Journal of Chromatography, 285 (1984) 231–234
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,384

Note

Separation of malt and hop proanthocyanidins on Fractogel TSK HW-40 (S)

G. DERDELINCKX* and J. JERUMANIS

Laboratory of Brewing, Université Catholique de Louvain, Place Croix du Sud 3, 1348-Louvain-la-Neuve (Belgium)

(Received October 19th, 1983)

Because of the biological importance of polyphenols, many authors have been interested in their separation and identification. Of the currently used separation techniques, we shall only consider column chromatography in this paper.

Silica gel[1], Amberlite[2], polyamides[3], Sephadex G-25 fine[4] or superfine[5], and Sephadex LH-20[6–8] have been used in the column chromatographic separation of polyphenols. A new type of gel which allows an extensive separation has recently been described[9]: Fractogel TSK consists of porous beads with a polyvinylic structure that confers on the gel a high physical stability. Separation is based on the principles of molecular sieve and affinity chromatography. Fracotgel TSK has been successfully used for the separation of several biomolecules[10]. Dorner[11] recently compared the possibilities for different types of Fractogel and Sephadex. He noted that Fractogel TSK HW-40 (S) can separate molecular weights from 10^2 to 10^4 daltons. These exclusion limits are suitable for polyphenol separation by column chromatography.

We have developed a method for the separation of malt and hop proanthocyanidin dimers and trimers for collected fractions after chromatography on Sephadex LH-20, exemplified here by the separation on Fractogel TSK HW-40 (S) of the fraction containing the procyanidin dimer B_3 and the separation of the fraction containing prodelphinidin trimers.

EXPERIMENTAL

Malt and hop polyphenols were extracted as described by Jerumanis[12], except that for the extraction of the aqueous layer we used methyl ethyl ketone, which allows a better extraction of polymerized polyphenols as described by the same author[13]. The polyphenolic extracts are separated on a Sephadex LH-20 column (80 × 2.5 cm I.D.) with 100% methanol. The proanthocyanidin fractions obtained are chromatographed on a column of Fractogel TSK HW-40 (S) (60 × 2.2 cm I.D.), also with 100% methanol as eluent.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of a hop polyphenolic extract on a Sephadex

0021-9673/84/$03.00 © 1984 Elsevier Science Publishers B.V.

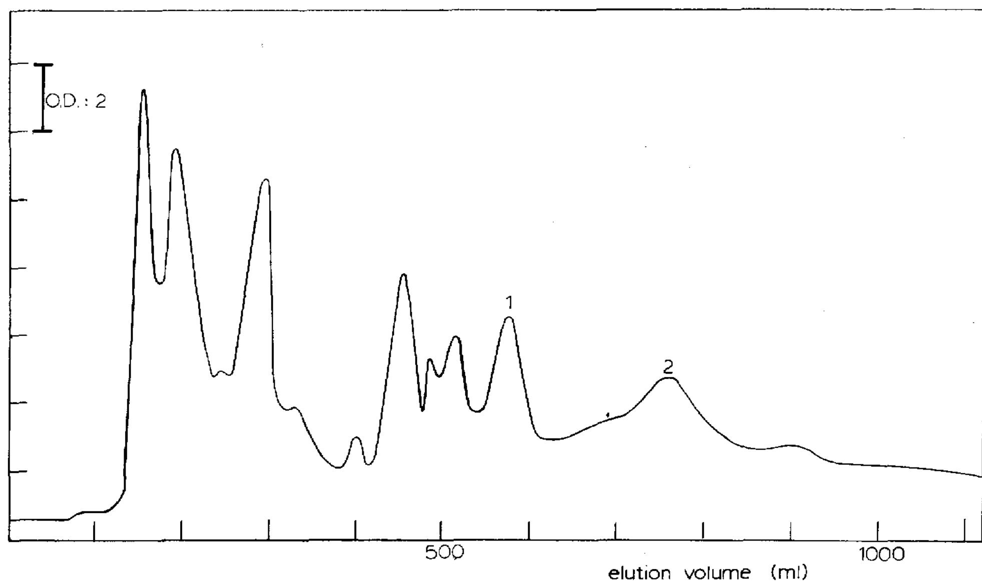

Fig. 1. Separation of an hop polyphenolic extract on Sephadex LH-20 column; eluent, methanol; flow-rate, 60 ml h^{-1}; absorbance, 280 nm. Peaks: 1 = procyanidin B_3; 2 = procyanidin C_2.

