90

Which polyphenols are involved in aged beer astringency? Assessment by HPLC and time-intensity method

Delphine Callemien, Meriam Bennani, Christine Counet & Sonia Collin

Université catholique de Louvain, Unité de Brasserie et des Industries Alimentaires, Croix du Sud 2, bte 7, B-1348 Louvain-la-Neuve, Belgium   (e-mail: callemien@inbr.ucl.ac.be, collin@inbr.ucl.ac.be)

SUMMARY

For a long time, we have investigated in our laboratory the evolution of beer stale flavours such as *trans*-nonenal, dimethyltrisulfide, or ß-damascenone. The present work tries to assess the impact of storage on other active compounds, most probably involved in beer astringency and bitterness. We optimised an extraction procedure for recovering beer proanthocyanidins. The resulting extracts were then concentrated and analysed by HPLC-ESI-MS/MS. The antioxidant efficiency was measured on various beers. The time intensity method was used to "quantify" the astringency. As the degree of polymerisation increases, bitterness decreases while astringency and reduction power significantly increase.

INTRODUCTION

Beer staling has been for a long time of prime concern for most brewers. Through storage, flavour appears to deteriorate greatly with time at a rate depending on beer composition and storage conditions (1,7,8,10). Improvement of beer stability requires a better knowledge of all chemicals involved.

Beer polyphenols, from malt and hop, are known to contribute to several characteristics of beer flavour, notably astringency but also bitterness, body, and fullness (4). Reduced polyphenols impart to beer a note of freshness, while their oxidized counterparts give it an "aged flavour" (3). The impact of polyphenols on flavour during ageing has been especially highlighted because of their susceptibility to oxygen in the package (10,14). Using the stable non-radioactive oxygen isotope $^{18}O_2$, Noël *et al.* (1999) have found, after natural (9 months at 20 °C) or accelerated ageing (5 days at 40 °C), ^{18}O to have been incorporated into respectively 0.61 and 6.48 % of the polyphenol molecules. Moreover, the incorporation of ^{18}O into water confirmed that high amounts of polyphenols are also oxidized into quinone derivatives (especially during natural ageing).

Oxygen and pH are two of the main factors influencing the astringency perception. Oxidation enhances the polymerisation degree of polyphenols (12), and consequently increases their astringent properties (figure 1a). Concerning pH, it is accepted that astringency is intensified at low pH (figure 1b) in model solutions, wine (9), and beer (6), although pH 4,4 is the optimum for beer colloidal instability (15). When beer at pH 3,0 is mixed with saliva, pH increases to around 4.4. The astringency intensification observed with decreasing pH is consistent with the fact that at pH 4.4, interactions between polyphenols and proline-rich proteins are strengthened (6).
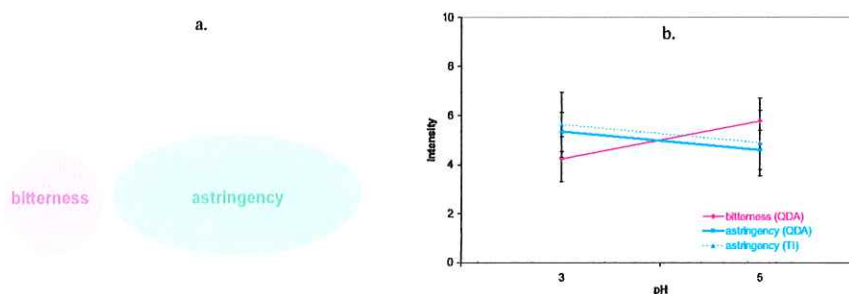

Figure 1: Bitterness and astringency properties: a. influence of the polymerisation degree (P1 to P10) on the antioxidant efficiency measured with the AAPH method (5) and on bitterness and astringency; b. mean and confidence intervals (95 %) for intensity obtained by QDA and time-intensity analysis according to pH (3 or 5) (6).

