

Influence of heating temperature on the development of volatile compounds in bigeye tuna meat (*Thunnus obesus*) as assessed by E-nose and SPME-GC/MS

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Abstract

To explore the relationship between the composition of volatile compounds in bigeye tuna (*Thunnus obesus*) and different processing temperatures, we employed electronic nose (E-nose) and gas chromatography coupled with mass spectrometer (GC/MS) using headspace solid-phase micro-extraction (SPME) for sample preparation. This methodology detected the tuna meat's composition of volatile compounds before and after exposure to temperatures ranging from 70°C to 150°C. E-nose data were subjected to principal component analysis (PCA) and linear discriminant analysis (LDA). These results clustered samples into three distinct groups, untreated, 70-120°C and 150°C heat treated. First, E-nose was used to evaluate tuna meats treated with different heating temperatures. Based on the analysis of the E-nose, the untreated samples and samples heated to 100°C and 150°C were further analyzed using SPME-GC/MS. Different volatile compounds were detected in these selected samples. Compounds that contribute the fresh flavor of bigeye tuna meats were found in the untreated sample group. However, as heating temperature increased, the relative amount of aldehydes, ketones, alcohols decreased rapidly and hydrocarbons, heterocyclic compounds increased. This was also detectable with E-nose. In this study, using an E-nose and SPME-GC/MS combined analysis approach a more complete profile of volatiles in bigeye tuna meat has been created.

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Introduction

Tuna is an important economic commodity and food source. There are approximately forty species distributed in the Atlantic, Indian, and Pacific Oceans as well as the Mediterranean Sea. Worldwide, the annual production of tuna has been continuously increasing, from less than 0.6 million tons in 1950 to almost 9.5 million tons in 2010 (FAO, 2010). Bigeye tuna is one of the most popular types of raw fish eaten today, its meat is nutritious and flavorful.

Flavor is the defining quality of edible fish species. For this reason, sensory analysis is used to evaluate the fish quality. Each fish species has a delicate and unique flavor that can be affected by processing technology, post-processing procedures, and storage methods (Duflos *et al.*, 2010). Lipid-derived volatile compounds arise primarily from oxidative enzymatic reactions and autoxidation of lipids (Jacobo and Isabel, 2008). These are important flavor compounds in fish (Morita *et al.*, 2003). The composition of the volatile profile, which may include carbonyls, aldehydes, and ketones, can change depending on the handling conditions. Sensors that can detect these changes and equipment

that can optimize management of conditions during fish processing have become targets of innovation.

The electronic nose (E-nose) is an instrument used to detect and discriminate complex odors by means of a sensor array that is designed to respond to various volatile compounds. E-nose technology has widespread use in food analysis (Schaller *et al.*, 1998; Peris and Escuder-Gilabert, 2009; Berna, 2010). Moreover, these machines have received special attention for their assessment of fish freshness (Masot *et al.*, 2009; Liu *et al.*, 2010). They also have applications in predicting optimal storage time and shelf life (Dodd *et al.*, 2000; Limbo *et al.*, 2009). Traditionally, gas chromatography (GC) either alone or coupled with mass spectrometry (GC/MS) has been used for analysis of fish volatiles. This method, however, requires the extraction of volatile compounds. The traditional extraction method is time consuming. Solid phase microextraction (SPME) is a simple extraction method that does not use solvents (Buldini *et al.*, 2002). SPME has been applied in the assessments of fish quality. Edirisinghe *et al.* (2007) studied the volatile substances relevant to stored yellowfin tuna using SPME coupled with GC/MS analysis. Their findings highlight the possibility that

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a rapid, high quality means of evaluating fish using SPME-GC/MS may be developed.

We explored the relationship between volatile compounds of bigeye tuna with different processing temperatures to provide more reference material for further study and practical application. In this study, we combined E-nose and SPME-GC/MS analyses to detect changes in the volatile compound profile of bigeye tuna meats before and after various heat treatments.

Materials and Methods

Materials

Bigeye tuna specimens (*Thunnus obesus*) were obtained from Ningbo Today Food Corp. Ltd. (Zhejiang, China) as round fish. Before the analysis, fish were stored in cold storage. The fish were filleted and dorsal ordinary muscle was immediately harvested. The samples were then subjected to E-nose and GC/MS analysis, as detailed in the following.

