Evaluation of gas freshness indicator for determination of skate (*Raja kenojei*) quality during storage

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Abstract A gas freshness indicator consisting of the pH-sensitive dye bromothymol blue-phenol red (BTB-PR) was investigated for visible color changes of fish product quality based on the quantity of volatile amines. Chromaticity values of the gas indicator accurately tracked an increase in the ammonia content in the packaging headspace of fish products, especially skate (*Raja kenojei*). Gradual color changes of the gas indicator response correlated with the quality of fish including pH values during storage. Ammonia content was evaluated during the fish storage using headspace solid-phase microextraction analysis with gas chromatography-flame ionization detection. The validation results suggested that good linearity ($R^2=0.99$) and recovery of 84.00%. The limits of detection and quantification were 8.70 and 26.30 mg/L, respectively. A pH values of skate and chromaticity of gas indicator were measured and the correlation with ammonia content was evaluated. Results of this study could prove the application of gas indicator as intelligent package for improvement of food safety.

Keywords: gas indicator, ammonia, fish quality, freshness indicator, skate (*Raja kenojei*)

Introduction

Control of fish quality is a prominent concern because of increasing request by consumers for quality and hygienic products. Fish freshness affect the fish consumption and can be changed easily during storage and transportation of fish products. The fish freshness could be evaluated by sensory aspects, including check of the eyes color, scale condition, fillet elasticity and odor. Odor is one of the most important parameters of fish freshness and could be easily determined by consumers (1).

The total volatile basic nitrogen compounds (TVB-N) including dimethylamine (DMA), trimethylamine (TMA), and ammonia (NH) are the characteristic materials responsible for quality of fish products. The TVB-N content is recognized as an index of freshness of fish products, and volatile amines are directly correlated to the sensorial quality of fish products (1,2). Among TVB-Ns, the concentration of ammonia is effective parameter for quality of fermented fish especially skate (*Raja kenojei*).

Fermented skate (*Raja kenojei*) is a Korean traditional food and caught in most of the world's seas such as Japan, the east sea of China and the southwest coast of South Korea. Fermented skate has a unique ammonia-like flavor. In addition, the function and nutrition of skate have been reported. The population favored a unique flavor

and taste is gradually increasing. Skate has a lot of urea and urease inside for controlling its osmotic pressure in the deep sea (3,4). Depending on the fermentation progresses, urease decomposes the urea and then the ammonia is generated. These unique odors and tangy flavors are produced. The content of ammonia depends on the storage period. Therefore, the level of ammonia could be used for the determination of skate freshness, quality and fermentation level.

Furthermore, there is increasing interest in the development and study for the determination of food freshness and quality indicators (5). Especially, the development of freshness indicators for volatile compounds generated in fish spoilage have been conducted. One concept is the simple color indicator for freshness in the form of in package sensor that could be monitored quality in fish products. The indicator for fish spoilage on the presence of total volatile basic nitrogen (TVB-N) was developed by Diamond's group (6,7). They used the colorimetric dye-based sensor contained bromocresol green as a pH sensitive dye.

In this study, applying these development, gas indicator for skate was developed and evaluated. The ammonia contents are increasing during fermentation of skate, and the pH value of skate are changed depending on the concentration of ammonia. Bromothymol blue-phenol red (BTB-PR) were used as a pH dye. The method validation for ammonia was conducted, and then experimental applications for

gas indicator of skate are performed. The ammonia content was detected using headspace solid-phase microextraction (HS-SPME) analysis and a gas chromatography-flame ionization detector (GC-FID) system. HS-SPME was developed in the early 1990s to analyze volatile compounds and has demonstrated a unique capability to incorporate extraction and concentration in a single step (8). This method has the advantages of simple manipulation and compatibility with gas chromatography. The pH and ammonia content of skate (*Raja kenaei*) samples were evaluated during storage under three different temperature conditions (4, 10, and 20°C). The pH values and chromaticity difference of gas indicator were evaluated for correlation with ammonia content. The purpose of this study was evaluate the gas freshness indicator consisting of the pH-sensitive dye bromothymol blue-phenol red (BTB-PR) for determination of fish quality during storage.

Materials and Methods

Materials and chemicals A 30% ammonia solution was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Sodium hydroxide was obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). A total of 65 µm of polydimethylsiloxane divinylbenzene (PDMS-DVB) of solid-phase microextraction (SPME) fiber was purchased from Supelco (cat. no. 57310-U; Bellefonte, PA, USA). Headspace vials (20 mL) with screw caps and PTT septa were obtained from Supelco (cat. no. SU860097). Skates (*Raja kenaei*) were purchased from a local market in Seoul, Korea. All other miscellaneous chemical reagents were of analytical grade and were acquired from Burdick & Jackson (Muskegon, MI, USA).

