

Initial Freshness of Pacific Oyster (*Crassostea gigas*) Affects Its Quality and Self-life during Freezing Storage

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Abstract We investigated the effect of initial freshness of raw oysters on the quality and storage period of oysters during freezing storage. The expressible drips of the oysters were more effused as the freezing storage period increased. Also, the lower the initial freshness of the oysters, the more drips were released after thawing. The pH values decreased slightly during freezing storage at -20 °C for 12 months, but there was no significant difference ($p > 0.05$) according to initial freshness of raw oysters. The initial glycogen contents of oysters before freezing was between 722 and 585 mg/100 g whereas the glycogen contents of the oysters after freezing for 12 months ranged from 667 to 522 mg/100 g. The initial TVB-N value of S-1 with freshness of “good quality” was 3.9 mg N/100 g, but gradually increased during the freezing storage period and its value was 7.9 mg N/100g at 12 months of storage. S-7 of with an initial TVB-N value of 15.2 mg N/100 g increased sharply during freezing storage, reaching a maximum of 36.2 mg N/100g at 8 months after storage and then slightly decreased to 32.2 mg N/100g at 12 months of storage. S-5 with an initial PV of 12.6 meq/kg showed the highest value of 33.7 meq/kg at the 10 months of storage and S-7 with an initial PV of 17.5 meq/kg showed the highest value of 44.6 meq/kg at 8 months of storage. PL of oysters decreased with increasing storage period regardless of their initial freshness in all samples, while FFA increased during freezing storage. The scores of all sensory evaluation parameters of S-1, which is the freshest sample of raw oysters before freezing, showed little change until 4 months after storage, and their scores began to slowly decrease after 4 months of storage and still could be accepted (scores of more than 6.0) at the end of storage. Sensory evaluation scores on color, taste and odor of S-7 after 8 months of storage showed unacceptable score of 6 or less, and fishy and sour taste were slightly stronger at 12 months of storage. These results indicate that raw oysters with TVB-N and PV of 3.9 mg/100 g and 4.2 meq/kg can maintain oyster quality for more than one year at -20°C. On the other hand, it is suggested that raw oysters with TVB-N and PV of 15.2 mg N/100 g and 17.5 meq/kg may not be stored for more than 8 months at -20°C.

Keywords: oyster, shelf-life, quality assessment, initial freshness

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1. Introduction

Several oysters, such as Tropical oyster (*Crassostrea nipponica*), Lamellated oyster (*Ostrea denselamellosa*) and Pacific oyster (*Crassostrea gigas*), are produced in Korea, and Pacific oyster (*Crassostrea gigas*) are cultivated mainly in Korea. Korean Pacific oysters are generally harvested from October up to April of the following year. The oysters harvested between October and February of the following year are mainly sold in shucked form, is distributed in fresh state via iced seawater. The bulk of oysters harvested after February are frozen in an individual quick freezer and stored for a long period. Quality of frozen oysters is affected by several factors such as storage temperature and time, packaging and thawing conditions [1]. The pH of the oyster gradually changes during freezing storage at -20 °C and -35°C [2],

but moisture and crude protein contents do not change significantly during freezing storage at -20°C for 12 months [23]. The total volatile basic nitrogen (TVB-N) and peroxide value (PV), which are the indicators of freshness in oyster, increased during storage at -18°C [4]. Jeong *et al.* [2] also reported that the enzymatic degradation of phospholipids in frozen oysters was progressed during storage at -35°C, resulting in releasing of free fatty acids. However, deoxygenated frozen oysters showed little change in lipid deterioration during freeze storage for more than 12 months. Shelled oysters stored at chilled temperature can also undergo biochemical and physical changes related to freshness. Songsaeng *et al.* [5] reported that shelled oyster could be kept in air chilled temperature for 9 days in terms of microbiological and sensory characteristics.

Freshness of raw oysters is an important factor affecting the quality change of oysters during freezing storage. The self-life of fresh seafood depends on conditions such as

storage time and temperature. The stored oysters at 10 °C and 5 °C reached the unacceptable freshness scores on day 7 and 11, respectively, while the oysters stored at 0 °C maintained acceptable freshness scores until day 17 [6]. The sensory quality and bacterial level of stored oysters at 3°C remained acceptable until day 22, but oysters stored in the cold storage process should not exceed 15 days in consideration of all assessment items of oyster quality [7]. Generally, shucked oysters are not immediately frozen after harvest. Shelled oysters harvested from shellfish farms are transported to the land by boat, exposed to ambient temperature and sunshine, and then the shucked shellfish work is carried out. This process affects the initial freshness of raw oysters before freezing. Therefore, the present study deals with the investigating the effect of the initial freshness of oysters on the quality of oysters during freezing storage.

