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Short communication

High-performance liquid chromatographic determination of 1,3-diethyl-2-thiobarbituric acid–malonaldehyde adduct in fish meat

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Abstract

The reaction conditions of 1,3-diethyl-2-thiobarbituric acid (DETBA)–malonaldehyde (MA) adduct formation were examined in order to analyze MA in fish tissue by high-performance liquid chromatography. A reaction mixture containing 4 m*M* butyl hydroxytoluene was heated at 100°C for 150 min and the DETBA–MA adduct formed was separated by a Inertsil ODS column for 20 min. The detection limit was 5 pmol. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Malonaldehyde; 1,3-Diethyl-2-thiobarbituric acid

1. Introduction

Lipid peroxidation is a major cause of quality deterioration in meat products [1]. Fish is more prone to lipid peroxidation than meat, which is due to the high degree of unsaturation in fish lipids and to the high concentrations of metals in seafood [2]. In fish, lipid peroxidation is also known to cause several diseases [3–7]. For early diagnosis, therefore, it is necessary to routinely measure the lipid peroxidation levels in the tissue. 2-Thiobarbituric acid reactive substance (TBARS) values are widely used as an index of lipid peroxidation. Accurate TBARS values, however, can be obtained using only a few methods, because fish contain a large amount of polyunsaturated fatty acids which are very labile and are easily oxidizable in their tissue [8,9]. Therefore, we attempted to develop a sensitive, specific and simple high-performance liquid chromatography (HPLC) method for determining malonaldehyde (MA) content in fish tissue.

2. Experimental

2.1. Equipment

A Model BIP I pump unit (Japan Spectroscopic, Tokyo, Japan) with a VL-614 injector (Japan Spectroscopic) was used in this experiment. Chromatographic separation was performed using a Inertsil ODS column (5 μm particle size, 250×4.6 mm I.D.; GL Sciences, Tokyo, Japan) and the column eluent was monitored at 515 nm (excitation) and 555 nm (emission) by a Model FP-210 fluorescence detector (Japan Spectroscopic). A Model 805 GI graphic integrator (Japan Spectroscopic) was used for integration.

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2.2. Materials

1,3-Diethyl-2-thiobarbituric acid (DETBA) was purchased from Aldrich (Milwaukee, WI, USA), and butyl hydroxytoluene (BHT) was from Tokyo Kasei (Tokyo, Japan). Sodium dodecyl sulfate (SDS) and 1,1,3,3-tetraethoxypropane were from Wako Pure Chemicals (Osaka, Japan). All other chemicals used were of the purest grade commercially available.

2.3. Preparation of samples

The DETBA assay is based on the method of Therasse and Lemonnier [10] modified by Suda et al. [11] with some modifications. Fish were obtained commercially. The muscle was ground and immediately homogenized with nine volumes of an ice-cooled 0.9% NaCl solution in a Polytron homogenizer. The homogenate (0.5 ml) was transferred to a screw-capped tube containing 3.5 ml of 0.125 *M* phosphate buffer (pH 3, containing 0.4% SDS, 10 m*M* DETBA and 4 m*M* BHT). The solution was mixed and heated at 100°C for 150 min, and was then quickly cooled with running tap water. Four ml of ethyl acetate was added to extract the DETBA–MA adduct and the mixture was vigorously shaken. After centrifugation at 140 *g* for 10 min, 0.5 ml of the organic layer was transferred to another tube and evaporated to dryness in vacuo. The residue was dissolved in 200 μl of methanol, and 20 μl of the sample was analyzed by HPLC.

2.4. High-performance liquid chromatography

A 20-μl volume of the extract was injected onto the column. The mobile phase consisted of acetonitrile–0.1 *M* NaCl (75:25, v/v) and the flow-rate was 1 ml/min.

3. Results and discussion

Suda et al. [11] have analyzed the MA in various plant materials. The incubation conditions of DETBA–MA adduct formation were: heating temperature, 100°C, heating time, 15 min, BHT concentration of phosphate buffer, 1 m*M*. We analyzed the DETBA–MA adduct formed in meat or liver

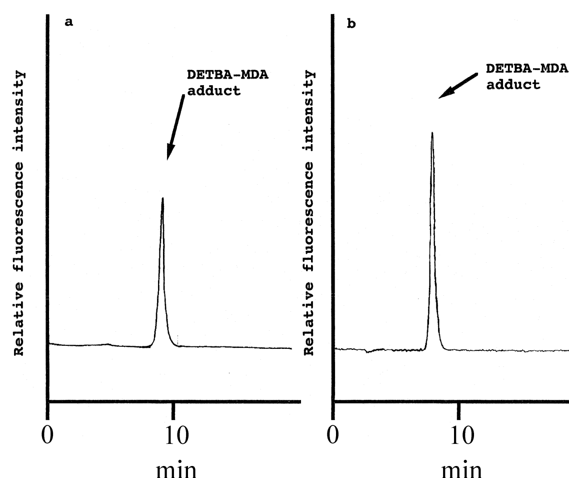

Fig. 1. HPLC chromatograms of DETBA–MA adduct. (a) DETBA–MA adduct formed from standard MA which was obtained from a hydrolysis of 1,1,3,3-tetraethoxy-propane (Wako Pure Chemicals). MA concentration was 15 nmol/ml. The incubation conditions of DETBA–MA adduct formation were: heating temperature, 100°C, heating time, 60 min, BHT concentration of phosphate buffer, 1 m*M*. (b) DETBA–MA adduct formed from meat of yellowtail. Incubation conditions of DETBA–MA adduct formation were as in (a).

