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Effects of packaging material and vacuum sealing packaging on TBARS values lipid oxidation in snakehead fish (*Channa striata*) sausages stored at 10 ± 2 °C

Pengaruh bahan kemasan dan pengemasan vakum terhadap nilai TBARS pada sosis ikan gabus (Channa striata) yang disimpan pada suhu 10 ± 2 °C

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Abstract

Lipid oxidation is a major cause of quality deterioration in processed fish products during storage. Snakehead fish (*Channa striata*) sausages contain unsaturated fatty acids that are susceptible to oxidative degradation. This laboratory experiment evaluated the effects of packaging and vacuum packaging on lipid oxidation in snakehead fish sausage. This experimental study was conducted between March and August 2025 at the Food Laboratory of the Department of Nutrition and Integrated Laboratory of Politeknik Kesehatan Kemenkes Jayapura, Jayapura, Indonesia. A 2×2 factorial design with two packaging materials (polyethylene [PE] and polypropylene [PP]) and two techniques (vacuum and non-vacuum) was used with seven independent production batches as replications stored at 10 ± 2 °C for 30 days. TBARS were measured on days 0, 5, 10, 15, 20, 25, and 30 and analyzed using two-way ANOVA. The TBARS levels increased progressively in all groups. Vacuum packaging significantly affected TBARS at all observation times ($p \leq 0.003$; $\eta p^2 = 0.317–0.826$). Packaging material had significant effects on days 5, 10, and 30 ($p \leq 0.008$), with PP yielding lower values than PE values. No interactions were detected. The lowest TBARS on day 30 was 0.374 mg MDA/kg in the PP + vacuum. In conclusion, storage at 10 ± 2 °C and vacuum sealing were associated with lower TBARS, whereas PP showed lower values on several days. These findings describe secondary lipid oxidation only and do not establish the microbiological or sensory shelf life.

Keywords: Malondialdehyde, lipid oxidation, vacuum packaging, polypropylene, snakehead fish sausage, vacuum packaging

Abstrak

Oksidasi lipid merupakan penyebab utama penurunan mutu produk olahan ikan selama penyimpanan. Sosis ikan gabus (*Channa striata*) mengandung asam lemak tak jenuh yang rentan terhadap degradasi oksidatif. Penelitian eksperimental laboratorium ini mengevaluasi pengaruh bahan kemasan dan pengemasan vakum terhadap oksidasi lipid pada sosis ikan gabus. Penelitian eksperimental ini dilakukan antara bulan Maret hingga Agustus 2025 di Laboratorium Pangan Departemen Gizi dan Laboratorium Terpadu Politeknik Kesehatan Kemenkes Jayapura, Jayapura, Indonesia. Rancangan faktorial 2×2 dengan dua bahan kemasan (polietilena [PE] dan polipropilena [PP]) dan dua teknik (vakum dan nonvakum) menggunakan tujuh batch produksi independen sebagai ulangan disimpan pada suhu 10 ± 2 °C selama 30 hari. Nilai TBARS diukur pada hari ke-0, 5, 10, 15, 20, 25, dan 30 serta dianalisis menggunakan two-way ANOVA. Nilai TBARS meningkat secara progresif. Pengemasan vakum berpengaruh signifikan pada seluruh waktu pengamatan ($p \leq 0{,}003$; $\eta p^2 = 0{,}317–0{,}826$). Bahan kemasan berpengaruh

signifikan pada hari ke-5, 10, dan 30 ($p \leq 0,008$), dengan PP menghasilkan nilai lebih rendah dibandingkan PE. Tidak ditemukan interaksi yang signifikan. Nilai TBARS terendah pada hari ke-30 adalah 0,374 mg MDA/kg pada PP + vakum. Kesimpulan, penyimpanan 10 ± 2 °C, pengemasan vakum berkaitan dengan nilai TBARS yang lebih rendah sedangkan kemasan PP menunjukkan nilai lebih rendah pada beberapa hari; temuan ini hanya menggambarkan oksidasi lipid sekunder dan tidak menetapkan keamanan mikrobiologis maupun kelayakan sensori produk.

