Aroma Profile Characterization of Mahi-Mahi and Tuna for Determining Spoilage Using Purge and Trap Gas Chromatography-Mass Spectrometry

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Abstract: Alcohols, aldehydes, ketones, amines, and sulfur compounds are essential aroma compounds related to fish flavor and spoilage. Gas chromatography-mass spectrometry (GC-MS) is an instrument that is widely used to identify and quantify volatile and semi-volatile compounds in fish products. In this research, a simple and accurate GC-MS method was developed to determine the aroma profile of mahi-mahi and tuna for chemical indicators of spoilage. In the developed GC-MS method, trichloroacetic acid (TCA) solution was used to extract analytes from homogenized fish samples. The purge and trap system was used for sample introduction, and the GC-MS with an RTX-Volatile Amine column was able to separate compounds without a derivatization procedure. The created purge and trap gas chromatography-mass spectrometry (PT-GC-MS) method could identify and quantify twenty aroma compounds in mahi-mahi (*Coryphaena hippurus*) and 16 volatile compounds in yellowfin tuna (*Thunnus albacares*) associated with fish spoilage. The amines (dimethylamine, trimethylamine, isobutylamine, 3-methylbutylamine, and 2-methylbutanamine), alcohols (2-ethylhexanol, 1-penten-3-ol and isoamyl alcohol, ethanol), aldehydes (2-methylbutanal, 3-methylbutanal, benzaldehyde), ketones (acetone, 2,3-butanedione, 2-butanone, acetoin), and dimethyl disulfide strongly statistically correlated with poorer quality tuna and mahi-mahi and were considered as the key spoilage indicators.

Keywords: aquatic product, spoilage indicator, GC-MS, volatile amines, amine column

Practical Application: A simplified and rapid purge and trap gas chromatography-mass spectrometry (PT-GC-MS) method developed in this research was able to identify and quantify important spoilage compounds in mahi-mahi and yellowfin tuna. This method is an efficient analytical method for determining volatile profiles of fish samples for industry analytical labs or the government. The identified analytical quality markers can be used to monitor the spoilage level of tuna and mahi-mahi.

Introduction

Fish spoilage means any change in the condition of fish resulted from microbial, enzymatic, and chemical reactions that leads to fish becoming less palatable or even poisonous (Ghaly, Dave, Budge, & Brooks, 2010; Prester, 2011; Silbande et al., 2016). In the process of fish spoilage, complex physical and chemical changes are involved and the primary concern of fish spoilage including the degradation of protein, lipid oxidation and hydrolysis of carbohydrate (Ashie, Smith, & Simpson, 1996; Hungerford, 2010). Each year, the total waste of spoiled fish is around 10 to 12 million tonnes and the economic impact due to fish spoilage cannot be ignored (FAO, 2010). Toxic biogenic amines, including histamine, cadaverine, and putrescine, can be formed in fish during spoilage and consumption of spoiled fish that contain significant amounts of histamine lead to fish poisoning (Bulushi, Poole, Deeth, & Dykes, 2009; Prester, 2011; Visciano, Schirone, Tofalo, & Suzzi, 2012). Tuna and mahi-mahi are identified as two major fish species responsible for histamine poisoning in the United States (Ahmed, 1991; Antoine, We, Littell, & Marshall, 1999; Bulushi et al., 2009). Therefore, monitoring the quality of tuna and mahi-mahi is helpful to prevent histamine poisoning.

Microbial action, enzymatic action, lipid oxidation, and other chemical reactions lead to the change of volatile components in fish meat and influence the organoleptic characteristic of fish products (Edirisinghe, Graham, & Taylor, 2007; Parlapani, Haroutounian, Nychas, & Boziaris, 2015). The changes of specific alcohols, carbonyls, acids, amines, sulfur compounds, aldehydes, and ammonia during fish spoilage have been reported (Ashie et al., 1996; Duflos, Coin, Cornu, Antinelli, & Malle, 2006). These volatile compounds have been considered as quality indicators of fish products and can be used to determine fish spoilage. Short-chain carbonyls, alcohols, and esters, such as ethanol, 1-penten-3-ol, 3-methyl-1-butanol, 1-butanol, and 1-octen-3-ol, 3-methylbutanal, 2-methylbutanal, ethyl acetate and ethyl butanoate, accumulate in spoiled fish due to microbial spoilage, enzymatic or non-enzymatic lipid oxidation and are responsible for the pungent, alcoholic, creamy, and fishy odors of spoiled fish (Duflos et al., 2006; Iglesias et al., 2009; Leduc et al., 2012; Olafsdottir, Jonsdottir, Lauzon, Luten, & Kristbergsson, 2005). Several studies have identified volatile amines, including trimethylamine (TMA), dimethylamine (DMA), and isobutylamine, as critical markers of fish freshness due to their gradual accumulation during the spoilage process and the contribution of characteristic fishy odor (Bene, Fornage, Luisier, Pichler, & Villettaz, 2001;

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Leduc et al., 2012; Ghaly et al., 2010; Gill, 1983; Dehaut et al., 2016). Sulfur compounds accumulate after fish landing due to microbial and enzymatic activity can give off unpleasant odors at extremely low concentrations (Duflos et al., 2006; Gram & Dalgaard, 2002; Ashie et al., 1996; Kawai, 1996). The variations of volatile acids, terpenes, alkanes, and alkenes have also been observed in spoiled fish (Aro, Tahvonen, Koskinen, & Kallio, 2003; Iglesias et al., 2009; Koutsoumanis & Nychas, 1999; Olafsdottir et al., 2005).

