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Shelf Life Prediction of Vacuum-Packaged Grilled Mackerel

Jae-Hee Park[1], Yong-Jun Cha[2], Seo-Jin Kim[1], Eun-Jin Lee[1], Jeong-Eon Lee[2], and Hye Kyung Moon[1,2]

[1]Interdisciplinary Program in Senior Human Ecology and [2]Department of Food and Nutrition, Changwon National University, Gyeongnam 51140, Korea

ABSTRACT: The current study aimed to establish the shelf life of vacuum-packaged grilled mackerel stored at 5, −5, and −20°C for 70 days. To this end, physicochemical analyses, which involved determining the pH, volatile basic nitrogen, amino nitrogen, trimethylamine (TMA), and thiobarbituric acid levels; microbiological analyses (aerobic plate count and coliform); and sensory quality determination were performed. Regression analysis on the relationship between physicochemical properties and storage time at various temperatures revealed TMA level was the most suitable parameter (R^2= 0.9769) for predicting changes in the quality of grilled mackerel during storage, with a quality limit value of 8.74 mg/100 g. The shelf life of vacuum-packaged grilled mackerel according to temperature was 21, 53, 62, and 75 days for 5, −5, −15, and −20°C, respectively, with the use-by date being 23 days at 5°C and 74 days at −5°C. In conclusion, TMA was the most suitable parameter for predicting changes in the quality of grilled mackerel during storage.

Keywords: mackerel, shelf life, trimethylamine, use-by date, vacuum

INTRODUCTION

The home meal replacement (HMR) market has grown rapidly in recent years as the patterns of eating out around the world underwent considerable changes due to the prolonged COVID-19 pandemic. Among the total HMR sales, ready-to-cook food (58.8%), ready-to-eat food (34.0%), fresh-cut products (5.3%), and meal kits (1.9%) accounted for the highest proportion of the market (Kim et al., 2020). Ready-to-cook food, which requires no preprocessing of food ingredients and complex cooking processes, is useful for single-person households, dual-earner parents, and seniors seeking convenience and diversity. Kim et al. (2004) established that institutional food services can have beneficial effects, such as cost reduction, efficient use of manpower, and increased food safety.

In addition, the seafood HMR product market has been growing at an average annual rate of 30% due to the development of efficient cold chain transportation systems and packaging technology in the meat-oriented HMR market. The annual per capita supply of seafood is 68.1 kg, which is higher than that of meat (64.3 kg), indicating a gradual increase in the proportion of seafood in our diet (Korea Maritime Institute, 2021). A survey on the most preferred seafood among Koreans showed that squid was most favored (13.0%) followed by mackerel (11.8%) (Korea Maritime Institute, 2022). As of 2020, Koreans have consumed around 101,288,000 kg of mackerel, with no indications of a decrease in consumption anytime soon (Ministry of Oceans and Fisheries, 2021).

Mackerel is rich in nutrients such as protein, lipids, and minerals (e.g., iron and selenium) (Joo et al., 2016). In particular, the average content of essential amino acids in mackerel is 48.5 g/16 g·N, which is superior to that of beef (47.9 g/16 g·N), a high-quality protein. Therefore, fish has been considered a good source of nutrients for growing adolescents and those lacking protein intake (Ryu, 2004). In addition, fish protein contains lesser stroma protein and has a higher digestive absorption rate than does meat (Ryu, 2004), making it a high-quality protein source for infants and seniors. In particular, mackerel contains more nutritionally superior polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, than do other external blue-colored fish, such as sardines and Spanish mackerel (Jeong et al., 1998). However, PUFAs are easily oxidized, and the free fatty acids produced by hydrolytic enzymes, such as lower carbonyl compounds and lipases, have not only a characteristic fishy smell but also poor quality as indicated by protein denaturation and nutritional degradation (Gwak and Eun, 2010; Lingnert and Eriksson, 1980).

Considering that current HMR-related research has

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Correspondence to Hye Kyung Moon, E-mail: hkmoon@changwon.ac.kr

mainly focused on meat, research on seafood products have been scarce. Some studies assessing the changes in the quality of fresh mackerel during refrigerated storage have been conducted to develop a freshness indicator (Park et al., 2016) and determine the effects of freezing storage temperature on the shelf life of mackerel fish (Joo et al., 2016). For cooked mackerel, several studies have mainly observed quality changes in the packaging state as determined by the quality of baked mackerel muscle during partially frozen storage (Lee et al., 1983) and by the effects of salt and soy sauce on lipid oxidation in broiled mackerel (Ryu et al., 2002). However, only a few studies have investigated quality changes during vacuum-packaging, which is widely used in HMR products.

