Development of freshness indicator for quality of skate (*Raja kenojei*) during storage

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Abstract There is much interest in the fishery industry in developing freshness indicator which can reflect the storage history of products and their quality. Freshness is one of the main considerations for the fishery industry and consumption. In this study, a freshness indicator of fish products, especially skate (*Raja kenojei*), was developed. The release of amines especially ammonia from decomposing fish is concentrated in headspace and they can be detected by pH-sensitive sensor unit. The sensor unit was organized with polymer matrix solution, which contains bromothymol blue-phenol red as pH-sensitive dye to monitor visible color changes from yellow to purple. We investigated the sensor unit formulation, which responds to the ammonia formation in fish products. The color change of the sensor unit was evaluated as well as the selection of the proper weight of fish products and packaging material. Results will be useful to establish a basis for development of a freshness indicator and to determine the suitability of freshness indicator formulations for intelligent food packaging applications.

Keywords: freshness indicator, ammonia, food quality, package, skate (*Raja kenojei*)

Introduction

Packaging plays an important role in the food industry and there is an increasing interest in the development of rapid methods for the determination of freshness and general quality of foods. The utility of food indicators would enable freshness to be confirmed at various stages along the distribution chain, and could be developed into “smart” packaging that provides information on the freshness of the packaged food to the consumer (1). Emphasis would reflect and account for the product’s history and their storage conditions from “harvest-to-home” (2).

The interest in fish and fish-based products has also increased sharply in recent years because of health concerns. Given this increasing demand, there is considerable interest from the fisheries industry in developing rapid methods to evaluate the real-time freshness of fish and seafood products (2). The freshness of fishery products may be determined by odor, oxidation, and microbiological criteria. Among these parameters, odor is one of the most important factors in easily detecting the freshness levels of fish by consumers. The odors of fishery products come from short-chain alcohols, acids, carbonyl, and amines, such as ammonia (NH_3). The levels of these materials, which are released according to fish storage, are effectual parameters for fish freshness. Therefore, volatile amines, such as trimethylamine (TMA), NH, and dimethylamine (DMA), are recognized

as indicators of fishery spoilage. The sensor unit of the indicator contains a chromoreactant dye (pH-sensitive) that indicates the presence of volatile spoilage compounds by a visible color change (2-4).

In some papers, freshness indicators for the volatile compounds produced in fish spoilage have been implemented. However, it takes a long time to leach the dye, which resulted in inexact fish freshness indicator responses. As a result, the occurrence of dye leaching needs to be investigated when this sensor unit system is used. Alternative methods are necessary for the determination of fish freshness using an indicator that is free from leaching, accurate, easy to use, low-cost, and rapid to evaluate fish freshness (5,6).

Furthermore, the study of a fish spoilage indicator is still limited. In this paper, the fish freshness indicator was applied to skate (*Raja kenojei*), a Korean traditional food. Skates are distributed in most of the world’s seas as a benthic fish, including the East Sea of China, the southwest coast of South Korea, and Japan. As the nutritional characteristics and function of fermented skate have been reported, interest in skates has increased, and the preference for a unique smell and taste is gradually increasing. Skate has a unique smell because it contains a lot of urea and urease inside for controlling osmotic pressure in the deep sea (7,8). The ammonia smell is formed during fermentation. The amount of ammonia generated is different depending on the storage period and ammonia content was used for

Fig. 1. Prototype of sensor unit for gas indicator

the determination of skate freshness and fermentation level of consumer acceptance. The main purpose of the present study was to evaluate the freshness indicator for quality of skate (*Raja kenojei*) During Storage.

Materials and Methods

Materials and chemicals Skates (*Raja kenojei*) were obtained from a local market in Seoul, Korea. A 30% ammonia solution was purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea) Bromothymol blue-phenol red (BTB-PR) was purchased from Ricca Chemical Co. (cat. no. 1385-16; Arlington, TX, USA), Dibutyl phthalate (DBP) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetone was obtained from Burdick & Jackson Labs (Muskegon, MI, USA). Sodium hydroxide and 2-butanone were obtained from Junsei Chemical Co. (Tokyo, Japan). Filter papers were obtained from Whatman GE Healthcare & Life Sciences (Seoul, Korea), and polytetrafluoroethylene (PTFE) as a gas-permeable membrane was obtained from Hyundai Micro., Ltd. (Seoul, Korea) All other miscellaneous chemical reagents were of analytical grade and were acquired from Burdick & Jackson.

