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Detection of malondialdehyde in processed meat products without interference from the ingredients

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ABSTRACT

Our aim was to develop a method for accurate quantification of malondialdehyde (MDA) in meat products. MDA content of uncured ground pork (Control); ground pork cured with sodium nitrite (Nitrite); and ground pork cured with sodium nitrite, sodium chloride, sodium pyrophosphate, maltodextrin, and a sausage seasoning (Mix) was measured by the 2-thiobarbituric acid (TBA) assay with MDA extraction by trichloroacetic acid (method A) and two high-performance liquid chromatography (HPLC) methods: i) HPLC separation of the MDA-dinitrophenyl hydrazine adduct (method B) and ii) HPLC separation of MDA (method C) after MDA extraction with acetonitrile. Methods A and B could not quantify MDA accurately in groups Nitrite and Mix. Nevertheless, MDA in groups Control, Nitrite, and Mix was accurately quantified by method C with good recovery. Therefore, direct MDA quantification by HPLC after MDA extraction with acetonitrile (method C) is useful for accurate measurement of MDA content in processed meat products.

1. Introduction

Products of lipid oxidation, such as aldehydes, ketones, alcohols, acids, and hydrocarbons, lead to undesirable changes in flavour, colour, and texture and a decrease in the nutritional value of foods (Frankel, 1996; Lee et al., 2015; St Angelo, 1996), thereby worsening overall quality of a food product. Lipid oxidation in foods is generally detected by measuring the concentration of malondialdehyde (MDA) because MDA is an abundant secondary product of lipid oxidation and relatively stable compared to lipid hydroperoxides, primary products of lipid oxidation, which readily decompose to other lipid oxidation products (Jung, Nam, Ahn, Kim, & Jo, 2013; Mendes, Cardoso, & Pestana, 2009; St. Angelo, 1996).

2-Thiobarbituric acid (TBA) is generally used for measurement of MDA content. Two molecules of TBA react with an MDA molecule under acidic conditions, and the MDA–TBA adduct forms a pink/red chromogen that can be detected on a spectrophotometer at 532–535 nm (Sinnhuber, Yu, & Yu, 1958; Ulu, 2004). The TBA assay is simple and reproducible, but TBA reacts with various carbonyl compounds in oxidised food; this situation leads to overestimation of MDA content (Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2012).

There are additional problems when the TBA assay is used for MDA quantification in meat products, especially cured meat. Nitrites are a major additive in cured meat products because nitrites provide cured meat colour and flavour as well as ensure control of *Clostridium botulinum* (Jung et al., 2015a, 2015b; Pegg & Shahidi, 2000). On the other hand, nitrites react with MDA under acidic conditions and lead to underestimation of MDA content when the TBA assay is applied to cured meat (Zipser & Watts, 1962). The latter authors suggested the use of sulfanilamide in the TBA assay of cured meat for prevention of condensation of a nitrite with MDA in a reaction of the nitrite with sulfanilamide before the reaction of the nitrite with MDA. Nevertheless, Kolodziejska, Skonieczny, and Rubin (1990) found no prevention effect of sulfanilamide after a complete reaction of a nitrite with MDA, and reported that sulfanilamide can only prevent the reaction of nitrite with MDA when added before the nitrite.

Moreover, formation of a yellow or orange chromogen is another problem in the TBA assay of meat products (Diaz, Linares, Egea, Auqui, & Garrido, 2014; Wang, Pace, Dessai, Bovell-Benjamin, & Phillips, 2002). The yellow or orange chromogen is formed in a reaction of TBA with various ingredients in meat products, such as sugars, water-soluble proteins and

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peptides, and pigments in spices and vegetables (Diaz et al., 2014; Shamberger, Shamberger, & Willis, 1977; Wang et al., 2002). This chromogen has absorbance at 532 nm, and consequently causes overestimation of MDA content (Diaz et al., 2014). The latter authors suggested that strongly diluted acid solutions of the TBA reagent and a heating time less than 1 h at 100 °C are the best conditions for the spectrophotometric TBA assay to minimise the interference of the yellow chromogen. Nonetheless, the method of Diaz et al. (2014) may not completely eliminate the interference of the yellow chromogen in the TBA assay of meat products containing sugar. Therefore, a specific method for quantification of MDA in meat products is needed.

To precisely measure MDA content present in foods and biological sample, various high-performance liquid chromatography (HPLC) methods have been suggested. Papastergiadis et al. (2012) precisely detected MDA in various oxidised foods by HPLC coupled with fluorescence detector based on the separation of MDA–TBA adduct after MDA extraction with trichloroacetic acid (TCA) or enzyme. Mendes et al. (2009) measured MDA content in fish by HPLC with an ultraviolet/visible (UV/VIS) detector after MDA extraction with TCA followed by derivatisation of MDA with TBA or 2,4-dinitrophenylhydrazine (DNPH). They showed that HPLC of the MDA–DNPH adduct has good specificity, recovery, and reproducibility. Tüközkan, Erdamar, and Seven (2006) measured MDA with good accuracy in human blood plasma and tissue of guinea pigs by detection of MDA–DNPH adduct with a HPLC-UV/VIS detector system after MDA extraction with acetonitrile (ACN). Karatas, Karatepe, and Baysar (2002) developed a simple HPLC method for MDA measurement in human serum; in this method, MDA is directly detected by HPLC with UV/VIS detector at 254 nm after MDA extraction with perchloric acid. On the other hand, these HPLC methods have not yet been applied to meat products.

