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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

MDA–DNPH adduct was passed through a 0.2-μm polyvinylidene difluoride (PVDF) syringe filter (Whatman), and the filtrate was collected in a vial. MDA–DNPH was analysed by HPLC (ACME 9000, Younglin Instruments Inc., Gyeonggi-do, South Korea). As for the analytical conditions of HPLC, an Atlantis T3 C18 RP column (4.6 × 250 mm, 5-μm particles, Waters Corp., Milford, MA) was used with the mobile phase consisting of water, ACN, and glacial acetic acid (55:45:0.2, *v*/*v*/*v*). The isocratic flow rate of the mobile phase was 1.0 mL/min, and the injection volume was 50 μL. The column temperature was maintained at 35 °C, and the UV/VIS detector was set at 310 nm. The concentration of MDA in a sample was expressed in mg MDA/kg meat product.

2.5. Direct quantification of MDA by HPLC

This procedure was conducted according to the method of Karatas et al. (2002), with modifications in the MDA extraction process and the mobile phase. For this analysis, MDA was extracted from the samples with ACN as follows. A meat product sample (3.0 g) was homogenised with 6 mL of DI water and 50 μL of 7.2% BHT in ethanol by means of 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 to room temperature, 1 mL of ACN was added into the tube, and the mixture was vigorously vortexed. The tube was centrifuged at 13,000*g* for 10 min (HM-150IV; Hanil). The clear upper part of supernatant served as the MDA extract. As an MDA standard, the TEP stock solution (3.2 mM, see above) was diluted with DI water to concentrations of 0.1, 0.2, 0.4, 0.8, and 1.6 μM. After that, 1 mL of the MDA extract, standard, or DI water (blank) was passed through a 0.2-μm PVDF syringe filter (Whatman) and the filtrate was collected in a vial. MDA was then analysed by HPLC (ACME 9000, Younglin Instruments Inc., Gyeonggi-do, South Korea). As for the analytical conditions of the HPLC, an Atlantis T3 C18 RP column (4.6 × 250 mm, 5-μm particles) was used with a mobile phase consisting of 30 mM K_2HPO_4 (pH adjusted to 6.2 with phosphoric acid). The isocratic flow rate of the mobile phase was 1.2 mL/min, and the injection volume was 50 μL. The column temperature was maintained at 35 °C and the UV/VIS detector was set to 254 nm. The concentration of MDA in a sample was expressed in mg MDA/kg meat product.

For validation of the method, the limit of detection (LOD), limit of quantification (LOQ), recovery, and accuracy were measured. LOD and LOQ were determined on the basis of the detector signal-to-noise ratio and a calibration curve (Dias, Camões, & Oliveira, 2008). LOD was determined by means of the expression "3.3 standard deviations (SD) ÷ slope" and was based on SD of the peak areas of the MDA standard solution at a concentration near the blank. LOQ was calculated by using the factor of 10 instead of 3.3 in the above expression. For assessment of analytical recovery, a 2-mL sample of hydrolysate (after incubation of 5-mL sample of the homogenate with 1 mL of 6 M NaOH in a water bath at 60 °C for 45 min) was mixed with 1 mL of 2 M HCl to adjust pH to neutral. After that, 1 mL of 1.6 μM TEP solution was added. The accuracy (reproducibility) was measured by determining reproducibility of analysis of the MDA standard solution (0.32 μM) and of the MDA extract from a sample. The reproducibility was expressed as the percentage of relative standard deviation (RSD %). Quantification of MDA in the MDA standard solution was repeated five times on the same day and additionally on five consecutive days to determine intraday and interday variations. To measure interday accuracy by means of the MDA extract from a meat product sample, the filtrate of MDA extract from the sample was collected into two vials. The amount of MDA in the second vial was determined 3 days after analysis of MDA in the first vial.

2.6. Statistical analysis

The MDA quantification in the samples and standards was performed in triplicate. Data were subjected to the analysis of variance procedure of SAS software (version 9.3; SAS Institute Inc., Cary, NC). Differences among the means were assessed by Tukey's multiple-range test. The results are reported as mean ± SD. Statistical significance was assumed at $p < 0.05$.