The Ministry of Food and Drug Safety (MFDS) has introduced quality standards in hopes of improving food safety in ready-to-eat/convenience foods. However, the MFDS only accounts for microbiological indicators. Although microbiological indicators are an important factor to consider when establishing food quality standards, physicochemical and physical indicators characteristic to each food group are equally as important. Therefore, the current study aimed to establish the shelf life of vacuum-packaged grilled mackerel stored and distributed at temperatures of 5, −5, and −20°C based on changes in physicochemical properties, microbiological properties, and sensual quality of the product. The presented results are expected to serve as fundamental data for various industries, such as food manufacturers and institutional food services.

MATERIALS AND METHODS

Material

This study used mackerel (*Scomber japonicus*) caught in Korea and auctioned on the day of the experiment at a local market in Changwon. Fresh raw materials were purchased and placed in an ice chest with ice and transported to the laboratory within 30 min. The head and intestines of the mackerel were removed, after which fish were filleted [13.71±2.47 cm (width), 8.64±0.94 cm (length); average, 150 g]. The fillets were then seasoned with 0.6 g of salt (NaCl 88%, *Kkotsogeum*, Sinyeong Corp.), sprinkled with 1 g of cooking oil, and baked in a preheated steam convection oven (RSCO-060E, Rinnai) for 20 min at 200°C. The internal temperature of the grilling mackerel was measured using a probe thermometer (Long Smart Thermometer, Foronemillion). The grilled mackerel was cooled at ambient temperature for 5 min, after which three fillets were vacuum-packaged together (HC-400S, Hansol Tech Corp.) using a multilayer film [nylon/polyethylene (12%/58%): 35 cm (height)×25 cm (width)×0.07 cm (thickness)] (Hansol Tech Corp.). The packaged fillets were stored for 70 days at three temperatures 5, −5, and −20°C. All experiments were performed in triplicate.

Proximate composition, pH, and salinity

Proximate compositions of the grilled mackerel were determined according to the procedure of the AOAC International (1995). Specifically, the air-oven method was used for the quantification of moisture, the semi-micro Kjeldahl method for crude protein, the Soxhlet extraction method for crude lipid, and the dry ashing method for crude ash. Carbohydrates were determined as a percentage by subtracting moisture, crude protein, crude lipid, and crude ash. pH was measured by adding 50 mL of distilled water to 5 g of the sample, homogenizing the mixture for 1 min using a homogenizer (T25 digital Ultra-Turrax, IKA), filtering it using a filter paper (Whatman no. 4), and measuring the filtrate using a pH meter (ST 3100, Ohaus) at 25°C. To measure salinity, 90 mL of distilled water was added to 10 g of the sample and homogenized for 1 min, after which the salinity of the filtrate was measured using a salinity meter (CSF-2500, CAS).

Aerobic plate count (APC) and coliform count

APC and coliform were quantitatively analyzed according to the microbial testing method of the food code (MFDS, 2021). Briefly, 10 g of the sample and 90 mL of sterile saline were combined in a sterilized filter bag (sample bag 1930F, 3M Microbiology), after which the solution was homogenized at 230 rpm for 120 s using a stomacher (Blender stomacher 400 circulator, Seward) and further diluted 10-fold before being used. For APC, 1 mL of diluted sample solution was inoculated into two sheets of 3M Petrifilm aerobic count plates (3M Microbiology) and cultured at 36°C for 48 h. The plate that generated the red colony was selected, and colonies were counted and presented as log colony-forming units (CFU)/g. Coliforms were inoculated with three Luria-Bertani broths (Difco) at each stage (1, 0.1, and 0.01 mL), cultured at 36°C for 24 h, and considered positive when gas was generated in the Durham tube. The coliform count was expressed as log most probable number (MPN)/g.

Volatile basic nitrogen (VBN)

The VBN of the sample was measured by modifying the micro diffusion method (Ministry of Health, Labour and Welfare, 1960). More specifically, 2 g of the sample was mixed with 2 mL of 20% perchloric acid and 16 mL of distilled water, left still for 10 min, and filtered through a filter paper (Whatman no. 4), after which the supernatant was used as the test solution. Thereafter, 1 mL of the sample solution and 1 mL of 50% saturated K_2CO_3 solution were combined in the outer wall of the Conway unit (Shibata Co. Ltd.), and 1 mL of boric acid absorbent was

added (H_3BO_3, 10 g; ethyl alcohol, 200 mL; mixing indicator, 10 mL) to the inner wall. The cover was immediately closed, and the unit was incubated at 37°C for 80 min and titrated with 0.01 N HCl.

Amino nitrogen (NH_2-N)

Amino nitrogen analysis was performed according to the Formol method (Sørensen, 1907). Briefly, 5 g of the sample were added into a 100 mL volumetric flask, diluted to volume with distilled water, and filtered through a filter paper (Whatman no. 4). After adding 0.1 N of NaOH to 20 mL of the filtrate, the pH was adjusted to 8.5 using a pH meter, and 20 mL of 35% formalin was then added and titrated with 0.1 N NaOH until the pH was 8.5.

