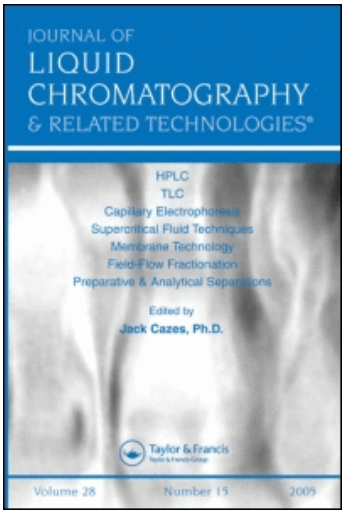


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# Determination of Glyoxal, Methylglyoxal, Diacethyl, and 2, 3-Pentanedione in Fermented Foods by High-Performance Liquid Chromatography with Fluorescence Detection

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# DETERMINATION OF GLYOXAL, METHYLGLYOXAL, DIACETHYL, AND 2,3-PENTANEDIONE IN FERMENTED FOODS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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ABSTRACT

A highly sensitive and rapid high-performance liquid chromatographic method for the determination of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione in fermented foods is described. After extraction of the compounds with methanol, the compounds in the extract are converted into the corresponding fluorescent derivatives by reaction with 1,2-diamino-4,5-methylenedioxybenzene, a fluorogenic reagent for α-dicarbonyl compounds. The derivatives are separated on a reversed-phase column (L-column ODS) with isocratic elution using acetonitrile - 0.5M ammonium acetate, and are detected fluorimetrically. The detection limits are 11.6 - 13.8 fmol per 10-μl injection for all the compounds at a signal to noise ratio of 3.

## INTRODUCTION

Diacethyl and 2,3-pentanedione are well known to be one of the important fragrant components in fermented foods such as alcoholic drinks and dairy products, and to be produced by microorganisms during fermented processes. Thus, it is very important to quantify the compounds in the final products for reasons of quality control. On the other hand, it is stipulated that glyoxal and methylglyoxal also are present in the fermented foods and are important for quality check of the foods. However, the compounds have never been successfully determined. This may be partially due to the lack of a sensitive and selective method for the simultaneous determination of the four α-dicarbonyl compounds described above.



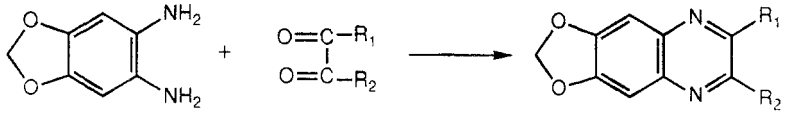
Some methods including gas chromatography (1-4), spectrophotometry (5-7), and high-performance liquid chromatography (HPLC) (8,9) have been developed for the determination of diacethyl and/or 2,3-pentanedione in fermented foods. However, the methods have limited sensitivity and do not allow the simultaneous determination of the four α-dicarbonyl compounds described above.

We previously developed 1,2-diamino-4,5-methylenedioxybenzene (DMB) as a highly sensitive and selective fluorogenic reagent for α-dicarbonyl compounds in HPLC (10). The reagent reacts with the α-dicarbonyl compounds in the presence of β-mercaptoethanol and sodium dithionite to produce the corresponding fluorescent quinoxalines (Fig. 1). In this paper, we applied the reaction to the simultaneous determination of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione which are very important for quality check of fermented foods.

## EXPERIMENTAL

### Reagents and solutions

All chemicals and solvents were of analytical-reagent grade, unless stated otherwise. Distilled water, purified with a Milli Q II system (Japan Millipore, Tokyo, Japan) was used for all aqueous solutions. Glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione were purchased from Wako Pure Chemicals




1,2-Diamino-4,5-methylenedioxybenzene (DMB) | α-Dicarbonyl compounds | Fluorescent derivatives

FIGURE 1. Derivatization of α-dicarbonyl compounds with 1,2-diamino-4,5-methylenedioxybenzene (DMB).



(Tokyo, Japan). DMB was prepared as described previously (11); it is now commercially available from Dojindo Labs. (Kumamoto, Japan). DMB solution (7.0 mM) was prepared in water containing 0.2 M β-mercaptoethanol and 0.25 M sodium dithionite. The DMB solution could be used for more than 1 week when stored in a refrigerator at 4 °C.