Fish samples (1.00 g) were minced and placed in 15 ml glass vials containing silica gel and sealed with Teflon caps. The samples were then incubated at 70°C, 80°C, 90°C, 100°C, 110°C, 120°C, or 150°C for one hour, and then cooled to room temperature for E-nose analysis. Untreated samples were analyzed directly. For GC/MS experiments, there are three groups of samples, untreated, 100°C heat treated, and 150°C heat treated. To perform data statistical correlation, e-nose measurements were taken at the same time that samples obtained for GC/MS.

E-nose analysis

Analyses were performed with a portable, E-nose (PEN3, Airsense Analytics GmbH, Schwerin, Germany). PEN3 uses a sensor array, and pattern recognition software (Win Muster v.2.1) to analyze and record data. The PEN3 was equipped with 10 different thermo-regulated (150-500°C) metal oxide semiconductors sensors that are sensitive to different classes of chemical compounds. The equipment works uses clean air (through an activated charcoal filter) as reference gas.

The E-nose analyses were recorded over the range of 0–110 s using an accumulation time of 1 s; the injection flow rate was 300 mL/min. Measurement procedure was controlled by a computer program. After each analysis, the sampling chamber was washed with clean air flow (600 mL/min) for 600 s. Six samples were analyzed from each temperature treatment group. Three replicates of each sample were performed to verify the signal stability and to get a sufficient number of data that considers all sample variations.

Chromatographic analysis

Volatile compounds were pre-concentrated using the solid-phase micro-extraction (SPME) method. A SPME fibre, coated with 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB; blue fiber; Supelco, U.S.), was employed to capture volatile compounds in sample vials which were placed in a 60°C water bath. The SPME fibre was manually inserted into the vial for 30 min. The SPME fiber collected volatile compounds emitted in the headspace above the samples. Next, the SPME fiber was analyzed by GC/MS, as described below.

The volatile compounds adsorbed to the fiber were desorbed for 2 min at 250°C using a splitless injection mode GC/MS (QP2010, Shimadzu, Japan). After each sample injection, fibres were kept inside the SPME needle to prevent possible contamination and were conditioned with helium at 250°C for 10 min before reuse. The compounds were separated in a VOCOL capillary column (60 m × 0.32 mm internal diameter, 1.8 µm film thickness, Supelco, U.S.). Helium was used as the carrier gas at a rate of 0.3 ml/min. The injection and the detection ports were operated at 210°C. The GC oven temperature program was started when the fiber was inserted and held at 35°C for 2 min, increased to 40°C at 3°C/min, maintained at 40°C for 1 min, heated to 210°C at 5°C/min, and maintained at 210°C for 25 min. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV. The detector temperature was 200°C, and the scan range of m/z was 45–1000 amu.

Chromatographic peaks were identified, and their gas chromatograph retention times and mass spectra were compared to those reported in NIST147, NIST27, and WILEY7 standard MS libraries. Peak area normalization method was used for quantitative analysis of volatile compounds.

Statistical analysis

An E-nose data set was built by calculating the sensor signal means in the signal constant range of 95–100 s recorded during analysis. Principal component analysis (PCA) and linear discriminant analysis (LDA) were conducted using the E-nose system Win Muster v.2.1 software package.

Results and Discussion

E-nose response to changes of bigeye tuna flavor

The evolution of signals from the E-nose sensors is presented in Figure 1. Each line represents the average signal variation of conductance in response to untreated and heat treated tuna samples, linking to the measurements of conductance. The conductance

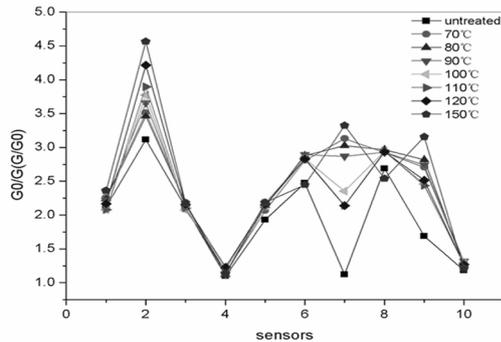


Figure 1. Relative conductivity of sensors on heat treated samples. The signals of the various samples obtained by sensors 1, 3, 4, 5 and 10 were very close. However, sensors 2, 7 and 9 were sensitive to changes because of its sensitivity to aldehydes, ketones, and heterocyclic compounds.

