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High Performance Liquid Chromatography (HPLC) Detection of Malonaldehyde-thiobarbituric Acid (MA-TBA) Complex in Ground Pork

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Abstract

For monitoring lipid oxidation development in cooked ground pork during refrigeration, malonaldehyde-thiobarbituric acid (MA-TBA) contents were measured using high performance liquid chromatography (HPLC). As the oxidation proceeded during refrigeration, TBA-reactive substances (TBARS) absorbances increased and the corresponding HPLC peak areas also increased proportionately. The correlation coefficient between the HPLC peak areas and MA-TBA absorbances were 0.9979. The treatment of cetrimide, an ion pairing agent, gave a complete resolution of the MA-TBA complex and the butanol extraction of the complex increased its recovery by 37.8%. Both cetrimide treatment and butanol extraction are essential steps for analyzing MA-TBA complex in ground pork with HPLC. A reliable and specific measurement of MA-TBA in ground pork was successfully performed using HPLC.

Key words: MA-TBA content, ground pork, high performance liquid chromatography, ion-pairing agent, butanol extraction

INTRODUCTION

Lipid oxidation is one of the major concerns to consumers and the food industry because it renders foods unacceptable and reduce their shelf lives (1). Moreover, lipid oxidation decreases the nutritional quality of foods and certain oxidized products produce toxic compounds (2). Although many methods have been devised for assesing the extent of oxidation development, thiobarbituric acid reactive substances (TBARS) values have been widely used for measuring oxidation of muscle foods. This method is simple, sensitive (3) and has high correlation with sensory evaluation results (3,4). It measures the intensity of a red chromogen produced from the reaction between malonaldehyde (MA, malondialdehyde, MDA) and TBA. The red complex produced has an absorption maximum at 532 nm, of which the optical densities were measured with spectrophotometer and reported as either TBARS absorbances or mg MA per kg of meat samples (4-7).

Even though this method is widely used, it has several disadvantages. It has been reported that TBARS is not specific because various other compounds interfere with the MA-TBA reaction and give higher values than the actual lipid oxidation level (7,8). More reliable and specific analyses of malonaldehyde were performed using high performance liquid chromatography (HPLC) (9-15) instead of measuring absorbances with a spectrophotometer. These methods were originally developed for measuring lipid oxidation in biological tissues such as plasma and liver (3). In a model system, a successful analysis of malonaldehyde produced from 1,1,3,3-tetraethoxypropane (TEP) was reported (16).

This study was carried out to apply the HPLC techniques that were found to be very successful in analyzing the content of malonaldehyde in biological tissues and a model system of a real food system, ground pork.

MATERIALS AND METHODS

Materials

1,1,3,3-Tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA) and antifoam were obtained from Sigma Chemical Co. (St. Louis, USA). Distilled water used in this experiment was all HPLC-grade. 2-Thiobarbituric acid was dissolved in distilled water. Methanol and butanol were HPLC-grade and were purchased from Merck (Darmstadt, Germany) and the ion-pairing agent, cetrimide (cetyltrimethylammonium bromide), was obtained from Sigma Chemical Co. for HPLC analysis. Fresh ground pork was purchased at a local butcher shop.

Release of MA from ground pork and formation of MA-TBA complex

Before the main experimental study, fresh ground pork as a test sample was prepared. In order to make oxidized pork, it was stored for 2 days in room temperature and MA-TBA analysis was performed both with spectrophotometer and HPLC. The major purpose of the test sample trial was to confirm the effectiveness of certrimide addition and butanol extraction on the improvement of MA-TBA resolution and recovery reported in the previous manuscript (16).

For the main experiment, fresh ground pork was cooked to an internal temperature of 60°C, wrapped with PVC film and stored in a refrigerator. At 0, 1, 2, 3 and 4 days during storage, the content of MA was measured by spectrophotometer and HPLC. Ground pork was homogenized and distilled

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following the addition of HCl (pH of homogenate is 1.5). Collected distillate was reacted with TBA in a boiling water bath for 35 minutes according to the method of Tarladgis et al. (4). For the measurement of MA-TBA complex, the red chromogen produced was analyzed with spectrophotometer and HPLC. Increases in the absorbance and peak height of MA-TBA in the distillate of ground pork during storage were monitored.

Spectrophotometric determination of TBARS absorbances

The absorbances of MA-TBA complex formed in ground pork were measured with the spectrophotometer (UVIKON 922, Kontron Instrument) at 532 nm.

HPLC analysis of MA-TBA complex

Analysis of MA-TBA was performed with a Young-Lin 930 HPLC. The system was equipped with an UV-VIS variable wavelength absorbance detector (Young-Lin M720) and the detection was made at 532 nm. The separation was carried out on a μBondapak C_{18} column (3.9 nm × 30 cm, 10 μm, Waters) with the mobile phase of methanol and distilled water mixture (75:25). Flow rate was 1 ml/min and column temperature was maintained at 30°C. Both solvents were filtered and degassing was performed with a degasser (DEGASYS DG 1310, UNIFLOWS, Tokyo, Japan).