Trimethylamine (TMA)

TMA was measured by modifying the method of AOAC International (2000). Briefly, 10 g of the sample were mixed with 20 mL of 7.5% TCA and homogenized with a homogenizer for 5 min, after which the homogenate was added into a 100 mL volumetric flask, diluted to volume with distilled water, and filtered with a filter paper (Whatman no. 4). Next, 4 mL of the filtrate, 1 mL of 20% formalin, 10 mL of toluene, and 3 mL of 50% saturated K_2CO_3 were placed into a separatory funnel, immediately stoppered, shaken well, and allowed to stand for 10 min to collect the toluene layer. The separated toluene layer and 1 spoon spatula of Na_2SO_4 were added to the test tube and dehydrated. The dehydrated toluene solution and 0.02% picric acid were mixed in a 1:1 ratio by volume. The mixed solution was quantitatively analyzed from the calibration curve obtained using a standard solution of TMA hydrochloride (Sigma-Aldrich Corp.) and the absorbance values at 410 nm with an ultra-violet/visible spectrophotometer (Libra S22, Biochrom Ltd.).

Thiobarbituric acid reactive substance (TBARS)

TBARS was measured using the steam distillation method of Tarladgis et al. (1960). Briefly, 2 g of the sample, 97.5 mL of distilled water, and 2.5 mL of HCl were placed into a Kjeldahl flask, and 50 mL of the distillate was collected within 30 min using a Kjeldahl distiller (C-KD6, Vision Lab Sciences). Thereafter, 5 mL of distillate and 5 mL of 0.02 M thiobarbituric acid regent were mixed, boiled in a water bath at 98°C for 35 min, and then cooled to ambient temperature. The TBARS was quantitatively determined using the absorbance values at 531 nm measured using a spectrophotometer, and the calibration curve was obtained using a 1,1,3,3-tetraethoxypropane (Sigma-Aldrich Corp.) standard solution.

Sensory evaluation

Sensory evaluation was conducted after receiving approval from the Institutional Review Board (IRB No. 7001066-202109-HR-037) of Changwon National University, and informed consent was obtained. In a sensory evaluation room equipped with individual partitions, sensory evaluations were conducted at 11 am or 4 pm by a panel of 17 trained individuals who had no objection to seafood for a total of 11 times over 70 days. Grilled mackerel was cut into 2.0×2.5 cm portions and presented in an opaque disposable plastic container (∅70) with a random three-digit number. Bottled water for rinsing the mouth was provided before each sample was tested to minimize the effects of the previous sample. During the sensory evaluation, the five items assessed were color, odor, taste, texture, and overall acceptance. Each item was evaluated using a 9-point hedonic scale (1 point, very unpleasant; 9 points, very good), and the standard value of the quality limit of the sensory evaluation was set to 4.0 points.

Quality index selection and prediction of shelf life

To predict the shelf life, regression analysis to determine the relationship between storage period and quality index was divided into zero-order and first-order reactions. The quality index with the highest coefficient of determination (R^2) was selected. The quality limit for the optimal quality index was set by substituting the sensual quality limit value into the regression equation for the selected quality index and the overall acceptance of the sensory evaluation. The absolute residual error (%) value between the predicted and experimental quality limit value for grilled mackerel was calculated and compared. In addition, the activation energy (*Ea*) value was obtained from equation [2], which modified the slope of the straight line [$-Ea/R$] when *InK* was the Y axis and *1/T* was the X axis according to equation [1], in which the Arrhenius equation was modified (MFDS, 2019).

$$InK = InA - \frac{Ea}{R}\left(\frac{1}{T}\right) \quad [1]$$

$$Ea = -Slope \times R \quad [2]$$

A: Arrhenius constant

Any temperature not tested was predicted through the reaction rate constant (*K*), activation energy (*Ea*, cal/mol), gas constant (*R*, 1.987 cal/mol), absolute temperature (*T*), and slope values obtained in the experiment. Finally, the shelf life was calculated using the reaction rate constant and Q_{10}-value equation [3].

$$Q_{10} = \frac{k_{T+10}}{k_T} \quad [3]$$

k: reaction rate constant
T: absolute temperature

Table 1. Proximate composition and salinity of raw and grilled mackerel fillets (unit: %)

	Moisture	Crude protein	Crude lipid	Crude ash	Total sugar[1)]	Salinity (w/v)
Raw	61.80±1.35	17.09±1.46 (44.74)	19.02±0.71 (49.80)	1.50±0.34 (3.93)	0.60	0.97±0.12
Grilled[2)]	49.55±1.10	26.29±0.47 (52.11)	21.80±1.04 (43.21)	1.85±0.12 (3.67)	0.51	1.46±0.21
t-Value	12.145***	−10.402***	−3.827*	5.904	−	−

Values are expressed as mean±SD (n=3).
Values in parentheses indicated dry basis.
[1)]100−(moisture+crude protein+crude lipid+crude ash).
[2)]Heated in the oven (200°C for 20 min).
*P<0.05 and ***P<0.001 via the *t*-test.
−, not measured.