3. Results and discussion

3.1. Detection of MDA by the spectrophotometric TBA assay

MDA content (TBA reactive substances value) in groups Control, Nitrite, and Mix as measured by the TBA assay was 2.200, 0.723, and 0.701 mg/kg meat product, respectively (Table 1). MDA concentrations in groups Nitrite and Mix were less than 30% of the control value ($p < 0.05$). MDA in foods exists as free or combined with protein and fat by means of aldol condensation (Vandermoortele & Meulenaer, 2015). Therefore, the extraction procedures of MDA from foods are involved with MDA detection. Acids such as TCA and perchloric acid are used for MDA extraction with the TBA assay, although acids could not completely release MDA, because TBA reacts with MDA under acidic conditions, and acids minimise interference from proteins (Kolodziejska et al., 1990; Ulu, 2004; Vandermoortele & Meulenaer, 2015). Nevertheless, nitrites react with MDA under acidic conditions (condensation reaction) and cause underestimation of MDA (Zipser & Watts, 1962). Kolodziejska et al. (1990) found that over 99.9% of MDA reacts with nitrite at pH < 3, and no MDA reacts with the nitrite at pH 6 when the molar ratio of MDA to nitrite is 1:5. Other studies showed that the reactive form of a nitrite for formation of the MDA-nitrite adduct is probably nitrous acid, whose pK_a is 3.4; therefore, less than 1% of the nitrite is in the form of nitrous acid at pH > 5.5 (Kolodziejska et al., 1990; Sebranek & Fox, 1985). After reaction of TBA with MDA extract, the yellow chromogen formed in the aqueous phase of Mix samples because Mix samples contained sugar and the sausage seasoning. Diaz et al. (2014) reported that sugar yields a yellow chromogen in a reaction with TBA and causes overestimation of MDA. The TBARS value of group Mix, however, was lower than that of the Control group and similar to that of group Nitrite in the present study. This result may be explained by the strong interference of the nitrite.

Table 1
Malondialdehyde (MDA; mg/kg meat product) in models of meat products quantified by the 2-thiobarbituric acid (TBA) assay and two high-performance liquid chromatography (HPLC) methods.

Methods	Meat product model[A]		
	Control	Nitrite	Mix
MDA-TBA adduct/spectrophotometer	2.200 ± 0.028[Ba]	0.723 ± 0.018[b]	0.701 ± 0.011[b]
MDA-DNPH adduct/HPLC	0.325 ± 0.001[a]	0.020 ± 0.005[c]	0.087 ± 0.010[b]
MDA direct/HPLC	0.354 ± 0.012[a]	0.274 ± 0.012[b]	0.290 ± 0.013[b]

[A] Control: ground pork without ingredients; Nitrite: ground pork with 0.01% sodium nitrite (*w*/*w*); Mix: ground pork with 0.01% sodium nitrite (*w*/*w*), 1% sodium chloride (*w*/*w*), 1% sodium pyrophosphate (*w*/*w*), 2% maltodextrin, and 1% sausage seasoning.
[B] Mean ± standard deviation ($n = 3$).
[a–c] Different letters within same row differ significantly ($p < 0.05$).

3.2. Detection of MDA by HPLC with the MDA–DNPH adduct

MDA content of the Control samples as measured by HPLC with the MDA–DNPH adduct was 0.325 mg/kg meat product (Table 1). This level was substantially lower than the MDA concentration detected by the TBA assay. Tüközkan et al. (2006) reported that the MDA concentrations in the kidney and liver of guinea pigs as measured by HPLC with the MDA–DNPH adduct are around 10% of the MDA concentration measured by the TBA assay. MDA concentrations in the Nitrite and Mix samples were 0.020 and 0.087 mg/kg meat product, respectively (Table 1), when measured by HPLC with the MDA–DNPH adduct. This result means that the MDAs in the Nitrite and Mix samples were not detected fully. In the present study, MDA was extracted from the samples with ACN to avoid the reaction of nitrites with MDA under acidic conditions. Then, the MDA extract solution was reacted with a DNPH solution. The DNPH solution was prepared in 2 M HCl because DNPH is soluble in acids, and derivatisation of DNPH with MDA proceeds under acidic conditions (Tüközkan et al., 2006). Therefore, it is likely that MDA reacts with nitrite during the derivatisation process under acidic conditions before the reaction of MDA with DNPH. Tüközkan et al. (2006) and Mendes et al. (2009) stated that HPLC with the MDA–DNPH adduct is a specific method for detection of MDA in biological samples and fish meat. On the other hand, this method cannot be used for quantification of MDA in meat products, especially cured meat products containing nitrite.

