DETERMINATION OF MALONALDEHYDE IN MEAT BY STIR BAR SORPTIVE EXTRACTION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Abstract – The traditional methods for analysis of malonaldehyde (MDA), such as thiobarbituric acid (TBA) assay, require strong acidic conditions at high temperature for derivatization and lack specificity under HPLC analysis. Stir bar sorptive extraction (SBSE) coupled with thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) with in situ derivatization using pentafluorophenylhydrazine (PFPH), under a relative moderate condition is an emerging technique for MDA analysis. MDA in meat was derivatized with PFPH at pH ~ 4 for 1 h at room temperature, forming a relative stable derivative of MDA-PFPH. The derivative of MDA-PFPH was simultaneously extracted using stir bar sorptive extraction. Following, MDA-PFPH was thermally released and quantitatively analyzed by GC/MS under selected ion monitoring (SIM) mode. The method of SBSE-TD-GC/MS for MDA analysis with in situ derivatization was optimized and validated with good linearity, specificity and limit of detection/quantification (LOD/LOQ). The SBSE-TD-GC/MS method was suitable to monitoring and analyzing MDA in meat samples at trace level.

I. INTRODUCTION

Lipid peroxidation is the major form of quality deterioration, including flavor, odor, taste, color, texture, and/or appearance, leading to spoilage in meat and fish products, even when lipid content is fairly low[1, 2]. Prolonged storage under unfavorable conditions can create rancid odors described from the products of autoxidation of unsaturated fatty acids (PUFAs), reacting with oxygen to produce a free radical. It further forms peroxyl radicals and reacts with other fatty acid, resulting in the propagation of a chain reaction. The hydroperoxides formed during propagation decompose and form secondary products, such as aldehydes, ketones, alcohols, acids and hydrocarbons. Among the secondary products, aldehydes, especially malonaldehyde, are largely responsible for rancid flavor development in meats. The degree of rancidity has been traditionally measured using an assay for the determination of malonaldehyde by its reaction with thiobarbituric acid (TBA). The detected threshold value of MDA reported at 1 - 2 mg/kg for rancidity by using TBA[3] and the sensory threshold was in a range of 0.5 to 1.3 mg/Kg for sensory tests in meat[4]. Due to harsh derivatization conditions of TBA (100 °C, pH ≤ 3) and also lack specificity of the TBA assay, the amount of malonaldehyde in some cases has been found to overestimate with artefactual MDA formation in sample preparation[5, 6].

A newly method of stir bar sorptive extraction (SBSE) coupled with thermal desorption (TD)-GC/MS has been widely used, as a simple and fast sampling method in food and environmental analysis[7, 8]. The aim of the present study is to develop a rapid, sensitive and solvent-less method for determination of MDA by using SBSE-TD-GC/MS with *in situ* derivatization with PFPH in meat.

II. MATERIALS AND METHODS

A. Chemicals and stir bars

2,6-Di-tert-butyl-4-methyphenol (BHT), 1,1,3,3-tetramethoxypropane (TEP), pentafluorophenylhydrazine (PFPH) and potassium phosphate monobasic solution (1M KH2PO4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of

analytical grade. The commercial stir bars [Twister TM], incorporated in a glass jacket and coated with polydimethylsiloxane (PDMS) (length: 10 mm; thickness: 0.5 mm), as well as the 10 mL vials and related equipment were purchased from Gerstel (Linthicum, MD, USA).

B. Optimization of SBSE-TD-GC/MS

The main factors of the method, such as pH value of derivatization (pH 2-5), extraction temperature (20 – 50 °C) and extraction time (0 – 300 min), were optimized. Five series of MDA-PFPH standards in a range of 10 - 1000 nM (n = 4) were prepared for linearity of standard curves in extraction solution (KH2PO4 buffer with pH ~ 4) and ground fish (1 ± 0.05 g) with 9 mL extraction buffer. Various amounts of MDA-PFPH were in solution and spiked in meat samples were validated for recovery and matrix effect of the method.

