate. Lager beers were in general more fully fermented (attenuated) than ales, and contain less residual carbohydrate. The results obtained in this work are in good agreement with others from literature.

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