Gas chromatography-mass spectrometry (GC-MS) is a highly effective analytical instrument that is widely used to separate, identify and quantify volatile and semi-volatile compounds in fish products (Sandra, Tienpont, & David, 2003; Wang, Lee, Lewis, Kamath, & Archer, 1999; Xu et al., 2014). In the GC-MS system, analytes are separated due to their different strengths of interaction with the stationary phase, and then the "fingerprint" information of molecules is sensitively identified by the mass spectrometer (Abraham, Poole, & Poole, 1999; Sneddon, Masuram, & Richert, 2007). A GC-MS method equipped with CAR/PDMS fiber and a BPX5 capillary column was able to investigate the freshness markers of whiting (*Merlangius merlangus*) (Duflos et al., 2010). An SPME-GC-MS method using a CP-Wax 52 column was developed to detect the volatiles identified as spoilage indicators of yellowfin tuna (*Thunnus albacares*) (Edirisinghe et al., 2007). An SPME-GC-MS method by Wierda, Fletcher, Xu, and Dufour (2006) using a ZB-Wax column was used to analyze the effect of storage on the volatile profile of fresh king salmon (*Oncorhynchus tshawytscha*). The application of the GC-MS method in determination of volatile compounds used as spoilage indicators of fish spoilage, including alcohols, acids, aldehydes, alkanes, ketones, trimethylamine, and sulfur compounds, has also been reported in other studies (Alasalvar, Taylor, & Shahidi, 2005; Leduc et al., 2012; Soncin, Chiesa, Panseri, Biondi, & Cantoni, 2009).

Most amines in fish products, such as isobutylamine, 3-methylbutylamine, and 2-methylbutylamine, are identified as important spoilage indicators and contribute to the fishy odor of spoiled fish (Eskin & Shahidi, 2013; Gill, 1983; Mayr & Schieberle, 2012; Prester, 2011; Takahashi, Nagayama, & Mori, 2004). However, using GC-MS instrument to determine short chain amines has some inherent challenges because of their high polarity, basic character, and high aqueous solubility. Complicated derivatization steps usually are needed to increase the volatility of amines when using GC (Du, Huang, Kim, Marshall, & Wei, 2001; Rogers & Staruszkiewicz, 1997; Staruszkiewicz & Bond, 1981). The objective of this study was to develop a simplified and accurate GC-MS method to determine amines and other important aroma compounds in a single GC run to serve as chemical indicators of spoilage without any complex derivatization step.

Materials and Methods

Fish samples and preparation

The mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) analyzed in this research were caught from South Pacific waters. More than five sensory experts in FDA and National Marine Fisheries Service (NMFS) applied the standard sensory grading system provided in the FDA ORA Laboratory Manual (Dole et al., 2016; FDA, 2013) to grade the fish filets of mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) into seven grades. The grading system used by FDA/NMFS experts depended on olfaction and were graded 1 to 7 to represent quality. Grade 1 represented high quality, while grade 7 represented very poor quality fish.

The individually packaged and graded fish filets from FDA/NMFS were shipped overnight, received frozen on dry ice, and then stored in a –20 °C freezer until analysis was performed. For each grade of fish samples, vacuum packaged frozen samples were defrosted overnight at room temperature, and then were chopped and homogenized by a blender (Blendtec, Orem, UT, USA) before extraction.

Standards and reagents

All chemicals used in this study were of analytical grade or higher. Isobutylamine, 3-methylbutylamine, and 2-methylbutylamine, trimethylamine, 1-nonanol, sodium bicarbonate, were supplied by Sigma–Aldrich (St. Louis, MO, USA). Dimethylamine was obtained from ACROS Organics (Geel, Belgium). Sodium hydroxide, ammonium hydroxide, trichloroacetic acid were supplied by Fisher Chemical (Pittsburgh, PA, USA).

For preparing the internal standard solution, 1-nonanol was dissolved in HPLC grade methanol at a concentration of 100 mg/L. For preparing external calibration curves of volatile amines, three different concentrations of a mixture of volatile amines containing isobutylamine, 3-methylbutylamine, 2-methylbutylamine, trimethylamine and dimethylamine were used: 10, 50, and 100 mg/L in HPLC grade water. In addition, spiking known amounts of volatile amines was applied to determine the effect of fish matrix on headspace volatile amines. A volatile amine mixture containing isobutylamine, 3-methylbutylamine, 2-methylbutylamine, trimethylamine and dimethylamine were spiked in triplicate into homogenized grade 1 of tuna samples to achieve the final concentration of each amine as 5, 10, 50, and 100 mg/kg fish.

Extraction procedures

The extraction procedures of Zhai et al. (2012) and Ruiz-Capillas and Horner, (1999) with modifications were used in this study. In brief, 6 g homogenized fish sample was added to a 50 mL centrifuge tube with 20 mL of 5% trichloroacetic acid (TCA) and then was vortexed for 15 min. Next the extract was centrifuged at 5000 × *g* for 10 min at 4 °C. After centrifugation, the supernatant was collected and the remaining solid was extracted using 20 mL of 5% TCA again by the same procedures as above, and the supernatant was collected. Both supernatants were combined and passed through a Whatman No. 1 filter paper. Fish extract was stored in a refrigerator at 4 °C and was analyzed by GC-MS within 24 hr. To prepare samples injected into PT-GC-MS, an amount of 4.5 mL 2 mol/L NaOH solution, 6.75 mL of saturated $NaHCO_3$ solution (adjusted the solution to basic pH 9 to increase volatility of amines) and 22.5 mL of filtered fish extract or standard solution were added into a 40 mL amber headspace vial (Thermo Scientific; Sunnyvale, CA, USA) and vortexed for 20 s. For each grade of mahi-mahi or tuna, the extraction and dilution procedures were performed in triplicate.

Purge and trap conditions

Volatile compounds in fish extract were collected and concentrated by the automated purge and trap unit AQUATek 100 (Teledyne Tekmar, Mason, OH, USA) prior to GC-MS analysis. The autosampler pulled 5 mL prepared fish extraction and 2 µL 100 mg/L 1-nonanol into the purge and trap unit. Fish extraction spiked with the internal standard was purged with ultrahigh purity helium gas at a flow rate 20 mL/min for 30 min at a purge

temperature of 55 °C. The headspace volatiles were adsorbed to a Tenax trap No. 1 (Teledyne Tekmar, Mason, OH, USA). Then the trapped compounds were desorbed at 180 °C for 3 min to the GC injection port.

GC-MS analysis

Identification and quantification of analytes were conducted on an Agilent Technologies 7890A Gas Chromatograph with 5975C Mass Spectrometer (Agilent, Santa Clara, CA, USA). The injected volatile compounds (split at 20:1) were separated on a 60 m RTX-Volatile Amine column (I.D. 0.32 mm, d_f 5 μm) (Restek Corporation, Bellefonte, PA, USA). The carrier gas was helium operated at a rate of 2 mL/min. The oven temperature program was as follows: hold 6 min at 40 °C; 40 to 175 °C at 10 °C/min; 175 to 240 °C at 15 °C/min; 240 to 260 °C at 20 °C/min; hold 5.2 min at 260 °C. The separated volatile compounds were transferred from GC to the MS to be scanned in the m/z range from 20 to 350.