The present work tries to assess the impact of storage on proanthocyanidins, most probably involved in beer astringency and bitterness. We report the optimisation of their extraction and HPLC-MS/MS analysis. Those data are further compared to antioxidant efficiency and total flavanoids for three different beers.

MATERIALS AND METHODS

Beer procyanidin extraction procedure
The solid phase extraction with Sephadex LH-20 resin allowed us to recover 100 % catechin (standard addition method) with water/acetone (30/70, v/v) elution. The eluates were concentrated by rotary evaporation under partial vacuum (30 °C) until dryness and dissolved in 2 ml methanol.

HPLC-ESI-MS/MS
A SpectraSystem (Finnigan Mat, San Jose, CA, USA) equipped with a SCM degasser, an AS3000 autosampler, a P4000 quaternary pump, and a diode array detector UV6000LP was used. The system was controlled with the Xcalibur software version 1.2 (Finnigan Mat). Procyanidins were separated on a Silica Alltech 5-μm normal-phase column, 250 x 2.1 mm i.d. (Alltech, Deerfield, IL, USA) at 25 °C. Separations were carried out at a flow rate of 0.2 ml/min with a linear gradient from A (dichloromethane) to B (methanol) and a constant 4 % level of C (acetic acid and water, 1:1 v/v). Gradient elution was 14-24 % B, 0-20 min; 24-35 % B, 20-50 min; 35-86 % B, 50-55 min; 55-60 min isocratic and return to the initial conditions for 15 min.
MS analyses were carried out using a LCQ Duo (Finnigan Mat) multipole mass spectrometer equipped with an ESI interface. ESI (in the negative ion mode) inlet

conditions were applied as follows: capillary voltage, -6 kV; capillary temperature, 200 °C; sheath gas (N_2), 20 psi; and discharge current, 1.8 μA. Collision-induced dissociation spectra were recorded at 37 %. Data were collected on a computer (Xcalibur software).

AAPH method
The reduction power of flavonoid fractions was measured by a method developed in our laboratory by Liégeois *et al.* (2000). The oxidation of linoleic acid was induced by 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) in an aqueous dispersion in the absence or presence of antioxidant. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. A Shimadzu UV-visible 240 spectrophotometer (Antwerp, Belgium) equipped with an automatic sample positioner allowed analysis of six samples per minute. In all cases, the measurements were run in duplicate against the buffer and compared with a separate AAPH-free control to check for any spontaneous oxidation.

Determination of the polymerisation degree
Total flavanoids were quantified by means of a colorimetric assay based on the reaction of their A-rings with *p*-dimethylaminocinnamaldehyde (4). All quantifications were performed in duplicate.

