

Effects of eco-friendly cold packs on the quality of vacuum-packed mackerel fillets during room temperature distribution

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Abstract

In this study, we evaluated the effectiveness of commercial cold packs (CC) and 3AC-developed cold packs (3AC) in expanded polystyrene containers (EPS+CC and EPS+3AC, respectively) for maintaining the freshness of vacuum-packed mackerel (*Scomber japonicus*) during distribution in EPS boxes. Temperature monitoring showed that EPS+3AC packaging maintained slightly lower temperatures (by over 1° C) than EPS+CC during 4–16 h of storage. The pH of both groups gradually increased over 72 h, with significant differences observed between 0 h and 72 h. The total volatile basic nitrogen content in EPS+CC exceeded the acceptable limit (≥ 35 mg/100 g) after 36 h, whereas EPS+3AC showed a slower increase. Odor intensity increased with storage time, with higher values observed in EPS+CC than in EPS+3AC at 72 h. Total aerobic bacteria counts exceeded 6 log CFU/g earlier in EPS+CC than in EPS+3AC. Overall, EPS+3AC packaging delayed quality deterioration and extended the freshness-related shelf-life indicators of vacuum-packed mackerel under the tested conditions.

Keywords: eco-friendly cold packs, microbial growth, shelf life, temperature control

Introduction

The dependence on online shopping has increased as contactless methods of shopping have become commonplace among consumers due to the influence of COVID-19 and the rise in single-person households. In addition, increased time spent at home has also raised the demand for delivered refrigerated and frozen food (Baker *et al.*, 2020). Frozen food can be defined as food that has undergone pretreatment

processes or raw materials that have been stored at temperatures below -18°C (Tribst *et al.*, 2018). Freezing is one of the most successful techniques used to prolong the storage life of food; it can prevent the loss of vitamins and minerals and maintain nutritional value close to that of fresh products without the need for additional preservatives (Tribst *et al.*, 2018). However, slight temperature fluctuations can alter the texture and flavor of food and affect its quality and integrity.

Fish and fish-based products are widely consumed owing to their high nutritional value and protein content, and they are also good sources of unsaturated fatty acids, particularly omega-3 fatty acids. However, they are vulnerable to food hygiene and safety issues because of their soft connective tissue and rapid protein denaturation, which cause them to spoil faster than other livestock products (Prabhakar *et al.*, 2020). Enzymatic autolysis after fish death breaks down proteins and other compounds, producing off-flavors and undesirable textures, while lipid oxidation, particularly in fatty fish, further affects taste, odor, and nutritional value (Kontominas *et al.*, 2021; Speranza *et al.*, 2021). In this context, fish quality is generally evaluated based on chemical, microbiological, and sensory characteristics that reflect freshness and safety.

According to the Korea Food Code, refrigerated or frozen products must be transported in facilities or vehicles that can maintain proper temperatures or by other means with equivalent or better effects (MFDS, 2023). Therefore, frozen food is typically hermetically packed with cold packs inside an insulated polystyrene box with an outer cardboard box. However, the temperature within the boxes is not consistently maintained; thus, the amount of refrigerant used to maintain the desired temperature range for the full duration of shipping must be considered. It is common to transport frozen food using a cold chain (temperature-controlled supply chain), but the current aquatic cold-chain infrastructure cannot consistently meet the freezing preservation temperature requirements of various fish.

Cold packs play a crucial role in the packaging industry by enabling the safe transport of temperature-sensitive goods. Traditional gel packs made from sodium polyacrylate (SAP) are effective but non-biodegradable, raising environmental concerns due to microplastic pollution (Jeon *et al.*, 2002; Anonymous, 2024). Water-based ice packs are safer and environmentally friendly but have lower cooling performance and can cause condensation damage. Recently, phase change material (PCM)-based cold packs have emerged as an eco-friendly alternative, providing controlled cooling for extended periods. Despite these advantages, PCM-based systems may still lead to condensation-related issues and may not always provide sufficient cooling performance under real distribution conditions. While previous studies have mainly focused on the thermal properties of PCMs, limited research has evaluated their practical application in maintaining the freshness of vacuum-packed seafood under simulated delivery environments. Thus, eco-friendly cold packs are designed to minimize environmental impact by using biodegradable or non-toxic materials while maintaining effective cooling performance.

