High performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in beer

8

Progress in Food Fermentation

VOLUME 2

Proceedings of Euro Food Chem VII
Valencia, Spain, September 20-22, 1993

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF 2-FURALDEHYDE AND 5-HYDROXYMETHYL-2-FURALDEHYDE IN BEER

F. LO COCO[1], C. VALENTINI[2], V. NOVELLI[1] and L. CECCON[3]

[1]Dipartimento di Scienze e Tecnologie Chimiche, Università di Udine, Via Cotonificio 108, 33100 UDINE (ITALY)

[2]Laboratorio Chimico delle Dogane di Venezia, Via Cà Marcello 15, 30172 MESTRE (ITALY)

[3]Dipartimento di Economia e Merceologia delle Risorse Naturali e della Produzione, Università di Trieste, Via Valerio 6, 34127 TRIESTE (ITALY)

Abstract

A method of determination of 2-furaldehyde (F) and 5-hydroxymethyl-2-furaldehyde (HMF) in beer by high performance liquid chromatography is described. The method is based on the formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds and subsequent reversed-phase separation of these derivatives. The procedure offers a high specificity and a detection limit of the order of 10^{-8} mol/l. Recoveries from beer spiked at different levels are quantitative for both analytes. Reproducibility data are presented.

1. Introduction

The occurrence of 2-furaldehyde (F) and 5-hydroxymethyl-2-furaldehyde (HMF) in several food products is an indication of quality deterioration (1-4). Both F and HMF are formed during heat processing or storage at improper temperatures (3,5-7). A close relationship between flavour changes and F content exists, while HMF can give rise to browning reactions (3,6,7). Moreover, deterioration of the flavour of beer

during distribution and storage may occur with particular reference to the role of carbonyl compounds (8).

The classical methods for the quantitative determination of F and HMF are based on colorimetric measurements (1,2,4-7,9). These methods I) are time consuming, II) make use of toxic or anyhow hazardous chemicals, III) require a strict control of both reaction time and temperature, since the instability of the colored reaction product may lead to low recoveries and wide statistical variations of the results and IV) no one of the methods is specific (1,2,4-7). In recent years, high performance liquid chromatographic (HPLC) methods have also been proposed (1-7,10-13). These methods are less time consuming, offer improved accuracy, sensitivity and specificity as compared to the colorimetric procedures and utilize less hazardous reagents (3,6,7).

In this paper a HPLC method is described that is based on formation of the 2,4-dinitrophenylhydrazones (DNPH-ones) of carbonyl compounds. The DNPH-ones obtained are then separated by reversed-phase HPLC and determined with spectrophotometric detection.

2. Materials and methods

These were the same as already described (12,13) with small modifications: I) both the stock standard solution and working standard solutions were prepared in ethanol-water (5:95, v/v); II) beer was degassed, filtered and directly subjected to derivatization. The acetonitrile solution of the DNPH-ones of carbonyl compounds was centrifuged and injected into the HPLC system with a syringe attached to a Millex-LCR$_{13}$ (Millipore) to remove all particles larger than 0.5 µm.

3. Results and discussion

3.1 Optimization of the derivatization step

In recent years HPLC has been employed for the determination of F and/or HMF in several food matrices (1-7,10-13). Most HPLC methods so far proposed provide for the injection of the sample without derivatization (1-7,11). However, we made the sample to undergo derivatization in order to obtain the DNPH-ones of the carbonyl compounds present (10,12,13). The sensitivity of the method can therefore be improved.

The DNPH-ones are usually obtained by employing an excess of

2,4-dinitrophenylhydrazine (DNPH) aqueous solution in the presence of hydrochloric acid. The utilization by us of an acetonitrile DNPH solution in the presence of perchloric acid offers the advantage of obtaining a solution of the derivatives that may be injected directly into the HPLC system (12). The use of perchloric acid instead of hydrochloric acid is due to its higher solubility in acetonitrile (12).

The derivatization step was optimized by us with respect to three parameters: 1) the DNPH-to-analyte molar ratio, 2) the acidity of the medium and 3) the reaction time. For this purpose, the amounts of the derivatives obtained were evaluated on two standard solutions containing respectively F and HMF both 10^{-4} mol/l. The standard solutions were prepared by employing an ethanol-water mixture, which simulates natural matrixes as much as possible. The results obtained are shown in Figures 1-3. As may be seen, the derivatization reaction is quantitative when the reagent-to-analyte ratio is at least 2.5:1 for both analytes and the acidity of the medium, as evaluated with a pH-meter, is about 1. Under these conditions, both F and HMF are quantitatively converted into their DNPH-ones within 25 min. The derivatives obtained are stable at room temperature for at least 48 h.