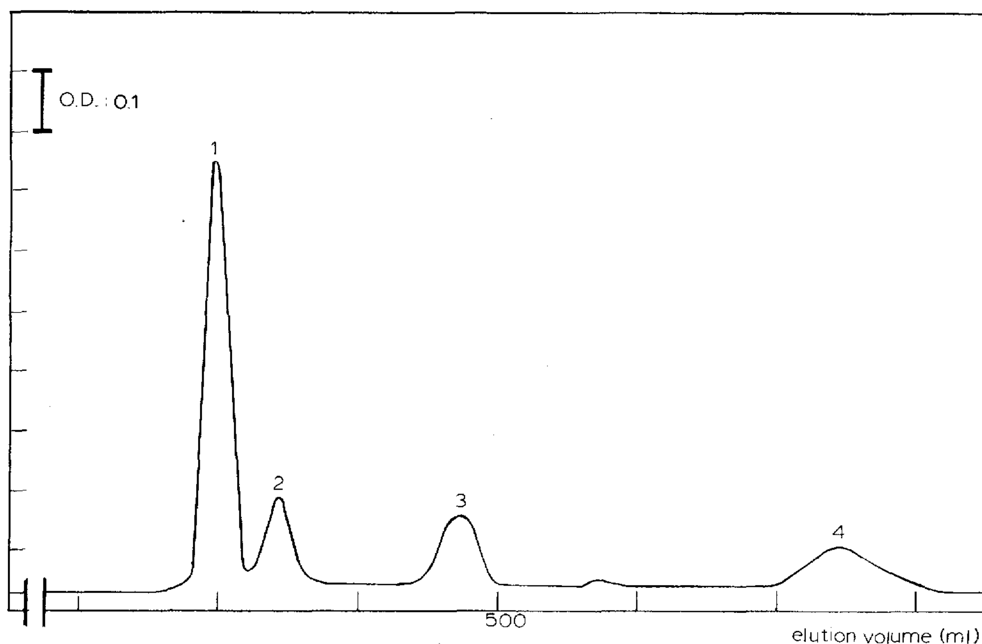

Fig. 2. Separation of peak 1 (see Fig. 1) on Fractogel TSK HW-40 (S) column; eluent, methanol; flow-rate, 28 ml h^{-1}; absorbance, 280 nm. Peaks: 1 = procyanidin (B_3) dimer ($\alpha C_{8-4},\beta C$); 2 = procyanidin (B_4) dimer ($\alpha C_{8-4},\beta EC$); 3 and 4 = procyanidin oligomers (unknown). C = (+)-Catechin; EC = (−)-epicatechin; GC = (+)-gallocatechin. We use the nomenclature suggested by Mulkay[14].

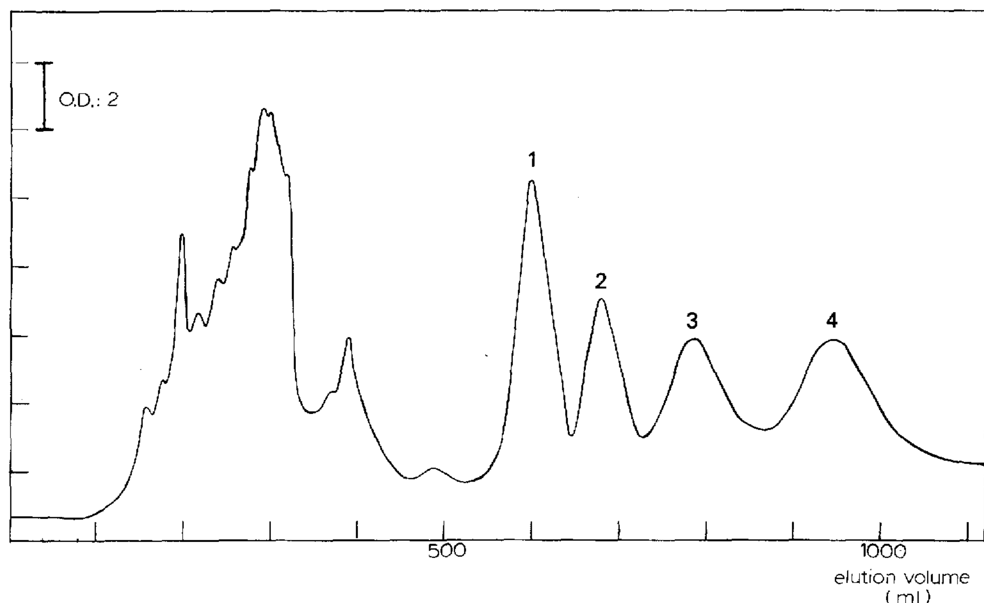

Fig. 3. Separation of a malt polyphenolic extract on Sephadex LH-20 column; eluent, methanol; flow-rate, 60 ml h^{-1}; absorbance, 280 nm. Peaks: 1 = procyanidin B_3; 2 = prodelphinidin dimer; 3 = procyanidin C_2; 4 = mixture of prodelphinidins.