Therefore, in this study, we aimed to evaluate the shelf life of vacuum-packed mackerel (*Scomber japonicus*) fillets supplied by 3AC, a company that has developed eco-friendly cold packs using food additive-based inorganic salts and thickeners with properties similar to seawater. Following the research of Lee *et al.* (2022), differences in freshness between the two storage methods were determined using chemical (pH and volatile base nitrogen content), physical (odor intensity), and microbiological (total aerobic bacteria count) methods. This study aims to provide practical insights into maintaining the quality and safety of vacuum-packed seafood during distribution.

Materials and Methods

Sample preparation

The vacuum-packed mackerel (*Scomber japonicus*) used as the raw material was purchased from the Tongyeong Traditional Market in South Korea. The head, tail, and intestines were removed from the fish, which was then cut into fillets, vacuum-packed using a vacuum packaging machine (FM2080-071; Food Saver, Oklahoma City, OK, USA), and frozen at -18°C for 12 h in the freezing warehouse of the Fisheries Processing Center at Gyeongsang National University. Commercial cold packs (CC), made from 100% water, were purchased in Tongyeong, Korea. The 3AC-developed cold packs (3AC) were sourced from the 3AC company (Seoul, Korea) and contained mineral salts, xanthan gum, and water. A comparison of the physicochemical properties of the CC and 3AC cold packs is presented in Table 1.

To ensure equivalent experimental conditions, the number of CC packs used was adjusted to match the total content weight of the developed refrigerant pack. The 3AC cold pack had a total weight of 2,100 g, whereas the CC pack weighed 2,136 g.

Measurement of temperature changes over time

The storage temperature was selected based on the recent average ambient temperature in August in Korea (26.7°C , 2021) to simulate a severe summer temperature exposure scenario. Although the typical duration of home delivery is approximately 2 days, the experimental period was extended to 72 h to evaluate the progression of quality deterioration under worst-case temperature abuse conditions rather than to replicate standard commercial distribution. A 150 g vacuum-packed mackerel fillet and two cold packs were placed in an expanded polystyrene (EPS) container, sealed, and stored in a controlled warehouse environment at $27 \pm 0.5^{\circ}\text{C}$. The internal temperature of the EPS container was continuously monitored

Table 1. Comparative characteristics of the 3AC-developed cold pack and commercial cold packs.

Parameter	EPS + CC	EPS + 3AC
Composition	Water	Mineral salts, xanthan gum, water
Packaging material	PE film	Recycled PE film (120 μ m, 4-side heat sealed)
Filling weight (g)	500	500
Thickness (μ m)	40–60	120
PCM ($^{\circ}$ C)	> 5	–5 ~ 0
Cooling duration (h)	2–3	\geq 8
Eco-friendliness	Non-biodegradable	Biodegradable, reduced plastic use

EPS + 3AC, Expanded Polystyrene + 3AC-developed cold packs, EPS + CC, Expanded Polystyrene + Commercial cold pack, PCM, Phase change temperature.

using a wireless temperature data logger (EBI 11, Ebro Co., Germany), which was fixed to the inner surface of the container lid to measure the air temperature inside the package. This experimental design was intended to assess the relative temperature-moderation effect of cold packs and their influence on freshness parameters under extreme conditions.

Measurement of pH

For pH measurement, 12 mL of distilled water was added to 3 g of vacuum-packed mackerel fillets, stirred at room temperature for 10 min, and filtered through Whatman paper (Whatman Inc., Piscataway, New Jersey, USA). The pH of the supernatant was measured three times using a pH meter (Starter 3100; Ohaus, Parsippany, NJ, USA).

Measurement of total volatile basic nitrogen (TVB-N) content

TVB-N content was measured according to the method described by Kapute *et al.* (2012). Two milliliters of 20% (v/v) trichloroacetic acid solution was added to 2 g of vacuum-packed mackerel fillets, and the sample was adjusted to a volume of 16 mL by adding distilled water. The sample was then homogenized at 1000 rpm for 30 min and filtered through Whatman No. 1 filter paper. Subsequently, 1 mL of the filtrate was added to the outer circular wall of a Conway dish, 1 mL of 0.01 N H_3BO_3 was placed in the inner wall, and 100 μ L of Conway solution was added to the inner wall. The cover was opened slightly, and 1 mL of 50% K_2CO_3 was added to the outer diffusion chamber. The Conway dish was placed in an incubator at 37 $^{\circ}$ C for 2 h. After removing the lid, the boric acid was titrated with 0.01 N H_2SO_4 until the

solution turned pink (end point). The same process was followed without adding 1 mL of 50% K_2CO_3 as a blank test. The following equation was used to calculate TVB-N content. The values “a” and “b” indicate the volumes of 0.01 N NaOH used in the main and blank tests, respectively. The “f” value represents the titer of 0.01 N NaOH, “D” represents the dilution ratio, and “S” represents the sample amount. Notably, 1 mL of 0.1 N NaOH is equivalent to 0.14 g N.