HS-SPME analysis Finely homogenized skate samples (2.0 g) that were stored at different temperatures (4, 10, and 20°C) were weighed respectively in 20 mL vials equipped with a magnetic stirrer bar, and 40% sodium hydroxide (NaOH) (3 mL) was added. After that, the samples were heated for 15 min at 50°C to extract headspace ammonia gas. Finally, the fiber needles were immediately inserted into the GC injector in splitless mode and desorbed for 5 min (9).

Gas chromatography The chromatograph used was an Agilent 6890 gas chromatography-flame ionization detector (GC-FID) system fitted with an HP-1 (30 m 0.25 µm based dimethylpolysiloxane). The injector and detector temperatures were set at 250°C. Initially, the column temperature was maintained at 50°C for 5 min, then increased by 10°C/min to 150°C and maintained for 15 min (Table 1) (10).

pH measurement The pH measurements of skate samples were performed using a pH meter (Orion 3-star Meter; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at different temperatures of 4, 10, and 20°C. pH values were measured using 5 g of ground skate

samples with 5 mL of distilled water. The calibration of pH meter was performed using pH buffer solutions at pH values of 10.00±0.01, 7.00±0.01, and 4.01±0.01 at 25°C (8).

Gas freshness indicator Gas indicator (GI) spot was instilled by polymer matrix solution including pH sensitive dye, bromothymol blue-phenol red (BTB-PR). This GI spot was coated with polyethylene terephthalate (PET) film which had a 10 mm diameter hole on the bottom side to allow the gas to pass through. A hydrophobic gas permeable membrane was added to protect the GI from humidity, while allowing gaseous compounds to pass through (2).

Chromaticity analysis The chromaticity of gas indicator was performed by colorimeter (CR-300; Minolta, Tokyo, Japan) during 9 days. The gas indicator was placed on white in a closed box to avoid any disturbance of light from the surrounding (11). The Hunter color value, included L (lightness), a (redness), and b (yellowness), was used. The E value was calculated using the L, a, and b values.

Statistical analysis All experiments were performed in triplicate ($n=3$), and statistical analysis was conducted using the IBM SPSS Statistics 21 (IBM, Armonk, NY, USA) software package program for Windows. The values were expressed as mean±standard deviation (SD). A one-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using the Student-Newman-Keuls test with significance defined as $p<0.05$ for the ANOVA (10).

Results and Discussion

Validation of analysis The HS-SPME with PDMS-DVB fiber was prepared, and the fiber needles were inserted into the GC-FID to determine the ammonia content (10). Developed by Pawliszyn and Arthur (12,13), solid-phase microextraction is considered simple, efficient, and solvent-free. Headspace solid-phase microextraction (HS-SPME) is a non-invasive sampling mode of SPME for volatile analytes in the gas mixture. Al-Masri and Al-Bachir (14) identified 65 µm of polydimethylsiloxane-divinylbenzene (PDMS-DVB) as the most suitable SPME fiber to determine the amounts of the VBNS dimethylamine (DMA), TMA, and NH. According to Loughran and Diamond (15), the most commonly used method of determining volatile bases in fish samples is GC.

Method was validated for linearity, limit of detection (LOD) and limit of quantification (LOQ). Method validation was preceded by the use of standard solutions. The use of the five different concentrations (5,000, 10,000, 15,000, 20,000, 25,000 mg/L) is calibrated, and the R^2 of 0.99 shows a higher accuracy for linearity. The limit of detection (LOD) is defined as the lowest concentration leading to a signal-to-noise ratio of 3, whereas the limit of quantification (LOQ) is defined as the concentration leading to a signal-to-noise ratio of 10 (16). Furthermore, the recovery values are measured using the peak area

of stand and it varied between 80.00-120.00 %. In this research, the LOD, LOQ, and recovery of peak area are represented in Table 2.

Storage experiment (Experimental applications) The storage test for skate was preceded. It measured the generation rate of ammonia in the skate sample during the fermentation storage period of 9 days at room temperature (Table 3). The generation rate of ammonia in the skate sample tended to be higher when the storage period was long. Lee *et al.* (17) reported that the ammonia generation rate of domestic fermented skate ranged from 4.4 to 14.9 mg/g. These figures include the range of the ammonia generation rate in this research. The content of ammonia had the range from 1.7 to 26.2 mg/g during fermentation period of 9 days. In addition, the pH values of skate were measured during storage time of 9 days at 3 different temperatures (4, 10, and 20°C) (Table 4). At the initial of storage time, the pH values tended to be low, and it represented the fresh state of skate samples. At the longer fermentation time and the higher fermentation temperature, the pH level tended to be high and reached over pH values of 7.0 represented the base state. Kuswandi *et al.* (18) suggested that the degree of fermentation was estimated through the measurement of the concentration and pH value of ammonia in skate, and the degree of fermentation can be regulated. Therefore, the fermentation level can be identified from the color change of the gas indicator.