Calculations and statistics

The percentage recoveries of isobutylamine, 3-methylbutylamine, 2-methylbutylamine, trimethylamine, and dimethylamine for spiking standard method, external standard method and the internal standard method were quantified by dividing the back-calculated concentration of each amine by its theoretical amount. The true concentrations of five amines in fish samples were quantified by using back-calculated concentrations from three methods (spiked standard curves, external standard curves, and addition of internal standard) divided by the percentage recoveries of each amine for each method, respectively.

To compare the amount of each compound in different grades of mahi-mahi or tuna, One-way analysis of variance (one-way ANOVA) was carried out with SAS statistical software (SAS, Cary, NC, USA.). The significance level (α) of the ANOVA test was set at 0.05. A Fisher's least significant difference (LSD) test was used to assess which means were significantly different. Pearson's correlation coefficients (r) was determined using SAS to investigate the relationships between determined volatile components and different grades of fish samples. The variables were the grade of mahi-mahi (tuna) and the concentrations of different volatile compounds. The significance level (α) of the Pearson's correlation test was set at 0.05.

Results and Discussion

Development of the PT-GC-MS method

To obtain a simplified and accurate GC-MS method that can determine the aroma profile of mahi-mahi and tuna without derivatization, different extraction and injection methods (solid-phase microextraction, liquid injection, purge, and trap), split ratios (10:1 and 20:1), types of GC columns (ZB-Wax plus column, 30 m, 0.25 mm ID; ZB-5MS plus column, 60 m, 0.25 mm ID; Rtx-Volatile Amine Column, 60 m, 0.32 mm ID), four different temperature programs, carrier gas flow rates (1.2 and 2 mL/min), were compared. The optimized purge and trap gas chromatography-mass spectrometry (PT-GC-MS) method created in this study was applied to the analysis of volatile compounds of different grades mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) and several volatile compounds were able to be determined in these two species of fish samples. To identify spoilage indicators, peak responses of each compound of different grades of mahi-mahi or tuna were compared to determine which compounds increased, declined or remained the same during fish spoilage (Table 1 and 2). Figure 1 shows the separation for relevant chemical indicators of spoilage in a grade 7 mahi-mahi sample.

The purge and trap method applied in this GC-MS method provided accurate and precise analysis. It has been reported that the purge and trap method can lower the limit of detection (LOD) value, extract volatile compounds in a higher amount and is more sensitive than a static headspace method (Beltran et al., 2006; Lucentini et al., 2005; Povolo & Contarini, 2003). The Tenax trap No. 1 used in this dynamic extraction method works well to trap nonpolar compounds along with extracting a low amount of water due to the hydrophobic property of the trap. Fukami et al. (2002) used a Tenax to trap volatile compounds after the fish sauce was purged for sixteen minutes, and twenty-three compounds were determined by the GC-MS method. The Tenax trap No. 1 has also been applied in a GC-MS method to concentrate volatile compounds of Sea Bream (*Sparus aurata*) and seventy-eight compounds, including aldehydes, ketones, carboxylic acids, terpenes, and amines were able detected (Alasalvar et al., 2005). The application of purge and trap to concentrate volatile compounds from fish meat, such as sea bream and sockeye salmon, has been well documented (Alexi, Fountoulaki, & Grigorakis, 2017; Girard & Durance, 2000; Jonsdottir, Olafsdottir, Chanie, & Haugen, 2008; Leduc et al., 2012).

The RTX-Volatile Amine column applied in this PT-GC-MS worked well for the separation of volatile amines. Determination of small chain amines by gas chromatography (GC) has inherent problems due to their physicochemical properties, including high polarity, basic character, and high aqueous solubility (Abalos, Mas, Suc, & Bayona, 2001; de Zeeuw, Stricek, & Stidsen, 2011). Strong adsorption of amines on the column leads to tailing peaks. In order to overcome this problem, complicated derivatization steps are commonly needed to convert amines to less polar derivatives to be more appropriate for the GC determination. Stationary phase deactivation technologies have been applied to modify the fused silica surface of amine column to make the columns more stable for determination of volatile amines (Abalos et al., 2001). Amine columns usually have low or mid polarity phases and can be determined without any complicated derivatization steps due to its inertness, water tolerance, and loadability (de Zeeuw et al., 2011). Applying amine columns in GC-MS can improve the separation of basic compounds and avoid tailing of these compounds. A GC-MS equipped with a base-deactivated RTX-Volatile Amine column has been used to quantify the amount of trimethylamine in standard solutions or marine sediments without any derivatization procedure (Steinkamp, DeGreeff, Collins, & Rose-Pehrsson, 2016; Zhuang et al., 2017). A SPME-GC-FID method with RTX-5 Amine column or PoraPLOT Amine column was able to analyze short-chain volatile amines C1 to C9, including dimethylamine, trimethylamine, monoethylamine, isopropylamine, and others, in standard solution without derivatization (Abalos et al., 2001).

Table 3 shows the volatile amines content in different grades of mahi-mahi and tuna samples calculated by the spiking standard method and the external standard method. The quantification results of each volatile amine in fish samples by spiking standard method were higher than those calculated by external standard method (Table 3). These observed differences were likely due to the matrix effect of the fish. It was observed that for each volatile amine concentration level of standard curve, the GC-MS peak response of each amine for the spiking standard method was lower than that for the external standard method, which led to the slopes of the spiking standard curves being lower than those of external standard curves. These observations were due to the matrix of

Table 1–Volatile compounds associated with spoilage in seven grades of mahi-mahi calculated by internal standard method, (ng/g) fish sample, $n = 3$.

