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HPLC Detection of Free Malonaldehyde for Rapid Measurement of Lipid Oxidation Development

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Abstract

Various concentrations of free malonaldehyde were prepared from 1,1,3,3-tetraethoxy propane (TEP). Spectrophotometric determination and HPLC analysis of free malonaldehyde instead of malonaldehyde-thiobarbituric acid (MA-TBA) complex were conducted. Malonaldehyde was well separated on a μBondapak C_{18} column. The absorbances at 254 nm and the HPLC peak areas of free malonaldehyde increased with the increase in its concentration. The correlation coefficient between absorbances and peak areas was 0.998. The total time elapsed to conduct the whole procedure was less than 15 minutes. This method directly measured the amount of free malonaldehyde in a short period of time successfully. This procedure is expected to be used as a rapid, accurate and specific means to determine the development of lipid oxidation in food.

Key words: free malonaldehyde, spectrophotometric determination, HPLC analysis

INTRODUCTION

The 2-thiobarbituric acid (TBA) value has been one of the most widely used methods for measuring the development of lipid oxidation in muscle food (1). It measures the intensity of a red chromogen produced from the reaction between malonaldehyde (MA), a secondary oxidation product of fatty acids, and TBA. The development of oxidative rancidity is usually expressed in terms of TBA number (mg of MA per kg of sample) or TBA reactive substances (TBARS) absorbances (2-5).

The traditional TBA method usually includes steps liberating free malonaldehyde from lipids and forming an MA-TBA complex in an acidic heating process. The optical densities of this complex is measured with the spectrophotometer at 532 nm (2-5). Despite its widespread use, this method has been criticized because the main reaction is nonspecific and raises the possibility of generating MA artifactually during heating (6,7). New techniques, however, that are specific and precise using high performance liquid chromatography (HPLC) for measurement of the MA-TBA complex were successfully conducted in a model system (8), biological tissues such as plasma and liver (9-15) and ground pork (16). Although this new TBA technique was successfully performed in various samples mentioned above, this procedure still requires the step of the MA-TBA complex formation resulting in the delayed termination of the whole procedure. If the MA-TBA complex formation step is omitted and free MA can be measured directly, the time consumed for performing the whole procedure could be shortened to a greater extent.

This study was undertaken to devise a rapid method using HPLC for the direct measurement of free malonaldehyde obviating the MA-TBA complex formation, thus shortening the time consumed for the analysis.

MATERIALS AND METHODS

Materials

1,1,3,3-tetraethoxypropane (TEP), cetrimide (cetyltrimethylammonium bromide) and antifoam were obtained from Sigma Chemical Co. (St. Louis, USA) and hydrochloric acid was purchased from Duksan Pure Chem. Co. (Ansan, Korea). The reagents were dissolved in HPLC-grade distilled water before use. HPLC-grade acetonitrile and acetic acid were purchased from Merck (Darmstadt, Germany) and J.T. Baker (Phillipsburg, USA), respectively.

Release of free MA from TEP

Ten μl of TEP solution was diluted to 10 ml with 0.1 N HCl. This solution was immersed in a boiling water bath for 5 minutes to facilitate the release of MA and cooled in tap water. One ml of this solution was rediluted to 10 ml and used as a stock solution. The concentration of MA of this working stock solution was approximately 6.07×10^{-4} M. To 0, 0.5, 1.0, 1.5, and 2.0 ml of this solution, distilled water was added to 5 ml and a spectrophotometric (UVIKON 922, Kontron Instrument) measurement and a HPLC (Young-Lin 930) detection of MA present were performed.

Spectrophotometric measurement of free malonaldehyde

Malonaldehyde in aqueous solution was reported to have a UV absorption maximum near 260 nm (17). When the UV spectra of the malonaldehyde solution prepared in this experi-

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ment were scanned, the absorption peak was observed at 254 nm. The UV absorption of the standard MA solutions prepared above was monitored at 254 nm.

HPLC analysis of free MA

The free malonaldehyde released from TEP were analyzed with a Young-Lin 930 HPLC. The system was equipped with a UV-VIS variable wavelength absorbance detector (Young-Lin, M720) and the detection was made at 254 nm. The separation was conducted on a μBondapak C_{18} column (3.9 mm × 30 cm, 10 μm, Waters) with the mobile phase of acetonitrile and 10% acetic acid mixture (75:25). Flow rate was 1 ml/min and the column temperature was maintained at 30°C. Both solvents were filtered and degassing was made with a degasser (DEGASYS DG 1310, UNIFLOWS, Tokyo, Japan).

UV-VIS DA (diode array) spectrophotometric analysis of HPLC eluant of free MA

The HPLC eluant fraction representing free MA was collected in several run and the UV spectra were measured with the UV-VIS DA spectrophotometer (Hewlett-Packard 8453, Waldbronn, Germany). These spectra were compared with those of the MA solutions prepared.

RESULTS AND DISCUSSION

Concentration of MA vs increase in its absorbance

Malonaldehyde solutions were prepared from TEP and the absorbances at 254 nm were measured. As shown in Fig. 1, absorbances of malonaldehyde solutions prepared from TEP increased linearly with the increase in the concentration of the solution.