Statistical analysis

Statistical analyses to determine the means and standard deviations were performed using SPSS version 27.0 (IBM Corp.). Significance (P<0.05) was established using Duncan's multiple range test after one-way ANOVA.

RESULTS AND DISCUSSION

Proximate composition of raw and grilled mackerel

The proximate composition of mackerel is detailed in Table 1. After heating, we observed a decrease in the moisture content of grilled mackerel decreased from 61.80% to 49.55% (P<0.05), an increase in the crude protein and crude lipid contents (P<0.05), and no significant change in crude ash content. In terms of dry matter, the crude lipid content in grilled mackerel decreased from 49.80% to 43.21% after grilling, presumably due to the outflow of moisture and lipids resulting from muscle contraction caused by protein denaturation during heating. Consequently, we observed a decreased in the ratio of moisture to crude lipid content with a relative increase in crude protein content.

The salinity of the grilled mackerel evaluated herein was 1.46% (350.4 mg of sodium/60 g of grilled mackerel). Park (2007) previously reported a salinity of 2.75% in 50 g of grilled mackerel per person provided by the institutional food services. Kwon and Kim (2015) also reported a salinity of 624.6 mg of sodium/100 g of grilled mackerel and 456.9 mg of sodium/100 g of low-salt mackerel provided by institutional food services. Therefore, the grilled mackerel evaluated in the current study can be considered a low-sodium food suitable for institutional food services.

Changes in pH, APC, and coliform count

Generally, the pH of fresh fish after death varies between 5.5 and 6.5 (Arnold and Brown, 1978), with our raw mackerel having a pH of 6.08 (Table 2). Over time, the pH increased from 6.10 to 6.23～6.26 on the 6th day of storage at 5°C, 12th day of storage at −5°C, and 20th day of storage at −20°C (data not shown). Thereafter, the pH decreased to 6.09～6.12 on the 9th day of storage at 5°C, 20th day of storage at −5°C, and 27th day of storage at −20°C. However, the pH remained stable (ranging from 6.09 to 6.26) throughout the 70 days of storage independent of the temperature.

Raw mackerel had an APC of 3.30 log CFU/g and coliform count of 0.78 log MPN/g (Table 2). The APC and coliform count could not be detected in grilled mackerel, regardless of temperature, for the entire 70 days of storage (data not shown). These results suggest a reduction in the microorganisms when the internal temperature of grilled mackerel exceeded 85°C for 17 min.

Changes in VBN and NH_2-N

In general, studies have shown that extremely fresh, normal fresh, and spoiled fish have a VBN content of 5～10, 15～25, and 50 mg or more/100 g (Kim et al., 1998). The VBN content of raw mackerel in the current study was 3.95 mg/100 g, indicating that the samples used in our experiments were fresh (Table 2). Changes in VBN con-

Table 2. Quality properties of raw and grilled mackerel fillets

	pH	VBN (mg/100 g)	NH_2-N (mg/100 g)	TMA (mg/100 g)	TBARS (mg/kg)	APC (log CFU/g)	Coliform (log MPN/g)
Raw	6.08±0.04	3.95±0.53	90.56±5.88	2.10±0.10	0.05±0.02	3.30±0.14	0.78±0.46
Grilled[1)]	6.10±0.08	19.84±0.24	110.42±1.58	5.55±0.22	0.11±0.00	ND	ND
t-Value	−0.481	−47.182***	−5.648**	−24.476***	−5.214**	41.576***	2.975

Values are expressed as mean±SD (n=3).
[1)]Heated in the oven (200°C for 20 min).
**P<0.01 and ***P<0.001 via the *t*-test.
VBN, volatile basic nitrogen; NH_2-N, amino nitrogen; TMA, trimethylamine; TBARS, thiobarbituric acid reactive substance; APC, aerobic plate count; ND, not detected.

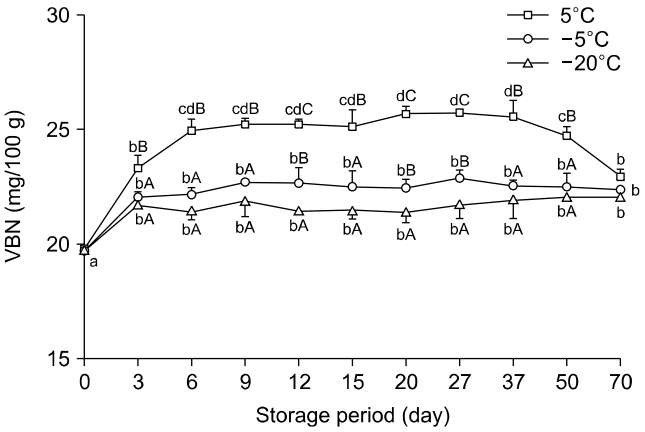

Fig. 1. Changes in volatile basic nitrogen (VBN) contents of grilled mackerel fillet during storage at 5, −5, and −20°C, respectively. Different letters within a day (A-C) and temperature (a-d) are significantly different by Duncan's multiple range test (P< 0.05).