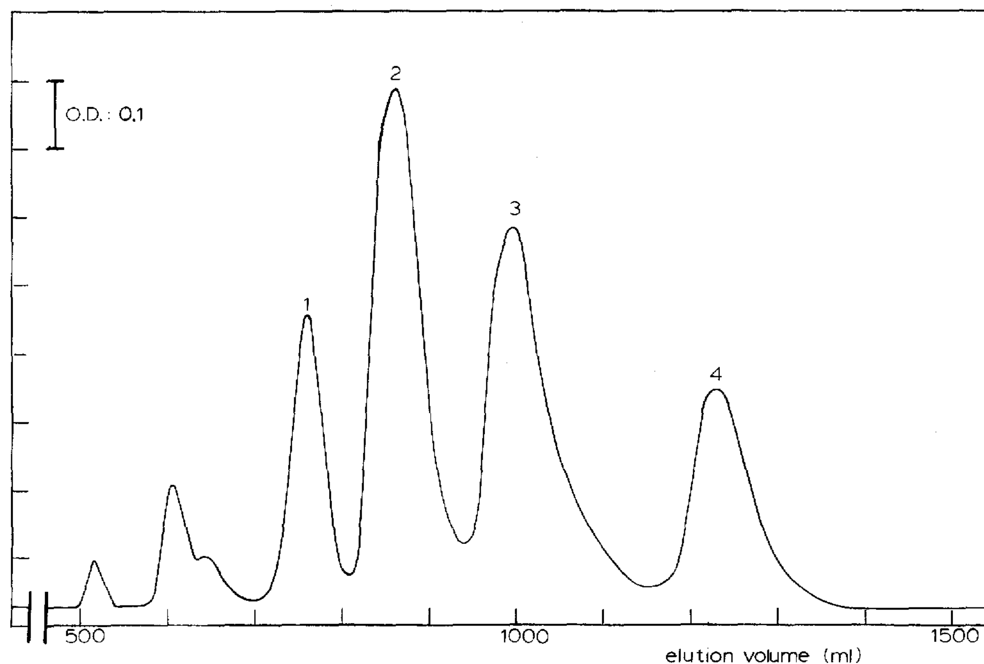

Fig. 4. Separation of peak 4 from Fig. 3 on Fractogel TSK HW-40 (S) column; eluent, methanol; flow-rate, 28 ml h^{-1}; absorbance, 280 nm. Peaks: 1 = mixture of four proanthocyanidin trimers; 2 = prodelphinidin trimer ($\alpha C_{8\text{-}4}, \beta C_{8\text{-}4}, \gamma GC$); 3 = prodelphinidin trimer ($\alpha C_{8\text{-}4}, \beta GC_{8\text{-}4}, \gamma GC$); 4 = procyanidin oligomer (unknown).

LH-20 column. The peaks labelled were rechromatographed on a Fractogel TSK HW-40 (S) column. The trace obtained for peak 1, which contains procyanidin B_3, is shown in Fig. 2. Four major peaks, corresponding to the procyanidin dimers (B_3 and B_4) and to two unknown procyanidin oligomers, were isolated.

Figs. 3 and 4 summarize the results obtained for the corresponding analysis of the polyphenolic extract of malt. As shown in Fig. 4, peak 4 of Fig. 3 also consists of four major peaks: a mixture of four proanthocyanidin trimers, two procyanidin trimers and an unknown procyanidin oligomer.

CONCLUSION

These results show that Fractogel TSK HW-40 (S) is an appropriate support for high-performance chromatography at low pressure. It has allowed us to separate and obtain the proanthocyanidins in an advanced state of purity. The unknown procyanidin oligomers are being studied in order to obtain a complete characterization.

ACKNOWLEDGEMENTS

We thank Dr. Dufour for his contribution to the use of Fractogel TSK, and the Centre of Research and Development TEPRAL (Champigneulles, France) for financial aid.

REFERENCES

1 A. E. Bradfield and M. Penney, *J. Chem. Soc.*, (1948) 2249.
2 R. Vancraenenbroeck, A. Rogirst, H. Lemaire and R. Lontie, *Bull. Soc. Chim. Belg.*, 72 (1963) 619.
3 L. Horhammer, H. Wagner and W. Leeb, *Naturwissenschaften*, 44 (1957) 513.
4 T. C. Somers, *Nature (London)*, 209 (1966) 308.
5 L. Debeer, R. Vancraenenbroeck and R. Lontie, *Arch. Int. Physiol. Biochim.*, 74 (1966) 312.
6 I. McMurrough, *J. Chromatogr.*, 218 (1981) 683.
7 J. A. Delcour, L. L. Verhulst, R. Geys, H. Delhiver and P. Dondeyne, *J. Inst. Brew.*, 87 (1981) 391.
8 P. Mulkay, R. Touillaux and J. Jerumanis, *Cerevisia*, 1 (1981) 29.
9 R. Wernicke, *Kontakte (Merck)*, 1 (1982) 3.
10 M. Gurkin and V. Patch, *Amer. Lab.*, 14 (1982) 64.
11 W. G. Dorner, *GIT Fachz. Lab.*, 27 (1983) 380.
12 J. Jerumanis, *Eur. Brew. Conv. Proc.*, (1979) 309.
13 J. Jerumanis, *Bull. Anciens Et. Brass. Louvain*, 64 (1969) 223.
14 P. Mulkay and J. Jerumanis, *Cerevisia*, 1 (1983) 29.