Kata Kunci: Malondialdehida, oksidasi lipid, pengemasan vakum, polipropilena, sosis ikan gabus

Introduction

Lipid oxidation is a common cause of quality deterioration in processed fish products, particularly those containing high levels of unsaturated fatty acids (Geng et al., 2023; Sheng & Wang, 2021). Fish sausages, typically produced from minced fish and emulsified fat components, are particularly susceptible to oxidative changes during storage (Dragoev, 2024; You et al., 2022). FAO (2022) reported that oxidative deterioration contributes substantially to quality loss in processed fish products during storage and distribution.

Lipid oxidation is a common cause of quality deterioration in processed fish products, particularly those that are rich in unsaturated fatty acids (Geng et al., 2023; Wu et al., 2022). Fish sausages, produced from minced fish and other formulation components, are susceptible to oxidative changes during storage (Dragoev, 2024; You et al., 2022). Oxidative deterioration can adversely affect the flavor, nutritional quality, and overall product acceptability during storage and distribution. Snakehead fish (*Channa striata*) is widely used in processed fish products because of its high protein content and favorable nutritional profile (Rosmawati et al., 2023). The lipid fraction of snakehead fish contains both saturated and unsaturated fatty acids, including docosahexaenoic acid (DHA), arachidonic acid (ARA), and oleic acid (Puteri & Febriansyah, 2023; Sasongko et al., 2025). The presence of polyunsaturated fatty acids increases the susceptibility of fish lipids to oxidative reactions during storage, which may lead to the formation of rancid compounds and a decline in product quality (Liu et al., 2024; Puteri & Febriansyah, 2023).

Lipid oxidation in fish products generally occurs through a sequence of reactions involving initiation, propagation, and termination (Farhoosh, 2021; Geng et al., 2023). In the early phase of oxidation, unsaturated fatty acids undergo radical formation upon oxygen contact. The resulting lipid radicals further interact with oxygen to generate hydroperoxides, which subsequently break down into secondary compounds, including aldehydes and ketones, leading to off-flavor development in fish products. (Cyprian et al., 2017; Wu et al., 2022). Some oxidation products, including malondialdehyde, are commonly used as indicators of lipid oxidation in food systems (Rizzo, 2024; Wu et al., 2022).

Lipid oxidation in fish products generally proceeds through initiation, propagation, and termination reactions (Farhoosh, 2021; Geng et al., 2023). During initiation, unsaturated fatty acids form lipid radicals that react with oxygen to generate hydroperoxides. These primary oxidation products subsequently decompose into secondary compounds, including aldehydes and ketones, which contribute to off-flavors in fish products (Cyprian et al., 2017; Wu et al., 2022). Malondialdehyde is a secondary oxidation product commonly used as an indicator of lipid oxidation in food systems (Rizzo, 2024; Wu et al., 2022). Several approaches have been investigated to reduce oxidative changes in fish products during their storage. Packaging methods that limit oxygen exposure, such as vacuum packaging, are typically used in fish processing. Previous studies have reported lower lipid oxidation levels in vacuum-packaged fish products than in those stored aerobically (Babic Milijasevic et al., 2023; Nguyen et al., 2023). The selection of packaging materials can also affect the diffusion of oxygen into packaged foods. Compared to polyethylene (PE) films, polypropylene (PP) films generally exhibit lower oxygen permeability, which may improve the oxidative stability of packaged products (Tuárez-Párraga et al., 2024).

This study examined the effects of polyethylene versus polypropylene packaging and vacuum versus non-vacuum sealing on TBARS values in snakehead fish sausages stored at 10 ± 2 °C. Previous studies have primarily evaluated the effects of packaging on fresh fish or fish fillets; however, evidence for emulsified snakehead fish products remains limited. Small-scale processors often rely on affordable and readily available packaging systems owing to equipment and cost constraints (Abdulla et al., 2025). Therefore, the evaluation of practical oxygen-limiting strategies is relevant. This study examined the main and interaction effects of packaging material (PE vs. PP) and technique (vacuum vs. non-vacuum) on TBARS values in snakehead fish sausages stored at 10 ± 2 °C. We hypothesized that vacuum and PP packaging would reduce TBARS formation and that their interaction would provide additional protection against lipid oxidation.