The aim of this study was to develop a method for accurate quantification of MDA in meat products without interference from the ingredients that may be naturally present or added for specific purposes. To this end, two HPLC–UV/VIS detector systems; i) analysis of the MDA-DNPH adduct and ii) direct quantification of MDA, were used for MDA analysis after MDA extraction with ACN from several models of meat products. Then, the results were compared with those of spectrophotometric TBA assay.

2. Materials and methods

2.1. Reagents

2,6-Di-*tert*-butyl-4-methylphenol (BHT, PubChem CID: 31404), 37% hydrochloric acid (HCl, PubChem CID: 313), dibasic potassium phosphate (PubChem CID: 24450), and 1,1,3,3-tetraethoxypropane (TEP) were purchased from Sigma-Aldrich (St. Louis, MO). DNPH (PubChem CID: 3772977) and phosphoric acid (PubChem CID: 1004) were purchased from Fluka (Buchs, Germany). HPLC-grade ACN (PubChem CID: 6342) and glacial acetic acid (PubChem CID: 176) were purchased from J.T. Baker Co. (Center Valley, PA). Sodium hydroxide (NaOH, PubChem CID: 14798), TBA (PubChem CID: 2723628) and TCA (PubChem CID: 6421) were purchased from Alfa Aesar Co. (Ward Hill, MA).

2.2. Preparation of the models of meat products

Pork hind leg was purchased at a local market (Daejeon, Korea). The meat was trimmed to remove visible fat and connective tissue and then ground using a meat grinder (M-12S; Hankook Fugee Industries Co., Ltd., Hwaseong, Korea) with a 6-mm plate. Ground meat was subdivided into three groups: 1) ground pork without additives (Control group); 2) ground pork with 0.01% sodium nitrite (*w*/*w*; group Nitrite); and 3) ground pork with 0.01% sodium nitrite (*w*/*w*), 1% sodium chloride (*w*/*w*), 1% sodium pyrophosphate (*w*/*w*), 2% maltodextrin (*w*/*w*), and 1% sausage seasoning (*w*/*w*; group Mix). The ground meat was mixed in a food mixer (FPP230; Kenwood Ltd, Havant, UK) for 2 min after supplementation with the additives. After mixing, aliquots of meat batters (100 g) were individually vacuum-packed (−650 mmHg) in 20 × 15 cm low-density polyethylene/nylon vacuum bags (oxygen permeability 22.5 mL m^{-2} d^{-1} at 60% relative humidity (RH) and 25 °C; water vapour permeability of 4.7 g m^{-2} d^{-1} at 100% RH and 25 °C). The specimens of packaged meat batters were cooked in an 85 °C water bath for 30 min, and cooled in tap water for 30 min. The specimens were weighed in test tubes depending on the method used, and the test tubes were stored at −70 °C until analysis.

2.3. Detection of the MDA–TBA adduct on a spectrophotometer

MDA in meat product samples was detected by the TBA assay with spectrophotometry according to the method of Mendes et al. (2009) with a minor modification. MDA was extracted from the samples with a 7.5% TCA solution as follows. A sample of a meat product (3.0 g) was homogenised with 9 mL of the 7.5% TCA solution and 50 μL of 7.2% BHT in ethanol using a homogeniser (T25b; Ika Werke GmbH & Co. KG, Staufen, Germany) at 16,000 rpm for 1 min. The homogenate was centrifuged at 2090*g* for 15 min (Union 32R;, Hanil Co., Ltd., Incheon, South Korea), and filtered through a Whatman No. 4 filter paper (Whatman, Maidstone, UK). This filtrate was used as the MDA extract. TEP (the MDA standard) was accurately diluted with 0.1 M HCl to a concentration of 3.2 mM (stock solution), and then kept for 2 h at room temperature in the dark. After hydrolysis, the TEP stock solution was diluted with 7.5% TCA solution to the concentration of 1, 2, 4, 8, 16, or 32 μM. After that, 1 mL of MDA extract, standard, or 7.5% TCA solution (blank) was transferred into a screw-cap tube, and 1 mL of a 20 mM TBA solution was added. The tubes were heated in a boiling water bath at 90 °C for 30 min and cooled in tap water for 10 min. Absorbance of the MDA-TBA adduct was measured at 532 nm on a spectrophotometer (DU®530; Beckman Coulter Inc., Brea, CA). The concentration of MDA in a sample was expressed in milligrams of MDA per kilogram of meat product (mg MDA/kg meat product).

2.4. Detection of the MDA–DNPH adduct by HPLC

This procedure was conducted according to the method described by Tüközkan et al. (2006) and Mendes et al. (2009). MDA was extracted from the samples with ACN as follows. A sample of a meat product (3.0 g) was homogenised with 6 mL of deionised (DI) water and 50 μL of 7.2% BHT in ethanol using a homogeniser (T25b) at 16,000 rpm for 1 min. Next, 500 μL of the homogenate were transferred into an Eppendorf tube, and 100 μL of 6 M NaOH solution (final concentration 1 M) were added for alkaline hydrolysis of protein-bound MDA. The tubes were incubated in a water bath at 60 °C for 45 min. After cooling at room temperature, 1 mL of ACN was added into each tube, and the mixture was vigorously vortexed. The tube was centrifuged at 13,000*g* for 10 min (HM-150IV; Hanil Co., Ltd., Incheon, South Korea). The clear upper part of the supernatant served as the MDA extract. As the MDA standard, TEP stock solution (3.2 mM, see above) was diluted with DI water to the concentration of 0.5, 1, 2, 4, 8, and 16 μM. After that, 1 mL of the MDA extract, standard, or DI water (blank) was transferred into an Eppendorf tube, mixed with 100 μL of 5 mM DNPH in 2 M HCl, and incubated for 10 min at room temperature for derivatisation. The solution containing