RESULTS AND DISCUSSION
As few procyanidin standards exist, we have used a chocolate procyanidin extract previously obtained in our laboratory for the optimisation (2). The resulting chromatograms obtained for chocolate and beer (LH-20 extraction) are shown in figure 2.
Three types of lager beers of the same brand have been compared through storage: I. beer stabilized with silica gel, II. beer stabilized with PVPP, and III. beer stabilized with PVPP and bottled with a scavenger (figure 3).
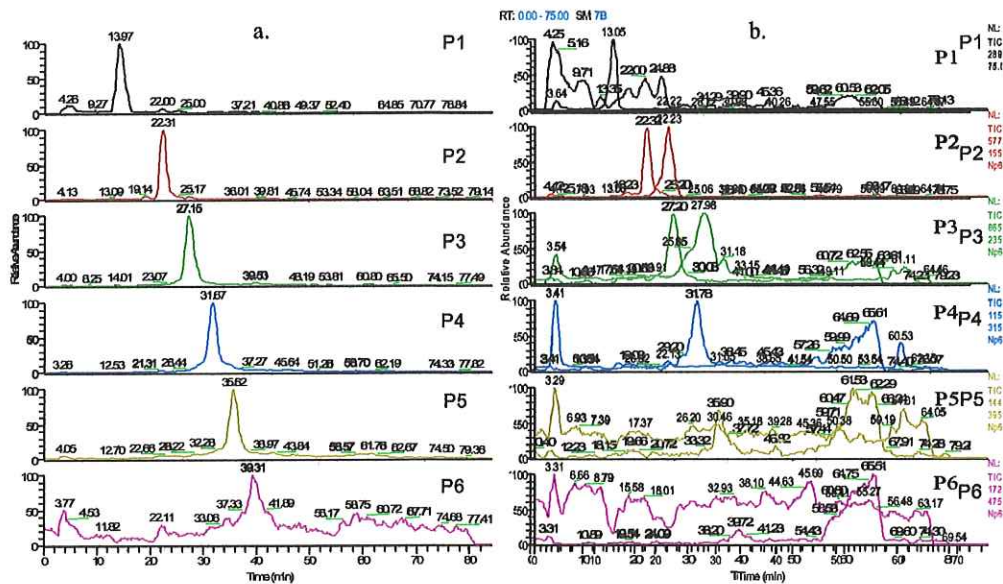
Figure 2: Comparison between procyanidin extracts from chocolate (a) and beer stabilized with silica gel (b). P1, m/z = 289.1; P2, m/z = 577.1; P3, m/z = 865.1; P4, m/z = 1153.1; P5, m/z = 1441.0; P6, m/z = 1729.0.
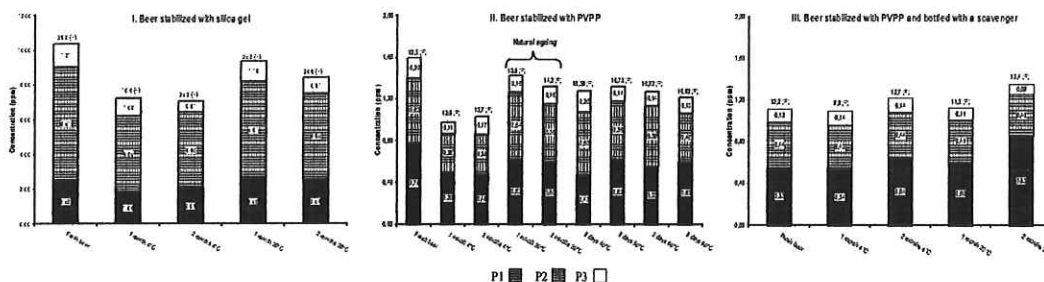

Figure 3: Procyanidin (P1, P2, and P3) concentrations (ppm) in different lager beers before and after ageing. (F) = total flavanoids (equivalent catechin, ppm) in the sample.

The AAPH method (11) has been applied on fresh and 20 °C stored samples (table 1).

Table 1: Inhibition time (Tinh) obtained with the AAPH method (7) for different lager beers before and after ageing. [a] in min for a beer diluted 400 x; [b] assay in duplicate.

Beer type	Storage	Tinh[a] (CV %)[b]
I. Stabilized with silica gel	Fresh	78.8 (3.79)
	1 month at 20 °C	40.5 (0.75)
	2 months at 20 °C	56.2 (0.79)
II. Stabilized with PVPP	Fresh	71.8 (0.55)
	1 month at 20 °C	30.2 (0.22)
	2 months at 20 °C	61.3 (1.23)
III. Stabilized with PVPP and bottled with a scavenger	Fresh	57.2 (0.41)
	1 month at 20 °C	33.3 (0.13)
	2 months at 20 °C	48.6 (0.97)

4

As expected, the beer stabilized with silica gel (I) revealed much more concentrated in procyanidins (P1-P3 = 10.3 ppm against 1.6 ppm and 1.1 ppm in II and III, respectively). Similar conclusions derive from total flavanoid measurements. A very different P2/P1 ratio can be also emphasized (2.5 in I against 0.8 for II and III).