Compound name	M1	M2	M3	M4	M5	M6	M7
Methanol	1696 ± 338^{A}	2942 ± 609^{A}	2532 ± 833^{A}	3365 ± 2800^{A}	1305 ± 394^{A}	1296 ± 349^{A}	1093 ± 374^{A}
DMA	n.d.	4086 ± 7070^{C}	5277 ± 232^{C}	n.d.	26410 ± 4970^{AB}	23500 ± 5860^{B}	33850 ± 4730^{A}
TMA	14800 ± 4140^{C}	18020 ± 589^{C}	14830 ± 2520^{C}	14800 ± 4850^{C}	138100 ± 16200^{B}	119700 ± 10800^{B}	243000 ± 48100^{A}
Acetone	206.8 ± 123^{B}	231.8 ± 6.18^{B}	884.7 ± 337^{A}	1038 ± 193^{A}	907 ± 87.9^{A}	1011 ± 114^{A}	764.9 ± 30^{A}
tert-butanol	1045 ± 179^{A}	1129 ± 54^{A}	1003 ± 309^{A}	1014 ± 60.4^{A}	994.5 ± 91.4^{A}	996.2 ± 162^{A}	888.9 ± 113^{A}
2-methylpropanal	71.19 ± 9.25^{B}	n.d.	80.21 ± 31.3^{B}	61.65 ± 14.8^{B}	116.5 ± 28.5^{A}	79.24 ± 3.04^{B}	73.89 ± 4.3^{B}
2,3-Butanedione	152.1 ± 41.6^{C}	404.3 ± 81.5^{A}	97.45 ± 17.8^{CD}	132.1 ± 41^{CD}	243.3 ± 66.9^{B}	92.89 ± 3.99^{CD}	53.99 ± 11.8^{D}
2-Butanone	100.6 ± 9.37^{A}	125.9 ± 26.6^{A}	136.4 ± 7.11^{A}	145.7 ± 24.3^{A}	191 ± 52.1^{A}	145.6 ± 33.1^{A}	138.9 ± 15.4^{A}
Isobutylamine	n.d.	n.d.	n.d.	n.d.	953.8 ± 1030^{B}	2394 ± 565^{A}	975 ± 242^{B}
3-methylbutanal	173.3 ± 15.6^{C}	123.2 ± 26.4^{C}	174.5 ± 16.7^{C}	147.4 ± 11.2^{C}	274.9 ± 61.6^{B}	240.6 ± 9.66^{B}	473.9 ± 64.8^{A}
2-methylbutanal	n.d.	n.d.	153 ± 23^{AB}	107.6 ± 15.7^{B}	129 ± 116^{B}	151.1 ± 9.38^{AB}	229.6 ± 30.5^{A}
1-Penten-3-ol	n.d.	455.9 ± 47.5^{B}	n.d.	87.82 ± 11.9^{D}	182.5 ± 16^{C}	690.6 ± 32.7^{A}	52.51 ± 11.4^{E}
3-methylbutylamine	n.d.	n.d.	n.d.	n.d.	2108 ± 1750^{B}	7369 ± 1160^{A}	1200 ± 249^{BC}
2-methylbutylamine	n.d.	n.d.	335.2 ± 380^{C}	364.3 ± 631^{C}	1333 ± 1080^{B}	3343 ± 464^{A}	756.4 ± 126^{BC}
isoamyl alcohol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	238.7 ± 9.35
Pyridine	36.2 ± 4.89^{B}	72.48 ± 16.4^{A}	n.d.	n.d.	n.d.	n.d.	n.d.
Dimethyl disulfide	n.d.	n.d.	n.d.	24.33 ± 21.1^{B}	n.d.	3.34 ± 5.79^{C}	128.4 ± 10.8^{A}
Hexanal	22.96 ± 20.8^{C}	131.4 ± 20.4^{A}	n.d.	10.15 ± 17.6^{C}	32.89 ± 32.9^{C}	97.21 ± 16.2^{B}	n.d.
Benzaldehyde	88.92 ± 5.93^{E}	256.2 ± 15.7^{B}	122.6 ± 3.21^{D}	186.6 ± 16.5^{C}	162.9 ± 8.88^{C}	344.6 ± 9.43^{A}	273.8 ± 35.7^{B}
2-ethylhexanol	316.5 ± 10.7^{A}	300.9 ± 4.12^{A}	60.03 ± 34.8^{D}	86.63 ± 11.5^{CD}	109.6 ± 56^{BC}	136.1 ± 8.2^{B}	129.7 ± 1.47^{BC}

Different letters within the same row indicate significant differences according to an LSD means separation test ($P < 0.05$). n.d. indicates not detected.

Table 2–Volatile compounds associated with spoilage in seven grades of tuna calculated by internal standard, (ng/g) fish sample, $n = 3$.