C. Preparation of meat samples

Pork samples (loin) were purchased from a local supermarket (Lacombe, AB, Canada). Chops were cooked on the preheated grill (250 °C) to an internal temperature of 35.5 °C, then turned over and cooked to a final temperature of 71 °C. Temperature was monitored by using a 10 cm spear point temperature probes inserted to the midpoint of the chop (Hewlett Packard HP349701A Data Logger, Hewlett Packard Co., Boise, ID, USA). Raw and cooked meat were packed and stored under condition (2 ± 1°C) for every day of analysis. Before analysis, meat samples were cut into small pieces for grounding using a Mini-Prep Chopper/Grinder (Cuisinart®, Canada). Ground meat (1 ± 0.05 g) was put into 10 mL amber vial with 5 mL water and 3.5 mL KH2PO4 buffer (0.5 M) with pH value around 4. BHT 200 µL (1 mM) with final concentration 20 µM was added to prevent oxidation in the process of derivatization and extraction. Then 200 µL aqueous PFPH (5 mg/mL) was added to derivatize MDA in meat samples. After vortex of the derivatization solution for 1 min, the samples were stirred and extracted by stir bars at room temperature for 60 min.

D. TD-GC/MS and conditions

Stir bars were placed in the desorption tube and inserted in the thermal desorption unit (TDU), where they were thermally desorbed by programming the TDU from 30 °C (held for 0.5 min) to 260 °C (held for 3 min) at 240 °C/min. Transfer temperature was fixed at 275 °C. The TDU for desorbing was in the splitless mode into the cryogenic trap (CIS 4) for focusing and concentrating the analytes prior to their transfer to the capillary column. The desorbed compounds were cryogenic focussed in the CIS 4 with a glasswool notched liner at −100 °C. After desorption, the CIS 4 was programmed from 280 °C (held for 3 min) at 12 °C/s to inject the trapped compounds onto the analytical column. Injection was performed in the programmable temperature vaporization (PTV) solvent vent mode, and purge flow to split vent was 36 mL/min at 1 min.

All analyses were performed on Agilent 7890A GC system coupled with 5975C mass spectrometry with MSD. The separations were carried out on a HP-5ms fused-silica capillary column, 30 m (length) × 250 µm (I.D.) × 0.25 µm (film thickness) (Agilent Technologies, Mississauga, ON, Canada). The oven temperature was programmed from 50 °C (held for 0.5 min) to 150 °C (held for 0.5 min) at 25 °C/min, then to 280 °C (held for 1 min) at 30 °C/min, and total run time was around 10 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Quantitative analysis was performed by using selected ion monitoring (SIM) mode with the characteristic ion at m/z 234.

III. RESULTS AND DISCUSSION

A. Optimization of SBSE-TD-GC/MS with in situ derivatization

We conducted a series of experiments to test the suitable conditions (pH value and reaction temperature) for the derivatization reaction in meat matrix. Results of pH effect on derivatization of MDA were shown in the range of 2 -5 at ambient temperature for 1 h (Figure 1). SBSE is by nature an equilibrium technique, the extraction is controlled by the coating material (e.g. PDMS) ratio and the partitioning coefficients with the octanol-water distribution ($K_{o/w}$; $\log K_{o/w}$of MDA-PFPH: 2.82).