Odor intensity

Odor intensity was measured using the method described by Kang *et al.* (2014). Each prepared vacuum-packed mackerel fillet (10 g) was placed in a 50 mL conical tube (30 \times 150 mm; SPL Life Science Co. Ltd., Pocheon, Korea). The suction port of the odor-concentration meter (XP-329R; New Cosmos Electric Co., Ltd., Osaka, Japan) was inserted into the conical tube and sealed with parafilm to enable odor retention. Subsequently, the odor intensity of each vacuum-packed mackerel fillet was measured, and the measurement mode of the odor concentration meter was set to batch, with volatile component intensity (VCI) used as the unit of odor intensity.

Microbiological analysis

Total aerobic bacterial analysis was performed in accordance with the guidelines of the Korea Food Code published by the Korea Ministry of Food and Drug Safety (MFDS, 2023), with some modifications. Vacuum-packed mackerel fillets (25 g) were transferred to a stomacher bag, followed by the addition of 225 mL of 0.85% saline solution before mixing in a stomacher (BagMixer 400; Interscience, Saint-Nom-la-Bretèche, Arpents, France)

for 2 min. The homogenized sample solution (1 mL) was diluted with 9 mL of 0.85% saline solution in a step-wise manner. The samples were then analyzed using the pour plate method. Subsequently, diluted samples from each stage were inoculated onto agar plates (PCA; BD Difco, Sparks, MD, USA) and incubated at 37 °C for 48 h. Thereafter, the number of colonies was counted.

Statistical analysis

All experiments were performed in triplicate and the results are presented as the mean \pm standard deviation (SD). One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used to compare mean values at each sampling time. Statistical significance was set at $P < 0.05$.

Results and Discussion

Temperature maintenance of cold packs in EPS

Mackerel (*Scomber japonicus*) is the most popular commercially sold fish species in South Korea, with an average consumption of 3.25 g per person per day (Korea Health Industry Development Institute, 2020). It is typically consumed in a processed raw state, such as frozen or salted (Bak *et al.*, 2014). However, mackerel is highly perishable and contains a substantial amount of trimethylamine oxide (TMAO), which can be bacterially reduced to trimethylamine (TMA). TMA has a distinctive, stale, and unpleasant "fishy" odor (Wu and Bechtel, 2008). In particular, scombroid fish such as mackerel are highly sensitive to the formation of histamine during storage; therefore, the presence of high amounts of histamine during processing and distribution can produce biogenic amines that cause food poisoning (Sone *et al.*, 2019). These problems can be exacerbated by storage conditions, particularly temperature and environmental humidity. Therefore, fresh raw fish must be handled and distributed correctly in summer, when temperatures inside delivery vehicles increase significantly. This study used packaging comprising EPS containers with CC packs (EPS+CC) and 3AC-developed cold packs (EPS+3AC) to measure temperature. To ensure comparability, the number of CC packs was adjusted such that their total weight (2136 g) was approximately equal to that of the 3AC packs (2100 g). Thus, the observed differences in temperature and quality may be associated with differences in early-stage temperature profiles and cumulative thermal exposure rather than differences in pack weight. Table 2 shows the results of measurements taken at 4 h intervals up to 72 h after the packs were stored at approximately 27 ± 0.5 °C. The temperature inside EPS containers was initially recorded as 4.89 °C for EPS+CC

packaging. After 4 h of storage time, the temperature increased to 9.56 °C, which is similar to the recommended "refrigerated" temperature range of 0–10 °C as specified by the MFDS (2023).