from yellowtail using their method; however the peak of the DETBA–MA adduct was not detected in the chromatogram. Fig. 1 shows a HPLC chromatogram of the DETBA–MA adduct formed in meat from yellowtail after 60 min of incubation. The DETBA–MA adduct peak is indicated with a retention time of 8.5 min; however, as shown in Fig. 2, the longer the reaction time, the more the DETBA–MA adduct was formed. The amount of DETBA–MA adduct increased during 5 h of incubation. This may indicate that the BHT concentration is too low to prevent new MA formation in the meat during the incubation period. As shown in Fig. 3, the increase in the amount of DETBA–MA adduct stopped after 150 min of incubation in the reaction mixture containing 4 m*M* BHT. As shown in Fig. 4, the amounts of DETBA–MA formed were same in the reaction mixture containing more than 4 m*M* BHT after 150 min of incubation. From these results, we determined that incubation time was 150 min and BHT concentration was 4 m*M*. The calibration graph was linear in a range of 0–40 nmol/ml. The relative standard deviations (RSDs) in the ordinary muscle of

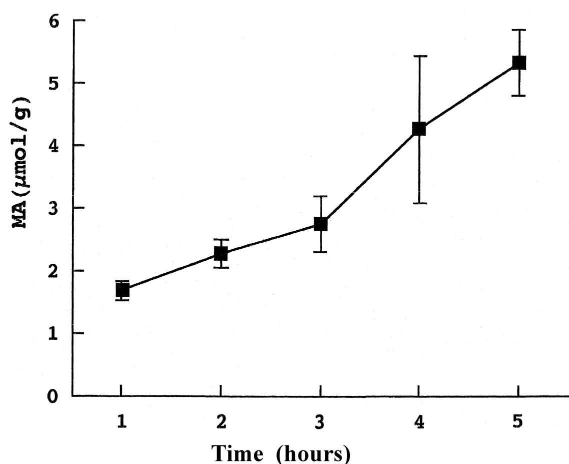

Fig. 2. Changes in the DETBA–MA formation in meat from yellowtail incubated under the following conditions: heating temperature, 100°C, BHT concentration of phosphate buffer, 1 m*M*.

yellowtail (0.08 μmol/g) and the red muscle (1.3 μmol/g) were 10.5 and 8.2%, respectively (n=4 each). Recoveries of 0.45 and 4.5 nmol of the MA added to ordinary muscle from yellowtail were 94.7±8.6 and 102.3±13.5%, respectively (n=6 each) and detection limit was less than 5 pmol. The MA content in the ordinary of some fish is determined using this method and distillation method of Yamauchi et al. [12] (Table 1). Yamauchi's method is thought to be one of the method from which accurate 2-thiobarbituric acid reactive substances values may be obtained [3,9,13]. Similar

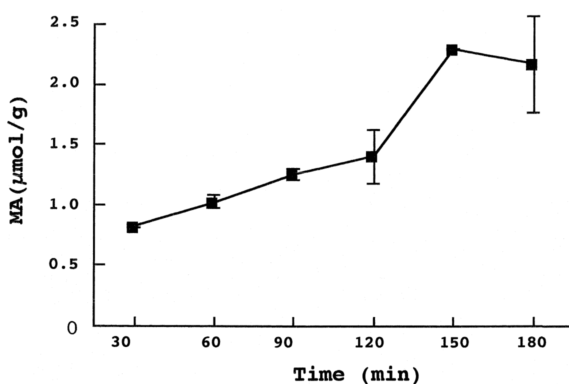

Fig. 3. Changes in the DETBA–MA formation in yellowtail meat incubated under the following conditions: heating temperature, 100°C, BHT concentration of phosphate buffer, 4 m*M*.

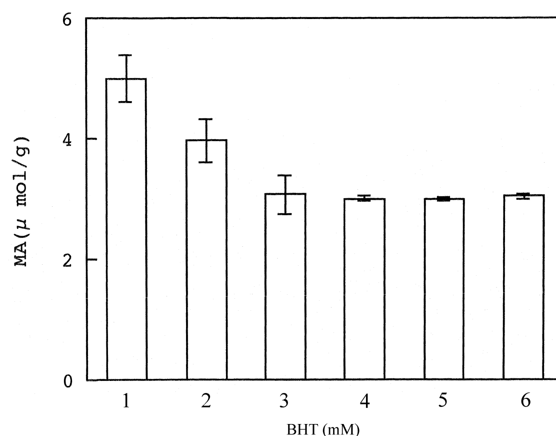

Fig. 4. Effect of BHT concentration on the DETBA–MA formation in yellowtail meat incubated under the following conditions: heating temperature, 100°C, heating time 150 min.

results were obtained from both method. However in contrast to Yamauchi's distillation method which requires more than 10 g of tissue and more than 15 h for incubation time, our method requires 0.5 g of tissue and 150 min for incubation time and may be useful in studying lipid peroxidation of fish tissue.

Acknowledgements

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Table 1
MA content measured by present method and TBARS values measured by Yamauchi's distillation method in the muscle of some fish

Species (Scientific name)	MA content[a] (nmol/g)	TBARS value[a] (nmol MA/g)
Flying fish (*Cypselurus agoo agoo*)	16.0	15.1
Tuna (*Thunnus thynnus*)	11.2	12.7
Japanese carsngid fish (*Truchurus japonicus*)	8.8	10.1
Yellowtail (*Seriola quinqueradiata*)	21.6	17.1
Red sea bream (*Pagrus major*)	44.4	48.0

[a] Average of duplicate analysis.

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