3.2 Calibration

The calibration graphs were obtained by employing standard solutions of both F

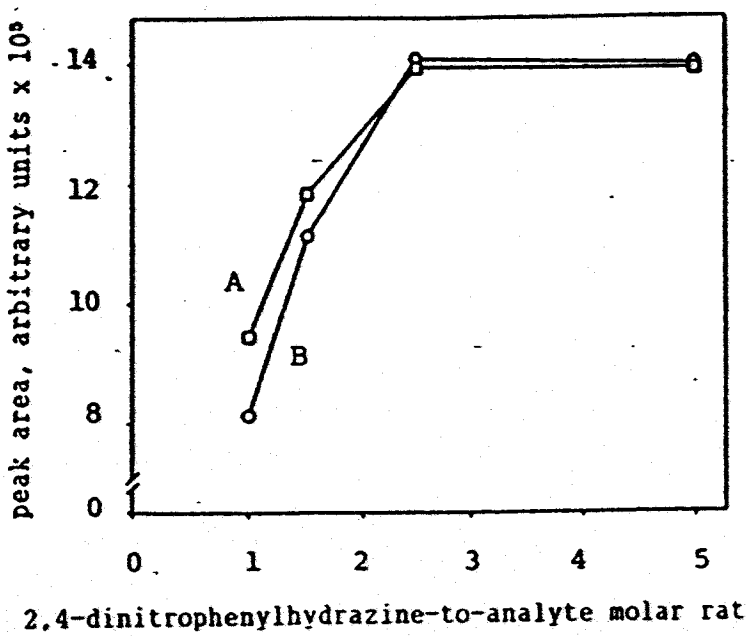

FIGURE 1. Conversion of 2-furaldehyde (A) and 5-hydroxymethyl-2-furaldehyde (B) to their 2,4-dinitrophenylhydrazones as a function of the 2,4-dinitrophenylhydrazine-to-analyte molar ratio. pH of the medium = 1; reaction time = 30 min.

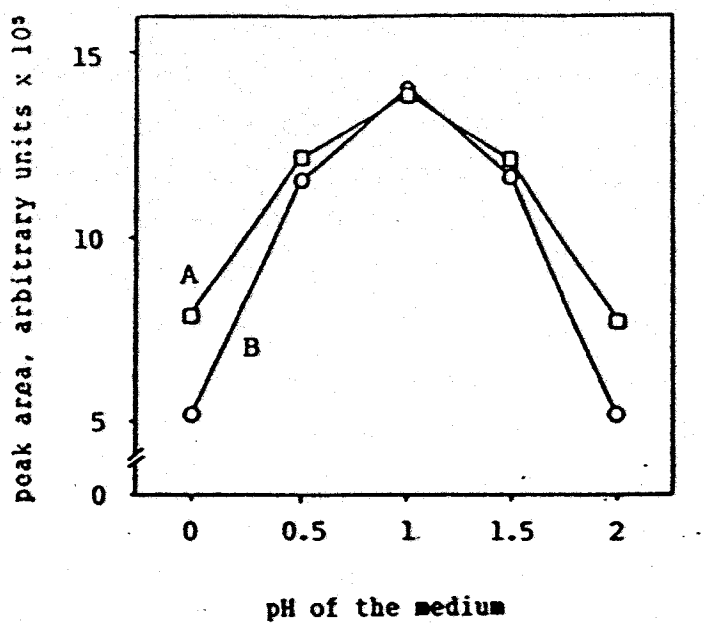

FIGURE 2. Conversion of 2-furaldehyde (A) and 5-hydroxymethyl-2-furaldehyde (B) their 2,4-dinitrophenylhydrazones as a function of the acidity of the medi 2,4-Dinitrophenylhydrazine-to-analyte molar ratio = 2.5; reaction time = 30 min.

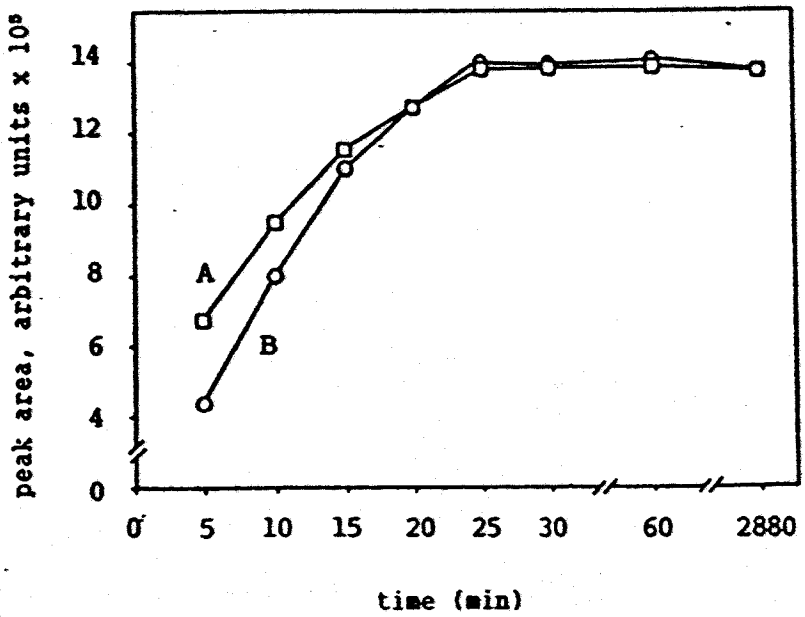

FIGURE 3. Conversion of 2-furaldehyde (A) and 5-hydroxymethyl-2-furaldehyde (B) their 2,4-dinitrophenylhydrazones as a function of reaction time. 2,4-Dinitrophe hydrazine-to-analyte molar ratio = 2.5; pH of the medium = 1.

