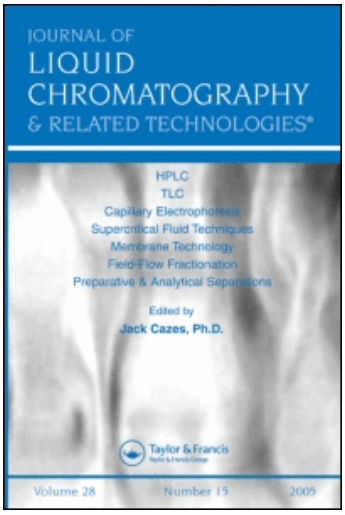

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DETERMINATION OF GLYOXAL, METHYLGLYOXAL, AND DIACETYL IN SELECTED BEER AND WINE, BY HPLC WITH UV SPECTROPHOTOMETRIC DETECTION, AFTER DERIVATIZATION WITH o-PHENYLENEDIAMINE

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ABSTRACT

The α-diketones glyoxal, methylglyoxal, and diacetyl were determined in selected beer and wine using a procedure involving the use of C_{18} solid phase extraction columns to remove interferences and derivatization of the compounds with o-phenylenediamine to form quinoxalines, which are separated by HPLC and detected using UV spectrophotometric detection.

Interferences were more difficult to remove in the case of beer, due to the higher complexity of the matrix and because the concentrations of the compounds were lower (higher for methylglyoxal and lower for diacetyl, but all in the 10^{-7} M region). The determination was easier to implement in the case of wine as the typical concentrations of the compounds were about ten times higher, with methylglyoxal being the more abundant compound found.

INTRODUCTION

Glyoxal, methylglyoxal, and diacetyl are important dicarbonyl compounds (R_1-CO-CO-R_2), usually present in food products obtained by fermentation processes,[1] like wine, brandy, yoghurt, cheese, vinegar, or butter. Diacetyl (R_1= R_2=CH_3) is the more important of those compounds in food technology, because it has a characteristic odour and taste that can affect the organoleptic quality of foods. For instance, in the brewing industry diacetyl must be controlled during important parts of the production of beer,[2] due to its unpleasant butter like taste. Methylglyoxal (R_1=CH_3, R_2=H) is a compound with biological relevance. Indeed, it is the substrate of an enzymic system involving glyoxalase I and glyoxalase II, found widespread in all living forms, but not yet well understood.[3]

The reaction with 2,4-dinitrophenylhydrazine is the basis for several UV spectrophotometric methods of determination of α-dicarbonyl compounds,[4] including HPLC methods. The use of this reaction has, however, a tendency to produce high results, because aldehydes and ketones can interfere; also, the high temperatures required for the reaction can induce changes on the sample.

A more convenient HPLC method involves derivatization with o-phenylenediamine derivatives and UV detection of the resulting quinoxalines.[5,6] This method is specific for α-dicarbonyl compounds and the changes on the sample are minimised as derivatization reaction is accomplished at low temperature and in aqueous conditions.

Gas chromatography with headspace sampling and electron capture detection is another method that has been applied to the determination of diacetyl in beer.[7] However, it cannot be applied to the determination of methylglyoxal, due to the decomposition of this compound at the high temperatures needed in GC. Another problem is that α-diketones can complex quite easily with other species, like sulphite ion, decreasing their vapour pressure when the GC method with headspace sampling is used.[8]

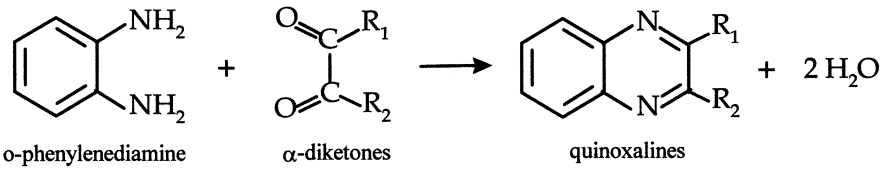

Figure 1. Derivatization reaction of α-diketones with o-phenylenediamine to form quinoxalines. Glyoxal: R_1=R_2=H; methylglyoxal: R_1=CH_3, R_2=H; diacetyl: R_1=R_2=CH_3.