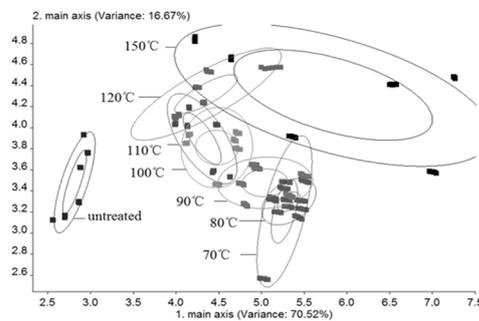


Figure 2. Principal component analysis (PCA) score plot of volatile aromatic compounds in bigeye tuna meats. The score plot in the area defined by the first two principal components (87.19% of the total variance) revealed two clusters according to temperature.

of sensors 1, 3, 4, 5 and 10 did not show significant change. Sensors 2 (broad range, polar and NO_x), 7 (sulphur-chlorinate), and 9 (sulphur and aromatic organic) displayed the greatest changes, implying these compounds importance in bigeye tuna flavor (see GC/MS analysis).

PCA and LDA of E-nose data

E-nose data collected from the samples during 95-100s were analyzed by PCA to evaluate the ability of PEN3 to detect changes in the volatile compound profiles of tuna meat after heat treatments. PCA was performed in a covariance matrix to achieve a partial visualization of the data set in a reduced dimension (Benedetti *et al.*, 2008). Examination of the score plot (Figure 2) in the area defined by the first two principal components (87.19% of the total variance) revealed two clusters of bigeye tuna with a clear separation according to temperature along the first principal component (PC1, 70.52%). The second principal component (PC2) explained 16.67% of the variance. Untreated samples were separated on PC1 axis from all heat treated samples. Although treatment

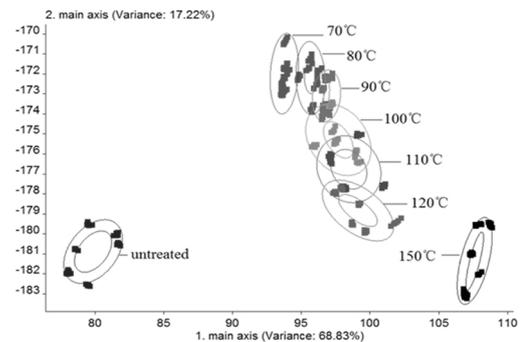


Figure 3. Linear discriminant analysis (LDA) of heat treated bigeye tuna meats. LDA analysis of the data sets separated the samples into three distinct classes, untreated, heat treated (70–120°C), and heat treated 150°C.

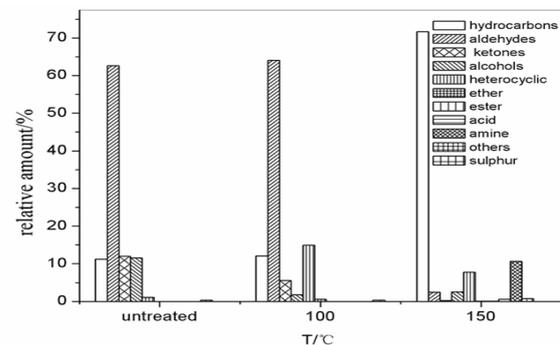


Figure 4. Bigeye tuna volatile compound profile determined by GC/MS. As heating temperature increased, the relative amount of aldehydes, ketones, alcohols decreased rapidly. Whereas, the relative amount of hydrocarbons, heterocyclic compounds increased.

groups overlapped with each other, a particular trend correlating with treatment temperature is visible (bottom to top on PC2 axis) for all the experimental temperatures except for the 150°C heat treated samples. LDA analysis of the data sets separated the samples into three distinct classes, untreated, heat treated (70–120°C), and heat treated 150°C. LDA function 1 (LD1) and function 2 (LD2) accounted for 68.83% and 17.22% of the variance, respectively. The total variance (86.05%) is represented in the score plot (Figure 3).