and HMF under optimum experimental conditions as described in the preceding section. A straight line was obtained for both analytes over the range of concentrations from 10^{-3} to 10^{-7} mol/l, which represent values typically found in real samples. By setting the detector wavelength at the maximum absorbance of the derivatives of both F and HMF, it is possible to determine the detection limit as 3σ/S (14), where S is the sensitivity, which is 1.39×10^{10} for F and 1.26×10^{10} for HMF as obtained from the calibration graphs, and σ is the peak thresold of the integrator, which was set by us at 100. The detection limits are therefore 2.2×10^{-8} mol/l for F and 2.4×10^{-8} mol/l for HMF .

3.3 Specificity, recovery and reproducibility

The method shows a high specificity because, under the described conditions, the derivatives of both F and HMF are well separated with respect to the other carbonyl compounds present in the sample under examination.

DNPH must be at least 20 times more concentrated than the analytes to be determined in the analyses of real samples, as an aliquot of the reagent is employed in the derivatization of the other carbonyl compounds present. In all the samples so far examined, a 1:20 ratio was sufficient, as I) a large peak of the DNPH excess appears in the chromatogram and II) area increments were not obtained for the two analytes of interest by utilizing a 1:50 analyte-to-reagent ratio.

Recoveries were determined by adding known amounts of both analytes to a sample of beer. The sample was selected on the basis of its low content of both F and HMF, two of the lowest levels among those which we found in real samples. Recoveries ranged from 96 to 99% for concentrations of 10^{-7}-10^{-3} mol/l of both F and HMF added.

Reproducibility was evaluated by carrying out the determination six times on the same sample of beer over a period of 48 h. The average concentration of HMF was 3.7×10^{-3} mol/l, with a standard deviation of 5.5×10^{-7} mol/l and a relative standard deviation of 2%. It was not possible to define reproducibility in the determination of F, as this analyte was not detectable in the sample under examination.

3.4 Application

The procedure was applied to the determination of F and HMF in different commercial samples of beer; each sample was analyzed in duplicate. The results are summarized in Table 1. As may be seen, in all the samples analyzed F was not detectable, while the amount of HMF was of the order of 10^{-3} mol/l.

Table 1: Concentrations of 5-hydroxymethyl-2-furaldehyde and 2-furaldehyde found in some commercial beers (n.d. = not detectable).

Sample n.	Concentration of 5-hydroxymethyl-2-furaldehyde (mol/l)	Concentration of 2-furaldehyde (mol/l)
1	3.7×10^{-5}	n.d.
2	5.3×10^{-5}	n.d.
3	5.5×10^{-5}	n.d.
4	6.8×10^{-5}	n.d.
5	3.8×10^{-5}	n.d.
6	2.6×10^{-5}	n.d.
7	3.1×10^{-5}	n.d.
8	6.2×10^{-5}	n.d.
9	5.2×10^{-5}	n.d.
10	3.5×10^{-5}	n.d.

4. References

1. LEE H.S., ROUSEFF R.L. and NAGY S., J. Food Sci. 51 (1986), 1075
2. LI Z.-P., SAWAMURA M. and KUSUNOSE H., Agric. Biol. Chem. 52 (1988), 2232
3. DAUBERTE B., ESTIENNE J., GUERERE M. and GUERRA N., Annls Falsif. Expert. Chim. 83 (1990), 231
4. KIM H.-J. and RICHARDSON M., J. Chromatogr. 593 (1992), 153
5. JEURING H.J. and KUPPERS P.J.E.M., J. Assoc. Off. Anal. Chem. 63 (1980), 1215
6. MARCY J.E. and ROUSEFF R.L., J. Agric. Food Chem. 32 (1984), 979
7. MIJARES R.M., PARK G.L., NELSON D.B. and McIVER R.C., J. Food Sci. 51 (1986), 843
8. NARZISS L., MIEDANER H. and GRAF H., Monatsschr. Brauwiss. 38 (1985), 396
9. WINKLER O., Z. Lebensm. Unters. Forsch. 102 (1955), 161
10. PUPUTTI E. and LEHTONEN P., J. Chromatogr. 353 (1986), 163
11. LEUBOLT R. and KLEIN H., Monatsschr. Brauwiss. 42 (1989), 207
12. LO COCO F., CECCON L., VALENTINI C. and NOVELLI V., J. Chromatogr. 590 (1992), 235
13. LO COCO F., VALENTINI C., NOVELLI V. and CECCON L., J. Liquid Chromatogr. (in press)
14. MASSART D.L., DIJKSTRA A. and KAUFMAN L., Evaluation and Optimization of Laboratory Methods and Analytical Procedures, Elsevier, Amsterdam, 1978