3.3. Analysis of MDA directly by HPLC

The method for direct quantification of MDA in biological samples was developed by Karatas et al. (2002). This method cannot be used directly for analysis of MDA in models of meat products because those authors extracted MDA from biological samples using perchloric acid and used a mild acid mobile phase (pH 4) for the operation of HPLC. Therefore, the extraction method and mobile phase were modified in the present study. To prevent the reaction of nitrite with MDA under acidic conditions, MDA in our models of meat products was extracted with ACN after hydrolysis of the sample homogenates with 1 M NaOH (final concentration), according to the method of Tüközkan et al. (2006), and 30 mM K_2HPO_4 (pH 6.2) served as the mobile phase in HPLC.

The chromatograms that we obtained with the MDA standards or samples are presented in Fig. 1. MDA peaks were identified by means of the standards at the retention time of 3.106 min. The calibration curves obtained in the MDA concentration range 0.1, 0.2, 0.4, 0.8, and 1.6 μM showed good linear regression ($y = 38.316x - 0.2538$; $r^2 = 0.9989$). MDA compounds in the Control, Nitrite, and Mix samples were clearly separated in the chromatogram. The areas of the MDA peak from groups Control, Nitrite, and Mix corresponded to 0.354, 0.274, and 0.290 mg/kg meat product, respectively (Table 1). MDA content of Control group was similar to that of the control samples analysed by the method of the MDA–DNPH adduct. Although the MDA concentrations in groups Nitrite and Mix were significantly lower than the MDA level of the Control group, the similarity of the MDA concentrations among the three groups appeared to be higher than the similarity observed with the TBA assay and the MDA–DNPH method. The lower MDA content in groups Nitrite and Mix may be explained by the antioxidant activity of the nitrite in the Nitrite samples and the nitrite and phosphate in the Mix samples (Sebranek, 2009).

For validation of this method, LOD, LOQ, recovery, and accuracy were measured. LOD and LOQ were 1.4 and 4.1 ng MDA/mL, respectively (0.019 and 0.057 μM MDA; data not shown). These values were lower than those of the spectrophotometric TBA assay and of the HPLC method with the MDA-DNPH adduct reported in another study (Mendes et al., 2009): LOD and LOQ were found to be 0.16 and 0.23 μM MDA, respectively, for the spectrophotometric TBA assay and were 0.20 and 0.26 μM MDA for the HPLC method with the MDA–DNPH adduct (Mendes et al., 2009).

Recovery of MDA in our method was measured after the MDA standard was added to the hydrolysate of control, Nitrite, and Mix samples. The recovery ranged between 101.9 and 108.4% (Table 2). Mendes et al. (2009) reported that the recovery of spectrophotometric TBA assay is poor (66–71%), but the HPLC method with MDA–DNPH adduct shows good recovery ranging between 90 and 112%. It is likely that the method for direct analysis of MDA by HPLC after MDA extraction with ACN yields good recovery.

Accuracy (reproducibility) was studied by determining reproducibility of analysis of the MDA standard solution and of the MDA extract from a meat product sample. RSD% of intraday and interday analysis of the MDA standard solution was 3.26 and 3.83, respectively (data not shown). To measure the interday accuracy of analysis of the MDA extract from a sample, MDA content of the same sample was remeasured 3 days after the first quantification. There were no significant differences in the MDA concentration of groups Control, Nitrite, and Mix between the first and second quantification (Table 3). Mendes et al. (2009) reported that RSD% of interday analysis of a fish sample by HPLC with the

