Effect of frozen-storage period on quality of American sirloin and mackerel (*Scomber japonicus*)

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Abstract This study aimed to study the effect of frozen-storage period on the quality of sirloin and mackerel (*Scomber japonicus*). The samples were evaluated after being kept in frozen storage at -17.9°C for different periods of time (1, 8, 15, 22, and 29 days). The frozen storage resulted in increase in ice crystal formation on the surface of both sirloin and mackerel. Frozen-storage period had an effect on the increase in the drip loss of both sirloin and mackerel with a positive correlation ($p < 0.05$) as well as on the decrease in the hardness of sirloin with a negative correlation ($p < 0.05$). During the frozen-storage period, the 2-thiobarbituric acid reactive substance level was increased in mackerel while the level in sirloin was maintained; both levels were within safe limits. Consequently, a 29-day freezing period is postulated to have little effect on the quality of sirloin and mackerel.

Keywords Frozen-storage period · Sirloin · Mackerel · Quality · Pearson correlation coefficient

Introduction

Owing to the compositional abundance of proteins and lipids in beef sirloin and mackerel, they are considered wholesome food items [1, 2]. In particular, mackerel is known to be effective in reducing cardiovascular risk [3]. However, at room temperature, they tend to perish owing to high lipid content. The presence of unsaturated fatty

acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [4] along with a large amount of pro-oxidant molecules of fish species are detrimental to food quality. Furthermore, off-flavors, discoloration, deteriorated texture, and loss of nutrients are some of the undesirable consequences [5]. Frozen storage is one of the easiest methods to extend the food shelf-life. Freezing has an effect not only on controlling enzymatic activation but also on inhibition of microbial growth in foods. Thus, freezing is envisaged to be a useful approach for preserving sirloin and mackerel as well [6, 7]. However, complete prevention of deterioration of food materials through freezing may not be always possible [8]. Even the quality of frozen sirloin and mackerel may be deteriorated owing to several factors such as lipid oxidation, protein denaturation, drip loss, discoloration, pH, tenderness, changes in moisture holding ability, microbial count, and water evaporation [9, 10]. Moreover, in case of sirloin, the ratio of monounsaturated fatty acid to saturated fatty acid can change during frozen storage. This is an important factor while perceiving the flavor and texture of lipids by consumers [7].

The freezing technology is mainly categorized into two groups, “quick-freezing” and “slow-freezing”. Usually, the home electric freezer is a slow-freezing system. Unfortunately, the zone of maximum ice-crystal formation (-5 to -1°C) of the slow-freezing system is greater than that of the quick-freezing system. This is associated with serious consequences, including changes in the structure and biochemistry of a variety of food materials under long duration of frozen storage [9, 11]. In general, with longer time required for attaining the zone of maximum ice-crystal formation, larger ice crystals are formed, and consequently, non-uniform distribution of ice crystals occurs in food items [12]. In addition, it has been found that

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frozen-storage temperature, a critical factor in the formation of ice crystals [13] as well as the degree and frequency of temperature fluctuation [10] dictate meat quality. Thawing also plays a key role in controlling many detrimental factors of the freezing process. Thawing time is also known to significantly affect microbial growth in foods. In general, thawing is classified into three categories, namely, ambient thawing, conventional thawing (in a refrigerator at 3–4 °C), and rapid thawing (e.g., ohmic, high-pressure, and acoustic thawing) [9, 10, 14]. However, the ambient thawing of food items is generally not preferred because of the high possibility of air-borne microbial spoilage [10]. Of late, new attempts such as vacuum packaging and modified atmosphere packaging have been made to prevent various kinds of microbiological and physicochemical spoilage mentioned above [15]. However, household application of such technologies is not widely seen. Moreover, the effect of the behavior of the customer while using a freezer (e.g., opening of the freezer door) on the quality of frozen sirloin and mackerel remains unresolved. Consequently, in this study, we tried to evaluate the effects of the freezing period (for 29 days) on changes in the quality of sirloin and mackerel by considering general door open behavior of consumers, through analyses of external appearance, drip loss, TBARS level, color, and hardness.

Materials and methods

Materials and chemicals

A household refrigerator was provided from a local market and the freezer in the refrigerator was only used for this experiment. Sirloin (imported from the United States) and pacific mackerel (*Scomber japonicus*), the beef cuts [16] and fish species [17] most in demand were purchased from a local market on the start date of this experiment for freshness. A TBARS parameter assay kit (sensitivity: 0.055 µM) was purchased from R&D Systems (Minneapolis, MN, USA) and double ziplock bags with low-density polyethylene (LDPE) film were bought from a local market.