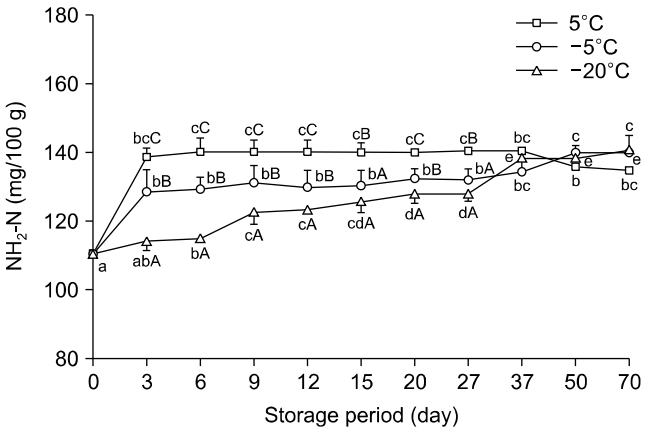

Fig. 2. Changes in amino nitrogen (NH_2-N) contents of grilled mackerel fillet during storage at 5, −5, and −20°C, respectively. Different letters within a day (A-C) and temperature (a-e) are significantly different by Duncan's multiple range test (P<0.05).

tent during the storage of grilled mackerel are summarized in Fig. 1. The VBN content of grilled mackerel stored at 5°C was significantly higher than that of grilled mackerel stored at −5°C and −20°C (P<0.05). Immediately after storage at 5°C, the VBN content continued to increase from 19.84 to 24.94 mg/100 g on day 6 of storage, thereafter maintaining a gentle progression curve. Those stored at −5°C and −20°C showed an in increase in VBN content to 22.05 and 21.75 mg/100 g on day 3 of storage, respectively, and remained at a constant level until the end of the 70 days of storage (P<0.05). However, those stored at 5°C showed a decrease in VBN content after reaching 25.60 mg/100 g on day 37 of storage. These results were similar to those of Lee et al. (1983) on the preservation of the quality of baked mackerel stored at refrigeration, freezing, and partially freezing temperatures, which showed that the VBN content of baked mackerel increased from 16 mg/100 g at the beginning of storage to 22 mg/100 g on day 4 of storage and kept constant thereafter. Evidence suggests that once the protein is completely decomposed, the amount of VBN, such as ammonia nitrogen and amine, is reduced.

The changes in NH_2-N content during storage of grilled mackerel are presented in Fig. 2. Before storage, the NH_2-N content was 110.42 mg/100 g, which increased rapidly to 138.74 mg/100 g on day 3 of storage at 5°C and then maintained a constant value until day 37 of storage. Storage at −5°C promoted a lesser increase in NH_2-N content compared to that at 5°C; however, NH_2-N content increased rapidly to 128.63 mg/100 g on day 3 of storage at −5°C and remained at a constant level thereafter. On the other hand, storage at −20°C promoted a gradual increase in NH_2-N content until day 70 of storage. Notably, the NH_2-N content decreased from day 37 of storage at 5°C, which is expected to impact the VBN content.

Changes in TMA

Most fish produce odorless TMA oxide during metabolic processes, which can afterwards be decomposed into TMA by reduction reactions catalyzed by autolytic enzymes or enzymes secreted by bacteria, leading to a characteristic fishy smell (Lee et al., 2016). Usually, the onset of spoilage occurs when the TMA content exceeds 3∼4 mg/100 g (Song et al., 2005). The TMA content of the sample used in this experiment was 2.10 mg/100 g, which corresponds to fresh fish (Table 2). The changes in TMA content during the storage of grilled mackerel are detailed in Fig. 3. As storage progressed, the TMA content increased regardless of temperature, with grilled mackerel stored at 5°C, in particular, showing the largest values. The TMA content of grilled mackerel before storage was 5.55 mg/100 g but increased significantly to 6.77 mg/100 g on the 3rd day of storage at 5°C, to 6.30 mg/100 g on the 6th day at −5°C, and to 6.41 mg/100 g on the 27th day of storage at −20°C (P<0.05). Park et al. (2016) reported that the TMA value of fresh mackerel

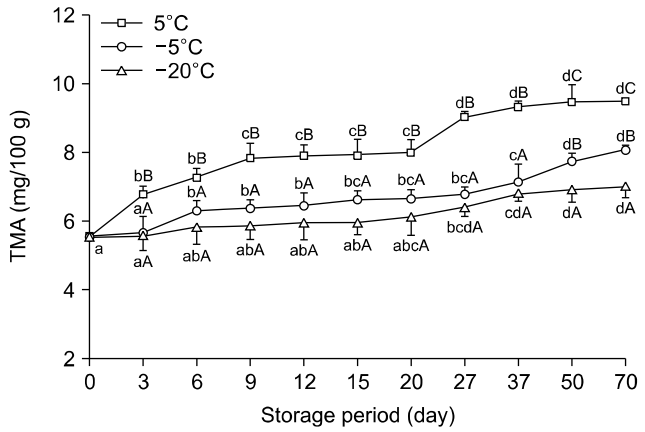

Fig. 3. Changes in trimethylamine (TMA) contents of grilled mackerel fillet during storage at 5, −5, and −20°C, respectively. Different letters within a day (A-C) and temperature (a-d) are significantly different by Duncan's multiple range test (P<0.05).