Surprisingly, the storage at 4 °C does not allow protecting polyphenols against oxidation (except in the case of beer III with a scavenger). 27-36 % P1, 33-41 % P2, and 21-44 % P3 have been destroyed after one month.

In beers I and II, the natural ageing at 20 °C produces a slight loss of the P2 (24 and 10 % after 2 months) and P3 (31 and 19 %) fractions (also P1 in beer II). Unexpectedly, both the inhibition time and the total flavanoid content increase between one and two months.

Five days at 50 °C well mimics the natural ageing.

After 2 months, the scavenger (beer III) induces a strong increase of the P1 fraction (depolymerisation of higher fractions?).

ACKNOWLEDGEMENT

LITERATURE CITED

1. Chevance, F., Guyot-Declerck, C., Dupont, J. & Collin, S. Investigation of the ß-damascenone level in fresh and aged commercial beers, Journal of Agricultural and Food Chemistry, 2002, 50, 3818-3821.

2. Counet, C. & Collin, S., Effect of the number of flavanol units on the antioxidant activity of procyanidins fractions isolated from chocolate, Journal of Agricultural and Food Chemistry, 2003, 51, 6816-6822.

3. Dadic, M. & Belleau, G., Polyphenols and beer flavor, Proceedings of the American Society of Brewing Chemists, 1973, 4, 107-114.

4. Dalgliesh, C.E., Flavour stability, Proceedings of the European Brewery Convention Congress, Amsterdam, 1977, 623-659.

5. Delcour, J.A. & Janssens de Varebeke, D., A new colourimetric assay for flavanoids in pilsner beers, Journal of The Institute of Brewing, 1985, 91, 37-40.

6. François, N., Guyot-Declerck, C., Hug, B., Callemien, D., Govaerts, B. & Collin, S., Beer astringency assessed by time intensity and quantitative descriptive analysis: influence of pH and accelerated aging, Food Quality and Preference (submitted).

7. Gijs, L., Perpète, P., Timmermans, A. & Collin, S., 3-Methylthiopropionaldehyde as precursor of dimethyltrisulfide in aged beers, Journal of Agricultural and Food Chemistry, 2000, 48, 6196-6199.

8. Gijs, L. & Collin, S., Effect of the reducing power of a beer on dimethyltrisulfide production during aging, Journal of the American Society of Brewing Chemistry, 2002, 60, 68-70.

9. Guinard, J.-X., Pangborn, R.-M. & Lewis, M.J., Preliminary studies on acidity-astringency interactions in model solutions and solutions, Journal of the Science of Food and Agriculture, 1986, 37, 811-817.

10. Lermusieau, G., Noël, S., Liégeois, C. & Collin, S., Nonoxidative mechanism for development of trans-2-nonenal in beer, Journal of the American Society of Brewing Chemists, 1999, 57(1), 29-33.

11. Liégeois, C., Lermusieau, G. & Collin, S., Measuring antioxidant efficiency of wort, malt, and hops against the 2,2-azobis (2-amidinopropane) dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid, Journal of Agricultural and Food Chemistry, 2000, 48, 1129-1134.

12. Noble, A.C., Astringency and bitterness of flavonoid phenols. In P. Given, and D. Paredes, Chemistry of taste. Mechanisms, behavior and mimics (pp. 192-201). New York: ACS Symposium Series 2002.

13. Noël, S., Metais, N., Bonte, S., Bodart, E., Peladan, F., Dupire, S. & Collin, S., The use of oxygen 18 in appraising the impact of oxidation process during beer storage, Journal of The Institute of Brewing, 1999, 105(5), 269-274.

14. Noël, S., Liégeois, C., Lermusieau, G., Bodart, E., Badot, C. & Collin, S., Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle, Journal of Agricultural and Food Chemistry, 1999, 47(10), 4323-4326.

15. Siebert, K.J. & Chassy, A.W., An alternate mechanism for the astringent sensation of acids, Food Quality and Preference, 2003, 15, 13-18.