MEASUREMENT OF MALONDIALDEHYDE IN CURED MEAT PRODUCTS WITHOUT INTERFERENCE FROM THE INGREDIENTS

Samooel Jung[1], Ju Ri Lee[1], Cheol Woo Lee[1], Ki Chang Nam[2] and Cheorun Jo[3,*]

[1]Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Korea; [2]Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, South Korea; [3]Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, South Korea

[*]Corresponding author email: cheorun@snu.ac.kr

Abstract – Our aim was to develop a method for accurate quantification of malondialdehyde (MDA) in meat products. MDA content of uncured ground pork (Control group); ground pork cured with sodium nitrite (Nitrite group); and ground pork cured with sodium nitrite, sodium chloride, sodium pyrophosphate, maltodextrin, and a sausage seasoning (Mix group) 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. Therefore, direct MDA quantification by HPLC after MDA extraction with acetonitrile (the method C) is useful for accurate measurement of MDA content in processed meat products.

Key Words – lipid oxidation, MDA, meat products, nitrite, HPLC

I. INTRODUCTION

2-Thiobarbituric acid (TBA) is generally used for measurement of MDA content. The TBA assay is simple and reproducible, but TBA reacts with various carbonyl compounds in oxidized food; this situation leads to overestimation of MDA content [1]. 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. However, nitrites react with MDA under acidic condition and lead to underestimation of MDA content when the TBA assay is applied to cured meat [2]. Moreover, formation of a yellow or orange chromogen is another problem in the TBA assay of meat products [3]. 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 peptides, and pigments in spices and vegetables [3]. This chromogen has absorbance at 532 nm, and consequently, causes overestimation of MDA content [3]. 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 [4, 5, 6]. Previous studies reported that HPLC method has good specificity, recovery, and reproducibility for MDA determination. 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.

II. MATERIALS AND METHODS

Preparation of the models of meat products

Ground pork meat was subdivided into three groups: 1) ground pork without additives (control group); 2) ground pork with 0.01% of sodium nitrite (w/w; group Nitrite); and 3) ground pork with 0.01% of sodium nitrite (w/w), 1% of sodium chloride (w/w), 1% of sodium pyrophosphate (w/w), 2% of maltodextrin (w/w), and 1% of a sausage seasoning (w/w; group Mix). The ground meat was mixed in a food mixer for 2 min after supplementation with the additives. After mixing, aliquots of meat batters (100 g) were individually vacuum-packaged (-650 mmHg) in 20 x 15 cm vacuum bags. 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 in a freezer at -70°C until analysis.

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. [3].

Detection of the MDA-DNPH adduct by HPLC

MDA in meat product samples was extracted according to the method described by Tüközkan et al. [6]. The 1 mL of the MDA extract mixed with 100 μL of 5 mM DNPH in 2 M HCl, and incubated for 10 min at room temperature for derivatization. The MDA-DNPH adduct was analyzed by HPLC according to the method described by Mendes et al. [4].

Direct quantification of MDA by HPLC

This procedure was conducted according to the method of Karatas et al. [5] 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 homogenized with 6 mL of DI water and 50 μL of 7.2% BHT in ethanol by means of a homogenizer at 16,000 rpm for 1 min. Next, 500 μL of the homogenate was transferred into an Eppendorf tube, and 100 μL of 6 M NaOH solution (final concentration 1 M) was added for alkaline hydrolysis of protein bound MDA. The tubes were incubated in water bath at 60°C for 45 min. After cooling at 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. The clear upper part of supernatant served as the MDA extract. As an MDA standard, the TEP stock solution (3.2 mM) was diluted with DI water to the concentration 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, and the filtrate was collected into a vial. MDA was then analyzed by HPLC. As for the analytical conditions of the HPLC, an Atlantis T3 C18 RP column (4.6 × 250 mm, 5-μm particles) was used with the mobile phase consisting of 30 mM K_2HPO_4 (pH was 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 UV/VIS detector was set to the wavelength of 254 nm. The concentration of MDA in a sample was expressed in mg MDA/kg meat product.

Statistical analysis

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

III. RESULTS AND DISCUSSION

Detection of MDA by the spectrophotometric TBA assay

MDA content (TBA reactive substances [TBARS] 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). Acids such as TCA and perchloric acid are used for MDA extraction with the TBA assay, although acids could not completely release MDA from protein and fat, because TBA reacts with MDA under acidic

condition, and acids minimize interference from proteins [7, 8]. Nevertheless, nitrites react with MDA under acidic condition (condensation reaction) and cause underestimation of MDA [2]. Kolodziejska et al. [9] found that over 99.9% of MDA reacts with nitrite at pH < 3. 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. Díaz et al. [3] 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.