Methods

Design

In this study, a 2×2 factorial experimental design was used to evaluate the effects of packaging material and vacuum packaging on TBARS values in snakehead fish (*Channa striata*) sausages stored at 10 ± 2 °C. The study was conducted from March to August 2025 at the Food Laboratory of the Department of Nutrition and Integrated Laboratory of Politeknik Kesehatan Kemenkes Jayapura, Jayapura, Indonesia. The two experimental factors were (1) packaging material (low-density polyethylene [PE] or cast polypropylene [PP]) and (2) packaging technique (vacuum or non-vacuum). These factors were combined to form four treatment groups: PE + vacuum, PE + non-vacuum, PP + vacuum, and PP + non-vacuum.

Seven independent replicates were used, each representing a separate production batch. Within each batch, the sausage batter was equally divided across the four packaging treatment combinations and six post-packaging observation time points (days 5, 10, 15, 20, 25, and 30). Each time point within each treatment group was represented by seven packages, one from each batch, which were analyzed destructively. A shared baseline measurement was obtained from each batch prior to packaging allocation (day 0), yielding seven independent baseline observations. In total, 175 sample units were analyzed: 168 treatment packages and seven baseline samples. Packages were coded by batch, treatment, and time point, and allocated to packaging combinations and rack positions using stratified randomization by batch. The package positions were rotated daily following a predetermined schedule to minimize microenvironmental variations within the storage chamber. The factorial design allowed the assessment of the main effects of packaging material and vacuum packaging, as well as their interaction, at each observation time point. The TBARS values were monitored on days 0, 5, 10, 15, 20, 25, and 30.

Materials and samples

Fresh snakehead fish (*Channa striata*) were obtained from Lake Sentani in Jayapura, Indonesia. Fish weighing approximately 500–700 g were selected based on freshness indicators, including bright red gills, clear eyes, and firm flesh. The fish were transported to the laboratory in ice-filled insulated containers (0–4 °C) within six hours of harvesting. A total of 56 snakehead fish (eight fish per batch across seven production batches) were used in this study. Within each batch, the fish were pooled and minced together to produce a single homogeneous batter, which was then divided across all packaging treatment combinations and observation time points.

The fish were subsequently washed with running water, descaled, eviscerated, and filleted to remove the bones and heads. The fish meat was minced using a stainless-steel meat grinder to obtain a uniform texture of the fish meat.

The sausage formulation consisted of 70% minced snakehead fish meat and 28% tapioca flour as a binder, combined with seasonings such as salt (1.5%), garlic powder (0.3%), and white pepper (0.1%). The reported proportions sum to 99.9% owing to rounding and were normalized to 100% of the total batter weight. The mixture was blended in an industrial mixer for approximately 15 min to form a stable emulsion. The batter was then stuffed into collagen casings with a diameter of 22 mm.

After stuffing, the sausages were steamed at approximately 90–95 °C until the internal temperature reached at least 72 °C, as verified using a probe thermometer inserted at the

center of the sausage, which required approximately 20 min. Sausages were then immediately cooled in an ice-water bath for approximately 15 min, surface-dried, and held at 4 °C until the product temperature fell below 10 °C. Packaging was completed within two hours of the end of steaming.

Two food-grade plastic films were used as packaging materials: low-density polyethylene (LDPE, 50 μm) and cast polypropylene (CPP, 50 μm) films. The specific oxygen and water vapor transmission rates were not measured under defined test conditions and were therefore not reported. Vacuum packaging was performed using a chamber vacuum sealer (DZ-400/2E) at approximately −0.9 bar gauge pressure with a sealing time of 30 seconds. Seal integrity was verified by visual inspection and manual compression, and packages with visible defects were repackaged. The residual oxygen within the packages was not measured instrumentally.