Freezing condition and sample preparation

Sirloin and mackerel were stored in a freezer at -17.9 ± 0.4 °C, regarded as ideal temperature to store foods, for 1, 8, 15, 22, and 29 days; a zero day test was set as the beginning of the experiment. The sirloin samples were prepared from a whole cow in order to prevent errors from differences between diverse species of cows. Sirloin was cut into even 0.8 cm thick slices in order to uniform the measurement of drip loss, Hunter color value, and

hardness. Mackerel was sliced into three pieces (head, body, and tail); among them, body and tail were used to measure drip loss and Hunter color value, respectively. To investigate the change in lipid oxidation (TBARS level) of sirloin and mackerel, both samples were ground separately using a mixer for obtaining homogenized samples. All samples were wrapped in the double ziplock bag and the air inside the bag was removed to the maximum. In this study, with a modification of average door opening times of household refrigerator (approximately 40 times per day) [18] by considering an experimental environment, a freezer door was opened 10 times a day.

Appearance and drip loss

Appearance of both frozen sirloin and mackerel was observed shortly after taking samples out of a freezer using a digital camera in the studio under light emitting diode (LED) light. After that, all samples were put in the plastic container and the lid was closed. Subsequently, these containers were stored in the refrigerator at 4.0 °C for 24 h for the thawing process. After 24 h, the samples were taken out of the refrigerator, and they were dried with a paper towel and weighed (W_2) using an electronic scale. The drip loss was calculated as follows with respect to the weight of the samples (W_1) before freezing:

$$\text{Drip loss} = \frac{W_1 - W_2}{W_1} \times 100 (\%)$$

Lipid oxidation measurement

Lipid oxidation degree (TBARS level) was analyzed using the TBARS parameter assay kit. Basically, 10 g of ground sirloin and 5 g of ground mackerel were mixed with 10 mL deionized water and shook intensely by hand. Subsequently, the samples were centrifuged using a centrifugal separator (Centrifuge 5810R, Eppendorf AG, Hamburg, Germany) with an F-34-6-38 rotor at 10,000 rpm for 10 min. Then, 100 µL of the supernatant was mixed with 100 µL of TBARS acid reagent in a micro-centrifuge tube. The mixtures were incubated at room temperature for 15 min. Thereafter, centrifugation was applied using a micro-centrifugal separator (Smart R17, Hanil science medical Co., Ltd., Daejeon, Korea) at 12,000g for 4 min. Subsequently, 75 µL of the supernatant and 37.5 µL of the thiobarbituric acid (TBA) reagent were mixed in a 96-well plate (SPL Life Science Co., Ltd., Pocheon, Korea), and immediately, the absorbance of the mixtures (the initial absorbance) was measured at 532 nm using a spectral scanning multimode reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). The mixtures were then incubated in a water bath (SWB-25, Han Yang Scientific Equipment

Co., Ltd., Seoul, Korea) at 47.5 °C for 2.5 h. The absorbance of the mixtures after reaction (the later absorbance) was measured along the same method as mentioned earlier. The total TBARS content of the samples was calculated by a standard curve obtained for a 0.26–16.70 µM of TBARS standard solution. Finally, the TBARS content was expressed in terms of milligram of malondialdehyde (MDA)/kilogram of materials (mg MDA/kg).

Color measurement

Color changes of sirloin and mackerel depending on the frozen-storage period (brightness; L, redness; a, and yellowness; b) were investigated using a Hunter Lab colorimeter (CR-300, Minolta Co. Ltd., Osaka, Japan). Thawed sirloin and mackerel were used for color measurement and calibration was carried out using a standard white tile ($L = 98.07$, $a = -0.11$, $b = 1.55$).

Hardness measurement

Changes in the hardness of sirloin depending on the frozen-storage period were measured using a texture analyzer (TMS-Pro, Food Technology Co., Sterling, VA, USA), which was equipped with a 25 N load cell and a circular probe of 5.0 cm in diameter. Thawed sirloin samples (15 mm × 15 mm × 8 mm) were compressed by up to 70% of its original height. Hardness of the samples calculated using the texture expert software (TMS-Pro, Food Technology Co.).

Statistical analysis

All measurements were performed at least in triplicate. Some data requiring statistical comparison were analyzed by two-way ANOVA with Tukey's HSD post hoc analyses ($p < 0.05$) using SPSS software (ver. 19, IBM Corp., Armonk, NY, USA). In addition, a Pearson correlation analysis was performed to determine the interrelation between the frozen-storage period of sirloin and mackerel and the quality parameters (drip loss, TBARS, Hunter color value, and hardness) by utilizing the SPSS software.