2. Materials and Methods

2.1. Materials

Pacific oysters (*Crassostrea gigas*) were purchased in February 2015 from an average oyster weighing 342±12.8g in Tongyeong Bay, Republic Korea. Immediately after harvest, the shelled oysters were shucked and placed in ice under 5 °C and transported to the laboratory within 2 hours and then some sample (S-1, very good quality) were soon frozen in an individual quick freezer (IQF). Frozen oysters were immediately glazed in water for 15 s, packed in polyethylene bag and stored at -20 °C. The other shucked samples were kept at 5 °C and frozen to IQF at 48-hours intervals for freshness assessment, and the samples were named S-3 (good quality), S-5 (medium quality) and S-7 (poor quality). After IQF, S-3, S-5 and S-7 samples were glazed and stored in the same method as that of S-1 sample.

2.2. Expressible Drip

The shucked oyster samples weighing (A) were placed between the Whatman No. 4 filter papers with two filter papers on top and three filter papers below. The samples were weighed at 3.5 kg and allowed to hold the pressure for 3 min. The samples were then removed and weighed (B). The drip under pressure was determined as $[(A-B)/A] \times 100$ [8].

2.3. pH

Ten grams of the shucked oyster samples were homogenized with 20 mL of distilled water at 12,000 rpm for 1 min under 5°C. The pH of the homogenate was measured using a pH metre.

2.4. Glycogen

Glycogen content of oyster samples was measured as described previously [9]. One gram of the shucked oyster samples was homogenized with 10 mL of 30% KOH and then heated in a 95°C water bath for 20 min. After cooling sample, 1 mL of saturated Na₂SO₄ solution and 10 mL of

ethanol were added to the samples and heated in a 95°C water bath for 15 min, and then it was then centrifuged at 3,000 rpm for 10 min. The precipitate was dissolved in 4 mL of distilled water and 5 mL of ethanol and then centrifuged at 3,000 rpm for 10 min. After evaporation of the ethanol in the precipitate in a hot bath, 4 mL of 5 M HCl were added to the collected precipitate. The solution was neutralized with 0.5 M NaOH and adjusted to 50 mL with distilled. Ten milligrams of the solution was added to 20 mL of 0.2% anthron-sulfate solution and heated a 95°C water bath for 15 min. After cooling the solution, it was measured on spectro-photometer (UV mini-1240, Shimadzu, Tokyo, Japan). The glycogen content in the samples was determined by multiplying the glucose concentration by 0.9.

2.5. Total Volatile Basic Nitrogen (TVB-N)

Two grams of the shucked oyster samples were homogenized with 8 mL of 4% trichloroacetic acid (TCA). The sample was kept at ambient temperature for 30 min and then centrifuged at 3,000 rpm for 10 min. The supernatant was made up to 10 mL with 4% TCA and determined for TVB-N according to the method of Hasegawa [8].

2.6. Lipid Extraction and Peroxide Value (PV)

Total lipids were extracted from 30 g of the shucked oyster samples with chloroform/methanol according to the method of Bligh and Dyer [10]. Peroxide value was determined with the ferric thiocyanate method [11]. The PV was calculated and expressed as milliequivalents of ferric ion/kg lipid.

2.7. Analysis of Phospholipid (PL) and Free Fatty Acid (FFA)

The lipid composition of oysters was determined using a thin layer chromatography/flame ionization detection analyzer (Iatroscan MK5 TLC/FID analyzer, Iatron Laboratories, Inc., Tokyo, Japan). One µL of lipid sample was spotted onto the scanned quartz rod (Chromarod S-IV, Iatron Laboratories, Inc., Tokyo, Japan) and separated using a mixture of benzene: chloroform: acetic acid (50: 20: 0.7 v/v/v) for 30 min. The developed sample was dried for 5 min in an 105°C oven and immediately scanned with the TLC/FID analyzer with a scanning speed of 30 s/scan. The lipid composition standards containing palmitic acid, oleic acid, phosphatidylcholine, monopalmitin, monoolein, diolein, dipalmitin, triolein and tripalmitin (Sigma, St. Louis, USA) were used to identify chromatographic peaks of the samples. Each lipid composition was calculated, based on peak area ratio and expressed as g / 100 g oil [12].