Instrumentation

A Hitachi (Tokyo, Japan) 655-A11 high-performance liquid chromatograph equipped with a sample injector (10-μl loop) was used. A Shimadzu (Kyoto, Japan) RF-535 fluorescence spectromonitor fitted with a 12-μl flow-cell operating at an excitation wavelength of 350 nm and emission wavelength of 390 nm. The column was a L-column ODS (100x4 mm i.d.; particle size, 5 μm)(Chemical Inspection and Testing Institute, Tokyo, Japan). The mobile phase was acetonitrile - 0.5 M ammonium acetate (35:65, v/v). The flow-rate was 1.0 ml/min. The column temperature was ambient (ca. 25 °C). Uncorrected fluorescence excitation and emission spectra of the eluates were measured with a Hitachi 650-60 fluorescence spectrophotometer fitted with a 20-μl flow-cell; the spectral bandwidths were 5 nm in both the excitation and emission monochromators.

Food samples

Portions (ca. 0.1 g or 0.1 ml) of yoghurt, beer, and wine were diluted with 5.0 ml of methanol, and the mixtures were centrifuged at 1000 g for 5 min. The supernatants were used as food sample solutions.

Derivatization procedure

A portion (100 µl) of a sample solution in a screw-capped 1-ml vial was diluted with 10-µl of water and 100 µl of the DMB solution. The vial was tightly closed and warmed at 60 °C for 40 min in the dark. After cooling, 10 µl of the resulting mixture were injected into the chromatograph.

The amounts of α-dicarbonyl compounds were calibrated by means of the standard addition method: water (10 µl) added to the sample solution was replaced by the standard solution (10 µl) containing 1.0 - 150 pmol each of the α-dicarbonyl compounds. The net peak heights in the chromatogram were plotted against the concentrations of the individual α-dicarbonyl compounds spiked.



## RESULTS AND DISCUSSION

The derivatization conditions were the same as described previously (10).

HPLC conditions

The separation of the DMB derivatives of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione was studied on a reversed-phase column (L-column ODS) using methanol, acetonitrile, water, 0.5 M ammonium acetate and their mixtures as mobile phase. The best separation was achieved using acetonitrile - 0.5 M ammonium acetate (35:65, v/v). The individual α-dicarbonyl compounds tested gave single peaks. Figure 2 shows a typical chromatogram obtained with a standard mixture of the four α-dicarbonyl compounds [retention time (min): glyoxal, 6.1; methylglyoxal, 7.7; diacethyl, 9.2; 2,3-pentanedione, 17.6].

Determination of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione in food samples

Representative chromatograms obtained with yoghurt and beer are shown in Figs. 3(A) and (B), respectively. The components of peaks 1, 2, 3, and 4 (Figs. 3) were identified as the DMB derivatives of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione, respectively, on the basis of their retention times and fluorescence excitation and emission spectra. This was achieved by comparison

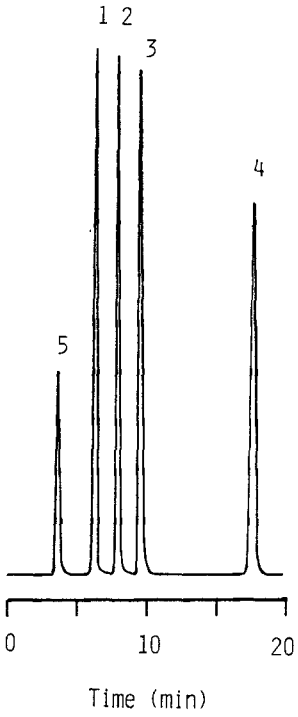



FIGURE 2. Chromatogram obtained with a standard mixture of α-dicarbonyl compounds. A portion (100 µl) of a standard mixture of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione ($1.0 \times 10^{-5}$ M each) was treated as in the procedure. Peaks: 1=glyoxal, 2=methylglyoxal, 3=diacethyl, 4=2,3-pentanedione, 5=DMB.

of the spectra with the standards, and also by co-chromatography of the standards and the foodstuff samples with aqueous 10 - 80 % acetonitrile or methanol as the mobile phase. Peak 6 may be due to the endogenous unknown α-dicarbonyl compounds in foodstuffs. This was suggested by the following results. Peaks 6 increased in height in proportion to the sample size of the foods. No peaks were detected in the chromatograms when the foodstuff samples were treated without DMB. Moreover, all the eluate from peaks 6 exhibited fluorescence excitation and emission maxima around 350 and 390 nm, respectively, almost identical with those of the DMB derivatives of the four α-dicarbonyl compounds tested.