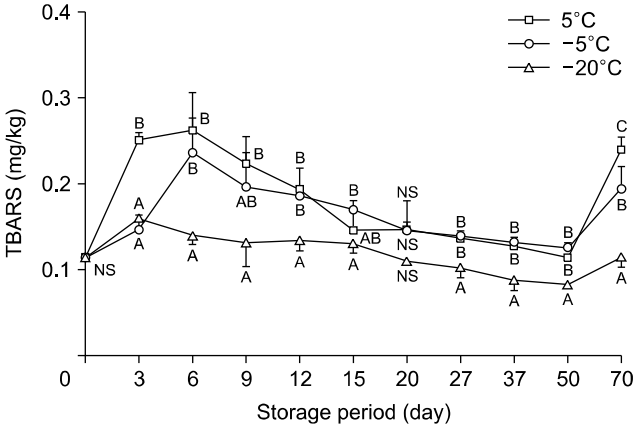

Fig. 4. Changes in thiobarbituric acid reactive substance (TBARS) value of grilled mackerel fillet during storage at 5, −5, and −20°C, respectively. Different letters within a some period (A-C) are significantly different by Duncan's multiple range test (P<0.05). NS, not significant.

stored at 4°C increased rapidly between 3 and 6 days of storage. Jo et al. (1988) also found that the TMA content increased rapidly after 4 days of storing vacuum-packaged mackerel at 5°C, indicating a difference in the TMA content compared to control. In summary, our results suggest that TMA was mostly affected by temperature, followed by the packaging method.

Changes in TBARS

Changes in TBARS content during storage of grilled mackerel are presented in Fig. 4. The TBARS contents of grilled mackerel stored at 5°C and −5°C were significantly higher than those of grilled mackerel stored at −20°C (P<0.05). The TBARS content increased to 0.25 and 0.23 mg/kg on day 3 of storage at 5°C and day 6 of storage at −5°C, respectively, and then continuously decreased to 0.11 mg/kg until day 50 of storage. Storage at −20°C promoted a relative smaller change in TBARS content that did storage at 5°C and −5°C. However, regardless of the storage temperature, TBARS content tended to increase from day 50 to day 70 of storage. Ryu et al. (2002), who analyzed the effects of seasoning on the lipid oxidation safety of roasted mackerel, reported that salt promoted lipid oxidation during the storage period and that the fatty acid composition decreased after heating. In the current study, the change in TBARS content occurred at the beginning of storage due to initial lipid acidification caused by heating and salt. Moreover, we found that PUFA was oxidized and malonaldehyde was rapidly generated but that malonaldehyde generation decreased as PUFA decreased. Future additional experiments are required, in particular, due to the observation that TBARS content tended to increase from day 50 to day 70 of storage.

Sensory evaluation

The results for the sensory evaluation of grilled mackerel according to storage temperature and period are presented in Table 3. Except for texture, the other four items, namely color, odor, taste, and overall acceptance,

Table 3. Sensory evaluation of grilled mackerel fillets during storage at 5, −5, and −20°C