2.8. Sensory Evaluation

The sensory properties of oysters were carried out by seven panelists of staff from the Department of Food Science & Engineering, Pukyong National University of Korea, using appropriately modified oyster guideline as shown in Table 1 [3,6,7]. The panelists were asked to

score the intensity of each characteristic describing appearance, color, odor and texture using an unstructured scale ranging from 0 to 10: 1, unacceptable; 2, extremely

poor; 3, very poor; 4, poor; 5, slightly poor; 6, acceptable; 7, good; 8; very good; 9, extremely good and 10, excellent. Frozen oysters were thawed at 5 °C for 12 hours.

Table 1. Description of the attributes used for sensory assessment

Appearance	Odor	Color	Taste
clear, amount of debris, cloudy	Hay, sea-weedy Fishy, mud, putrid smell	cream white white, tan, yellow/brown	sour, sweeten, bitter, salty, astringency

Table 2. Physico-chemical properties according to the freshness of raw oysters

Sample	Expressible drip (%)	pH	TVB-N (mg N/100g)	Glycogen (mg/100 g)	PV (meq/kg)
S-1	9.8±1.5 ^a	6.36±0.16 ^c	3.9±0.5 ^a	722±14 ^c	4.2±1.8 ^a
S-3	9.2±3.1 ^a	6.27±0.12 ^{bc}	6.6±1.8 ^a	702±12 ^{bc}	10.4±1.2 ^b
S-5	15.9±2.2 ^b	6.01±0.20 ^{ab}	11.4±1.2 ^b	682±18 ^b	12.6±1.8 ^b
S-7	21.6±1.8 ^c	5.89±0.10 ^a	15.2±2.2 ^c	586±16 ^a	17.6±2.2 ^c

Significant at P < 0.05. The result of relationship analysis about expressible drip, TVB-N, glycogen, PV, and the freshness of raw oysters.

2.9. Statistical Analysis

The results from three replications of two trials were subjected to analysis of variance (ANOVA) and Duncan’s multiple range test for significant differences at p < 0.05 [13].

3. Results and Discussion

3.1. Expressible Drip

The occurrence of drip after thawing of frozen food is probably due to damage of cell structure and tissue by ice crystal growth during freezing storage. Generally, the expressible drip of frozen food differs depending on its freezing and thawing method and the freshness of the food before freezing. The initial expressible drips of raw oyster before freezing storage increased as the freshness of raw oysters decreased, with values of 9.8 % (S-1), 9.2 % (S-3), 15.9 % (S-5), and 21.6 % w/w (S-7), respectively (Table 2).

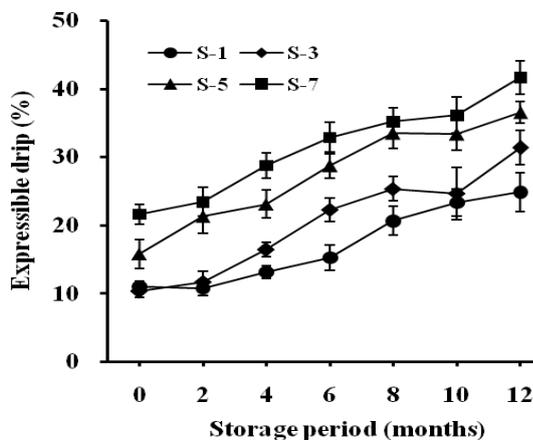


Figure 1. Changes in expressible drip during storage at -20°C of frozen oysters by the initial freshness.

The results of relationship analysis about expressible drip and frozen storage period in samples were significant differences according to Duncan’s test (P < 0.05).

Changes in expressible drip during freezing storage are shown in Figure 1. The expressible drip in all samples increased with increasing freezing storage period (p < 0.05), suggesting that ice crystals may have resulted in tissue damage and leakage of various organelles [14].

Furthermore, as the freshness of the initial raw oysters decreased, the drips of frozen oysters increased during freezing storage, and the freshness of raw oyster before freezing storage may have influenced the maintenance of protein water holding capacity.

3.2. pH and Glycogen

The pH has been used as one of the important factors determining the freshness and quality of oysters during the distribution of oysters. The pH levels of raw oysters by freshness were 6.36 for S-1, 6.27 for S-3, 6.01 for S-5 and 5.89 for S-7 (Table 2). These were similar to the results that the decomposition of glycogen was promoted as the freshness of raw oysters decreased.

