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

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Abstract

A method for the determination of 2-furaldehyde (F) and 5-hydroxymethyl-2-furaldehyde (HMF) in fruit juices by high performance liquid chromatography is described. The method is based on the formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds and subsequent separation of these derivatives. The procedure offers a high specificity and a detection limit of the order of 10^{-8} mol/l. Recoveries of 93-96% were obtained from an orange juice spiked at different levels with both analytes. The reproducibility (mean of six determinations) is $\pm 2\%$ for F and $\pm 3\%$ for HMF.

1. Introduction

The occurrence of 2-furaldehyde (F) and 5-hydroxymethyl-2-furaldehyde (HMF) in fruit juices is an indication of quality deterioration (1-5). Fruit juices undergo flavor, taste, color and nutritional changes when stored at warm temperatures and/or for prolonged periods of time (4,6-9). Both F and HMF are formed during thermal processing or storage at improper temperatures; for this reason both are useful indicators of temperature abuse in fruit juices (3,4,7,9-12). In particular, F is virtually absent in fresh juices, whereas large amounts have been found in juices stored at improper temperatures (6,7,12,13). A close relationship between flavor changes and F content has been demonstrated; for this reason the F content is useful as an off-flavor indicator (6-10,12). On the other hand, HMF is correlated with

browning reactions (4,9,10,12).

The classical methods for the quantitative determination of F and/or HMF in fruit juices are based on spectrophotometric measurements (6,8,10,11,13). 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 reaction product may lead to low recoveries and wide statistical variations of the results and (IV) no one of the methods is specific (1,2,7,10,11,13). In recent years, high performance liquid chromatographic (HPLC) methods have been proposed (1-5,7,10,12). These methods are less time consuming, offer improved accuracy, sensitivity and specificity and utilize less hazardous reagents (4,7,10).

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

2. Materials and methods

These were the same as already described in previous papers (14-16).

3. Results and discussion

3.1 Optimization of the derivatization step

The HPLC methods so far proposed for the determination of F and/or HMF in fruit juices provide for the injection of the sample without derivatization (1-5,7,10,12). However, we made the juice to undergo derivatization in order to obtain the DNPH-ones of the carbonyl compounds present. The sensitivity and selectivity of the method can therefore be improved. This type of derivatization has been already employed for the determination of F and/or HMF in other kinds of food matrices (14-16).

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 derivative solution that may be injected directly into the HPLC system. Long and tedious steps, such as filtration and washing of the derivatives obtained and preparation of a derivative solution in a suitable solvent before the HPLC determination, may therefore be avoided. The use of perchloric acid instead of hydrochloric acid is due to its higher solubility in acetonitrile.

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. 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 under optimum experimental conditions as described in section 3.1. A straight line was obtained for both analytes over the range of concentrations from 10^{-9} 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 $3\sigma/S$ (17), where S is the sensitivity, which is 1.39×10^6 for F and 1.26×10^6 for HMF as obtained from the calibration graphs, and 0 is the peak threshold 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, since the derivatives of both F and HMF are well separated from the other carbonyl compounds present in the sample.

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 1) a large peak of the DNPH excess appears in the chromatogram and 2) 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 orange juice. The amount found in respect of the sum between the amount added and that originally present represents the recovery. Recoveries ranged from 93 to 96%.

Reproducibility was evaluated by carrying out the determination six times on the same sample of peach juice over a period of 48 h. The average concentration of F was 7.43×10^{-6} mol/l, with a standard deviation of 1.4×10^{-7} mol/l and a relative standard deviation of 2%; the average concentration of HMF was 9.82×10^{-6} mol/l, with a standard deviation of 2.9×10^{-6} mol/l and a relative standard deviation of 3%.

The derivatization step was carried out on the same sample of apricot juice for 30, 60, 90 and 120 min to verify that no artifactual amounts of either of the analytes are produced during the preliminary sample processing under acidic

conditions. A slight increase of the amount of F was obtained at 60 min, but this was followed by a decrease at both 90 and 120 min to levels even just lower than at 30 min. As far as the HMF amount is concerned, a slight, progressive increase was observed. However, no one of the values obtained for both F and HMF was significantly different ($p < 0.05$) with respect to the amount observed at 30 min.

3.4 Application

The procedure was applied to the determination of F and HMF in different commercial samples of fruit juices. In all the samples analyzed, the amount of HMF (range: 10^{-4} – 10^{-3} mol/l) was one order of magnitude greater than the amount of F (range: 10^{-5} – 10^{-6} mol/l).

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