The storage temperature increased progressively, reaching 11.40 °C at 8 h and 24.90 °C at 20 h. There was no significant difference ($P > 0.05$) in temperature between 24 h and 72 h, which ranged from 26.04–26.75 °C. The initial temperature in EPS+3AC packaging was 4.77 °C, which was comparable to that of EPS+CC (4.89 °C). However, on average, temperatures in EPS+3AC packaging remained more than 1 °C lower than those in EPS+CC packaging during 4–16 h of storage. The temperature differences between EPS+CC and EPS+3AC at each time point [(9.56–9.15 °C), (11.40–10.52 °C), (14.20–13.60 °C), and (21.28–19.17 °C)] were averaged to calculate this value. These results show that EPS+3AC packaging can prolong temperature retention time and maintain a lower temperature than EPS+CC packaging, indicating a modest temperature-moderation effect during the initial storage period under the tested conditions. In practical distribution chains, such packaging systems may be applied to short-distance delivery or insulated transport conditions where temperature control is partially maintained. However, their performance may vary depending on factors such as ambient temperature fluctuations, handling conditions, and packaging configurations. Although the temperature difference between the two systems was modest (approximately 0.5–1 °C) and limited to the early storage period, it may have influenced cumulative thermal exposure during the initial phase of storage. Therefore, the observed differences in quality should be interpreted with caution and cannot be attributed solely to short-term temperature variations.

Changes in pH of vacuum mackerel fillets

The pH is an indicator of fish freshness and early spoilage. The post-mortem pH of fresh fish is approximately 5.5–6.5, depending on the fish species, and the pH value at the initial stage of decay of mackerel is 6.2–6.4, with the pH limit for fish consumption generally being above 6.5 (Jo *et al.*, 2013). However, pH depends on various factors, such as species, diet, season, level of activity or stress during catching, and the type of muscle (He and Xiao, 2016). The initial pH of the fish used in our study was approximately 5.96, which was slightly lower than that reported by Somjid *et al.* (2017) for the same fish species (Table 3). The pH of the fish fillets in the EPS+CC and EPS+3AC packaging ranged from 5.98–6.40 and 5.98–6.38, respectively, after 12–72 h of storage. The pH was 6.22–6.24 at 48 h of storage, exceeding the initial decay pH of 6.20. In addition, significant differences ($P < 0.05$) were

Table 2. Temperature changes of samples stored in an EPS+CC and EPS + 3AC at 27 ± 0.5 °C.

Time (hours)	Temperature (°C)		
	Room-temperature	EPS + CC	EPS + 3AC
0	27.57	4.89 ± 0.17 ^{gA}	4.77 ± 0.05 ^{hA}
4	27.60	9.56 ± 0.42 ^{fA}	9.15 ± 0.25 ^{gA}
8	27.57	11.40 ± 0.05 ^{eA}	10.52 ± 0.25 ^{hB}
12	27.55	14.20 ± 0.18 ^{dA}	13.60 ± 0.69 ^{eB}
16	26.55	21.28 ± 0.58 ^{cA}	19.17 ± 0.52 ^{dB}
20	27.57	24.90 ± 0.48 ^{bA}	23.77 ± 0.60 ^{cB}
24	27.57	26.04 ± 0.22 ^{aA}	25.59 ± 0.22 ^{bB}
28	27.57	26.39 ± 0.35 ^{aA}	25.62 ± 0.62 ^{bB}
32	27.59	26.50 ± 0.28 ^{aA}	25.75 ± 0.58 ^{bB}
36	27.58	26.52 ± 0.33 ^{aA}	25.70 ± 0.59 ^{bB}
40	27.59	26.52 ± 0.26 ^{aA}	26.05 ± 0.25 ^{abB}
44	27.58	26.52 ± 0.07 ^{aA}	26.04 ± 0.24 ^{abB}
48	27.59	26.56 ± 0.31 ^{aA}	26.04 ± 0.21 ^{abA}
52	27.58	26.63 ± 0.31 ^{aA}	26.04 ± 0.23 ^{abB}
56	27.29	26.69 ± 0.32 ^{aA}	26.10 ± 0.21 ^{abB}
60	27.57	26.69 ± 0.27 ^{aA}	26.21 ± 0.45 ^{abB}
64	27.58	26.70 ± 0.20 ^{aA}	26.59 ± 0.41 ^{aA}
68	27.59	26.75 ± 0.34 ^{aA}	26.61 ± 0.54 ^{aA}
72	27.58	26.75 ± 0.20 ^{aA}	26.70 ± 0.44 ^{aA}

Values are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences (P < 0.05). EPS+3AC, Expanded Polystyrene + 3AC-developed cold packs. EPS+CC, Expanded Polystyrene + Commercial cold pack.