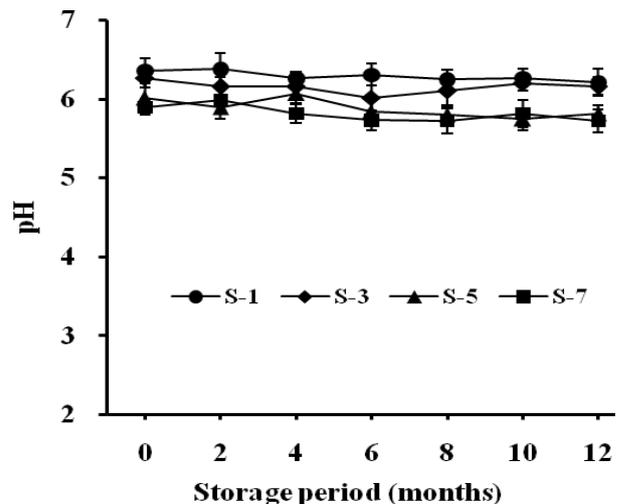


Figure 2. Changes in pH during storage at -20°C of frozen oysters by the initial freshness

The results of relationship analysis about pH and frozen storage period in samples were no significant differences according to Duncan’s test (P < 0.05).

The initial pH levels of shucked oysters before freezing was between 5.89 and 6.36, whereas the pH levels of the oysters after freezing for 12 months ranged from 5.72 to 6.21. The pH levels of the oysters during freezing storage showed almost no change regardless of the freshness of the initial raw oysters (Figure 2). Similar results have been reported that no significant changes in pH were observed during storage at -20°C for 12 months [2,5].

The initial glycogen content of S-1, which is of very good quality, was 722 mg/100 g, but the initial glycogen content of S-7, which is of poor quality, was 586 mg/100 g. Glycogen contents in all frozen oysters remained relatively constant with the freshness of each sample during the frozen storage for 12 months (Figure 3). Ratio of change on glycogen decomposition as well as pH during freezing storage did not represent significant difference according to the initial freshness of raw oysters.

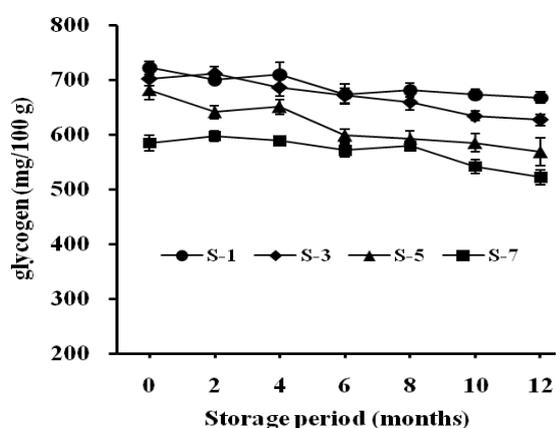


Figure 3. Changes in glycogen contents during storage at -20°C of frozen oysters by the initial freshness

The results of relationship analysis about glycogen contents and frozen storage period in samples were significant differences according to Duncan's test ($P < 0.05$).

3.3. Total Volatile Basic Nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) resulting from degradation of protein and non-protein nitrogenous compounds has been represented as an indicator of the freshness on oyster [3,15,16]. The initial TVB-N values of raw oysters according to the freshness were 3.9 (S-1), 6.6 (S-3), 11.4 (S-5) and 15.2 mg N/100g (S-7), respectively (Table 2). TVB-N values in S-1 sample almost remain changed, while S-3 slightly increased during freezing storage for 12 months. However, S-5 and S-7, with initial freshness of medium and poor, remarkably increased during frozen storage period for 12 months (Figure 4). TVB-N in S-5 sample recorded the highest value at 10 months of freezing storage, while S-7 sample was the highest at 8 months. The increase rates of TVB-N values during freezing storage compared to the initial contents of TVB-N before freezing storage were almost constant in all samples regardless of the initial freshness of the raw oysters. However, after 12 months of freezing storage, the TVB-N content of the S-7 was about four times higher than that of S-1. The TVB-N values of fish during freezing storage were generally reported to increase linearly with increasing storage period [17,18]. The TVB-N value could be increased due to the decomposition of the Trimethylamine

oxide (TMAO), because the bacterial count and activity are gradually decreased under freezing conditions [16]. This decomposition promotes the leakage of dimethylamine (DMA) and formaldehyde (FA) by the endogenous enzyme present in the muscle and consequently can increase the TVB-N value [16,19].