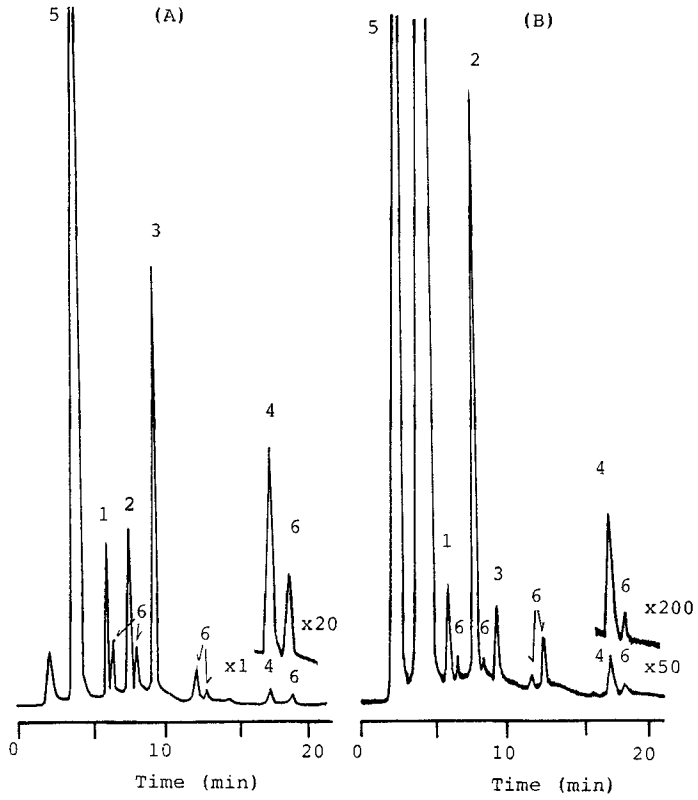



FIGURE 3. Chromatograms obtained with foodstuffs. Portions (100 µl) of (A) yoghurt and (B) beer sample solutions were treated as in the procedure. Peaks: 1 - 5 = see in Fig. 2, 6=unknown.

Many substances such as carboxylic acids, alcohols, sugars, aldehydes, ketones, phenols, amines, and amino acids gave no fluorescent derivatives under the described conditions. On the other hand, DMB reacts with α-keto acids (α-ketoglutaric, pyruvic, α-ketoisovaleric, α-ketoisocaproic, and α-keto-β-methylvaleric acids) to produce fluorescent derivatives. However, the DMB derivatives of α-keto acids have fluorescence excitation (maximum, 367 nm) and emission (maximum, 446 nm) spectra different from those of the α-dicarbonyl compounds and were eluted at retention times of 3 - 5 min. Thus,

α-keto acids did not interfere with the sensitive determination of the four α-dicarbonyl compounds tested.

Linear relationships were observed between the net peak heights and the amounts of the individual α-dicarbonyl compounds spiked to foodstuff samples, up to at least 90 nmol/g (or ml) of the foods. The correlation coefficients of the calibration curves were higher than 0.998 for all the compounds. The detection limits for glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione were 260, 270, 400, and 330 pmol/g (or ml) of foods (12.0, 12.5, 13.8, and 11.6 fmol/10 μl injection volume), respectively, at a signal to noise ratio of 3. The within-day precision of the methods were established by repeated determination (n=10) using the individual food samples. The relative standard deviations did not exceed 4.0 % for all the compounds in all food samples. The recoveries of the α-dicarbonyl compounds added to 100 μl of the individual food samples in the amounts of 5.0 nmol were 94.2 - 99.6 %.

The amounts of the four α-dicarbonyl compounds in foodstuffs were determined by this method (Table 1). The mean values of diacethyl and 2,3-pentanedione in beer, wine, and yoghurt were not very different from those listed by other workers (4,7,8,12-14). The levels of glyoxal and methylglyoxal in foodstuffs are first determined by the present method.



TABLE 1

Concentration (nmol/ml or g) of α-Dicarbonyl Compounds in Foodstuffs

| Foodstuffs | | Glyoxal | Methylglyoxal | Diacethyl | 2,3-Pentanedione |
|---|---|---|---|---|---|
| Yoghurt | A | 15.8 | 17.7 | 10.5 | 5.4 |
| | B | 12.3 | 14.4 | 22.5 | 6.2 |
| | C | 10.9 | 8.4 | 25.9 | 4.8 |
| Beer | D | 0.6 | 3.3 | 0.5 | 0.4 |
| | E | 0.4 | 1.3 | 0.6 | 0.4 |
| | F | 0.7 | 1.2 | 0.6 | 0.4 |
| Wine | (red) | 12.8 | 12.6 | 32.0 | 1.1 |
| | (white) | 8.7 | 40.2 | 11.2 | 1.4 |

This work provides the first fluorimetric HPLC method for the simultaneous quantification of glyoxal, methylglyoxal, diacethyl, and 2,3-pentanedione in fermented foods. This method has a satisfactory sensitivity in the determination of the compounds in a small amount of the foods. This is of advantage in the measurement of the α-dicarbonyl compounds for the quality control of fermented foods such as alcoholic drinks and dairy products. This method is rapid and simple to perform and can, therefore, be applied to routine analyses in the investigations of fermentation technology and food chemistry.

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