Gas indicator Fabrication Gas indicator sensor unit spots were prepared by coating with a bromothymol blue and phenol red (BTB-PR) polymer formulation. In this study, BTB-PR were used as a pH dye, and a yellow was displayed below pH 6.0 and a purple was displayed above pH 8.4. A total of 0.6 g of cellulose acetate was prepared in 20 mL of 1:1 acetone:butanone. After full dissolution using an ultrasonic bath (JAC 2010; Kodo Technical Research Co. Ltd., Hwaseong, Korea), dibutyl phthalate (0.62 g) was added (3). In this study, bromothymol blue and phenol red (BTB-PR) were used as a pH dye, and a yellow was displayed below pH 6.0 and a purple was displayed above pH 8.4. The optimum dose of BTB-PR was added to the mixture for a polymer matrix solution. The gas indicator sensor unit was produced by dispensing 6–6.4 μ L of this polymer matrix

solution with an automatic micropipette onto paper discs. To ensure that the change in the color of the sensor unit looked good, the discs were punched out with a common office hole puncher, put over the sensor unit to provide a white background above, and coated with a PET film drilled with holes to allow the gas to pass through. Furthermore, a PTFE as a hydrophobic gas-permeable membrane was added to protect the gas indicator from humidity, while allowing gaseous compounds to pass through (4,5). Figure 1 shows the prototype sensor unit for gas indicator.

pH measurement The pH values of skate samples were obtained using a pH meter (Orion 3-star Meter; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at three different storage temperatures (4, 10, and 20°C) and different weights of samples (1, 5, 10, 20, 30, and 40 g). The pH meter was calibrated using the buffer solutions of pH (10.00 $\pm$ 0.01, 7.00 $\pm$ 0.01, and 4.01 $\pm$ 0.01 at 25°C) (9).

Chromaticity analysis The chromaticity of the sensor unit was measured by placing the probe of a portable colorimeter (CR-300; MINOLTA, Tokyo, Japan) on each sensor unit during 20 days of storage. During the measurement, the sensor unit was placed on white in a closed box to avoid any light disturbance from the surrounding environment (10). The measured values, the Hunter color value, showed L (lightness), a (redness), and b (yellowness), and the values are the mean of triplicate determinations. In addition, the ΔE value was calculated using the following equation:

$$\Delta E = \sqrt{(L-L')^2 + (a-a')^2 + (b-b')^2}$$

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. Values were expressed as mean $\pm$ 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 (11).

Results and Discussion

Sensor unit in response to ammonia standard solution Figure 2 shows the color changes of the gas indicator in response to ammonia levels in fish fermentation simulations by using diluted ammonia standard solutions in sequence at room temperature. The sensor unit with 500 and 1,000 mg/L of ammonia showed slight color changes to dark yellow. The sensor units with 5,000 and 10,000 mg/L of ammonia, indicating the early state of fish fermentation, showed color changes to slight red. The sensor units with concentration levels above 50,000 mg/L of ammonia showed color changes to purple that were easily recognizable to the naked eye, indicating fish

fermentation. The chromaticity values of the sensor units in response to ammonia standard solution are shown in Table 1. As the figure of ΔE has been gradually changed, it can be found that the gas indicator with the use of BTB-PR is suitable for the indicator of ammonia. We expect pH-sensitive gas indicator to be applicable for enhancing the traceability system of the freshness indicator and Hazard Analysis Critical Control Points (HACCP) systems for food industry (12).

Evaluation of the relation between sensor unit and sample weight

To evaluate whether the weight of the skate sample impacts on gas indicator, changes of pH and chromaticity based on the period of its storage were measured. The minimum weight of no influence on the

Fig. 2. Changes in color of the gas indicator in response to content of ammonia

Table 1. Chromaticity of the sensor unit in response to ammonia standard solution

	Concentration of ammonia standard solution (mg/L)						
	500	1,000	5,000	10,000	20,000	5,000	100,000
L ¹⁾	90.34±0.49	90.44±1.34	87.00±1.34	82.92±0.35	70.63±0.86	61.85±0.63	60.71±0.94
a	-2.14±1.28	-2.21±1.28	1.79±0.72	4.40±0.64	8.45±2.14	22.21±0.65	22.83±1.82
b	20.93±2.31	18.08±1.58	12.76±1.81	6.67±0.21	-19.61±0.80	-22.67±0.26	-23.37±1.22
ΔE	0.00	2.85	9.66	17.35	46.30	57.49	58.86

¹⁾L, a, b values are mean±standard deviation of triplicate determination.