Results and discussion

A change in appearance of sirloin and mackerel

Changes in the appearance of sirloin and mackerel as a function of frozen-storage time in a freezer are shown in Fig. 1. Surface color of sirloin after taking out of the freezer was dark red to the naked eye (Fig. 1A). It is well known that muscle pigment of beef is controlled by

hemoglobin and myoglobin. However, in reality, the color of beef is mostly controlled by myoglobin because most of the hemoglobin is removed by the process of exsanguination. Myoglobin in muscle consists of R·Mb (reduction type), O₂Mb (oxygen type), and MMb (oxidation type); when beef is sliced, R·Mb transforms majorly into myoglobin, resulting in a color change in beef from bright red to dark red [19]. Furthermore, complex interactions between pH, temperature, oxygen-partial pressure, lipid oxidation, and microorganisms may be forwarded as possible reasons for the change in meat color [8]. During the entire frozen-storage period, it was observed that the ice crystal growth was slow until 22 days, but the growth rate dramatically increased in the last 7 days, resulting in ice crystals covering a large proportion of the sirloin surface at the end of the 29-day freezing period. Even though ziplock bags with the LDPE film have been used as moisture barrier material [20], the LDPE film did not completely block moisture permeations between the inside and outside of the meat. This may be postulated as the possible cause for the formation of ice crystals on the surface of sirloin in this study. On the other hand, we were not able to sense a huge change in the surface color of mackerel with our naked eyes (Fig. 1B). However, ice crystal growth on the surface of mackerel showed a relatively progressive change compared to that of sirloin during the entire frozen-storage time. Even if the area of ice crystal formed on the mackerel surface increased evidently after just 1 day, the increase was stopped for 14 days thereafter. However, the area of ice crystal increased again; eventually, the area reached its peak after the 29-day freezing period. Approximately, the amount of ice crystal on the mackerel surface was more than that on the sirloin surface, which is considered a consequence of the difference in initial water content between sirloin and mackerel. Nazemroaya et al. and Aranda et al. have shown that the moisture content in mackerel ranges roughly between 73 and 75% [21], while it is between 74 and 78% in whole jack mackerel [22]. The content is relatively higher than that in beef loins imported from the United States, which ranges from approximately 67.36 and 70.76%. In general, it is known that the moisture content in Korean sirloin is much lower than that in imported sirloin [23, 24]. From our experimental results, we can postulate that Korean sirloin may show much lesser ice crystal content, if used as a specimen during the same frozen-storage period.

Drip loss of sirloin and mackerel

Drip loss is one of the most important quality parameters for a variety of food materials. It has been known that drip loss by freezing is the result of decrease in the moisture-holding capacity due to protein denaturation [8]. Although water

Fig. 1 A change in the appearance of sirloin (A) and mackerel (B) during the frozen-storage period

content in food materials has been known as one of the main factors of microbial growth and food spoilage during storage, it also plays a positive role in the sensory aspects of food materials, including silky texture and moistness. Thus, drip loss has been considered to be a main part in understanding the quality of certain food materials for many consumers. The changes in the drip loss of sirloin and mackerel are presented in Fig. 2. During the 29-day frozen-storage time, the drip loss of sirloin did not increase dramatically but showed a moderate increase; eventually, it increased up to approximately 5% (Fig. 2A). Previous research has indicated that the drip loss of sirloin (1 cm thick) thawed at 4 ± 1 °C after freezing at -20 °C for 1 month was increased up to roughly 1% [8], which seems to be a result of differences in the thickness of samples and the degree of temperature fluctuation during frozen-storage time. In our study, the freezer door was opened about 10 times per day during the frozen-storage period, which seems to have caused higher drip loss, since frequent door opening can cause high temperature fluctuation, which could in turn have

a negative effect on the shelf-life of foods [25]. A previous study revealed that the drip loss of a boneless beef strip loin (2.8 cm thick) thawed at 3–4 °C after freezing at -20 °C for 1 week was increased up to 3.9% [14]. In fact, in this study, the drip loss of sirloin had a significant positive correlation with frozen-storage time (correlation coefficient 0.591) (Table 2), which demonstrates that increase in frozen-storage time would affect the growth of ice crystals inside sirloin as well as the increase in the drip loss of sirloin. On the other hand, mackerel showed a drip loss of approximately 7% after the 29-day frozen-storage period, although there were few changes in the drip loss of mackerel during 22-day frozen storage (Fig. 2B). We observed that the drip loss of mackerel had a significant positive correlation with frozen-storage time (correlation coefficient 0.571) (Table 2). As a result, the drip loss of both sirloin and mackerel was increased during the 29-day frozen storage; in addition, the drip loss of mackerel being higher than that of sirloin is likely a result of relatively higher moisture content in mackerel than that in sirloin.