Table 3. Change in pH during storage at 27 ± 0.5 °C in mackerel fillets by EPS+CC and EPS+3AC

Time (hours)	pH	
	EPS + CC	EPS + 3AC
0	5.96 ± 0.02 ^{gA}	5.96 ± 0.02 ^{gA}
12	5.98 ± 0.00 ^{fA}	5.98 ± 0.00 ^{fA}
24	6.05 ± 0.02 ^{eA}	6.05 ± 0.02 ^{eA}
36	6.16 ± 0.00 ^{dA}	6.16 ± 0.00 ^{dA}
48	6.24 ± 0.02 ^{cA}	6.22 ± 0.02 ^{cB}
60	6.34 ± 0.00 ^{bA}	6.34 ± 0.00 ^{bA}
72	6.40 ± 0.01 ^{aA}	6.38 ± 0.01 ^{abB}

Values are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences (P < 0.05). EPS+3AC, Expanded Polystyrene + 3AC-developed cold packs. EPS+CC, Expanded Polystyrene + Commercial cold pack.

observed between the pH of fish fillets in the EPS+CC and EPS+3AC packaging (approximately 0.02 pH units) after 72 h of storage. Based on these results, EPS+3AC packaging had a greater cooling effect than EPS+CC packaging. Toe *et al.* (2019) suggested that the increase in pH may be caused by bacterial accumulation and

the effects of temperature on extracellular proteolysis. Peptides, amino acids, and amines are produced from the breakdown of fish proteins by microorganisms, which can also increase the pH (Shin *et al.*, 2006). These results are similar to those of Sardar *et al.* (2015), who reported that, in sardines, the pH increased

during storage from 6.10 (day 0) to 6.50 (day 18). In addition, Yu *et al.* (2019) observed that the pH of cutlassfish ranged from 6.39 to 6.70–6.80 with increasing storage time. Our findings are also consistent with those of Kim *et al.* (2023), who found that the pH of fish flesh generally tends to increase with storage time. Based on these results, EPS+3AC packaging showed a slightly lower increase in pH compared to EPS+CC packaging. However, the difference in pH between EPS+3AC and EPS+CC was relatively small (~0.02 units after 72 h) and should be interpreted with caution in relation to the limited temperature differences observed.

Changes in TVB-N content in vacuum-packed mackerel fillets

TVB-N includes compounds such as ammonia, various basic amines, and TMA. TVB-N content increases as the freshness of the fish decreases. TVB-N is extremely low in the meat of freshly caught fish and is therefore used to determine the freshness of fish meat (Sulfiana *et al.*, 2022). The TVB-N content in fresh fish is typically in the range of 5–10 mg/100 g and 15–25 mg/100 g in moderately fresh fish. When the fish starts to deteriorate, TVB-N content increases to 30–40 mg/100 g and exceeds 50 mg/100 g in spoiled fish (Tsai *et al.*, 2018). In Korea, the upper limit of TVB-N content in fishery processing materials is 20 mg/100 g (Kim *et al.*, 2012). Table 4 presents the changes in the TVB-N content of vacuum-packed mackerel stored in EPS+CC and EPS+3AC packaging. For fish stored in EPS+CC, the initial TVB-N content was 14.25 mg/100 g and increased to 30.93, 40.26, and 44.40 mg/100 g after 24, 36, and 48 h, respectively. These results indicate that both storage time and temperature significantly affected TVB-N formation. In contrast, the initial

TVB-N content of mackerel stored in EPS+3AC was 13.22 mg/100 g and increased to 22.60–28.88 mg/100 g after 12–24 h, which corresponds to moderately fresh fish. Notably, spoilage onset occurred more rapidly in fish stored in EPS+CC packaging than in EPS+3AC packaging. Based on these findings, vacuum-packed mackerel fillets exceeded the acceptable TVB-N limit (≥ 35 mg/100 g) after 36 h of storage, rendering them unsuitable for human consumption. The increase in TVB-N content with storage time was mainly due to the production of ammonia.