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. 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 derivatization of DNPH with MDA is proceeded under acidic conditions [6]. Therefore, it is likely that MDA reacts with nitrite during the derivatization process under acidic conditions before the reaction of MDA with DNPH. Therefore, this method cannot be used for quantification of MDA in meat products, especially cured meat products containing nitrite.

Analysis of MDA directly by HPLC

The method for direct quantification of MDA in biological samples was developed by Karatas et al. [5]. 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 the 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. [6], 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 were corresponded to 0.354, 0.274, and 0.290 mg/kg meat product, respectively (Table 1).

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

	Meat product model[1]		
Methods	Control	Nitrite	Mix
MDA-TBA adduct /spectrophotometer	2.200 ± 0.028[2a]	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]

[1]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.
[2]Mean ± standard deviation (n=3).
[a,b]Different letters within same row differ significantly ($p < 0.05$).

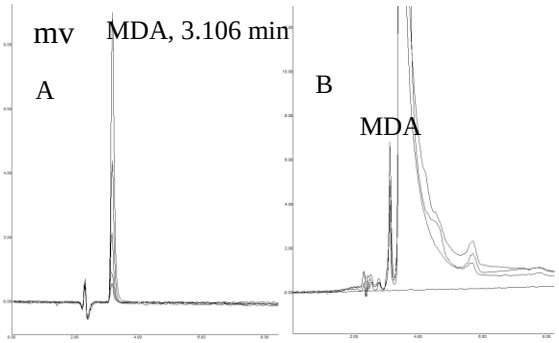

Fig 1. HPLC traces of (A) the malondialdehyde (MDA) standard at the concentrations 0.1, 0.2, 0.4, 0.8, or 1.6 μM and (B) samples of meat product models

MDA content of control group was similar to that of the control samples analyzed 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 [10]

IV. 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. Therefore, the method with the direct MDA analysis by HPLC after MDA extraction by ACN is accurate and useful for measurement of MDA concentration in processed meat products.

ACKNOWLEDGEMENTS

This study was supported by High Value-added Food Technology Development Program (115014-03-1-HD020), Ministry of Agriculture, Food and Rural Affairs (MAFRA) Republic of Korea.

REFERENCES

1. Papastergiadis, A., Mubiru, E., Van Langenhove, H., & De Meulenaer, B. (2012). Malondialdehyde measurement in oxidized foods: Evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. Journal of Agricultural and Food Chemistry, 60, 9589-9594.
2. Zipser, M. W., & Watts, B. M. (1962). A modified 2-thiobarbituric acid (TBA) method for determination of malonaldehyde in cured meats. Food Technology, 16, 102-104.
3. Diaz, P., Linares, M. B., Egea, M., Auqui, S. M., & Garrido, M. D. (2014). TBARs distillation method: Revision to minimize the interference from yellow pigments in meat products. Meat Science, 98, 569-573.
4. Mendes, R., Cardoso, C., & Pestana, C. (2009). Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. Food Chemistry, 112, 1038-1045.
5. Karatas, F., Karatepe, M., & Baysar, A. (2002). Determination of free malondialdehyde in human serum by high-performance liquid chromatography. Analytical Biochemistry, 311, 76-79.
6. Tüközkan, N., Erdamar, H., & Seven, I. (2006). Measurement of total malondialdehyde in plasma and tissues by high-performance liquid chromatography and thiobarbituric acid assay. Firat Tip Dergisi, 11, 88-92.
7. Ulu, H. (2004). Evaluation of three 2-thiobarbituric acid methods for the measurement of lipid oxidation in various meats and meat products. Meat Science, 67, 683-687.
8. Vandermoortele, A., & Meulenaer, B. D. (2015). Behavior of malondialdehyde in oil-in-water emulsions. Jorunal of Agricultural and Food Chemistry, 63, 5694-5701.
9. Kolodziejska, I., Skonieczny, S., & Rubin, L. J. (1990). Malondialdehyde-nitrite interactions in meat and model systems. Journal of Food Science, 55, 925-928.
10. Sebranek, J. G. (2009). Basic curing ingredients. In R. Tarte (Ed.), Ingredients in meat products: properties, functionality and applications, (pp. 1-24). New York: Springer.