GC/MS analysis of volatile compounds

Based on the above results, untreated tuna samples and two heat treated samples (100°C and 150°C) were selected for further GC/MS analysis to determine their volatile compound profiles which are relevant to flavor.

A total of 113 kinds of volatile compounds were identified across the three treatment groups by GC/MS analysis. These compounds include: 60 hydrocarbons, 24 aldehydes, 8 ketones, 7 alcohols, and 6 heterocyclic compounds (Table 1). The volatile

Table 1. Volatile compounds of bigeye tuna (*Thunnus obesus*)

Retention time (min)	Compound name	Relative amount (%)		
		Untreated	100°C	150°C
Aldehydes				
6.508	Propanal	0.69	—	—
20.142	2,4-Pentadienal	31.74	11.95	—
24.317	Heptanal	4.88	6.14	0.03
24.525	(Z)-4-Heptenal	0.91	0.87	—
28.158	Octanal	5.63	9.63	—
28.458	Benzaldehyde	2.21	6.88	0.05
29.317	(E)-2,4-Heptadienal	2.16	—	—
30.525	2-Octenal	0.74	0.47	—
31.675	Nonanal	5.79	15.44	—
33.883	(E)-2-Nonenal	0.31	0.38	—
34.033	2,6-Nonadienal	0.61	—	—
34.942	Decanal	0.65	1.84	—
35.325	4-(2-Methylenecyclopropyl)butanal	0.63	—	—
35.467	4-Ethylbenzaldehyde	0.89	3.54	—
37.033	(E)-2-Decenal	—	0.57	2.36
38.008	Undecanal	0.05	1.12	—
38.317	2,4-Nonadienal	0.65	—	—
39.100	2,4-Decadienal	0.61	—	—
39.292	2-Pentyl-2-nonenal	2.89	—	—
39.975	2-Dodecanal	—	0.34	—
40.925	Dodecanal	0.59	1.39	—
44.350	Tridecanal	—	1.13	—
48.783	Tetradecanal	—	0.95	—
62.983	Hexadecanal	—	1.45	—
Ketones				
15.417	3,3-Dimethyl-2-butanone	0.93	—	—
23.850	2-Heptanone	—	0.62	0.08
26.267	6-Methyl-2-heptanone	—	0.62	—
27.667	2-Octanone	—	0.31	0.02
27.308	2-Methyl-3-octanone	5.8	—	—
31.217	2-Nonanone	2.4	0.77	0.15
32.125	3,5-Octadien-2-one	2.13	0.26	—
37.575	2-Undecanone	0.74	2.95	—
Alcohols				
14.233	1-Penten-3-ol	3.72	0.42	—
26.858	1-Octen-3-ol	7.8	1.03	—
29.358	2-Isopropyl-5-methyl-1-heptanol	—	—	0.61
31.683	1-Decanol	—	—	0.19
36.450	Isotridecanol	—	—	1.23
37.467	2-Methyl-5-(1-methylethyl)-cyclohexanol	—	—	0.38
37.767	5-Methyl-2-(1-methylethyl)-1-hexanol	—	—	0.1
Heterocyclic compounds				
15.092	2-Ethyl-furan	0.38	3.77	—
27.375	2-Pentylfuran	—	4.67	0.08
28.033	(E)-2-(2-Pentenyloxy)furan	0.25	2.97	—
35.308	1,2-Epoxy-5-cyclodecene	—	1.08	—
36.750	Naphthalene	—	0.50	7.61
37.450	6-(5-Methyl-2-furyl)-2-hexanone	—	1.07	—
38.475	8-Methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane	0.43	—	—
39.617	1-Methyl-2,4-imidazolidinedione	—	—	0.1
42.108	1,2-Epoxyhexadecane	—	0.92	—
Ether				
15.508	Allyl ethyl ether	—	0.58	—
26.250	Allyl octyl ether	—	—	0.04
Sulphur compounds				
4.333	Methanethiol	—	0.29	—
Ester				
26.667	Acetic acid, cyclohexyl ester	—	—	0.04
Acid				
34.467	Benzoic acid	—	—	0.54
Amine				
4.217	N,N-dimethyl-Methylamine	—	—	10.7

Note: —, not detected

compound profiles for these samples determined by GC/MS agree with the E-nose data collected specifically from sensor 2 (broad range, polar and NOx).