Ammonia is produced by the autolytic degradation of adenosine monophosphate (AMP), as well as by bacterial deamination of amino acids (Shakila *et al.*, 2003). In the present study, the initial TVB-N content of mackerel stored in EPS+CC packaging was 14.25 mg/100 g, which increased to 44.40 mg/100 g after 48 h of storage. These values are consistent with those observed by Cho *et al.* (2023), who reported an initial TVB-N content of 17 mg/100 g that progressively increased during 48 h of storage at 0 °C, despite differences in storage conditions. Similarly, Pandey *et al.* (2018) observed that mackerel stored at 3–6 °C exhibited an initial TVB-N content of 15.87 mg/100 g, which increased to 33.13 mg/100 g after 6 days. Collectively, these results indicate that storage temperature and duration are critical determinants of TVB-N accumulation and, consequently, fish freshness. In contrast, Cheng *et al.* (2022) reported that the initial TVB-N content in bigeye tuna (*Thunnus obesus*) transported with a cold pack in an EPS container was 13.30 mg/100 g and increased to 26.04 mg/100 g after 40 h of storage, which corresponds to a moderately fresh state. Although the fish species and experimental conditions differed from those used in the present study, their results similarly highlight the influence of packaging and cooling methods on the rate of TVB-N

Table 4. Change in TVB-N during storage at 27 ± 0.5 °C in mackerel fillets by EPS + CC and EPS + 3AC

Time (hours)	TVB-N (mg/100 g)	
	EPS + CC	EPS + 3AC
0	14.25 \pm 0.01 ^{aA}	13.22 \pm 0.01 ^{aB}
12	22.67 \pm 0.02 ^{1A}	22.60 \pm 0.02 ^{1B}
24	30.93 \pm 0.01 ^{eA}	28.88 \pm 0.01 ^{eB}
36	40.26 \pm 0.00 ^{dA}	35.67 \pm 0.00 ^{dB}
48	44.40 \pm 0.02 ^{cA}	43.09 \pm 0.02 ^{cB}
60	55.77 \pm 0.02 ^{bA}	55.37 \pm 0.02 ^{bB}
72	82.98 \pm 0.00 ^{aA}	69.19 \pm 0.00 ^{aB}

Values are expressed as mean \pm standard deviation (n = 3). Different letters indicate significant differences (P < 0.05). EPS+3AC, Expanded Polystyrene + 3AC-developed cold packs. EPS+CC, Expanded Polystyrene + Commercial cold pack.

accumulation. Taken together, these comparisons suggest that EPS+3AC packaging showed a tendency to delay TVB-N accumulation compared to EPS+CC packaging. However, given the relatively small temperature differences observed, these results should be interpreted as indicative of relative trends rather than definitive differences in spoilage progression.

Changes in odor intensity of vacuum-packed mackerel fillets

Fishes are highly perishable because of their susceptibility to oxidation, which causes off-odors and flavors, ultimately leading to spoilage during transportation and storage (Ocaño-Higuera *et al.*, 2011). Therefore, it is necessary to improve the preservation and transportation of fish and fishery products to enhance their quality and prolong their shelf life. The odor intensity of mackerel was 120 and 110 for fish in EPS+CC and EPS+3AC packaging, respectively (Figure 1). The odor intensity of vacuum-packed mackerel fillets in EPS+CC packaging ranged from 120–793 at 12, 24, 36, 48, and 60 h of storage, whereas that of EPS+3AC ranged from 110–805; in both packaging systems, odor intensity increased significantly with storage time ($P < 0.05$). Our findings are consistent with those of Lee *et al.* (2022), who investigated effective freshness indicators for seafood during room-temperature distribution using EPS+CC packaging and EPS alone and reported that the odor intensity of mackerel stored at room temperature increased from 199 to 251, 352, 336, 481, and 793 during 0–60 h of storage. In addition, Lee (2011) identified 60 volatile compounds in mackerel, including aldehydes, ketones, esters, alcohols, acids, sulfur-containing compounds, and amines, among which aldehydes, sulfur-containing compounds, and TMA increased significantly with prolonged storage and higher temperatures, showing a close relationship with changes in odor intensity. After 72 h of storage, the odor intensity of mackerel stored in EPS+CC packaging reached 1227, whereas that of mackerel stored in EPS+3AC packaging reached 1192. These results suggest that EPS+3AC may inhibit the formation of certain volatile compounds, thereby delaying deterioration in terms of odor intensity compared to EPS+CC. In Korea, frozen seafood is frequently transported using EPS and ice packs, which do not provide the same level of preservation as a cold chain system. It should be noted that odor intensity in this study was measured instrumentally and is presented as a relative indicator of volatile compound accumulation rather than a direct measure of human sensory perception, as no sensory evaluation was conducted. Therefore, the observed differences should be interpreted as comparative trends between packaging systems rather than

definitive sensory acceptability thresholds. Based on these relative measurements, EPS+3AC packaging showed a tendency to delay increases in instrumental odor intensity compared to EPS+CC.