Storage and Treatment Procedures

After packaging, all sausage samples were stored in a controlled refrigeration chamber maintained at 10 ± 2 °C and 70 ± 5% relative humidity for a total storage period of 30 days. These conditions were selected to simulate potential temperature abuse conditions that may occur in small- and medium-scale fish processing and distribution, where reliable refrigeration infrastructure is not consistently maintained. Samples from the four treatment groups were randomly arranged on stainless-steel racks to ensure adequate air circulation.

The samples were periodically rotated during storage to minimize potential microenvironmental differences in the chamber. Storage temperature and humidity were monitored daily using digital sensors checked against a reference thermometer prior to use to ensure consistent storage conditions. Before chemical analysis, the samples were removed from cold storage and allowed to equilibrate at room temperature for approximately 30 min.

Lipid Oxidation Analysis

Lipid oxidation was assessed using the thiobarbituric acid reactive substances (TBARS) method to quantify malondialdehyde (MDA), a secondary product of lipid oxidation. Approximately 5 g of each sausage sample was homogenized with 25 mL of 7.5% trichloroacetic acid (TCA) solution using a high-speed homogenizer (T18 digital ULTRA-TURRAX, IKA-Werke GmbH & Co. KG (Staufen, Germany) for 2 min at low temperature to minimize further oxidation during sample preparation. The homogenate was centrifuged at 4,000 × g for 15 min at 4 °C (Eppendorf 5810 R, Eppendorf SE, Hamburg, Germany), and the supernatant was collected for analysis.

An aliquot of the supernatant was mixed with 0.02 M thiobarbituric acid (TBA) reagent. The mixture was then heated in a water bath at 95 °C for 40 min to promote TBA-MDA complex formation. After incubation, the samples were rapidly cooled in an ice bath. The absorbance was measured at 532 nm using a UV–visible (UV–Vis) spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan). Nonspecific turbidity was corrected by measuring the absorbance at 600 nm and subtracting this value from the primary reading at 532 nm. The corrected absorbance was calculated as follows:

$$\text{The corrected Absorbance} = Abs_{532} - Abs_{600}.$$

A calibration curve was constructed using 1,1,3,3-tetraethoxypropane (TEP) standard solutions ranging from 0–10 μg/mL. TBARS values were expressed as mg of MDA per kilogram of sample (mg of MDA/kg). Quality control included reagent blanks, duplicate analyses, and standard curve verification. Each sample was analyzed in duplicate, and the mean of the two analytical replicates was used as the TBARS value for that package. The TBARS concentration was calculated as follows: $MDA\ (mg/kg) = (C \times V \times DF) / W$, where C is the MDA concentration derived from the calibration curve (μg/mL), V is the total extract volume (25 mL), DF is the dilution factor, and W is the sample weight (5 g). The calibration curve was linear over the range of 0–10 μg/mL; however, formal validation parameters, including recovery, intra-assay precision, limit of detection, and limit of quantification, were not determined and are reported as analytical limitations.

Statistical Analysis

Statistical analyses were performed using SPSS version 21. Descriptive statistics are presented as mean ± standard deviation. The normality of the data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Lipid oxidation values were analyzed using two-way

analysis of variance (ANOVA) for each observation day, with packaging material (PE vs. PP) and packaging technique (vacuum vs. non-vacuum) as fixed factors. This model allowed the evaluation of (1) the main effect of packaging material, (2) the main effect of the vacuum technique, and (3) the interaction between these factors at each time point. Effect sizes were estimated using partial eta squared (ηp^2) and interpreted according to Cohen's criteria (small = 0.01, medium = 0.06, large = 0.14). Statistical significance was set at $p < 0.05$. Separate two-way ANOVAs were conducted on each observation day to examine the treatment effects at specific time points, rather than modeling storage time as a factor, because the primary interest was in identifying the days on which packaging material and vacuum packaging produced significant differences. To control the family wise error rate across the six observation days, a Bonferroni correction was applied; therefore, the adjusted significance threshold was $p < 0.008$ (0.05/6).