Chromaticity of gas indicator The 20.00 g sample was put into each acryl container, and the change of color was observed by attaching the indicator to the top of the container. It measured the chromaticity of the indicator, followed by the fermentation storage period (Table 5). The indicator was gradually changed to red from yellow and switched to purple, after which no change occurred. There was a difference of the color change rate due to the difference

Table 1. Conditions of GC-flame ionization detection (GC-FID) analysis for volatile compounds in skates

GC analysis condition	
Instrument	Agilent Technologies model 6890
Carrier gas	10 mL/min nitrogen (constant flow)
Column	HP-1 (30 m×0.32 mm×0.25 µm; J&W Scientific, Folsom, CA, USA)
Temperature Gradient	50°C hold for 5 min, 10°C/min to 150°C Hold for 15 min
Injector	250°C
Injection mode	Splitless mode

degree of sample fermentation due to the temperature, and it can be found that there was a distinction in the color change for each temperature from the change of E.

Evaluation of gas indicator In terms of the ammonia standard solution with five different concentrations (5,000, 10,000, 15,000, 20,000, 25,000 mg/L), the correlation is represented with the pH and E value at room temperature (Fig. 1). The correlation between change of pH values during storage period and concentration of ammonia was confirmed, and the results came out the squared correlation coefficient of determination ($R^2=0.98$). In addition, the ammonia content increased in direct proportion with an increasing chromaticity difference of gas indicator during storage period of skate. In other words, the results represent an intimate correlation. The increase in ammonia emissions depends on the fermentation progresses and it makes the base state of skate samples. Therefore, the base state of skate produce the chromaticity difference from yellow to purple. It can be concluded that ammonia as a gas indicator index material, that is, BTB-PR, possesses a strong correlation

Table 2. The limit of detection (LOD), limit of quantification (LOQ), recovery, and linearity of ammonia

LOD (mg/L)	LOQ (mg/L)	Recovery (%)	Calibration equations	Linearity (R^2)
8.70	26.30	84.00±4.50	$y=0.0078x+138.3700$	0.9932

Table 3. Ammonia content of skate during fermentation period at room temperature (unit: mg/kg)

Storage time (day)				
1	3	5	7	9
1720.51±11.27 ¹⁾	4875.64±29.39	8585.90±34.17	14192.31±87.56	26183.33±152.43

¹⁾All values are mean±standard deviation of triplicate determinations.

Table 4. The pH value of skate during fermentation period at different temperatures (4, 10, and 20°C)

Temp (°C)	Storage time (day)				
	1	3	5	7	9
4	6.70±0.00 ^{1),d,2)}	6.77±0.01 ^c	6.81±0.00 ^b	6.84±0.00 ^a	6.82±0.01 ^{ab}
10	6.71±0.00 ^e	6.78±0.00 ^d	6.83±0.00 ^c	7.04±0.00 ^b	7.70±0.00 ^a
20	6.72±0.00 ^e	6.81±0.00 ^d	6.98±0.00 ^c	7.48±0.00 ^b	8.03±0.01 ^a

¹⁾All values are mean±standard deviation of triplicate determinations.

^{2)a-f}Means with different capital letters in the same column are significantly different ($p<0.05$) by Student–Newman–Keuls test.

Table 5. Chromaticity of the sensor in response to ammonia according to fermentation of skate at different temperatures (unit: ΔE value)

Temp. (°C)	Storage time (day)				
	1	3	5	7	9
4	0.00 ^{1),a2)}	2.17±1.66 ^b	3.76±1.70 ^b	22.36±1.07 ^c	34.17±1.88 ^d
10	0.00 ^a	3.50±0.85 ^b	22.09±0.27 ^c	31.47±2.10 ^d	41.53±0.68 ^e
20	0.00 ^a	2.67±0.92 ^b	11.50±2.11 ^c	13.47±2.08 ^c	18.11±1.23 ^d

¹⁾All values are mean±standard deviation of triplicate determinations.

^{2)a-f}Means with different capital letters in the same column are significantly different ($p<0.05$) by Student-Newman-Keuls test.

Fig. 1. (A) Correlation between the pH value and ammonia concentration; (B) Correlation between E value and ammonia concentration.

through the former outcome.

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