Changes in total aerobic bacteria (TAB) in vacuum-packed mackerel fillets

TAB in fish are generally not associated with food safety hazards but may be useful as indicators of quality and shelf life. Although TAB are not pathogenic, individuals with weakened immune systems are susceptible to infection (Mehta *et al.*, 2014). Additionally, an increase in microbial growth theoretically decreases freshness and initiates spoilage (Mohammed *et al.*, 2021). Changes in the TAB count of vacuum-packed mackerel fillets in EPS+CC and EPS+3AC packaging during storage time are shown in Figure 2. The initial TAB count in fish in EPS+CC and EPS+3AC packaging was typically 2.90–3.05 log CFU/g; a TAB count < 4 log CFU/g generally indicates that the fish is fresh. The initial TAB count was similar to that reported by Zheng *et al.* (2020), who investigated TAB counts in Spanish mackerel (approximately 3.90 log CFU/g), and Otero *et al.* (2018), who investigated TAB counts in Atlantic mackerel (*Scorpaenopsis scorpaenoides*) (approximately 4.00 log CFU/g). Moreover, the TAB counts of all samples increased gradually as storage time increased ($P < 0.05$). The International Commission on Microbiological Specifications for Foods considers an aerobic plate count of 10^5 to 10^7 CFU/g acceptable for fresh and frozen fish (ICMSE, 1986). Our results showed that after 36 h of storage, fish samples in both packaging types had TAB counts below the acceptable limit of 6 log CFU/g. After 48 h, fish stored in EPS+CC had a TAB count of 6.33 log CFU/g, whereas fish stored in EPS+3AC had a TAB count of 5.11 log CFU/g. EPS+3AC packaging

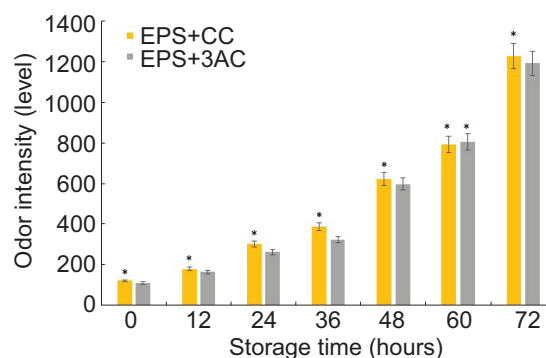


Figure 1. Change in odor intensity of mackerel fillets during storage in EPS+CC and EPS+3AC packaging at $27 \pm 0.5^\circ\text{C}$. EPS+3AC: expanded polystyrene containers with 3AC-developed cold packs; EPS+CC: expanded polystyrene containers with commercial cold packs. Asterisks (*) indicate significant differences between EPS+CC and EPS+3AC ($P < 0.05$, t-test).

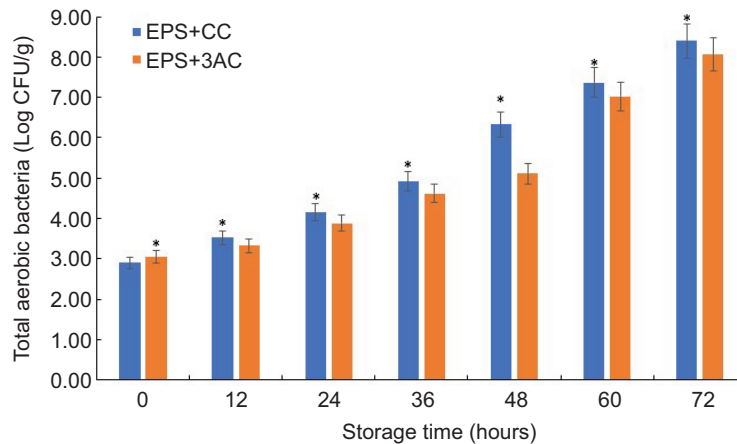


Figure 2. Change in total aerobic bacteria of mackerel fillets during storage in EPS+CC and EPS+3AC packaging at 27 ± 0.5 °C. EPS+3AC: expanded polystyrene containers with 3AC-developed cold packs; EPS+CC: expanded polystyrene containers with commercial cold packs. Asterisks (*) indicate significant differences between EPS+CC and EPS+3AC ($P < 0.05$, t-test).