Result and Discussion

TBARS Values During Storage

Table 1 shows the TBARS values (mg MDA/kg) measured during the 30-day storage period for the four packaging treatments: PE + vacuum, PE + non-vacuum, PP + vacuum, and PP + non-vacuum. On day 0, a shared baseline measurement was taken from each production batch prior to packaging allocation; the mean baseline TBARS value was 0.071 ± 0.005 mg MDA/kg. This value is presented in all four treatment columns as a common reference point and was not included in the statistical comparison between the treatment groups. A consistent progressive increase in TBARS was observed across all treatment groups from day 0 to the 30-day storage period.

Table 1. TBARS values (mg MDA/kg) during storage

Storage Day	PE + Vacuum	PE + Non-Vacuum	PP + Vacuum	PP + Non-Vacuum
Day 0	0.071 ± 0.005	0.071 ± 0.005	0.071 ± 0.005	0.071 ± 0.005
Day 5	0.121 ± 0.006	0.141 ± 0.009	0.111 ± 0.007	0.128 ± 0.010
Day 10	0.132 ± 0.008	0.150 ± 0.005	0.127 ± 0.006	0.138 ± 0.011
Day 15	0.176 ± 0.009	0.188 ± 0.011	0.171 ± 0.006	0.183 ± 0.012
Day 20	0.226 ± 0.008	0.260 ± 0.019	0.226 ± 0.006	0.244 ± 0.007
Day 25	0.313 ± 0.012	0.351 ± 0.014	0.306 ± 0.012	0.354 ± 0.008
Day 30	0.396 ± 0.008	0.436 ± 0.014	0.374 ± 0.013	0.424 ± 0.008

Note: Values are presented as mean ± SD (n = 7). Day 0 represents a shared baseline measured prior to packaging allocation and was not included in the between-group statistical comparisons.

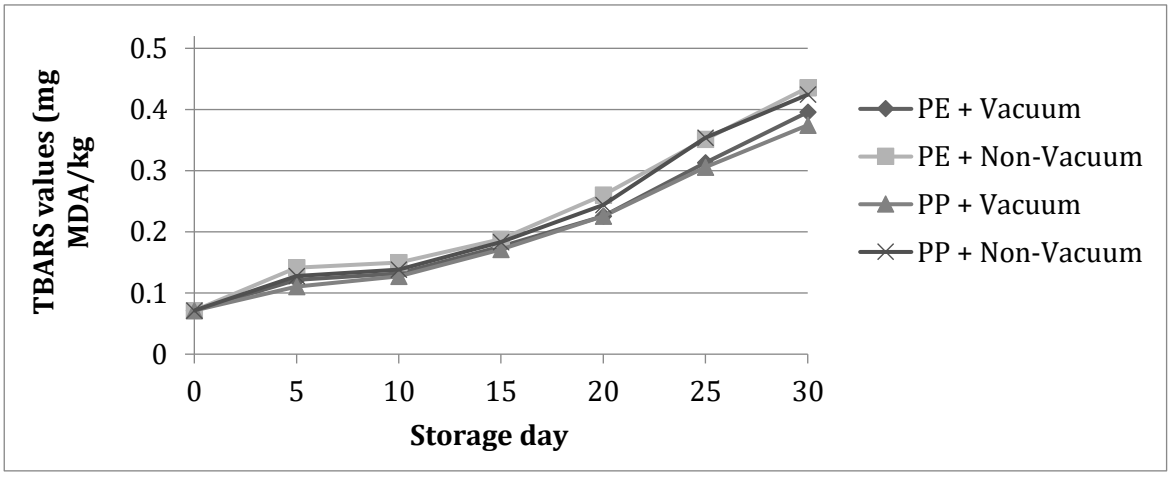

Figure 1. TBARS values (mg MDA/kg) in snakehead fish sausages by packaging treatment for 30 days at 10 ± 2 °C. Values are presented as mean ± SD (n = 7)

Throughout the storage period, the PP + vacuum group generally exhibited the lowest numerical TBARS values among the four treatments. The TBARS level in this group increased from 0.071 mg MDA/kg on day 0 to 0.374 mg MDA/kg on day 30. The PE + non-

vacuum group had the highest value at the end of storage, reaching 0.436 mg MDA/kg on day 30. On most observation days, the vacuum-packaged samples had lower TBARS values than their corresponding non-vacuum-packaged groups.