3.4. Peroxide Value (PV)

The frozen oysters is dried generally on the surface due to inadequate packaging or glazing, and then the oyster lipids are placed in conditions that can be easily oxidized, which results in rancidity called freezer burn. Because oysters contain phospholipids such as lecithin, fatty acids and various sterols, which have health-beneficial functional properties, oxidation of these lipids in oyster is considered as an indicator of oyster quality [2,12,20].

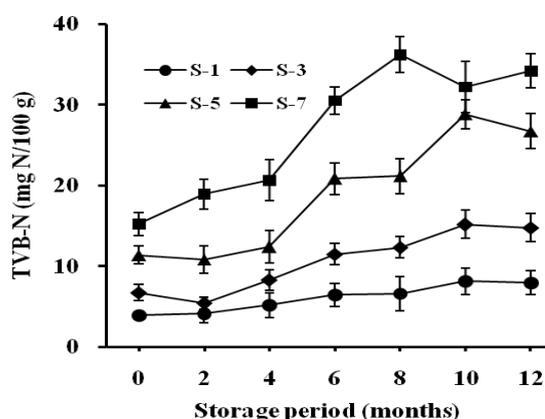


Figure 4. Changes in total volatile basic nitrogen (TVB-N) during storage at -20°C of frozen oysters by the initial freshness

The results of relationship analysis about TVB-N and frozen storage period in samples were significant differences according to Duncan's test ($P < 0.05$).

The basic mechanism for lipid oxidation during storage of food is commonly described as autoxidation. It is designed as a complex set consisting of initiation step initiated by free radicals activated by oxygen, light and metals, etc, propagation step in which reactions are accelerated by autocatalytic action, and termination step that produce carbonyl compounds such as aldehydes and ketones.

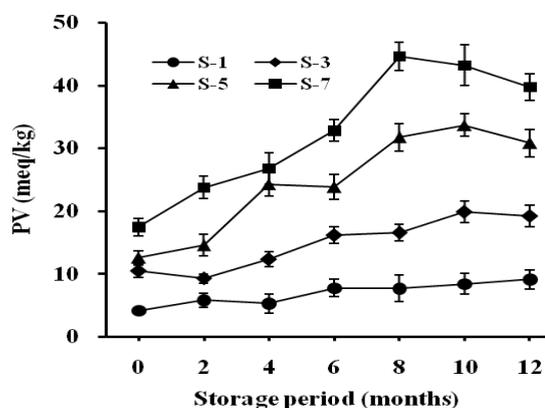


Figure 5. Changes in peroxide value (PV) during storage at -20°C of frozen oysters by the initial freshness

The results of relationship analysis about PV and frozen storage period in samples were significant differences according to Duncan's test ($P < 0.05$).

Table 3. Changes in phospholipid (PL) and free fatty acid (FFA) (g/100g oil) of oysters during freezing storage

Storage periods (months)	S-1		S-3		S-5		S-7	
	PL	FFA	PL	FFA	PL	FFA	PL	FFA
0	43.4±1.1 ^a	0.8±0.1 ^a	41.2±2.2 ^a	2.1±0.1 ^a	38.1±1.4 ^a	3.2±0.2 ^a	36.5±1.6 ^a	4.7±0.3 ^a
2	43.2±0.8 ^a	1.2±0.1 ^b	40.2±0.9 ^{ab}	3.8±0.2 ^b	39.4±1.0 ^{ab}	4.2±0.3 ^b	34.2±1.8 ^{ab}	6.8±0.2 ^b
4	41.9±1.6 ^{ab}	1.4±0.3 ^b	38.9±1.3 ^{abc}	3.6±0.1 ^b	37.2±0.8 ^{abc}	4.4±0.3 ^b	34.3±0.8 ^{ab}	7.3±0.4 ^b
6	40.6±1.2 ^{bc}	2.5±0.2 ^c	38.3±2.0 ^{bc}	4.5±0.2 ^c	35.5±2.1 ^{bc}	6.2±0.2 ^c	32.0±1.8 ^{bc}	8.2±0.4 ^c
8	40.2±1.0 ^{bc}	3.1±0.1 ^d	38.1±0.4 ^{bc}	5.7±0.2 ^d	34.6±1.8 ^{cd}	7.6±0.4 ^d	32.2±2.4 ^{bc}	8.8±0.3 ^{cd}
10	38.1±1.5 ^c	4.6±0.2 ^e	36.4±1.1 ^{cd}	6.6±0.3 ^e	32.4±0.8 ^{de}	9.1±0.4 ^e	31.4±2.2 ^{bc}	9.1±0.5 ^d
12	38.8±1.8 ^c	5.2±0.2 ^f	35.2±1.2 ^d	7.1±0.4 ^f	31.6±1.6 ^e	9.5±0.3 ^e	29.3±1.8 ^c	10.7±0.4 ^e