Compound Name	T1	T2	T3	T4	T5	T6	T7
DMA	3988 ± 747^{BC}	2133 ± 1030^{B}	3201 ± 1120^{BC}	5729 ± 2420^{AB}	8209 ± 4150^{A}	1768 ± 927^{C}	4141 ± 1040^{BC}
TMA	12390 ± 3770^{D}	16470 ± 1220^{D}	15260 ± 932^{D}	23730 ± 3450^{C}	36180 ± 4740^{A}	28890 ± 2640^{B}	33620 ± 613^{AB}
Ethanol	33.77 ± 6.86^{A}	46.9 ± 6.21^{A}	39.4 ± 4.96^{A}	118.1 ± 13.5^{A}	174.3 ± 36.2^{A}	1211 ± 1510^{A}	486.5 ± 29.9^{A}
Acetone	1389 ± 1720^{A}	2839 ± 687^{A}	1399 ± 706^{A}	1902 ± 1460^{A}	1268 ± 119^{A}	700 ± 598^{A}	2619 ± 272^{A}
tert-butanol	646.7 ± 111^{A}	467.6 ± 105^{A}	650 ± 14.2^{A}	534.4 ± 151^{A}	686.1 ± 136^{A}	559.4 ± 62.6^{A}	566.1 ± 19.2^{A}
2-methylpropanal	7.983 ± 7.04^{E}	19.97 ± 1.88^{DE}	30.92 ± 17.3^{CD}	52.91 ± 2.17^{B}	54.27 ± 6.28^{B}	103.5 ± 17.7^{A}	45.59 ± 4.65^{BC}
2,3-Butanedione	n.d.	n.d.	n.d.	404.4 ± 45.3^{B}	637.8 ± 13.7^{A}	30.4 ± 11.5^{D}	64.19 ± 5.8^{C}
2-Butanone	24.59 ± 11.7^{C}	40.64 ± 4.92^{B}	27.76 ± 6.32^{C}	49.32 ± 4.94^{AB}	57.02 ± 5.13^{A}	43.18 ± 9.63^{B}	46.59 ± 3.57^{AB}
Isobutylamine	n.d.	n.d.	n.d.	677.8 ± 161^{A}	n.d.	n.d.	n.d.
3-methylbutanal	71.3 ± 30.2^{D}	93.76 ± 20.1^{D}	100.9 ± 20.2^{D}	205.7 ± 18.8^{B}	168.2 ± 7.62^{C}	288.4 ± 26.7^{A}	149 ± 8.95^{C}
2-methylbutanal	26.27 ± 17.1^{D}	40.23 ± 10.4^{D}	45.26 ± 5.44^{D}	96.45 ± 10.2^{B}	76.14 ± 8.98^{C}	157.9 ± 13^{A}	75.15 ± 7.04^{C}
Acetoin	n.d.	n.d.	n.d.	935.2 ± 88.5^{B}	1567 ± 69.4^{A}	n.d.	51.11 ± 5.47^{C}
3-methylbutylamine	n.d.	n.d.	n.d.	1820 ± 365^{A}	n.d.	n.d.	n.d.
2-methylbutylamine	n.d.	n.d.	n.d.	751.4 ± 172^{A}	n.d.	n.d.	n.d.
Benzaldehyde	23.97 ± 14.6^{E}	78.54 ± 13.4^{D}	23.82 ± 3.66^{E}	106.1 ± 4.64^{B}	123.7 ± 3.56^{A}	88.61 ± 11.8^{CD}	94.82 ± 11.8^{BC}
2-ethylhexanol	53.73 ± 26.5^{AB}	71.76 ± 8.75^{A}	53.65 ± 2.17^{AB}	50.11 ± 4.63^{BC}	52.34 ± 5.3^{AB}	52.63 ± 2.35^{AB}	31.47 ± 1.55^{C}

Different letters within the same row indicate significant differences according to an LSD means separation test ($P < 0.05$). n.d. indicates not detected.

fish changing the equilibrium distribution of an analyte between the sample phase and the gas phase (essentially binding analyte), which led to the reduction of amines in the headspace. The lower instrument response for the standard curves in the spiked standard method led to the larger calculated amine amount in fish samples.

Chemical indicators in different spoilage grades of mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*)

As the fish spoilage grade increased, the detected levels of trimethylamine (TMA) and dimethylamine (DMA) in both mahi-mahi and tuna samples increased significantly (Table 1, 2, and 3). The accumulation of DMA and TMA in spoiled fish have been well documented by several studies (Bene et al., 2001; Leduc et al., 2012; Prester, 2011). Significant positive correlations between TMA, and DMA concentrations with increasing spoilage grade of fish were observed for mahi-mahi and tuna (Table 4). Both DMA and TMA are potent odorants giving off a characteristic fishy odor and contribute to the off-flavor of spoiled fish (Olafsdottir et al., 2005; Prester, 2011). The odor threshold values of DMA and TMA are 0.0846 and 0.0008 mg/L, respectively (Ruth, 1986). The precursor of DMA and TMA is identified as trimethylamine oxide (TMAO), which is an odorless osmolyte naturally presenting in marine fish and protecting fish from dehydration (Ghaly et al., 2010). TMA can be produced from TMAO by gram-negative bacteria during the process of fish spoilage, and the maximum level of TMA in fish was found to be 680 mg/kg (WHO, 2006). The level of TMA in fish has been considered as a marker of microbial deterioration in fish (Alasalvar et al., 2005; Alexi et al., 2017; Bene et al., 2001; Chan et al., 2006; Ghaly et al., 2010; Jorgensen, Huss, & Dalgaard, 2001; Leduc et al., 2012; Olafsdottir et al., 2005; Zhang, Li, Luo, & Chen, 2010). Dimethylamine (DMA) can be produced from the degradation of trimethylamine oxide (TMAO) by intrinsic enzyme activity during the spoilage (Chan et al., 2006; Ashie et al., 1996). Chan et al. (2006) observed that there was an increase in the level of DMA in freshwater grouper (*Siniperca*