Table 2. Changes of pH values of skate rays according to different weights during storage at 4, 10, and 20°C

Temp. (°C)	Weight (g)	Storage time (day)								
		1	3	5	7	9	11	13	15	17
4	1.00	6.48±0.00 ^{1),e,2)}	6.81±0.00 ^d	6.88±0.00 ^c	6.70±0.00 ^f	6.67±0.00 ^f	6.72±0.00 ^f	6.81±0.00 ^f	6.88±0.00 ^e	6.92±0.00 ^f
	5.00	6.71±0.01 ^c	6.78±0.00 ^e	6.82±0.00 ^d	6.76±0.00 ^e	6.79±0.00 ^d	6.81±0.00 ^e	6.85±0.00 ^e	6.91±0.00 ^d	6.97±0.00 ^e
	10.00	6.65±0.00 ^d	6.68±0.00 ^f	6.72±0.00 ^e	6.84±0.00 ^d	6.73±0.00 ^e	6.82±0.00 ^d	6.88±0.00 ^d	6.90±0.00 ^d	6.98±0.00 ^d
	20.00	6.88±0.01 ^b	6.95±0.00 ^c	6.98±0.00 ^b	6.99±0.00 ^b	7.03±0.01 ^a	7.11±0.01 ^a	7.09±0.00 ^b	7.10±0.01 ^c	7.07±0.00 ^c
	30.00	6.90±0.01 ^a	6.98±0.01 ^a	7.01±0.00 ^a	7.03±0.00 ^a	7.02±0.00 ^b	7.03±0.00 ^b	7.15±0.01 ^a	7.18±0.00 ^a	7.20±0.00 ^a
	40.00	6.88±0.00 ^b	6.96±0.00 ^b	6.99±0.00 ^b	6.98±0.00 ^c	7.01±0.00 ^c	7.01±0.00 ^c	7.02±0.00 ^c	7.16±0.00 ^b	7.19±0.00 ^b
10	1.00	6.38±0.00 ^d	6.57±0.00 ^e	6.88±0.00 ^b	7.14±0.00 ^c	7.31±0.00 ^c	7.36±0.00 ^c	7.47±0.00 ^d	7.55±0.00 ^f	7.63±0.00 ^f
	5.00	6.33±0.00 ^e	6.60±0.01 ^d	6.68±0.00 ^e	6.70±0.01 ^f	7.01±0.00 ^e	7.29±0.00 ^e	7.46±0.00 ^e	7.61±0.00 ^e	7.65±0.00 ^e
	10.00	6.72±0.00 ^a	6.68±0.00 ^b	6.72±0.00 ^d	6.83±0.00 ^e	6.92±0.00 ^f	7.25±0.00 ^f	7.44±0.00 ^f	7.66±0.00 ^d	7.71±0.00 ^d
	20.00	6.57±0.00 ^c	6.67±0.00 ^c	6.82±0.00 ^c	7.04±0.00 ^d	7.20±0.00 ^d	7.32±0.00 ^d	7.76±0.00 ^c	7.86±0.00 ^c	8.02±0.00 ^b
	30.00	6.72±0.00 ^{ab}	6.82±0.00 ^a	6.93±0.00 ^a	7.31±0.00 ^b	7.42±0.00 ^b	7.53±0.00 ^b	7.89±0.00 ^a	7.93±0.00 ^b	8.01±0.00 ^c
	40.00	6.71±0.00 ^b	6.81±0.00 ^a	6.88±0.00 ^b	7.36±0.00 ^a	7.53±0.00 ^a	7.67±0.00 ^a	7.88±0.00 ^b	7.98±0.00 ^a	8.06±0.00 ^a
20	1.00	6.69±0.00 ^f	6.85±0.00 ^c	7.12±0.00 ^d	7.48±0.00 ^f	7.52±0.00 ^f	7.69±0.00 ^f	7.74±0.00 ^f	7.88±0.00 ^e	7.92±0.00 ^f
	5.00	6.71±0.00 ^e	6.82±0.00 ^d	6.98±0.00 ^f	7.51±0.00 ^e	7.63±0.00 ^e	7.78±0.00 ^e	7.80±0.00 ^e	7.93±0.00 ^d	7.98±0.00 ^e
	10.00	6.75±0.01 ^d	6.81±0.00 ^d	7.01±0.00 ^e	7.59±0.00 ^d	7.82±0.00 ^d	7.85±0.00 ^d	7.89±0.00 ^d	7.95±0.00 ^c	8.01±0.00 ^d
	20.00	7.22±0.00 ^a	7.51±0.00 ^b	7.76±0.00 ^c	7.79±0.00 ^c	7.83±0.00 ^c	7.97±0.00 ^c	7.98±0.00 ^c	7.96±0.00 ^c	8.07±0.00 ^c
	30.00	7.17±0.00 ^b	7.68±0.00 ^a	7.89±0.00 ^b	7.93±0.00 ^b	7.91±0.00 ^b	8.02±0.00 ^b	8.02±0.00 ^b	8.04±0.00 ^b	8.16±0.00 ^a
	40.00	6.97±0.00 ^c	7.51±0.00 ^b	7.91±0.00 ^a	8.05±0.00 ^a	7.99±0.00 ^a	8.07±0.00 ^a	8.11±0.00 ^a	8.10±0.00 ^a	8.14±0.00 ^b