Significant at P < 0.05. The result of relationship analysis about PL, FFA, and frozen storage period in samples.

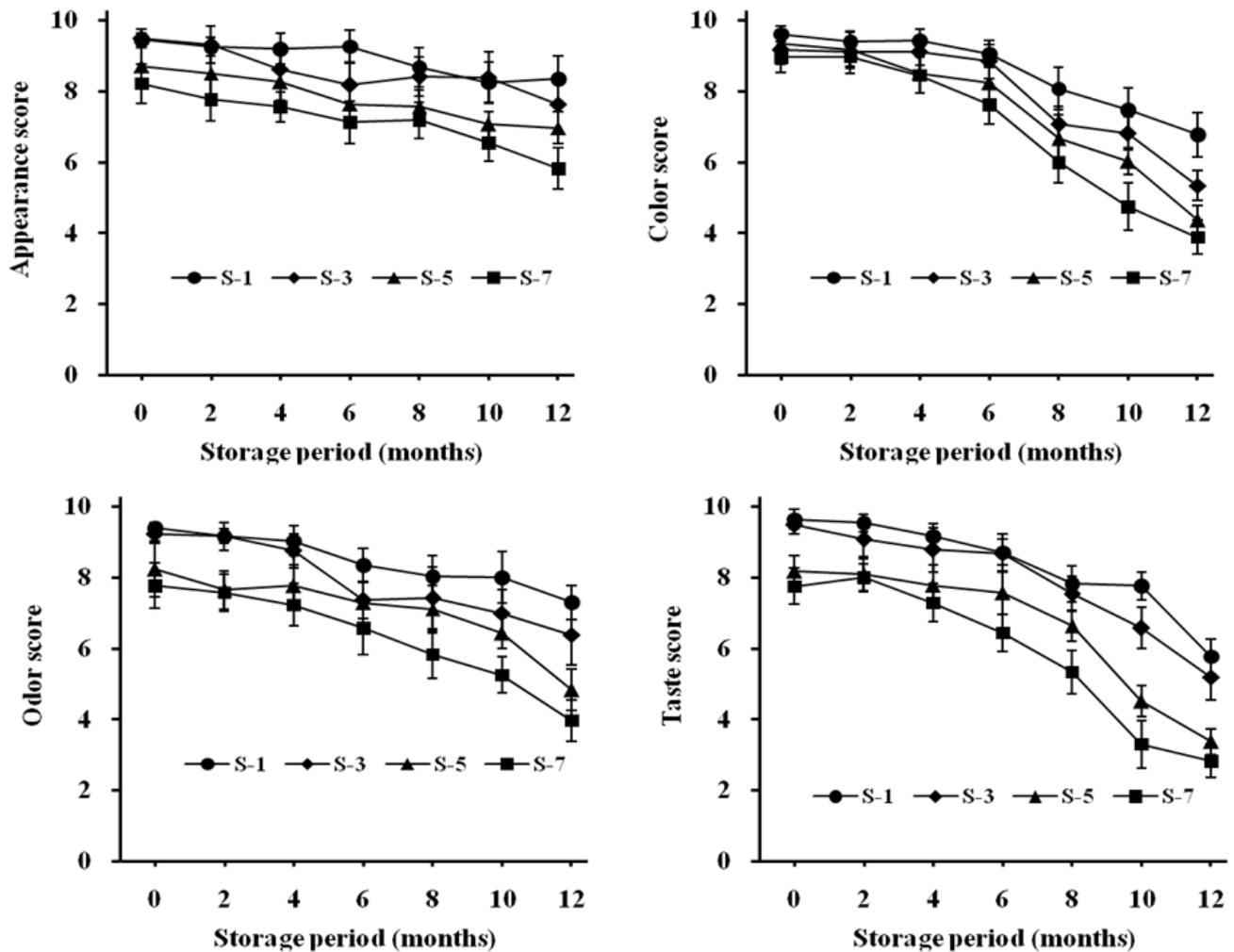


Figure 6. Changes in sensory scores during storage at -20°C of frozen oysters by the initial freshness

Significant at P < 0.01 except S-5 of appearance score (P < 0.05). The results of relationship analysis about sensory evaluation and frozen storage period in samples were significant differences according to Duncan's test.