In this work an adaptation of the procedure proposed by Verhagen et al.[8] is used, allowing the determination of glyoxal, methylglyoxal, and diacetyl in beer and in wine. The compounds are derivatized with o-phenylenediamine to produce quinoxalines (Figure 1), which are separated by HPLC, and detected using UV spectrophotometry. The use of solid phase extraction as a clean-up step prior to liquid chromatographic analysis is a common procedure.[9] The determination in beer is more problematic, as the concentration of the compounds is less than 10^{-6} M (slightly higher than 10^{-7} M for diacetyl) and interferences in the matrix must be eliminated carefully. The determination in wine is easier, because the matrix effect is not so pronounced and the concentration of the compounds is significantly higher.

EXPERIMENTAL

Instrumentation

A Gilson HPLC instrument was used, consisting of a 307 pump, a 115 UV variable wavelenght detector, selected to 315 nm, an injection valve with a 20 μL loop and a 25 cm x 4.6 mm, 5 μm particle size, reverse phase C_{18} column from Phase Separation Ltd. Results were registered in a Spectra Physics Data Jet integrator. Isocratic elution was used, with a flow rate of 0.8 mL/min. A mixture 80:20 acetonitrile/0.04 M acetate buffer pH=4.5 was used as eluent, after being degassed with a Schleicher & Schuell GV 050/0 vacuum filter holder, equipped with 0.2 μm S & S NL 16 membrane filters.

Before injection in the HPLC column, samples were passed through a Gelman Acrodisc 13 CR PTFE 0.45 μm syringe filter. Solid phase extraction columns of 200 mg (3 mL) and 500 mg (6 mL) were Bond Elut from Varian and the elution was facilitated using a Vac Elut system.

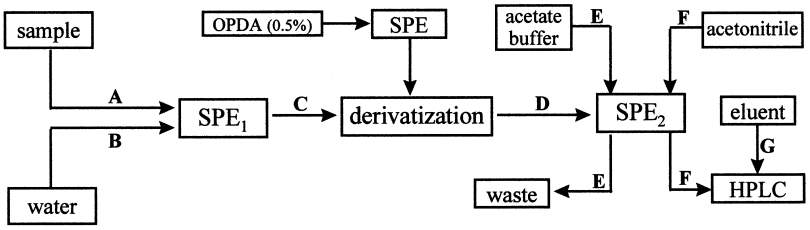

Figure 2. Schematic representation of the procedure used in the HPLC/UV determination of glyoxal, methylglyoxal, and diacetyl in beer and in wine.

Reagents and Solutions

Solutions of glyoxal, methylglyoxal, and diacetyl were prepared from the commercial products obtained from Aldrich. All other chemicals used were of analytical grade. Distilled water purified in a Millipore system was used for preparation of solutions.

The o-phenylenediamine (OPDA) derivatization solution was prepared fresh before use by dissolution of the convenient amount of this compound (Merck) in the buffer solution used for derivatization. This solution should be kept in a dark place and handled carefully since o-phenylenediamine is toxic and may cause allergenic reactions.

The samples of beer and of wine were directly purchased in a store.

Procedures

The procedure developed for the determination of the compounds was adapted from Verhagen et al.[8] and is represented in Figure 2. After being degassed with magnetic stirring, a volume of 1 mL of sample (A) is passed through a 500 mg solid phase (C-18) extraction column SPE_1 and washed with 10 mL of water (B). As will be seen in the discussion of the results, these conditions (volume of sample and capacity of the extraction column SPE_1) were optimized for beer. Less polar interferences are retained and the more polar α-diketones are collected (C), derivatized during 30 minutes with 5 mL of 0.5% o-phenylenediamine (previously passed through another solid phase extraction column to remove interference impurities), and diluted with pH=4.5 acetate buffer to 25 mL in a volumetric flask. These 25 mL of reactant mixture (D) are passed through the extraction column SPE_2 (200 mg of capacity), where the quinoxalines are retained (they are less polar than the α-dicarbonyl compounds

from which they were formed) and o-phenylenediamine (more polar, as in acetate buffer it is protonated) is washed out almost completely with 2 mL acetate buffer (E). This buffer is removed by forcing air through the column with a syringe. Then, 1 mL of acetonitrile (F) is injected into the column to extract the quinoxalines; the extract is expelled from the column by forcing air again, and injected in the HPLC column, where the quinoxalines are separated using as eluent a solution of acetonitrile/aqueous acetate buffer (G) and detected spectrophotometrically at 315 nm.