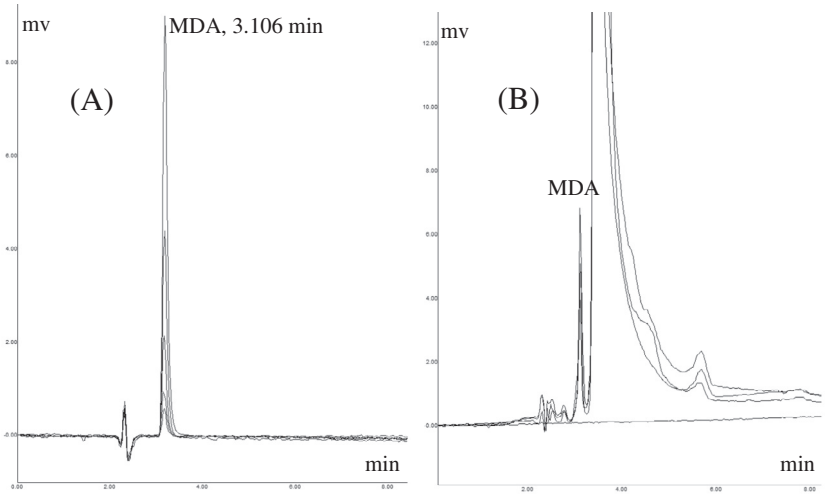

Fig. 1. High-performance liquid chromatography (HPLC) traces of (A) the malondialdehyde (MDA) standard at the concentrations 0.1, 0.2, 0.4, 0.8, or 1.6 μM and (B) samples of meat product models.

Table 2
Recovery of malondialdehyde (MDA) in samples in the method for direct quantification of MDA by high-performance liquid chromatography (HPLC).

Meat product model[a]	MDA content (mg/kg meat product)			
	MDA in sample	MDA added	MDA detected	Recovery (%)
Control	0.354 ± 0.012[b]	0.173	0.527 ± 0.012	102.4 ± 0.03
Nitrite	0.274 ± 0.012	0.173	0.447 ± 0.012	101.9 ± 0.04
Mix	0.290 ± 0.013	0.173	0.463 ± 0.013	108.4 ± 0.10

[a] Control: ground pork without ingredients; Nitrite: ground pork with 0.01% sodium nitrite (*w*/*w*); Mix: ground pork with 0.01% sodium nitrite (*w*/*w*), 1% sodium chloride (*w*/*w*), 1% sodium pyrophosphate (*w*/*w*), 2% maltodextrin, and 1% sausage seasoning.
[b] Mean ± standard deviation ($n = 3$).

Table 3
Interday accuracy of the method for direct quantification of malondialdehyde (MDA) by high-performance liquid chromatography (HPLC).

Meat product model[a]	MDA content (mg/kg meat product)		RSD (%)[c]
	MDA with first detection	MDA with second detection	
Control	0.354 ± 0.012[b]	0.375 ± 0.035	6.5
Nitrite	0.274 ± 0.012	0.289 ± 0.012	3.5
Mix	0.290 ± 0.013	0.287 ± 0.005	3.5

[a] Control: ground pork without ingredients; Nitrite: ground pork with 0.01% sodium nitrite (*w*/*w*); Mix: ground pork with 0.01% sodium nitrite (*w*/*w*), 1% sodium chloride (*w*/*w*), 1% sodium pyrophosphate (*w*/*w*), 2% maltodextrin, and 1% sausage seasoning.
[b] Mean ± standard deviation ($n = 3$).
[c] Relative standard deviation of the mean of the data from first and second detection.

MDA–DNPH adduct was 11.4%. In the present study, RSD% of the method for direct quantification of MDA by HPLC for groups control, Nitrite, and Mix was 6.5, 3.5, and 3.5, respectively (Table 3). These values were lower than the value obtained by the HPLC method with the MDA–DNPH adduct described by Mendes et al. (2009). Therefore, the method for direct measurement of MDA concentration by HPLC yields good reproducibility.

4. Conclusion

In this study, we tried to develop a method for accurate measurement of MDA content of meat products. According to the results of this study, the TBA assay and the HPLC method with the MDA–DNPH adduct are not applicable to MDA quantification in our models of meat products because these two methods involve an acid in the analytical procedure and the nitrite in the models of meat products reacts with MDA under acidic conditions. Therefore, a method that does not involve an acid in the analytical procedure from MDA extraction to MDA quantification is preferred. Accordingly, MDA was extracted from our models of meat products with ACN, and then MDA was directly quantified by HPLC with a UV/VIS detector at 254 nm and phosphate buffer (pH 6.2) as a mobile phase. This method shows good sensitivity for MDA in our models of meat products. In addition, this method yields good recovery and accuracy. Therefore, the method with direct MDA analysis by HPLC after MDA extraction by ACN is accurate and useful for measurement of MDA concentration in processed meat products.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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