Effects of Packaging Material and Vacuum Packaging

The effects of packaging type and vacuum technique on the TBARS values were analyzed using two-way ANOVA for each observation day. The results are shown in Table 2.

Table 2. Results of two-way ANOVA on TBARS values by observation day

Observation Day	Variation	F value	Adjusted R^2	ηp^2 (Effect Size)	*p* value
Day 5	Packaging material (PE/PP)	16.130	0.654	0.402	0.001
	Vacuum technique	37.730		0.611	<0.001
	Packaging × Vacuum (Interaction)	0.223		0.009	0.641
Day 10	Packaging material (PE/PP)	8.336	0.538	0.258	0.008
	Vacuum technique	24.758		0.508	<0.001
	Packaging × Vacuum (Interaction)	1.362		0.054	0.255
Day 15	Packaging material (PE/PP)	1.495	0.263	0.059	0.233
	Vacuum technique	11.124		0.317	0.003
	Packaging × Vacuum (Interaction)	0.000		0.000	0.992
Day 20	Packaging material (PE/PP)	3.613	0.618	0.131	0.069
	Vacuum technique	39.468		0.622	<0.001
	Packaging × Vacuum (Interaction)	3.613		0.131	0.069
Day 25	Packaging material (PE/PP)	0.285	0.784	0.012	0.598
	Vacuum technique	99.689		0.806	<0.001
	Packaging × Vacuum (Interaction)	1.231		0.049	0.278
Day 30	Packaging material (PE/PP)	15.187	0.825	0.388	0.001
	Vacuum technique	113.943		0.826	<0.001
	Packaging × Vacuum (Interaction)	1.407		0.055	0.247

Vacuum packaging had a statistically significant effect on TBARS values at all observation times ($p \leq 0.003$). Packaging material had significant effects on days 5, 10, and 30 ($p \leq 0.008$). No significant packaging material × packaging technique interaction was detected at any of the observation times ($p > 0.05$).

Effect Size Across Storage Times

The effect sizes (ηp^2) for packaging material, packaging technique, and their interaction across storage periods are shown in Figure 2. The ηp^2 for vacuum packaging ranged from 0.317 on day 15 to 0.826 on day 30 and exceeded the corresponding packaging material effect size at every observation time. The interaction effect ranged from negligible to medium according to the stated criteria (ηp^2 = 0.000–0.131) but was not statistically significant at any of the observation times.

During 30 days of storage at 10 ± 2 °C. The PP + vacuum group showed the lowest mean TBARS values; however, because the interaction term was not significant, the findings do not demonstrate a synergistic effect of PP and vacuum sealing. Vacuum packaging consistently reduced TBARS values across the observation period, whereas the effect of the packaging material varied over time. The PP + vacuum group generally had the lowest numerical TBARS values; however, the nonsignificant packaging material × packaging technique interaction did not support a synergistic effect. These findings indicate that oxygen limitation through vacuum packaging is the dominant factor associated with improved oxidative stability under the tested conditions.