Effects of high temperatures on the volatile compound profile of bigeye tuna meat

Changes in the volatile compound profiles of bigeye tuna meat after heat treatment are summarized in Table 1 and Figure 4. In the untreated sample, volatile compounds primarily consisted of aldehydes (62.63%), such as 2,4-pentadienal (31.74%), heptanal (4.88%), octanal (5.63%), nonanal (5.79%). The next most prevalent compounds ketones (8.2%), such as 2-methyl-3-octanone (5.8%) and 2-nonanone (2.4%) were found at a far lower abundance. After heating for one hour at 100°C, aldehydes (64.09%) were still the most dominant volatiles detected, but the relative amounts of these compounds changed and new compounds were detected. Heterocyclic compounds (14.98%), such as 2-ethyl-furan (3.77%) and 2-pentylfuran (4.67%), were the second largest

volatile group in these samples. There were great changes in the 150°C heat treated samples' relative levels of carbonyls during the heating process. In particular, the amount of aldehydes, the main compounds detected in untreated and 100°C heat treated samples decreased sharply in these samples, and hydrocarbons became the most abundant.

Characterization of volatile compounds

Aldehydes make up the majority of volatile compounds in untreated tuna meats and meats heated to 100°C. Aldehydes are responsible for oxidized aromas of foods and are important in many food products. Twenty-four aldehyde species were identified in our analysis, including ten saturated aliphatic and fourteen unsaturated aliphatic species. Saturated aliphatic aldehydes, derived from oxidative degradation of polyunsaturated fatty acids (PUFA), normally have unpleasant, sharply pungent or irritating odors with oily and waxy topnotes (Baek and Cadwallader, 1997). The most abundant aldehyde, 2,4-pentadienal, found in the untreated samples produces a gravy flavor that contributes to the complex taste of bigeye tuna (Vincent *et al.*, 2007). Heptanal, octanal, and nonanal have also been detected in fresh fish (McGill *et al.*, 1977; Kawai, 1996; Edirisinghe *et al.*, 2007). These compounds were identified in untreated bigeye tuna meats. Interestingly these compounds increased to higher levels in the 100°C heat treated samples, however the concentrations plummeted to undetectable levels in the 150°C heat treated samples. Heptanal, octanal, and nonanal are characterized as having green-fatty and green-fruity aromas (Shibamoto and Horiuchi, 1997). Nonanal and octanal are the most powerful aroma-active aldehydes and contribute to the aroma profile of the grey mullet (Cayhan and Selli, 2011). In the 100°C heat treated samples tridecanal, tetradecanal, and hexadecanal were detected. The 150°C heat treated samples contained, heptanal, benzaldehyde, and (E)-2-decenal. The relative content of (E)-2-decenal increased from zero to 2.36% in high heat treated tuna samples. These aldehydes have been described in the literature as contributing the fatty aroma of ripened cod roe and the almond aroma of cooked grey mullet (Jonsdottir *et al.*, 2004; Cayhan and Selli, 2011). The relative content of (E)-2-decenal increased from zero to 2.36% in high heat treated tuna samples.

Eight ketones were detected in bigeye tuna meats. Ketones comprised 12% of total volatiles in untreated tuna meats, 5.53% in 100°C heat treated samples, and 0.25% in 150°C. Untreated tuna meats contain high concentrations of 2-methyl-3-octanone, 3,5-octadien-2-one, and 2-nonanone. The 100°C

heat treated samples contain 2-undecanone (2.95%), 2-heptanone (0.08%), 2-octanone (0.02%), and 2-nonanone (0.15%) were detected in 150°C heat treated samples. Generally, ketones with lower aroma thresholds result in greater contributions to overall fresh fish-like odors (Alasalvar *et al.*, 2005). Ketones may be produced by thermal degradation, oxidation of polyunsaturated fatty acids, degradation of amino acids, microbial oxidation, and by the Maillard reaction (Chung *et al.*, 2007).