Temperature (°C)	Storage period (day)					
	0	12	27	37	50	70
Color						
5	8.88±0.33	5.88±1.69^{a}	4.12±2.18^{a}	3.06±1.09^{a}	3.35±1.27^{a}	3.18±1.47^{a}
−5	8.88±0.33	5.88±1.54^{a}	6.18±1.59^{b}	5.76±1.52^{b}	4.47±1.81^{a}	5.35±1.84^{b}
−20	8.88±0.33	7.35±1.80^{b}	6.24±1.48^{b}	6.35±1.69^{b}	6.82±1.88^{b}	6.00±1.66^{b}
Odor						
5	8.88±0.33	5.71±1.72^{a}	3.00±1.90^{a}	2.82±1.07^{a}	3.00±1.17^{a}	1.65±0.79^{a}
−5	8.88±0.33	5.71±1.36^{a}	5.53±1.50^{b}	4.47±1.46^{b}	4.88±1.87^{b}	4.29±1.79^{b}
−20	8.88±0.33	7.53±1.42^{b}	6.18±1.59^{b}	6.59±1.54^{c}	6.41±1.84^{c}	6.24±1.60^{c}
Taste						
5	8.88±0.33	5.59±1.66^{a}	3.12±2.15^{a}	−	−	−
−5	8.88±0.33	6.65±0.93^{b}	6.12±1.65^{b}	4.76±1.52^{a}	4.71±1.69^{a}	4.18±1.81^{a}
−20	8.88±0.33	7.35±0.86^{b}	6.65±1.27^{b}	6.65±1.58^{b}	6.59±1.84^{b}	6.59±1.28^{b}
Texture						
5	8.94±0.24	6.24±1.56^{ns}	3.53±2.45^{a}	−	−	−
−5	8.94±0.24	6.65±1.73	6.59±1.50^{b}	5.71±1.69^{a}	5.24±1.89^{a}	4.76±2.36^{a}
−20	8.94±0.24	6.53±1.66	6.82±1.63^{b}	6.76±1.60^{b}	6.06±2.11^{a}	6.24±1.60^{b}
Overall acceptance						
5	8.88±0.33	6.00±1.46^{a}	3.12±2.26^{a}	3.00±0.79^{a}	2.82±1.13^{a}	1.76±0.66^{a}
−5	8.88±0.33	6.47±0.80^{a}	5.76±1.35^{b}	4.88±1.73^{b}	4.59±1.46^{b}	4.18±1.74^{b}
−20	8.88±0.33	7.47±1.07^{b}	6.71±1.10^{b}	6.65±1.54^{c}	6.65±1.84^{c}	6.47±1.28^{c}

Values are expressed as mean±SD (n=17).
Means with different letters in the same column (a-c) indicate significant differences using Duncan's multiple range test (P<0.05). ns, not significant; −, not measured.

changed significantly according to the storage temperature from day 12 of storage ($P<0.05$). No significant differences in the aforementioned items were observed according to storage period, but the preference decreased as the storage period increased. Compared to the scores for other items assessed, those for color and texture exhibited a slighter decrease due to the period of storage. In the case of odor, taste, and overall acceptance, a score of <4 was recorded on day 27 at 5℃, whereas the initial score of 4 was reached on day 70 at −5℃. In contrast, those stored at −20℃ maintained a score of 6 until day 70 of storage. These results show that the increase in TMA content during storage significantly affects sensory evaluation (Fig. 3).

Selection of quality index and prediction of shelf life

To select the quality index for grilled mackerel, microorganisms that were not detected during storage were therefore excluded from the analysis. The results for the regression analysis obtained for pH, VBN, NH_2-N, TMA, and TBARS according to storage temperature and period are detailed in Table 4. TMA and NH_2-N showed a strong correlation with an R^2 value of ≥0.8 in the zero-order and first-order reaction equations. In particular, changes in TBARS content during the storage period decreased after the initial increase; hence, regression analysis showed an R^2 value as low as 0.7771, which is an unreasonable quality index. However, regression analysis of TBARS content from days 6 to 50 of storage showed a decreasing trend, with an R^2 value in the first-order reaction equation of 0.9524, indicating a strong correlation (data not shown). This suggests that TBARS content can be used as a quality index for lipid oxidation in grilled mackerel, but TMA showed the strongest correlation, with an R^2 value of 0.9769 in the zero-order reaction equation. Thus, this parameter was selected as the quality index for predicting the shelf life of grilled mackerel. Yu et al. (2019), who measured the change in TMA content in cutlassfish during refrigerated storage, also reported that TMA, but not pH and VBN, were suitable fish freshness indicators.

The results of the regression analysis on TMA, a quality index of grilled mackerel, and overall acceptance were substituted into the zero-order reaction formula with the largest coefficient of determination (R^2=0.9641), and the quality limit of TMA was 8.74 mg/100 g. We verified that the suitability of the regression equation was pre-

Table 4. Correlation between storage period and quality index of grilled mackerel fillet across various storage temperatures

Quality index	Reaction order	Temperature (℃)	Regression equation	R^2	Activation energy (cal/mol)
pH	Zero-order	5	Y=0.0013X+6.1575	0.1574	4,149.05
		−5	Y=0.0008X+6.1680	0.0500	
		−20	Y=0.0006X+6.1602	0.0304	
	First-order	5	Y=0.0002X+1.8176	0.1576	3,488.97
		−5	Y=0.0001X+1.8193	0.0510	
		−20	Y=0.0001X+1.8181	0.0310	
VBN	Zero-order	5	Y=0.0549X+23.561	0.2367	4,596.33
		−5	Y=0.0261X+21.770	0.2247	
		−20	Y=0.0226X+21.087	0.3287	
	First-order	5	Y=0.0024X+3.1548	0.2329	5,892.45
		−5	Y=0.0012X+3.0792	0.2219	
		−20	Y=0.0008X+3.0523	0.3153	
NH_2-N	Zero-order	5	Y=0.1856X+133.45	0.0999	−6,031.14
		−5	Y=0.3486X+123.80	0.5309	
		−20	Y=0.5646X+114.33	0.8977	
	First-order	5	Y=0.0015X+4.8886	0.1049	−5,986.83
		−5	Y=0.0027X+4.8172	0.4969	
		−20	Y=0.0045X+4.7407	0.8824	
TMA	Zero-order	5	Y=0.0679X+6.6969	0.8089	4,485.45
		−5	Y=0.0381X+5.8429	0.8985	
		−20	Y=0.0292X+5.5797	0.9769	
	First-order	5	Y=0.0087X+1.9012	0.7435	3,287.49
		−5	Y=0.0058X+1.7682	0.8697	
		−20	Y=0.0047X+1.7223	0.9722	
TBARS	Zero-order	5	Y=−0.0021X+0.2087	0.3517	2,179.34
		−5	Y=−0.0010X+0.1761	0.1771	
		−20	Y=−0.0013X+0.1424	0.7192	
	First-order	5	Y=−0.0119X1.5993	0.3553	−747.57
		−5	Y=−0.0058X1.7622	0.1688	
		−20	Y=−0.0121X1.9366	0.7771	