Fig. 2 A change in the drip loss of sirloin (A) and mackerel (B) during the frozen-storage period

A change in TBARS levels of sirloin and mackerel

Figure 3 presents changes in the TBARS level of sirloin and mackerel during the 29-day frozen storage. In general, TBARS level indicates the degree of lipid oxidation, which generates malondialdehyde (MDA). MDA reacts with TBA, which leads to red coloration; eventually, deeper red indicates a higher level of lipid oxidation. The initial TBARS level of our sirloin samples was approximately 0.21 ± 0.01 mg MDA/kg, which was in close agreement with a previous study wherein it was documented that the initial TBARS level of sirloin imported from the United States was 0.35 ± 0.09 mg MDA/kg [23]. Furthermore, the TBARS level of sirloin was maintained at very low values (below 0.40 mg MDA/kg) during the 29-day frozen storage (Fig. 3A). Foods with a TBARS level less than 0.46 mg MDA/kg and more than 1.20 mg MDA/kg has been considered edible and thoroughly decayed, respectively [26], which means that the quality of the frozen sirloin was not affected during the 29-day frozen storage. On the other hand, the rate of TBARS level increase was relatively quicker than the results of other studies, which indicates that the TBARS level of sirloin thawed at 3 °C after freezing at -20 °C for 6 months was increased to about 0.37 ± 0.02 mg MDA/kg [7]. This phenomenon can be explained by temperature fluctuation caused by regular door-opening action in this study. As with the results mentioned above, it was indicated that the TBARS level of sirloin has no significant correlation with the freezing period (correlation coefficient 0.018) (Table 2). Akhtar et al. insisted that lipid oxidation can take place in meat frozen in a freezer at above -20 °C by biochemical reactions in unfrozen water [10]. On the other hand, in case of mackerel, the TBARS level was gradually increased during frozen storage (Fig. 3B). During the first day of storage, the

TBARS level was maintained at a low value but it sharply increased for 7 days thereafter. The level increased up to approximately 22.50 ± 2.03 μ mol/kg (3.24 ± 0.29 mg MDA/kg) until day 15, after which it stabilized. A previous study revealed that TBARS levels of mackerel after freezing at -15 °C for 0 and 1 month are 12.4 and 22.2 μ mol/kg, respectively [27], which is very similar to our result. Furthermore, another study showed that the TBARS level of Indian mackerel after storage in an air-blast freezer at -20 °C for 1 month was approximately 2.50 mg MDA/kg [28], which is also consistent with our results; the TBARS level in the previous study is slightly low, which seems to be a result of quick freezing. Lastly, it was demonstrated that the TBARS level of mackerel has a significant positive correlation with the freezing period (0.839), which means that frozen storage can have a strong influence on the deterioration of mackerel. It is known that mackerels die and decompose as soon as they are caught, which probably causes the much higher TBARS level for mackerel than for sirloin (Fig. 3).

A change in color of sirloin and mackerel

In this study, meat color was analyzed after thawing. Color is one of the most important factors that can affect the perception of quality of foods and purchasing decision by general consumers [29]. Table 1 shows change in color of thawed sirloin and mackerel after 29 days of frozen storage. As mentioned earlier, sliced fresh meat is affected by R-Mb, which leads to dark red coloration. However, on placing outside the freezer, the frozen beef comes in contact with oxygen, resulting in the change in a major myoglobin to O₂Mb, and consequent change in color from dark red to cherry red [19]. In this study, it was revealed that the L* value (lightness) of sirloin was slightly increased during

Fig. 3 A change in the TBARS levels of sirloin (A) and mackerel (B) during the frozen-storage period

Fig. 4 A change in the hardness of sirloin during the frozen-storage period

the 29-day frozen storage. Even though the lightness of sirloin right after freezing seemed to decrease to the naked eye, O_2Mb generated during the thawing process might have dictated the slight increase in the L^* value of sirloin. The phenomenon showing a moderate decrease in the a^* value (redness) of sirloin also seemed to be affected by the same process. Lastly, the change in the b^* value (yellowness) of sirloin was not statistically significant. The L^* and a^* values of sirloin had a positive correlation with the freezing period (correlation coefficients 0.275 and 0.414, respectively) and the b^* value of sirloin had a weak negative correlation with the freezing period (correlation coefficient -0.279) (Table 2). It has been previously reported that bright red color of meat is the most preferred by consumers [29]. Accordingly, it is postulated that there was no effect on the visual appearance of sirloin in this study because L^* and a^* values significantly increased during the 29-day frozen storage. On the other hand, all color parameters of mackerel did not show significant changes during the 29-day frozen storage (Table 1). Similarly, as shown in Table 2, there was no significant correlation between frozen-storage time and color parameters

(correlation coefficients 0.015, 0.146, and 0.020 for L^* , a^* , and b^* , respectively), which means that frozen-storage time within a month is not associated with quality of mackerel in terms of color parameters.