Seven alcohols were identified in bigeye tuna meats. These alcohols are generally minor contributors to food flavor because of their high aroma thresholds (Baek and Cadwallader, 1997), they are present in high concentrations and/or in unsaturated form. They possess primarily fragrant, plantlike, rancid, and earthy odors and they can contribute to smoother taste qualities (Cadwallader *et al.*, 1995). Some of the alcohols found in the samples (untreated and 100°C heat treated samples) that contribute to the meats complex taste include, 1-octen-3-ol, which has a mushroom-like aroma, and has been identified as one of the major volatile alcohols in sea bream and cooked alligator meat (Alasalvar *et al.*, 2005; Baek and Cadwallader, 1997). Jonsdottir *et al.* (2004) reported that 1-octen-3-ol is the most important compound contributing to ripened roe odor. Another important alcohol, 1-penten-3-ol, compound has a green, fishy odor (Iglesias *et al.*, 2009). This compound has also been found in refrigerated sardines and identified as the compound that provides major contribution to sardines' paint-like and chemical-like odor (Ganeko *et al.*, 2008).

Other compounds that contribute to tuna meat flavor that were identified by SPME are heterocyclic compounds, ether, sulfur, ester, acid, and amine, which mainly present in heated bigeye tuna meats (100°C and 150°C) (Figure 4). Among them, heterocyclic compounds especially 2-ethyl-furan and 2-pentylfuran were identified. Most furans have been reported to contribute to burnt, sweet, bitter, meaty, and coconut-like flavors in some foods. In addition, 2-pentylfuran has also been linked to the undesirable beany, grassy reversion flavors of soybean oil (Krishnamurthy *et al.*, 1967; Maga, 1979; Moon *et al.*, 2006; Xie *et al.*, 2008). In 150°C heat treated samples naphthalene and N,N-dimethyl-methylamine were detected at relatively high levels (7.61% and 10.7%). Naphthalene is present in tuna meats because of environmental pollution and overexploitation of certain natural resources (Hossain *et al.*, 2010). Its relative content increased as heat treatment temperatures increase. N,N-dimethyl-methylamine (also called trimethylamine), is naturally produced

during the cooking of fresh fish. When choosing sites and designing fish factories engineers should take into account that trimethylamine has an emission factor of 0.3 lb/ton of fresh fish cooked (Gavriel, 2001). Moreover, to avoid the accumulation of trimethylamine we recommend that the heating temperatures should be control below 150°C during fish processing.

Most of the volatile compounds identified in this study have already been identified in several freshwater and saltwater fish species. Aldehydes are the most dominant volatiles in the untreated bigeye tuna, accounting for the largest proportion of the total volatile compounds (Figure 4). Food matrix components (such as proteins and fats) are known to interact with flavor compounds, which can influence the retention of volatile compounds in chromatographic analysis (Chevance and Farmer, 1999). Heat treatment induces a series of changes in precursor substances (fats, proteins, and sugars) in bigeye tuna to form varied flavor compounds.

Conclusion

SPME-GC/MS is an effective method that has been used extensively for analysis of volatile compound profiles in foods. This methodology has been employed here to establish the effects of heating on bigeye tuna meat volatile compound profile. The heating process is known to cause changes in its complex flavor patterns of fish meats. These changes presumably arise from proteolytic and lipolytic reactions. After heating at 150°C for 1 hour, tuna meat emitted a significantly stronger amine odor than control samples. Here we have shown that data collected by E-nose support its use as a viable method for rapid detection of changes in the volatile compound profile of tuna meat after heat treatment. The changing volatile compound profile correlates to flavor changes observed in the tuna meat after heat exposure. The combination of E-nose and SPME-GC/MS provides a great deal of information and a more complete picture of bigeye tuna complex flavor. The detection of changes in tuna flavor after heating at different temperatures can ensure consistent quality for further processing and may help manufacturers to select tuna products of optimal quality.

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