Effect of addition of an ion pairing agent and butanol extraction on the resolution of MA-TBA

In order to confirm the effectiveness of addition of an ion-pairing agent on the resolution of MA-TBA complex (16), a test sample described above was prepared and 0.1% cetrimide was added to the moblile phase (w/v) and the resolution peaks were monitored.

Butanol extraction of MA-TBA, reported to increase the recovery of the complex and to protect the column (9), was employed and the recovery increase was monitored. The pH of the MA-TBA solution was reduced to less than 0.75 to maximize the recovery. One ml of butanol was added to the solution and the complex was extracted. After the butanol was evaporated with N_2 gas at a temperature of 35°C, the dried chromogen was resolubilized in distilled water and 20 μl was injected into the HPLC.

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

The HPLC eluant fraction representing MA-TBA complex was collected and the spectra at 400 to 700 nm were measured with a UV-VIS DA spectrophotometer (HP 8453, Waldbronn, Germany). The above spectra were compared with those of the chromogen in the MA-TBA solution produced both from the TEP model system and ground pork.

Release of MA from TEP and formation of MA-TBA complex

1,1,3,3-Tetraethoxypropane was chosen for a model system producing free malonaldehyde upon hydrolysis. 0, 2, 4, 6, 8 and 10×10^{-8} moles of TEP were prepared and TBA was added (final vol. 10 ml). This mixture was heated in a boiling water bath for 35 minutes resulting in the production of a red chromogen, MA-TBA complex.

RESULTS AND DISCUSSION

HPLC analysis of MA-TBA with a test sample

Distillates of test samples were reacted with TBA and solutions of red chromogens were analyzed. As shown in Fig. 1, without the addition of cetrimide to the mobile phase, MA-TBA in the distillate of ground pork coeluted with other compounds and gave an incomplete separation. When the MA-TBA solution was extracted with butanol, the recovery of the complex increased but still, the separation was not complete (data not shown).

However, Addition of cetrimide to the mobile phase, gave a complete separation of MA-TBA complex from the other compounds (Fig. 2). The retention time of the complex delayed from 4~5 to 8~9 minutes because the complex becomes neutral by the ionic interaction with an ion-pairing agent, cetrimide and the resultant decreased polarity makes the complex remain in the column for a longer time. The shape of MA-TBA peaks on the chromatogram in Fig. 2 is typical for a well-separated single compound. Therefore, for the main experimental analysis, it was decided that an ion-pairing agent, cetrimide, be added into the mobile phase.

Butanol extraction of MA-TBA increased its recovery by an average of 37.8% (data not shown). Both cetrimide addition to the mobile phase and butanol extraction of the complex are thought to be essential steps to be employed for a complete separation and analysis of MA-TBA in ground pork by HPLC.

Development of TBARS of cooked ground pork during refrigeration

The oxidation pattern of cooked ground pork during refrigeration was very similar to the experimental results reported previously (17). Absorbances of TBARS increased from

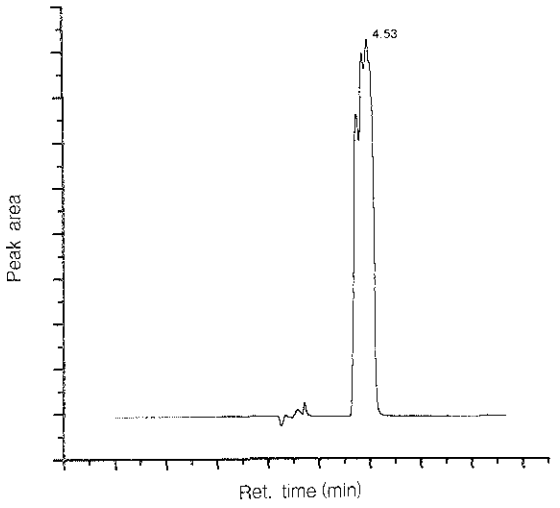

Fig. 1. HPLC elution profile of MA-TBA peak from oxidized ground pork (test sample) w/o the treatment of cetrimide (not extracted w/ butanol).

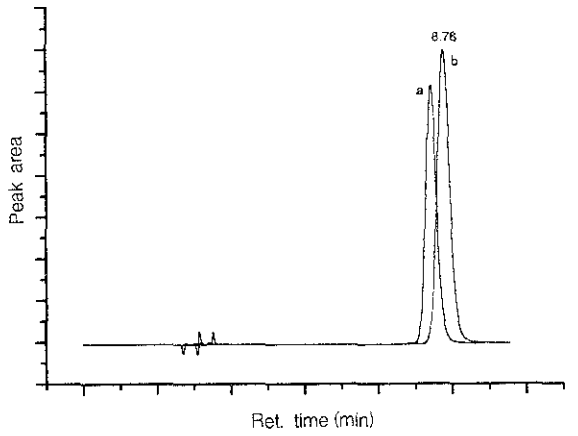

Fig. 2. HPLC elution profile of MA-TBA peaks from oxidized ground pork (test sample) w/ the treatment of cetrimide.
a: not extracted w/ butanol, b: extracted w/ butanol.