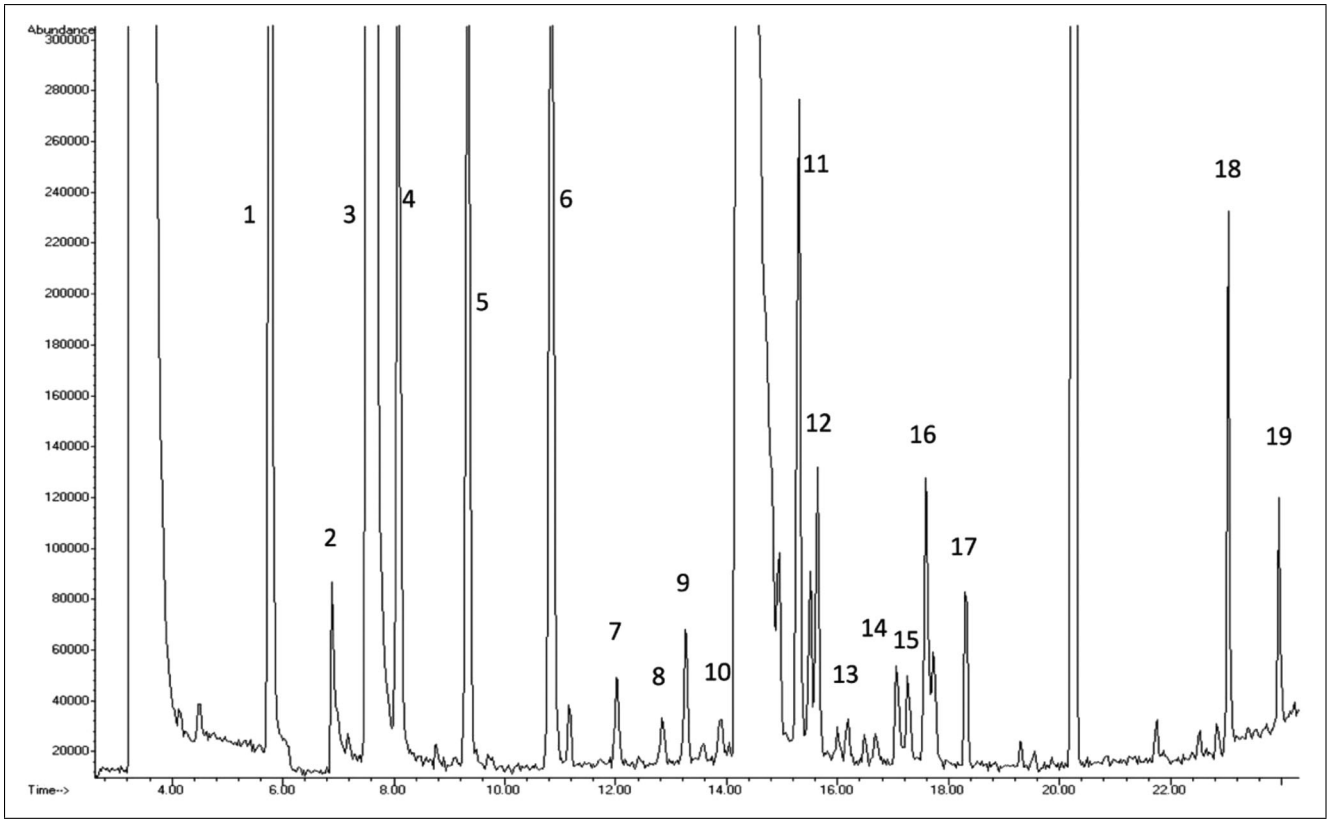

Figure 1–Example chromatogram (mahi-mahi grade 7). Nineteen chemical indicators were found: 1. Methanol 2. DMA 3. TMA 4. Ethanol 5. Acetone 6. tert-Butanol 7. 2-Methylpropanal 8. 2,3-Butanedione 9. 2-Butanone 10. Isobutylamine 11. 3-Methylbutanal 12. 2-Methylbutanal 13.1-Penten-3-ol 14. 3-Methylbutylamine 15. 2-Methylbutylamine 16. Isoamyl Alcohol 17. Dimethyl Disulfide 18. Benzaldehyde 19. 2-Ethylhexanol.

chuatsi) and mangrove snapper (*Lutjanus griseus*) from zero mg/kg in fresh fish to 5.6 and 2.98 mg/kg after a 28 days storage at 0 °C, respectively. The amount of DMA in fish tissue has been reported as an accepted measure of fish deterioration. Based on the TMA and DMA content detected in the seven grades of mahi-mahi and tuna (Table 1 and 2), the level of TMA above 20 mg/kg or DMA above 6 mg/kg in mahi-mahi or tuna means likely indicates poor quality.

Isobutylamine, 3-methylbutylamine, and 2-methylbutylamine were not detected in good quality mahi-mahi (e.g., Grade 1 and 2) and their level significantly increased in spoiled mahi-mahi (e.g., Grade 5, 6, 7). For all grades of tuna, these three biogenic amines were only detected in grade 4 of tuna. Therefore correlations between the levels of these three amines with the increasing spoilage grade of tuna were zero. Isobutylamine can give off an intense fishy odor even at low concentration and accumulation of this compound is as a result of microbial degradation of fish tissue (Gill, 1983). Free valine can produce isobutylamine in meat by the reaction of the bacterial valine decarboxylase during spoilage, and this reaction was determined to occur in spoiled marine fish (Eskin & Shahidi, 2013; Gill, 1983; Gruger, 1972). The compound 3-methylbutylamine, which also named as isopentylamine, can be formed in spoiled meat by microbial decarboxylation of leucine with the formation of fatty, unpleasant ammonia odor (Mayr & Schieberle, 2012; Takahashi et al., 2004). Isoleucine can be decarboxylated by decarboxylase activities of bacteria and produce 2-methylbutylamine that has a fishy odor (Mayr & Schieberle, 2012; Zufall & Munger, 2016). Tuna and mahi-mahi containing any of these three amines indicates spoilage occurred in the fish sample (Table 1 and 2).

Five alcohols detected in mahi-mahi and three alcohols detected in tuna changed as spoilage progressed (Table 1 and 2). The increasing spoilage grade of mahi-mahi was negatively correlated with the concentrations of 2-ethylhexanol, methanol, tert-butanol and was closely positively correlated with concentrations of 1-penten-3-ol and isoamyl alcohol in fish (Table 4). For tuna samples, the increasing spoilage grade of fish was negatively correlated with levels of 2-ethylhexanol and was positively correlated with levels of ethanol in fish (Table 4). The changes of alcohols during fish spoilage are mainly associated with the microbial activity and influence the organoleptic characteristic of the fish product (Duflos et al., 2006; Girard & Durance, 2000). Leduc et al. (2012) reported that the level of 2-ethylhexanol, which compound gives off pleasant fatty and fruity odor, in European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*) decreased to zero after ninety days frozen storage. Iglesias et al. (2009) also reported similar observation that 2-ethylhexanol decreased in gilthead sea bream during a 266 days frozen storage period. The loss of the compound 2-ethylhexanol may be useful as a quality marker to signify a decline in freshness of fish. Ethanol leads to a strong alcoholic smell and the formation of ethanol in spoiled fish is associated with the microbial decomposition of fish. The increase of ethanol in spoiled yellowfin tuna (*Thunnus albacares*), whiting (*Merlangius merlangus*), cod (*Gadus morhua*), and mackerel (*Scomber scombrus*), have been reported in several studies (Edirisinghe et al., 2007; Duflos et al., 2006; Soncin et al., 2009). The accumulation of isoamyl alcohol in spoiled Baltic herring (*Clupea harengus membras*) has been reported and isoamyl alcohol was identified as an indicator of microbial spoilage with fusel and alcoholic odor (Aro et al., 2003). The degradation of linoleic or

Table 3–Volatile amines contents in seven grades of mahi-mahi and tuna sample (ng/g) calculated by the spiked standard method and an external standard method, $n = 3$. Spiked: calculated levels of amines using spiked chemical standards (ng/g in fish). External: calculated levels of amines using an external calibration curve (ng/g in fish).