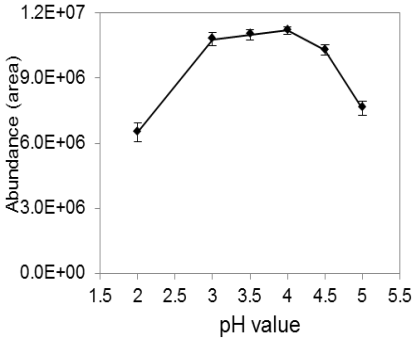

Fig. 1. pH value effect on the condensation reaction of MDA with PFPH

It is well known that extraction conditions, such as time, temperature and speed, are the main factors of variability prior to GC/MS analysis. In previous reports, extraction time of SBSE varied from several minutes to hours or even days depending on the properties of the target compounds and the experimental conditions[8]. SBSE of MDA-PHPF increased quickly from beginning to 1 h and then the extraction slowed down reaching a plateau state after 60 min (Figure 2). Therefore, a SBSE time of 60 min was chosen for all experiments. In the range of 20-50 °C for SBSE, temperature did not significantly affect MDA-PFPH extraction. Moreover, the extraction temperature at 50 °C or higher led to a slight decrease of extraction efficiency (data not show). Therefore, room temperature for 1 h was used for SBSE with stir speed of 1200 rpm. In the thermal desorption process, the TDU temperature of 280 °C and cryogenic temperature of -100 °C in CIS 4 were used without further test in the study.

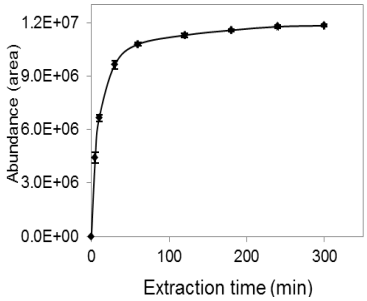

Fig. 2. Extraction time effect on SBSE-TD-GC-MS of MDA-PFPH

B. Validation of the method

To avoid the matrix effect, we used the standard curve plotted at a range of 10 – 1000 nM in meat matrix for following quantitative analyses. The linear ranges, precision data, limit of detection (LOD) and limit of quantification (LOQ) are showed in Table 1.

Table 1. Validation results of optimized SBSE-TD-GC-MS method.

Conc. (pM)	Recovery (%)	Range (nM)	R^2	LOD (nM)[c]	LOQ (nM)[d]
In solution[a]	96 -103	10 - 1000	0.9991	~ 0.4	~ 1
In meat[b]	86 - 108	10 - 1000	0.9998	~ 1	~ 2

[a] KH_2PO_4 buffer with pH ~ 4.
[b] In ground pork (1 ± 0.05 g) with 9 mL extraction solution.
[c] Limit of detection (S/N = 3, n = 4).
[d] Limit of quantification (S/N = 10, n = 4).

The calibration in solution and in meat displayed a good linearity with correlation coefficients (R^2) >0.999 (n = 4, RSDs < 5 %). LOD and LOQ were~ 0.4 nM and ~ 1 nM in solution and ~ 1 and 2 nM in meat matrix, by fortifying 0.2 - 2 nM (n = 4, RSDs < 7 %). Both LOD and LOQ of MDA by SBSE-TD-GC-MS were obviously improved, compared to previous reports of LC-UV/MS with TBA[9] and SPME-GC-MS with the same PFHF derivazation in urine [10].The recovery demonstrated the suitability of the method for the target analyte.

C. MDA analysis in meat

Many different strategies have been used for MDA analysis in food (meat, fish and formula) and biological samples (plasma, urine and saliva) by using GC and HPLC for separation. However, most of the methods need one and more times for extraction/concentration of MDA derivatives by lipid extraction with organic solvents, like hexane, followed by evaporation or precipitation[11, 12]. It is time-consuming and also causes loss of target compounds in each sampling steps. In our study, we extracted the derivative of MDA simultaneously in process of derivatization.

The optimized SBSE-TD-GC-MS method with *in situ* PFPH derivatization was applied to examine the MDA both raw and cooked pork. We monitored and evaluated the amount of MDA in both raw and cooked meat stored under retail condition at 2 °C and – 20 °C, respectively. The results of MDA measurement were shown in Table 2. In raw meat, MDA slightly increased

from 0.37 µmol/Kg to 0.51 µmol/Kg at - 20 °C after one week of storage.

Table 2. Amount of MDA determined in raw and cooked meat stored in different condition using the developed SBSE-TD-GC-MS method with *in situ* derivatization.