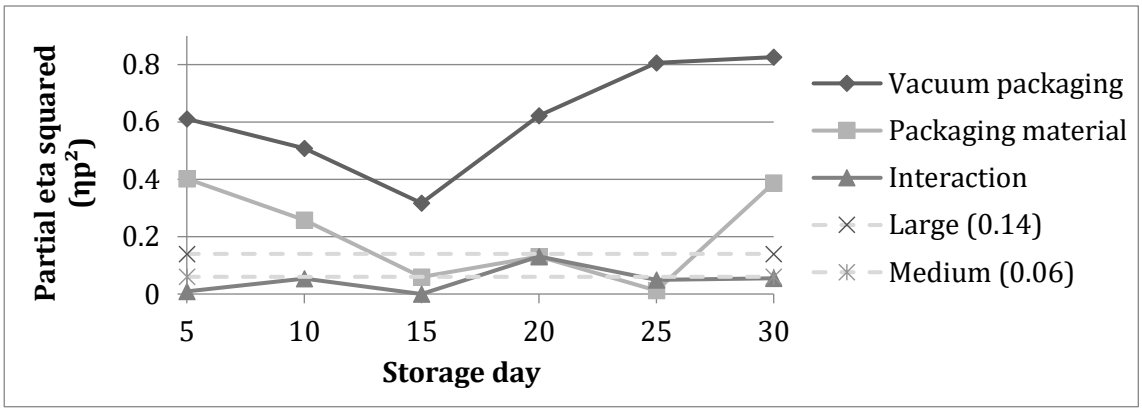

Figure 2. Partial eta squared (ηp^2) trends by storage day for packaging material, vacuum packaging, and their interaction. Dashed reference lines indicate medium (0.06) and large (0.14) effect sizes based on Cohen's criteria.

The differences in packaging materials may be related to the barrier properties of the films. Polypropylene generally has lower oxygen transmission rates than polyethylene does, thereby reducing oxygen diffusion into packaged foods. Tuárez-Párraga et al. (2024) attributed this difference to the relatively dense crystalline structure of polypropylene, which limits oxygen diffusion through the film. Nilsen-Nygaard et al. (2021) noted that the barrier performance of conventional polymeric packaging materials, including PP, plays a critical role in controlling oxidative deterioration in seafood products during storage. Laorenza et al. (2022) similarly highlighted that polymeric packaging selection is a primary determinant of lipid oxidation in seafood products.

The lower TBARS values observed in the vacuum-packaged samples are consistent with previous studies on fish and fishery products. By reducing the residual oxygen within the package, vacuum sealing limits the availability of oxygen needed to sustain oxidative reactions in lipid-rich food products. Nguyen et al. (2023) reported lower levels of lipid oxidation markers in vacuum-packaged snakehead fish fillets than in those stored under aerobic conditions. Similar findings have been reported for other fish products, where vacuum packaging slowed the development of lipid oxidation markers during storage (Ramadhan et al., 2025). Jakhar et al. (2026) further demonstrated that vacuum packaging significantly reduced TBARS values in pangas fish fillets during refrigerated storage, confirming the role of oxygen limitation in controlling secondary lipid oxidation.

The TBARS values increased progressively across all treatments during storage at 10 ± 2 °C. Several studies on fresh or processed fish products have reported comparable trends during refrigeration. Nguyen et al. (2023) reported increasing TBARS values in snakehead fillets during storage, with lower oxidation levels in vacuum-packaged samples than in those stored under aerobic conditions. Kawecki et al. (2021) reported progressive increases in TBARS values in vacuum-packed fish oil-enriched sausages during refrigerated storage, confirming that lipid oxidation in emulsified fish-based products follows a time-dependent pattern, regardless of the packaging method.

Babic Milijasevic et al. (2023) reported reduced lipid oxidation in rainbow trout fillets stored in CO_2/N_2 gas mixtures. However, vacuum packaging remains widely used in small-scale processing because it requires simpler equipment and lower operational costs (Abdulla et al., 2025). Modified atmosphere packaging has also been reported to reduce lipid oxidation in seafood. Babic Milijasevic et al. (2023) reported reduced lipid oxidation in rainbow trout fillets stored in CO_2/N_2 gas mixtures. However, because MAP was not included as a comparator in the present study, no direct equivalence could be inferred. Vacuum packaging may remain more feasible for small-scale processors because it requires simpler equipment and lower operational costs (Abdulla et al., 2025), as demonstrated in sausage products where vacuum packaging effectively limited oxidative and microbial deterioration during storage (Siddi et al., 2023).

Previous studies have also examined the influence of packaging materials on the microbial quality of fish products. Vuoso et al. (2022) reported that PP-based active packaging slowed microbial growth in fish burgers during

refrigerated storage, whereas Babic Milijasevic et al. (2023) reported that packaging type and gas composition significantly influenced microbial counts in rainbow trout fillets. Although microbiological parameters were not measured in the present study, these reports indicate that packaging materials may influence multiple aspects of product stability during storage.