	DMA		TMA	
Sample	Spiked	External	Spiked	External
M1	n.d.	n.d.	28700 ± 8030	13350 ± 3740
M2	4098 ± 7100	432.3 ± 749	34940 ± 11400	16250 ± 5310
M3	5293 ± 2330	558.4 ± 246	28760 ± 4900	13370 ± 2270
M4	n.d.	n.d.	28710 ± 9410	13350 ± 4380
M5	26490 ± 4980	2794 ± 525	267700 ± 31400	124500 ± 14600
M6	23570 ± 5870	2486 ± 620	232200 ± 20900	108000 ± 9730
M7	33960 ± 4750	3582 ± 501	471300 ± 93300	219200 ± 43400
T1	4000 ± 749	422 ± 79	24020 ± 7310	11170 ± 3400
T2	2139 ± 1030	225.7 ± 108	31940 ± 2370	14860 ± 1100
T3	3211 ± 1120	338.7 ± 118	29600 ± 1810	13770 ± 841
T4	5746 ± 2430	606.1 ± 256	46020 ± 6690	21400 ± 3110
T5	8234 ± 4160	868.6 ± 439	70180 ± 9190	32640 ± 4270
T6	1773 ± 931	187.1 ± 98.1	56020 ± 5120	26050 ± 2380
T7	4154 ± 1040	438.2 ± 110	65210 ± 1190	30330 ± 553

	Isobutylamine		3-methylbutylamine		2-methylbutylamine	
Sample	Spiked	External	Spiked	External	Spiked	External
M1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M3	n.d.	n.d.	n.d.	n.d.	336.4 ± 381	30.92 ± 35
M4	n.d.	n.d.	n.d.	n.d.	365.7 ± 633	33.61 ± 58.2
M5	958.1 ± 1040	83.71 ± 90.7	21050 ± 1740	147 ± 122	1338 ± 1080	123 ± 99.4
M6	24050 ± 568	210.1 ± 49.6	7361 ± 1150	514 ± 80.6	3356 ± 465	308.5 ± 42.8
M7	979.4 ± 243	85.57 ± 21.2	1199 ± 249	83.69 ± 17.4	759.2 ± 127	69.79 ± 11.6
T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T4	680.8 ± 162	59.48 ± 14.2	1818 ± 365	127 ± 25.5	754.2 ± 172	69.33 ± 15.8
T5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d: indicates not detected.

arachidonic acids has been reported as the major reason leading to the formation of 1-penten-3-ol with pungent odor in spoiled fish (Girard et al., 2000. For tert-butanol, pleasantly fruity, creamy, buttery odors have been identified. Based on the mean separation results (Table 1 and 2), Pearson correlations (Table 4), and the flavor descriptor of each compound for three alcohols (2-ethylhexanol, 1-penten-3-ol and isoamyl alcohol) in mahi-mahi and two alcohols (2-ethylhexanol, and ethanol) in tuna were used to identify key indicators that could contribute to spoilage. Based on the volatile alcohols determined in poor grades of fish (Table 1 and 2), the existence of isoamyl alcohol in mahi-mahi and the ethanol level in tuna above 0.1 mg/kg indicates the quality of fish is poor.

Four aldehydes, including 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde, significantly increased in spoiled mahi-mahi and tuna samples. These four aldehydes significantly positively correlated with spoiled fish, except 2-methylpropanal which only significantly correlated with tuna. The detected level of hexanal in mahi-mahi declined as the spoilage grade increased. The odor thresholds of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal are low and the range of their thresholds is from 2.24 to 40.7 ng/g (Shimoda, Peralta, & Osajima, 1996). The increase of these three aldehydes in spoiled fish is associated with the lipid oxidation, and deamination of amino acids due to microbial catabolic activities (Joffraud, Leroi, Roy, & Berdague, 2001; Shimoda et al., 1996). Duflos et al. (2006) reported a similar trend to this study in that the level of 2-methylpropanal, 2-methylbutanal, 3-methylbutanal in whiting (*Merlangius merlangus*), cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) increased after a ten days storage under 4 °C. Significant increases of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal in European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*), and salmon (*Salmo salar*) were reported after a ninety days frozen storage (Leduc et al., 2012). These three aldehydes have been identified as good markers of fish spoilage in several studies (Alasalvar et al., 2005; Duflos et al., 2010; Jorgensen et al., 2001; Mace et al., 2013; Olafsdottir et al., 2005). Benzaldehyde is an aroma with almond, fruity odor and Mace et al. (2013) observed the accumulation of benzaldehyde in *P. phosphoreum*-inoculated raw salmon (*Salmo salar*) after an eight days storage at 8 °C. Hexanal is a carbonyl compound with a "green" plant-like odor and is a volatile commonly present in fresh fish. Wierda et al. (2006) reported the decline of hexanal in spoiled king Salmon (*Oncorhynchus tshawytscha*) and identified hexanal as an indicator for salmon freshness. The level of hexanal in both Whiting (*Merlangius merlangus*) and Cod (*Gadus morhua*) decreased after stored the fish samples for 10 days at 4 °C (Duflos et al., 2006).

Three ketones, including acetone, 2,3-butanedione, 2-butanone, generally increased as the spoilage grade of mahi-mahi and tuna increased (Table 1 and 2). Significant positive correlations between levels of these three ketones with the spoilage grade of mahi-mahi and the significant correlation between 2-butanone level and grade of tuna were observed (Table 4). The residual glycogen catabolism and fatty acid catabolism are major reasons leading to the accumulation of these three ketones in spoiled fish

Table 4–Flavor descriptors of volatile compounds associated with spoilage in mahi-mahi and tuna samples. Pearson correlation coefficients between levels of volatile compounds with increasing spoilage grade of mahi-mahi and tuna.