HPLC analysis of MA

Free malonaldehyde was well separated on a μBondapak C_{18} column as shown in Fig. 2. The peak appeared in the chromatogram shows a well separated single compound pattern and the retention time of the peak was around 2.76 min. When the effect of the concentration (polarity) of acetic acid

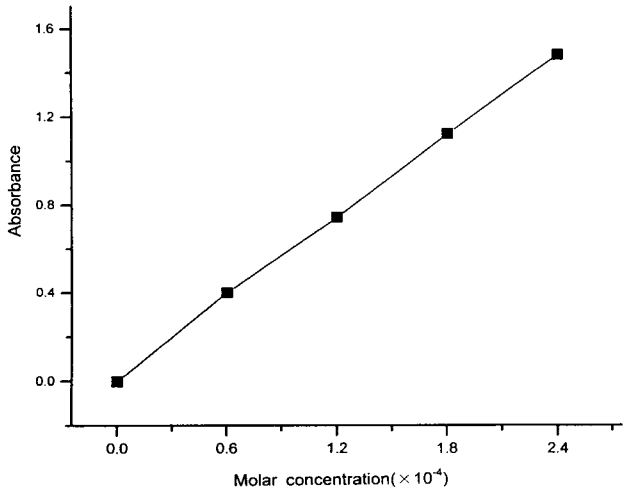

Fig. 1. Standard curve for free MA.

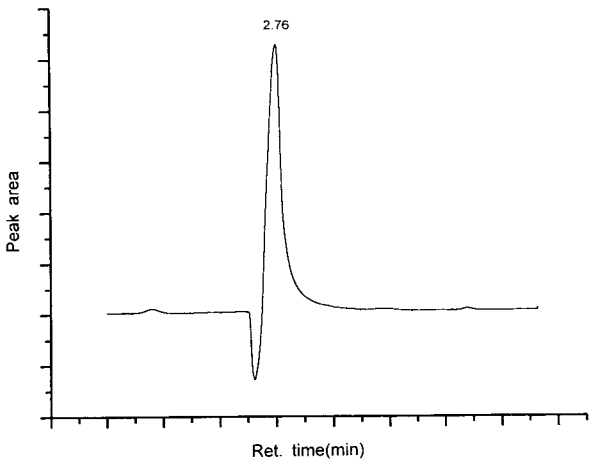

Fig. 2. HPLC elution profile of free MA (2.4×10^{-9}mole, inj.vol. 20 μl).

(1 to 100%) on the separation of MA was monitored, the resolution of malonaldehyde except 10% acetic acid solution was worse or very poor (data not shown). A higher concentration of acid is not recommended because it could damage the column (18), so acetic acid with higher concentrations for a long period of time should be avoided. It was determined that 75:25 of acetonitrile and 10% acetic acid mixture was the best concentration and polarity of the mobile phase to separate free malonaldehyde.

Peak areas of MA increased with the increase in its concentration (Fig. 3). As shown in the figure, when those two parameters were plotted, there was a linear relationship between the two. The correlation coefficient between the peak areas and the absorbances was 0.998. The treatment of cetrimide, an ion pairing agent, which is reported to improve the separation of MA on a reversed phase column (17,19), was not effective for that purpose in this experiment (data not shown).

UV-VIS DA spectra of MA

The HPLC eluants for free malonaldehyde were collected in several runs and the UV absorption spectra were measured with a UV-VIS DA spectrophotometer. The spectra for the

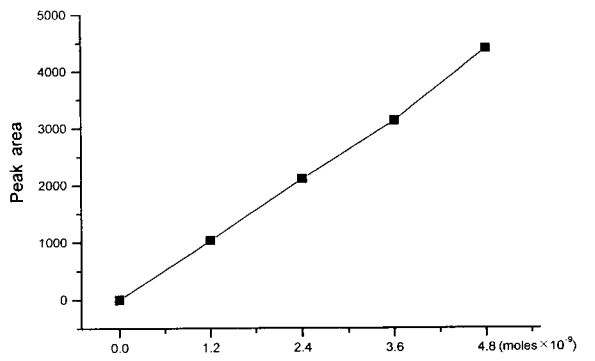

Fig. 3. Relationship between concentrations of MA and their corresponding HPLC peak areas.

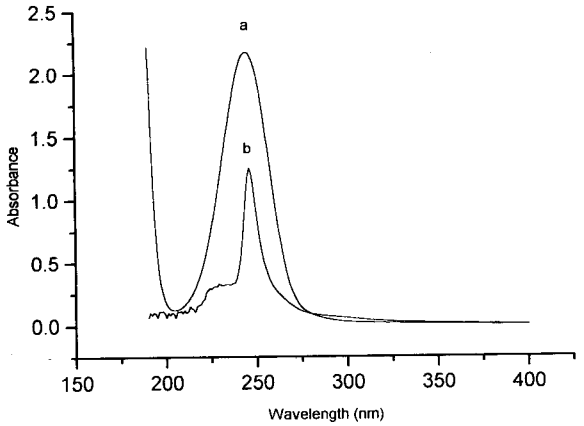

Fig. 4. UV-VIS DA spectra for (a) free MA solution and (b) HPLC eluant of free MA.

malonaldehyde solution itself were also measured and compared with the above ones. Both spectra had the same absorption maxima at 254 nm (Fig. 4). This observation clearly indicates that the MA peak eluted from HPLC is the same material contained in the MA solution absorbing maximum UV.

It was found that free malonaldehyde was successfully separated and quantitated on a reversed phase column. The most important finding is that the total time elapsed to conduct the whole procedure was less than 15 minutes. The time required for analysis was considerably reduced by obviating the step of MA- TBA complex formation. Since this method directly measured free malonaldehyde successfully in a short period of time, the determination of the development of lipid oxidation using this method is expected to be rapid, accurate and specific. Analyzing the content of free malonaldehyde in food needs to be developed in the future.

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