RESULTS AND DISCUSSION

In the application of the method described in reference[8] to the determination of glyoxal, methylglyoxal, and diacetyl in wine and in beer, some modifications had to be introduced. The volumes and pH values of the solutions used in the different phases of the process had to be optimised, in order to obtain a good compromise between sensitivity and clean up of the sample to eliminate matrix interferences. Instead of using chloroform to extract the quinoxalines from SPE_2, evaporating it to dryness and dissolving the residue before injection into the HPLC column, the process was simplified by extracting the quinoxalines with a small volume of acetonitrile, which was directly injected into the column.

Determination of Glyoxal, Methylglyoxal, and Diacetyl in Beer

In the conditions proposed in reference 8, a severe interference of the matrix of beer was found. Using as blank a situation without addition of the derivatizing agent, the influence of sample volume and of capacity of the extraction column on the level of interference was studied. As was to be expected, interference is diminished if the sample volume is decreased and the extraction column capacity is increased. This can be seen in Figure 3: in chromatograms A and B interference is significative, indicating that the sample volume is too large for the capacity of the extraction columns; in chromatogram C (volume of beer reduced to 1 mL and column capacity increased to 500 mg) interference is practically absent; in chromatogram D, obtained in the same conditions of C, but with derivatization, peaks 1, 2, and 3 are clearly distinguished and correspond to glyoxal, methylglyoxal, and diacetyl, respectively. Using the method of standard additions, the concentrations of glyoxal, methylglyoxal, and diacetyl found were 4.4×10^{-7} M, 1.0×10^{-6} M, and 1.3×10^{-7} M, respectively, results that are of the same order of magnitude of those indicated by Yamaguchi et al. In the analysis of three different beers, these authors obtained concentrations of respectively 6×10^{-7} M, 4×10^{-7} M, and 7×10^{-7} M for glyoxal, 3.3×10^{-6} M, 1.3×10^{-6} M, and 1.2×10^{-6} M for methylglyoxal and 5×10^{-7} M, 6×10^{-7} M, and 6×10^{-7} M for diacetyl.

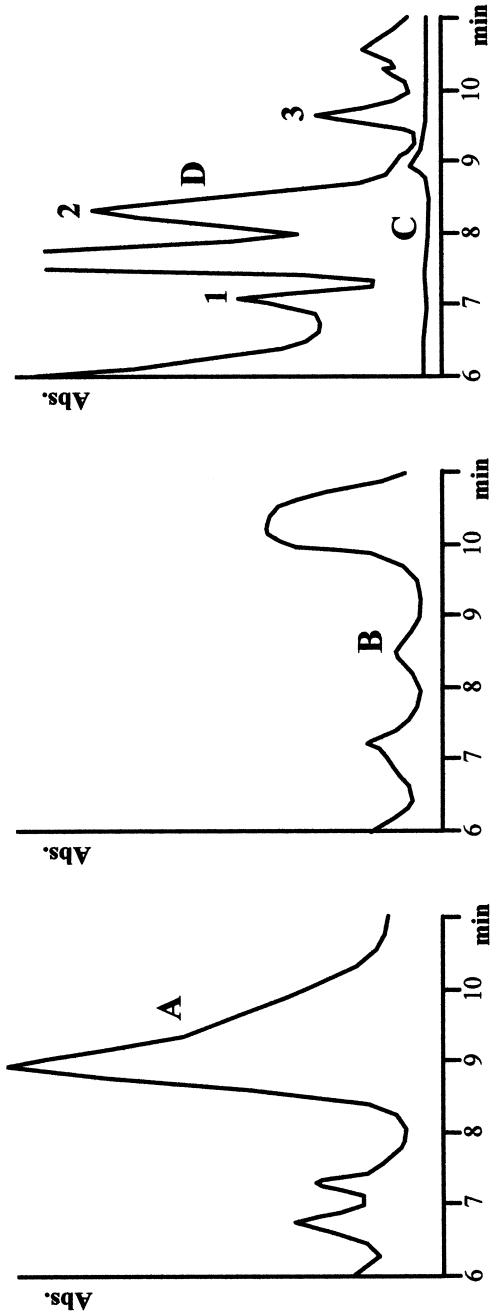

Figure 3. Chromatograms obtained in the analysis of beer using the proposed procedure. A - extraction of 5 mL of beer in a column of 200 mg; B - extraction of 3 mL of beer in a column of 500 mg; C, D - extraction of 1 mL of beer in a column of 500 mg. In situations A, B, and C the derivatization step was omitted. Peaks identified in chromatogram D: 1 - quinoxaline (derivatized glyoxal); 2 - methylquinoxaline (derivatized methylglyoxal); 3 - dimethylquinoxaline (derivatized diacetyl).