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 3. Changes of ΔE values of skate rays according to different weights at different temperatures (4, 10, and 20°C)

Temp. (°C)	Weight (g)	Storage time (day)								
		1	3	5	7	9	11	13	15	17
4	1.00	0.00	7.22±0.25 ^{1),a,2)}	9.42±0.27 ^b	13.04±0.53 ^b	14.38±1.07 ^b	12.91±0.64 ^a	15.46±0.29 ^b	16.19±0.69 ^c	13.43±0.96 ^a
	5.00	0.00	6.78±0.10 ^a	8.83±0.17 ^a	10.83±0.09 ^a	12.31±1.14 ^a	13.20±0.67 ^a	12.42±0.11 ^a	11.55±0.10 ^a	12.55±0.37 ^{ab}
	10.00	0.00	8.04±0.31 ^b	10.78±0.25 ^c	12.95±0.20 ^b	15.89±0.42 ^c	15.00±0.38 ^b	15.08±0.16 ^b	13.85±0.31 ^b	14.23±0.39 ^b
	20.00	0.00	8.85±0.31 ^c	14.92±0.13 ^e	18.69±0.18 ^e	17.71±0.42 ^d	17.45±0.56 ^c	17.57±0.41 ^c	17.47±0.34 ^d	20.07±0.17 ^d
	30.00	0.00	6.70±0.53 ^a	13.35±0.31 ^d	16.51±0.84 ^d	15.19±0.94 ^{bc}	15.22±0.57 ^b	15.35±0.84 ^b	17.75±0.74 ^d	18.86±0.98 ^c
	40.00	0.00	8.78±0.42 ^c	13.63±0.19 ^d	15.55±0.24 ^c	15.99±0.35 ^c	16.57±0.54 ^c	17.60±0.24 ^c	14.59±0.78 ^b	18.19±0.59 ^c
10	1.00	0.00	7.10±0.14 ^c	11.22±0.16 ^{bc}	13.69±0.21 ^b	18.23±0.11 ^b	19.28±0.27 ^{ab}	24.79±0.78 ^a	22.74±0.42 ^a	23.68±0.36 ^a
	5.00	0.00	5.21±0.14 ^b	8.76±0.18 ^a	12.25±0.17 ^a	13.98±0.10 ^a	17.55±0.08 ^a	31.59±0.53 ^b	33.50±0.73 ^b	35.11±0.38 ^b
	10.00	0.00	6.49±0.14 ^c	11.87±0.63 ^{bc}	17.00±0.42 ^d	17.42±0.19 ^b	20.59±0.63 ^b	34.24±0.17 ^c	36.22±0.89 ^c	36.98±0.29 ^c
	20.00	0.00	4.96±0.60 ^b	12.42±0.66 ^c	19.56±0.41 ^e	25.31±0.67 ^e	30.08±0.76 ^e	33.74±0.98 ^c	48.73±0.53 ^d	52.96±0.69 ^e
	30.00	0.00	3.36±0.68 ^a	10.61±1.18 ^b	15.84±1.02 ^c	21.18±1.13 ^c	25.30±1.64 ^c	30.76±0.80 ^b	48.78±0.93 ^d	50.49±0.83 ^d
	40.00	0.00	3.67±1.46 ^a	11.29±1.32 ^{bc}	17.51±1.01 ^d	23.72±1.63 ^d	28.02±1.83 ^d	34.94±1.31 ^c	51.81±1.10 ^e	53.33±1.48 ^e
20	1.00	0.00	1.98±0.37 ^b	3.58±0.13 ^b	5.28±0.11 ^c	7.81±0.53 ^b	7.06±0.24 ^c	10.11±0.03 ^a	33.16±0.58 ^e	33.81±0.12 ^d
	5.00	0.00	0.67±0.30 ^a	1.81±0.36 ^a	2.95±0.19 ^b	3.83±0.08 ^a	4.31±0.38 ^a	18.67±0.16 ^c	30.68±0.27 ^d	29.27±0.35 ^c
	10.00	0.00	0.84±0.41 ^a	2.22±0.59 ^a	2.21±0.35 ^a	3.50±0.51 ^a	5.83±0.36 ^b	17.92±0.80 ^c	34.89±0.52 ^f	36.17±0.67 ^e
	20.00	0.00	1.85±0.28 ^b	4.90±0.20 ^c	5.47±0.27 ^c	10.08±0.19 ^c	12.09±0.39 ^d	14.40±0.21 ^b	19.68±0.46 ^a	22.21±0.27 ^a
	30.00	0.00	3.31±0.61 ^c	6.15±0.27 ^d	9.73±0.48 ^e	12.80±0.46 ^e	15.61±1.12 ^f	17.86±0.61 ^c	22.17±0.95 ^b	25.25±0.67 ^b
	40.00	0.00	2.31±0.13 ^b	5.64±0.86 ^{cd}	7.89±0.38 ^d	11.13±0.58 ^d	14.34±0.58 ^e	18.00±0.30 ^c	24.09±0.57 ^c	25.75±0.22 ^b