VBN, volatile basic nitrogen; NH_2-N, amino nitrogen; TMA, trimethylamine; TBARS, thiobarbituric acid reactive substance.

Table 5. Validation of the predicted and experimental values for the TMA content of grilled mackerel fillets

Quality index	Temperature (˚C)	Storage period (day)	Predicted value (mg/100 g)	Experimental value (mg/100 g)	Error (%)
TMA	5	30.08	8.74	9.13±0.04	4.27
	−5	76.02		8.14±0.06	7.37

TMA, trimethylamine.

Table 6. Predicted shelf life of grilled mackerel fillets based on the quality index

Quality index	Temperature (˚C)	Reaction rate constant (K)	Activation energy (cal/mol)	Q_{10}-Value (−5˚C to −15˚C)	Storage period (day)	Predicted shelf life[1)] (day)	Measured shelf life[2)] (day)
TMA	5	0.0679	4,485.45	1.1687	30.08	21.06	23.82
	−5	0.0381			76.02	53.21	74.63
	−15	0.0326			88.84	62.19	−
	−20	0.0292			108.20	75.74	−

[1)]Storage period×0.7.
[2)]The overall acceptability of the sensory test is 4 points.
TMA, trimethylamine; −, not measured.

dicted to be <10% of the predicted TMA quality limit for each temperature storage period and the experimental value of TMA for grilled mackerel stored at 5°C and −5°C for 30 and 76 days, respectively (Table 5).

The predicted shelf life, which was calculated using the K value and Q_{10}-value for each temperature of TMA, and the measured shelf life obtained through the experiments are presented in Table 6. The non-experimental temperature was set to −15°C, which was higher than the freezing temperature (−17.8°C) of food distributed in supermarkets across Korea. Thereafter, using the Arrhenius equation, the K value at −15°C was found to be 0.0326. Therefore, this study established that grilled mackerel can be stored for 30.08 days at 5°C, 76.02 days at −5°C, 88.84 days at −15°C, and 108.20 days at −20°C. Importantly, when multiplying these numbers by a safety factor of 0.7, the predicted shelf life was 21.06 days at 5°C, 53.21 days at −5°C, 62.19 days at −15°C, and 75.74 days at −20°C. However, based on the sensory evaluation of this study, the measured shelf life was 23.82 days at 5°C and 74.63 days at −5°C, which were longer than the predicted values. These results show that the predicted value is the shelf life multiplied by a safety factor of 0.7, which indicates the allowed duration a product is to be sold to the consumer from the date of manufacture, and that the measured value is the use-by date with no safety concerns even if consumers consume it. Therefore, there was a large difference between the predicted (shelf life) and measured values (use-by date). Ko (2006), who examined consumers' perceptions of the food labeling standards, revealed that 84.8% of 500 consumers answered that they should not consume food that has passed the shelf life. The mentioned study also found that several consumers misunderstood the differences between shelf life and use-by date. In addition, Gim (2012), who conducted focus group interviews with people in their 20s to 80s, revealed that 67% of the respondents thought they could not eat expired food, which they discarded or used for other purposes given that they displayed a defensive attitude toward the lack of clear standards for how long a product can be consumed.

In conclusion, the current study showed that shelf life of vacuum-packaged grilled mackerel is 21 days at 5°C, 53 days at −5°C, 62 days at −15°C, and 75 days at −20°C, whereas the use-by date is 23 days at 5°C and 74 days at −5°C. Therefore, our finding suggest that displaying both the shelf life and use-by date alongside one another to inform customers regarding the actual deadline for consumption can reduce food waste and further contribute toward environmental protection by reducing greenhouse gas emissions.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: YJC, JHP. Analysis and interpretation: JHP, SJK, EJL, JEL. Data collection: JHP. Writing the article: JHP. Critical revision of the article: YJC. Final approval of the article: all authors. Statistical analysis: JHP. Overall responsibility: HKM.

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