inhibited TAB growth more effectively than EPS+CC packaging as storage time increased. Conversely, Alfaro *et al.* (2002) reported that the TAB count in Atlantic horse mackerel fillets (*Trachurus trachurus*) was 4 and 5 log CFU/g after being stored at 2 °C and 10 °C for 24 h, respectively. In addition, Kolodziejska *et al.* (2002) observed that the microbial quality of frozen-thawed smoked mackerel stored at 2 °C for 21 days did not change. The microbial quality of fish is influenced by multiple factors, including species, origin, processing methods, storage temperature, and water content. In particular, storage temperature has been identified as a principal determinant of microbial growth and enzyme inactivation. For example, Duarte *et al.* (2020) highlighted that the shelf life of fish is heavily dependent on storage time and temperature, with additional effects of species and pre-freezing stress. Similarly, Prabhakar *et al.* (2020) confirmed that storage temperature and duration, along with catch-related factors, significantly affect fish freshness. Therefore, improper storage temperatures during transport may lead to increased bacterial counts. All safety factors should be considered during delivery; in particular, the amount of gel ice packs, dry ice, or other cold sources should be adjusted depending on the amount of the product and its state (frozen or refrigerated). Product spoilage generally occurs within 12–24 h if it is not refrigerated or stored at temperatures < 5 °C (ICMSE, 1986). Our findings suggest that EPS+3AC packaging may serve as a useful option for the storage and transport of frozen seafood by providing improved temperature regulation during the initial stage of storage. However, considering that the observed temperature differences between packaging systems were relatively small, its effect should be interpreted as contributing to temperature stabilization rather than as the sole determining factor in quality preservation.

It should be noted, however, that the present study did not directly investigate microbial adaptation mechanisms or specific spoilage organisms. Therefore, the observed differences in bacterial growth should be interpreted as a relative response to temperature conditions rather than as evidence of specific inhibitory mechanisms.

This study has several limitations that should be considered when interpreting the results. Temperature was monitored at a single location inside the EPS container, which may not fully reflect potential spatial variations in temperature within the packaging system. In addition, minor variability among fish fillets, including differences in initial microbial load, moisture content, and biological characteristics, could have influenced the observed quality changes. Although efforts were made to standardize sample weight and storage conditions, such inherent variability is difficult to eliminate completely. Therefore, the findings should be interpreted as indicative of general trends under the tested conditions, and further studies using multiple temperature monitoring points and larger sample sets would help improve measurement accuracy and experimental robustness.

Conclusions

This study demonstrated that EPS+3AC packaging was more effective than EPS+CC packaging in maintaining the freshness of vacuum-packed mackerel during storage under simulated distribution conditions. EPS+3AC maintained slightly lower internal temperatures within EPS boxes during the early storage period and delayed increases in pH, TVB-N content, odor intensity, and

total aerobic bacteria counts compared to EPS+CC. The onset of quality deterioration, as indicated by TVB-N content and bacterial counts, occurred earlier in EPS+CC packaging. Overall, these findings indicate that EPS+3AC packaging can contribute to delaying freshness deterioration and extending quality-related shelf-life indicators of vacuum-packed mackerel under the tested conditions. Unlike previous PCM-based studies that primarily focused on thermal properties, this study provides a practical evaluation of cold pack performance in relation to food quality under simulated distribution conditions. It should also be noted that this study did not include a cost analysis of the packaging systems. As economic feasibility is an important factor for practical adoption in distribution chains, the lack of cost evaluation limits the direct applicability of these findings. Therefore, future studies should consider cost-performance analysis to support real-world implementation.

Mandatory Disclosure on Use of Artificial Intelligence

The authors declared that no generative AI or AI-assisted technologies were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

Data Availability Statement

All data generated or analyzed in this study are included in this published article.

Author Contributions

E.B.J: Data curation, Investigation, Writing – original draft; J.U.L, S.-Y.P., J.K: methodology; J.S.L., S.Y.P: Writing – review & editing, Funding. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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