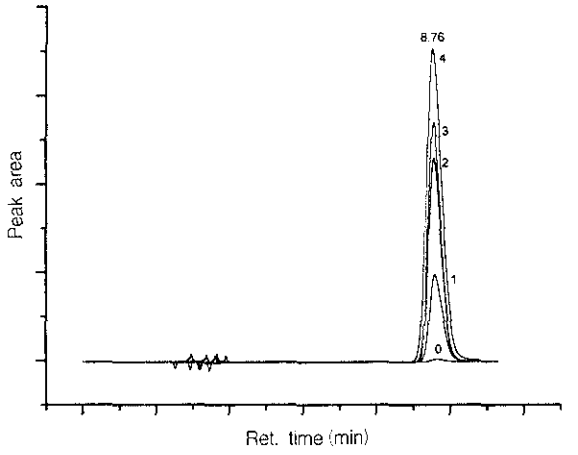

Fig. 4. HPLC chormatograms of MA-TBA peaks developed in ground pork during oxidation at 4°C. 0, 1, 2, 3, 4 (days)

0.0251 to 0.9081 during 4 days of refrigeration (Fig. 3). Cooked ground pork oxidizes rapidly even in refrigerated storage because of the catalytic action of iron which is freely released from myoglobin during the heating process (18). It was reported that cooked ground pork became rancid within 2 days during refrigeration (19,20).

HPLC chromatogram of increase in MA-TBA peak areas during oxidation

When the oxidation proceeded as shown by the increase in TBARS absorbances (Fig. 3) during refrigeration, the corresponding HPLC peak areas increased almost proportionately (Fig. 4). The correlation coefficients between HPLC peak areas and absorbances were 0.9979 for both non butanol-extracted and butanyl-extracted MA-TBA complex. This finding became very important because HPLC method was proven to be a very useful tool for measuring oxidation in a meat system. Since HPLC is a very specific and reliable analytical instrument, the application of the technique established in this study to other food systems should work very well.

Increase in the recovery of MA-TBA peaks (37.8%, data not shown) with butanol extraction and the increase in the peak areas with oxidation were also observed in this experiment. Butanol extraction seemed to have more effect in the recovery of the complex in foods than in a model system (29.4%) (16).

UV-VIS DA spectra of MA-TBA HPLC eluant

The HPLC eluant fraction collected for MA-TBA peak produced from ground pork was scanned with a UV-VIS DA spectrophotometer. The spectra of the chromogen produced from both TEP model system and ground pork were obtained and compared with the above spectra. As shown in Fig. 5, three components had the same absorption maxima at 532 nm. This confirms the fact that MA-TBA peak eluted from HPLC is the same compound with those chromogens produced from both TEP model system and oxidized ground pork.

It was concluded that the present method is very specific and reliable in measuring the development of lipid oxidation in meat. A successful application of HPLC technique used in biological samples and a model system to real food was established. A more rapid method of assessing free malonaldehyde needs to be studied in the future. The present method is expected to be utilized to analyze the malonaldehyde

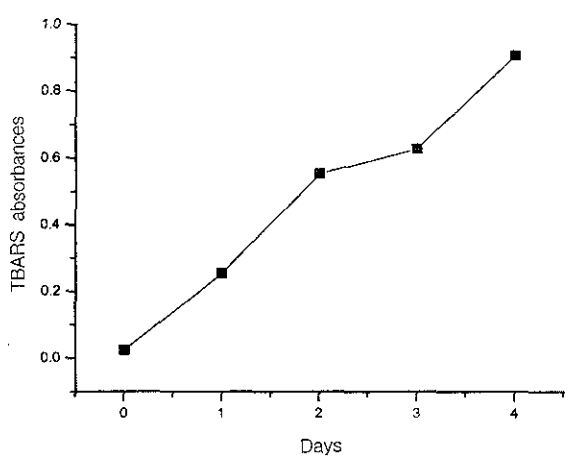

Fig. 3. Development of 2-thiobarbituric acid reactive substances (TBARS) absorbances of ground pork during storage at 4°C.

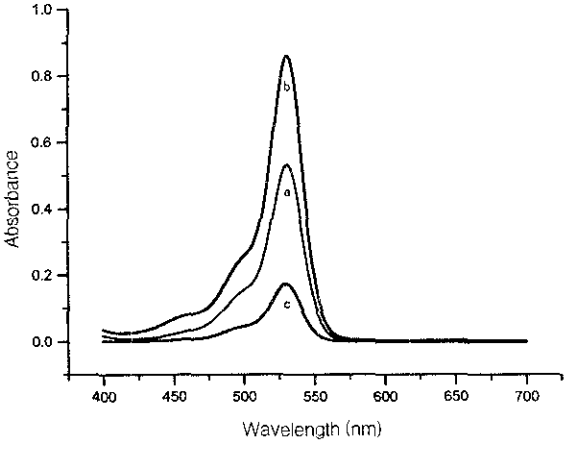

Fig. 5. UV-VIS DA spectra of MA-TBA chromogen produced from (a) a model system (b) ground pork and (c) those of the HPLC eluant fraction of MA-TBA from ground pork.

content in other lipid-containing foods.

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