	Amount of MDA (µmol/Kg)[a]		
	0-day	7-day[b]	
		2°C	-20 °C
Raw meat	0.37 ± 0.04	0.75 ± 0.07	0.51 ± 0.08
Cooked meat	1.45 ± 0.14	8.19 ± 0.13	1.82 ± 0.06

The amount of MDA measured in present study was lower than the previous reports of 2 –10 µmol/Kg via TBARS tests [13] and 0.5 – 10 µmol/Kg by HPLC analysis [13, 14]. After cooking, the amount of MDA increased dramatically to 1.45 µmol/Kg, due to the oxidation of PUFAs during cooking. After one week in storage, the amount of MDA slightly increased to 1.82 µmol/Kg at – 20 °C, but to 8.19 µmol/Kg at 2 °C. This means that oxidation happened more easily in cooked meat at normal storage [15]. During heating, free radicals were formed, increasing the rate of oxidation of PUFAs in cooked meat, and also free irons were released from globin proteins, acting as a catalyst in oxidation during storage of cooked meat [1].

IV. CONCLUSIONS

Under conditions optimized in this study, the simple, sensitive and solvent-less method of SBSE-TD-GC-MS with milder derivatization conditions can be considered as an appropriate technique for analysis of MDA in meat and meat products.

ACKNOWLEDGEMENTS

Dr. E. Ruan acknowledges the receipt of a Natural Sciences and Engineering Research Council (NSERC) fellowship funded through Agriculture Agri-Food Canada (AAFC) A-base program.

REFERENCES

1. Aalhus, J.L. & Dugan, M.E.R. (2004). In Encyclopedia of Meat Science, 2nd Edition. Eds. Elsevier: Oxford. p. 1330-1336.
2. Frankel, E.N. (2005). Lipid Oxidation. The Oily Press. p. 488.
3. Watts, B.M. (1962). Symposium on food lipids and their oxidation in Meat products. AVI Pub. Co. Inc.: Westport CT. p. 202.
4. Roncales, P. et al. 2013. Lipid oxidation in light lamb. Proceedings of the 59th International Congress of Meat Science and Technology. Turkey.
5. Fenaille, F. et al. (2001) Comparison of analytical techniques to quantify malondialdehyde in milk powders. J Chromatogr A, 921(2):237-45.
6. Giera, M., Lingeman, H. & Niessen, W.M.A. (2012). Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): A brief overview. Chromatographia, 75(9-10):433-440.
7. Sánchez-Rojas, F., Bosch-Ojeda, C. & Cano-Pavón, J. (2009). A Review of Stir Bar Sorptive Extraction. Chromatographia, 69(1):79-94.
8. Lancas, F.M., et al. (2009). Recent developments and applications of stir bar sorptive extraction. Journal of Separation Science, 32(5-6):813-824.
9. Moselhy, H.F., et al. (2013). A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC. J Lipid Res, 54(3):852-8.
10. Shin, H.S. & Jung, D.G. (2009). Sensitive analysis of malondialdehyde in human urine by derivatization with pentafluorophenylhydrazine then headspace GC-MS. Chromatographia, 70(5-6):899-903.
11. Zelzer, S., et al. (2013). Measurement of total and free malondialdehyde by gas-chromatography mass spectrometry-comparison with high-performance liquid chromatography methology. Free Radical Research, 47(8):651-656.
12. Sim, A.S., et al. (2003). Improved method for plasma malondialdehyde measurement by high-performance liquid chromatography using methyl malondialdehyde as an internal standard. Journal of Chromatography B, 785(2):337-344.
13. Papastergiadis, A., et al. (2012). Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. J Agric Food Chem, 60(38):9589-94.
14. Bergamo, P., et al. (1998). Measurement of Malondialdehyde Levels in Food by High-Performance Liquid Chromatography with Fluorometric Detection. Journal of Agricultural and Food Chemistry, 46(6):2171-2176.
15. Min, B.R., et al. (2008). Factors Affecting Oxidative Stability of Pork, Beef, and Chicken Meat, in Animal Industry Report, Iowa State University.