Compound name	Mahi-mahi Pearson coefficient (r)	Tuna Pearson coefficient (r)	Flavor descriptor
Methanol	−0.3571	n.f.	Alcoholic (Ruth, 1986)
DMA	0.849*	0.1289	Fishy (Ruth, 1986)
TMA	0.855*	0.8654*	Fishy (Olafsdottir et al., 2005)
Ethanol	n.f.	0.4451*	Alcoholic (Ruth, 1986)
Acetone	0.6569*	−0.0382	Apple pear (Ruth, 1986)
tert-butanol	−0.362	−0.0148	Camphor-like odor[w]
2-methylpropanal	0.4099	0.7246*	Fresh, floral (Girard et al., 2000)
2,3-Butanedione	−0.4688*	0.2689	Sweet, buttery[w]
2-Butanone	0.4391*	0.587*	Fruity, green[w]
Isobutylamine	0.6751*	0	Fishy (Gill, 1983)
3-methylbutanal	0.7689*	0.685*	Sweet, caramel, fishy (Olafsdottir et al., 2005)
2-methylbutanal	0.7967*	0.7021*	Musty (Girard et al., 2000)
1-Penten-3-ol	0.2353	n.f.	Pungent (Girard et al., 2000)
Acetoin	n.f.	0.2094	Buttery (Xiao et al., 2014)
3-methylbutylamine	0.5644*	0	Unpleasant ammonia (Takahashi et al., 2004)
2-methylbutylamine	0.6043*	0	Fishy (Zufall & Munger, 2016)
Isoamyl alcohol	0.612*	n.f.	Fusel, alcoholic (Komes et al., 2006)
Pyridine	−0.673*	n.f.	Fishy[w]
Dimethyl disulfide	0.6258*	n.f.	Sulfurous (Alasalvar et al., 2005)
Hexanal	−0.1482	n.f.	Green (Girard et al., 2000)
Benzaldehyde	0.6487*	0.6414*	Almond, fruity (Girard et al., 2000)
2-ethylhexanol	−0.6144*	−0.5408*	Fatty, fruity (Mahmoud et al., 2017)

*Significant below 0.05 level. n.f. signifies not found.
[w]Flavor descriptor obtained from the website reference: http://www.thegoodscentscompany.com/

and release a buttery, green odor (Joffraud et al., 2001). Wierda et al. (2006) reported the accumulation of acetone in spoiled king salmon. Significant increases of acetone and 2-butanone in sea bream (*Sparus aurata*) were observed after the freshly caught fish were stored on ice for 6 days (Soncin et al., 2009). Duflos et al. (2006) observed that the level of acetone in Cod (*Gadus morhua*), the levels of 2,3-butanedione, 2-butanone in Mackerel (*Scomber scombrus*) and Cod (*Gadus morhua*) increased after the fish samples was stored at 4 °C for 10 days. Similar results have also been reported by several studies and acetone, 2,3-butanedione, 2-butanone are identified as good markers to characterize freshness of fish due to their accumulation during fish spoilage (Alasalvar et al., 2005; Leduc et al., 2012; Olafsdottir et al., 2005; Prost, Hallier, Cardinal, Serot, & Courcoux, 2004).

Dimethyl disulfide is a sulfur compound with sulfurous odor and the formation of this compound was observed only in spoiled mahi-mahi samples. The level of dimethyl disulfide is initially low in freshly caught fish and accumulates after landing (Duflos et al., 2006). Dimethyl disulfide has an extremely low odor threshold, and thus can impact overall aroma of spoiled fish even at low levels. The generation of dimethyl disulfide is associated with the degradation of sulfur-containing amino acids and peptides. After fish samples were stored in ice for 23 days, the level of dimethyl disulfide increased from 20 to 560 ng/g in cultured sea bream (*Sparus aurata*) and from 0 to 617 ng/g in wild sea bream (*Sparus aurata*), respectively (Alasalvar et al., 2005). Similar results were also reported by Olafsdottir et al. (2005) and Leduc et al. (2012) that dimethyl disulfide increased in cod (*Gadus morhua*) as the spoiled degree increased. Any mahi-mahi sample containing dimethyl disulfide fell into the category of poor quality (Table 1).

The decline of pyridine in the tested spoiled mahi-mahi samples was observed in this study. Acetoin was only detected in grade 4, 5, and 7 tuna samples and the detected level of acetoin was highest in grade 5 tuna, and then followed by grade 4 tuna, grade 7 tuna. Acetoin is also named as 3-hydroxy-2-butanone and is formed by microbial activities occurring the fish spoilage imparting a buttery odor (Emborg & Dalgaard, 2006). It also has been reported that acetoin can be reduced to 2,3-butanediol as the spoilage progresses (Duflos et al., 2006). The relative level of acetoin in yellowfin tuna (*Thunnus albacares*) increased after fish samples were stored in ice for twenty days (Edirisinghe et al., 2007). Acetoin has been identified as a useful marker to characterize freshness of fish in previous studies (Duflos et al., 2010; Jonsdottir et al., 2008; Olafsdottir et al., 2005). The cut off level of acetoin for good quality tuna should be zero based on the GC-MS results of the different grades of tuna samples (Table 2).

Conclusions

A PT-GC-MS method with RTX-Volatile Amine column was developed for the analysis of mahi-mahi and tuna volatiles for chemical indicators of spoilage. Five volatile amines (dimethylamine, trimethylamine, isobutylamine, 3-methylbutylamine, and 2-methylbutanamine) strongly correlated with spoilage of both mahi-mahi and tuna. Three alcohols (2-ethylhexanol, 1-penten-3-ol and isoamyl alcohol), three aldehydes (2-methylbutanal, 3-methylbutanal, benzaldehyde), three ketones (acetone, 2,3-butanedione, 2-butanone), and dimethyl disulfide had good correlation with spoilage of mahi-mahi. Two alcohols (2-ethylhexanol, ethanol), four aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde), and three ketones (2,3-butanedione, 2-butanone, acetoin), strongly correlated with tuna spoilage. The purge and trap system applied in this method enriched the concentration of the analytes injected into GC-MS instrument. The application of RTX-Volatile Amine column made it possible to identify and quantify these five amines without any sample derivatization. This simplified and accurate PT-GC-MS method could be used to monitor the markers of tuna and mahi-mahi spoilage.

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Author Contributions

Jing Bai contributed to the study design and conducted the experimental work, analyzed data and drafted the manuscript. Paul J. Sarnoski designed this experiment, contributed to data analysis, and revised the manuscript. Shirley M. Baker, Renee M. Goodrich-Schneider, and Naim Montazeri contributed to the study design and the manuscript revision.

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