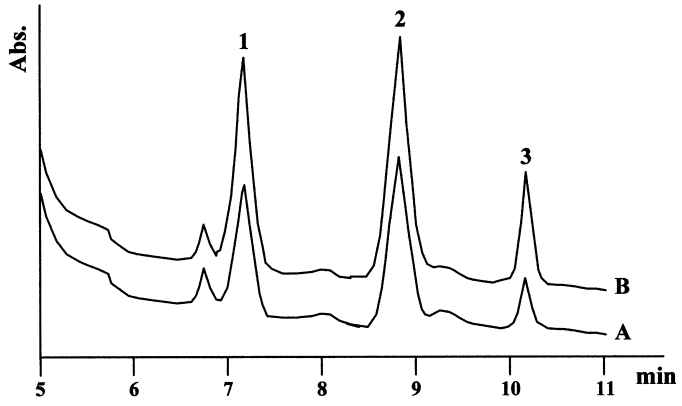

Figure 4. Chromatograms obtained in the analysis of wine using the proposed procedure. A - analysis of wine; B - analysis after a standard addition to the wine of 4 x 10^{-6} M of each of the three α-diketones: glyoxal (1), methylglyoxal (2) and diacetyl (3).

Table 1

Determination of Glyoxal, Methylglyoxal and Diacetyl in Two Brands of White Wine

Wine Brand	α-Diketone	Concentration (M)
Solouro	Glyoxal	6.2×10^{-6}
	Methylglyoxal	9.0×10^{-6}
	Diacetyl	3.1×10^{-6}
Pavão	Glyoxal	26×10^{-6}
	Methylglyoxal	26×10^{-6}
	Diacetyl	7.0×10^{-6}
White Wine of Reference 1	Glyoxal	8.7×10^{-6}
	Methylglyoxal	40×10^{-6}
	Diacetyl	11×10^{-6}

Determination of Glyoxal, Methylglyoxal, and Diacetyl in Wine

In the case of wine the problems with matrix interference were small, especially because the concentrations of the α-diketones are about ten times

higher than those found in beer, with no need to use a large volume of sample. Good results were obtained with the conditions optimized for beer (a volume of wine of 1 mL and a SPE_1 extraction column with a capacity of 500 mg).

The chromatograms obtained in the analysis of wine (Solouro) can be seen in Figure 4A. The chromatograms obtained after a standard addition of 4.0 x 10^{-6} M of each of the three α-diketones to the wine can be seen in Figure 4B. Two different brands of white wine were analysed and, again, the results are similar to those indicated in reference 1, as can be seen in Table 1. It is worthy to note that, as in beer, the more abundant of the three α-diketones is methylglyoxal.

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REFERENCES

1. M. Yamaguchi, J. Ishida, Z. Xuan-Xuan, M. Nakamura, T. Yoshitake, J. Liq. Chromatogr., **17**, 203-211 (1994).

2. T. Wainwright, J. Inst. Brew., **79**, 451-470 (1973).

3. P. J. Thornally, Biochem. J., **269**, 1-11 (1990).

4. K. Fung, D. Grosjean, Anal. Chem., **53**, 168-171 (1981).

5. A. C. McLellan, S. A. Phillips, P. J. Thornalley, Anal. Biochem., **206**, 17-23 (1992).

6. W. Bednarski, L. Jedrychowski, E. G. Hammond, Z. L. Nikolov, Journal Dairy Science, **72**, 2474-2477 (1989).

7. S. J. Dupire, J. Inst. Brew., **104**, 67-68 (1998).

8. L. C. Verhagen, R. L. de Jong, J. Strating, European Brewery Convention, Proceedings of the 21st Congress, Madrid, 615-622 (1987).

9. M. Moors, C. Teixeira, M. Jimidar, D. Massart, Anal. Chim. Acta, **255**, 177-186 (1991).

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