This reaction also can be influenced by pH, storage temperature, enzymes, salt and concentration of antioxidants [21,22]. The initial peroxide value (PV) of raw oyster (S-1) was 4.2 meq/kg and it increased to 10.4 (S-3), 12.6 (S-5), or 17.6 meq/kg (S-7) during the keeping at 5 °C (Table 2). The S-1 sample that was frozen immediately after harvest represented little change in PV during freezing storage. The S-5, which was medium quality, and the S-7, which was poor quality, increased linearly during freezing storage. The S-5 showed the highest value at the 10th month of storage, but

the S-7 reached the maximum value at 8 months after storage (Figure 5). These results suggest that the aggregation of hydroperoxide during freezing storage can be influenced by the initial concentration of hydroperoxide in raw oysters and that the rapid increases in PV of S-5 and S-7 may have led to a propagation step in the autoxidation process of lipids. In to a propagation step, free radical can then react with oxygen to form a peroxy radical, which can further react with another lipid molecule to generate a hydroperoxide and another lipid radical [23].

3.5. Changes in Lipid Composition

Changes in free fatty acid (FFA) and phospholipids (PL) contents during freezing storage are shown in Table 3. The contents of FFA and PL in raw oysters prior to the frozen were changed, depending on their initial freshness. During frozen storage period, PL contents in all samples decreased ($p < 0.05$), possibly due to the activity of phospholipase [24]. However, FFA contents increased ($p < 0.05$) with increasing storage period, which may be due to hydrolysis of PL and other triglycerides. Despite the difference in freshness of raw oysters, the decrease and increase of PL and FFA during storage were found to be almost independent of their initial freshness. Enzymatic hydrolysis of PL in fish progresses slowly at low temperature, such as at -20°C [2,25]. Our results also suggest that some phospholipases act on oysters during freezing, resulting in some degradation of PL, and more FFA may be a factor in increasing PV.

3.6. Sensory Evaluation

Sensory evaluation is a useful method for describing the sensory characteristics of food as a means of identifying the initial quality characteristics of food and any changes in the food storage process [26]. Therefore, the sensory evaluation is mainly used in the food industry and research as a method of examining the appearance, flavor, and texture of the product to determine the development properties of the product, the storage period of the product, and the correlation between the sensory and physical characteristics of the product. The scores of sensory evaluation parameters gradually decreased with storage period in all samples (Figure 6). The scores of all sensory evaluation parameters of S-1, which is the freshest sample of raw oysters before freezing, showed little change until 4 months after storage, and their scores began to slowly decrease after 4 months of storage and still could be accepted (scores of more than 6.0) at the end of storage. Sensory evaluation scores of S-7 showed less than 6 points which were unacceptable score after 8 months of storage in color, taste, and odor parameters, and showed somewhat fishy and sour taste at 12 months of storage.

4. Conclusions

The present study are undertaken to investigate the influence of initial freshness of oysters on the freezing storage period. In the initial quality of the oysters, S-1 with pH, TVB-N and PV of 6.36, 3.9 mg N/100g and 4.2 meq/kg and S-3 with 6.27, 6.6 mg N/100g and 10.4 meq/kg showed a freezing storage period of more than 12 months at -20°C . However, S-5 with the quality characteristics of pH, TVB-N and PV of 6.01, 11.4 mg N/100g and 12.6 meq/kg could be accepted only for up to 10 months storage at -20°C in sensory evaluation. S-7 sample with pH, TVB-N and PV of 5.89, 15.2 mg N/100g and 17.6 meq/kg respectively showed unacceptable quality after 8 months of storage at -20°C . Generally, frozen oysters are stored at -20°C for over a year, and the quality of the frozen oysters is maintained to a level that is commercially available. However, if the raw oysters were

stored at 5°C for more than 3 days and then stored frozen, the storage period of oysters could not be maintained for more than one year.

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