A change in hardness of sirloin

The hardness value of sirloin was found to decrease from 10.5 ± 1.2 to 7.4 ± 1.9 N during the 29-day frozen storage (Fig. 4). Decrease in the hardness value of sirloin during the frozen-storage period could be attributed to tissue destruction, resulting from ice-crystal formation and drip loss. In particular, a considerable decrease was noted for the initial 8 days. After that, there was no significant change in the hardness. This may be bracketed together with the idea that slow cooling (refrigeration) has a huge influence on ice-crystal formation for the initial first week. It is pertinent to note that the hardness value of sirloin had a slightly negative correlation (correlation coefficient -0.243).

In this study, the effect of the frozen-storage period on the quality of sirloin and mackerel was analyzed on the basis of the changes in appearance, drip loss, TBARS, Hunter color value, and hardness of the meat. The frozen storage resulted in dark red coloration of sirloin. Such observation, common for various frozen environments, may be linked to oxygen-mediated events. Furthermore, it was observed that the area occupied by the ice crystals on the surface of sirloin and mackerel increased with increasing frozen-storage time. Drip loss was noted for both sirloin and mackerel, particularly, the highest value for a 29-day frozen-storage period. However, the amount of drip loss was higher in mackerel than in sirloin, which is assumed to be a result of the difference between the initial moisture contents of the two samples. Although the TBARS level was measurable in both sirloin and mackerel (with higher values in the latter), the level was within the maximum acceptable limits for consumption: 0.46 mg MDA/kg for beef [26] and 8.0 mg MDA/kg for fish [30, 31]. Finally, it was verified that the hardness of sirloin

Table 1 Color lightness (L^*), redness (a^*), and yellowness (b^*) of sirloin and mackerel depending on frozen-storage time

Days	L^*		a^*		b^*	
	Sirloin	Mackerel	Sirloin	Mackerel	Sirloin	Mackerel
0	34.87 ± 2.46^b	34.80 ± 1.08^a	17.55 ± 2.33^{bc}	3.78 ± 0.80^{bc}	11.78 ± 1.06^a	5.66 ± 1.04^{bc}
1	36.40 ± 2.88^{ab}	35.88 ± 3.60^a	16.71 ± 2.64^c	3.31 ± 0.66^{cd}	12.21 ± 1.69^a	7.01 ± 1.14^a
8	34.94 ± 1.81^b	37.11 ± 2.86^a	18.02 ± 3.29^{abc}	4.53 ± 0.98^{ab}	9.05 ± 1.27^c	5.14 ± 1.36^c
15	36.66 ± 2.77^{ab}	36.09 ± 1.37^a	19.33 ± 2.11^{ab}	2.73 ± 0.99^d	10.39 ± 1.22^b	6.14 ± 1.58^{abc}
22	37.12 ± 3.20^a	36.04 ± 2.87^a	19.82 ± 2.66^a	3.06 ± 0.89^{cd}	9.97 ± 1.32^{bc}	5.77 ± 1.37^{bc}
29	37.28 ± 2.87^a	35.15 ± 1.41^a	20.03 ± 2.23^a	4.80 ± 1.22^a	10.57 ± 1.24^b	6.74 ± 1.16^{ab}

^{a-d} Means within same column with different superscripts are significantly different ($p < 0.05$)

Table 2 Correlation coefficients between frozen-storage time of sirloin and mackerel and quality parameters (TBARS, drip loss, hardness, and Hunter color value)

Pearson correlation (frozen-storage time)	Drip loss	TBARS	Hardness	Hunter color value		
				L*	a*	b*
Sirloin	0.591*	0.018	−0.243*	0.275**	0.414**	−0.279**
Mackerel	0.571*	0.839**	N ^a	0.015	0.146	0.020

^a Not investigated

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

decreased slightly (implying the loss of moisture-holding capacity and tissue deterioration) during the 29-day frozen-storage period. In conclusion, it is postulated that a 29-day freezing period might have little effect on the quality of sirloin and mackerel.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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