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.

gas indicator was determined. The period of storage lasted 17 days at 4, 10, and 20°C considering skate storage temperatures.

The pH values according to storage periods are indicated in Table 2. At the higher temperature and the longer storage period, the values of pH tended to be high, ranging from a minimum value of 6.48 to a maximum of 8.14. Kuswandi *et al.* (6) reported that the pH value of skate in the first day was 6.3 and 9.1 at 12 days during the fermentation storage period. The pH value of general food tends to be lower according to the storage period by microorganisms; in contrast, the pH value of skate showed an increase trend, since skate contains urea and the precursor of urea in the body to adjust its osmotic pressure. The pH of skate was dependent on storage environments and fermentation methods. In this research, at 4°C, there was a slight growth trend in all weights with a lesser change of pH. At 10°C, there was a stable growth trend in all weights, and a higher pH value was shown from above 20.00 g from 13 days. At 20°C, the pH value had a smaller change pattern after rapid growth at the weights of 1.00 to 10.00 g from 1 to 7 days, whereas the pH value at the weights of 20.00 to 40.00 g had a smaller change pattern after a sharp increase from 1 to 5 days. In other words, a different pattern was demonstrated from the weight of 20.00 g or more.

The L, a, b value, which are based on the storage period by sample weight, were measured by colorimeter. The L value, which represents the brightness of color, tended to be lower when the storage period was long, ranging from a minimum value of 61.35 to a maximum of 93.32. The a value, which represents the redness of color, tended to be higher when the storage period was longer, ranging from a minimum value of −2.81 to a maximum of 33.31. The b value, which

represents the yellowness of color, tended to be lower when there was a higher temperature and longer period of storage and ranged from a minimum value of −12.64 to a maximum of 29.66.

The chromaticity, ΔE value, was calculated using these L, a, b values (Table 3). At 10°C, there was a large color change. At 4 and 10°C, there was a trend in all weights with a large color difference in 3 days and 15 days. At 20°C, it appeared big change in 5 days. Furthermore, at all temperatures, the color was the most different between 10.00 g and 20.00 g, and at 20.00 g or more, there was a same trend in color change.

Comparing the difference of the average pH, L, a, and b values according to the storage period by each weight, the largest change rate was between 10.00 g and 20.00 g and the smallest change rate was above 20.00 g. Therefore, the minimum weight of 20.00 g was selected, as it does not impact the change of color on the gas indicator.

Evaluation of the relation between sensor unit and packaging material

The previous experimental result of the 20.00 g sample was put into each container, and the change of color was observed by attaching the indicator to the top of the container to evaluate the influence of packaging material on the change of sensor unit color. The packaging materials used for the product were polypropylene (PP), polycyclohexane dimethylene terephthalate (PCT), copolyester, and PET (polyethylene terephthalate), which were being sold on the market, and finally took about 1.1 L container. The storage period lasted for 9 days at 4, 10, and 20°C using the storage method of the skate sample.

Fig. 3. Change of BTB-PR sensor unit's ΔE values in response to ammonia content based on fermentation of skate rays for different packaging materials at 20°C

In the case of the L value, there was a decline trend in all temperatures when the storage period was longer and an increase trend when the temperature became higher. As the storage period became longer, there was an increase trend of a value and a decrease of the b value. Additionally, a bigger change rate was shown when both storage temperatures became higher. Though all of the L, a, and b values demonstrated different change rates for each different packaging material, it was found that there was a significant influence of the packaging material on the indicator (11).

With the use of the measured the L, a, and b values, there was a color difference, ΔE , for each packaging material, as shown in Fig. 3, according to storage period. A high slope for this graph implies a higher rate change. Hence, PET was selected for the packaging material, which has a clear distinction for the change of color.

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