Studies on active and modified atmosphere packaging have reported its effects on microbial growth in fish products. Vuoso et al. (2022) found that antimicrobial active packaging slowed microbial growth in fish burgers, whereas Babic Milijasevic et al. (2023) reported the effects of packaging atmosphere on microbial counts in rainbow trout. As microbiological outcomes were not assessed in the present study, these findings provide only contextual information and should not be used to infer shelf-life extension or microbiological safety. Statistical analysis revealed no significant interaction between the packaging material and vacuum sealing. These findings indicate that the effects of the two factors occurred independently under the experimental conditions used in this study. Similar independent effects of packaging treatments have been reported in other food packaging studies, although interactions may occur when additional preservation technologies are combined (Monteiro et al., 2020).

It should be noted that storage at 10 ± 2 °C represents a temperature abuse condition rather than standard commercial refrigeration. At this temperature, microbial growth may proceed at rates that could compromise product safety, independent of the lipid oxidation status. Therefore, the present findings describe only the chemical oxidative stability of the product under the tested conditions and should not be interpreted as evidence of microbiological safety, sensory acceptability, or overall shelf life.

Because no validated product-specific TBARS acceptability threshold, sensory assessment, or microbiological analysis was included, the present TBARS values cannot be used to infer consumer acceptability, safety, or shelf-life. Secondary lipid oxidation products, such as malondialdehyde, negatively affect both the sensory attributes and nutritional value of fish-based foods (Cyprian et al., 2017). Therefore, controlling oxidation during storage is important for maintaining product quality.

The TBARS values remained below 0.5 mg MDA/kg throughout the 30-day storage period. However, interpretation against sensory acceptability or advanced-oxidation thresholds requires a directly applicable, product-specific reference because thresholds vary by species, formulation, and analytical method. Secondary lipid oxidation products, including malondialdehyde, can adversely affect the sensory and nutritional quality of fish-based foods (Cyprian et al., 2017). Therefore, the present findings should be interpreted as relative differences in oxidative stability rather than as evidence of sensory acceptability or preserved nutrient content. Recent studies have explored alternative packaging technologies, including active packaging and bio-based materials. Mellinas et al. (2024) investigated antioxidant-enriched films, and Nilsen-Nygaard et al. (2021) examined bioplastic packaging materials for seafood preservation. Although such technologies may provide additional protection against oxidation, conventional packaging combined with vacuum sealing remains widely used because of its simplicity and cost-effectiveness. These considerations are particularly relevant for small-scale fish-processing operations.

This study has several limitations. First, samples were stored at 10 ± 2 °C, whereas temperature conditions may fluctuate during distribution in tropical environments. Second, the observation period was limited to 30 days; longer studies are needed to characterize the oxidation dynamics over extended storage periods. Third, lipid oxidation was assessed using only the TBARS method. Additional markers, such as peroxide value, hexanal, and fatty acid composition, would provide a more comprehensive evaluation. Finally, light exposure, microbial quality, sensory attributes, and nutrient retention were not evaluated. Future studies should evaluate these outcomes under controlled and fluctuating temperature conditions. Because shelf life is a multidimensional endpoint, it should not be inferred from the TBARS alone.

Conclusion

During storage at 10 ± 2 °C, TBARS values increased in all treatment groups during 30 d of storage at 10 ± 2 °C. Vacuum packaging was consistently associated with lower TBARS values across all observation times and showed large

effect sizes. Packaging material showed time-dependent effects, with PP generally yielding lower TBARS values than PE at selected time points. The non-significant interaction indicates that the effects of packaging material and vacuum packaging were independent under the tested conditions.

Under the tested condition of 10 ± 2 °C, vacuum-sealed PP packaging consistently produced the lowest numerical TBARS values, while PP packaging showed an effect value on selected observation days; no significant interaction was detected on day 30. These findings are limited to secondary support vacuum packaging as a practical approach for limiting lipid oxidation and should not be interpreted as evidence of microbiological safety in snakehead fish sausages. However, conclusions regarding shelf life, sensory acceptability, 30-day shelf life nutritional retention, or microbiological safety require additional outcome measures. Future studies should include peroxide value, fatty-acid or volatile fluctuating storage temperatures, complementary oxidation markers, sensory testing, microbiological quality, and sensory evaluation under standard refrigeration and temperature-abuse conditions.

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