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Original Research Article

# Physicochemical and Microbial Changes in Fish Cake during Storage Obtained by Sterilization and Oxygen Absorbers

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ABSTRACT

The main function of food packaging technology is to protect products from environmental contamination, particularly oxygen and microorganisms. Therefore, this research aimed to analyze and optimize the effects of sterilization and oxygen absorbers on physicochemical and microbiological changes in fish cake during storage. The experiment was conducted with two and three replications using Split-Plot Randomized Design (SPRD), with sterilization type (semi-aseptic fish cake (SACP) and sterilized fish cake (STERILE)) as the main plot and oxygen absorbers' presence (with or without) as the subplot. Fish cake samples were collected from branded retailers in the Kayuagung District, Ogan Ilir Regency, and several parameters were observed. These included pH, total volatile base nitrogen (TVB-N), total plate count (TPC), hardness (texture), texture profile analysis (cohesiveness, gumminess, and chewiness), and colour changes. The results showed that sterilization type significantly influenced pH (4.5–7.2), TVB-N (0.00–11.67 mg-N/100 g), TPC (3.50–6.25 log CFU/g), hardness (21.9–36.9 N), cohesiveness (0.82–0.95), gumminess (1745–5432 g), chewiness (1621–25,114 g), and colour changes (4.21–11.41 ΔE). Among all treatments, STERILE method had better quality and stability compared to others.

***Keywords:*** Palembang, Vacuum packaging, Sterilization, Oxygen absorbers, Shelf-life, Storage.

## Introduction

Fish cake (Pempek) is a popular traditional snack from Palembang, South Sumatra, Indonesia, with a limited shelf life.[1–6] At room temperature of 25°C, cylindrical-shaped fish cake can last for approximately one day.[2,7,8] This is due to limited shelf life caused by high moisture content ranging from 58.1% to 67.5%, alongside elevated protein and carbohydrate content of 4.9-9% and 24.5-35.4%, respectively.[9,10] The relatively high water and protein content made fish cake susceptible to microbial growth.[11] After contamination, microbes produce various metabolites or by-products that reduce quality, cause colour changes and texture, decrease pH, and increase both total volatile base nitrogen (TVB-N) as well as total plate count (TPC).[12]

During the storage of fish cake, several methods have been applied to inhibit microbial growth and minimize oxidative changes, including pretreatment, packaging, and processing.[13] This is because oxygen strongly influences the composition of microflora in fish cake as well as the rate of enzymatic reactions. Therefore, the use of packaging, oxygen absorbers, and sterilization plays an essential role in product protection.[14]

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Contemporary developments in packaging technologies for food products have enhanced the defense against environmental elements, such as moisture, pathogens, oxygen exposure, and light.[15–20] Multiple preservation methods, including sterilization pretreatments, incorporation of oxygen absorbers, vacuum sealing, and hot-fill vacuum packaging, have shown efficacy in prolonging product shelf stability.

Sardines maintained in vacuum packaging at 4°C showed lower production of Adenosine Triphosphate (ATP) derivatives, inhibited bacterial growth, decreased the formation of histamine, Trimethylamine (TMA), and TVB-N, thereby extending shelf life.[16] This suggests that vacuum packaging combined with low-temperature storage has been reported to extend shelf life of various food products.[21,22] However, vacuum packaging and low-temperature storage require additional protective packaging to maintain cold conditions, particularly during long-distance transportation. Previous research reported that vacuum-sterilized fish cake (STERILE) had a shelf life of approximately one year,[23] bulgogi sauce up to 90 days[24], and coconut milk without alteration in taste.[25] The texture of fish cake stored in vacuum-STERILE flexible packaging tended to harden during storage due to water diffusion from the product into the material. To address this limitation, controlling negative pressure through partial vacuum sterilization can reduce water evaporation and preserve texture. The shelf life of fish cake with improved textural quality can be achieved through the application of partial vacuum packaging combined with oxygen absorbers, which inhibit microbial growth and enzymatic reactions.

The incorporation of oxygen absorbers into flexible packaging effectively reduces oxygen concentration by 67.44% in 24 hours. Due to the significant potential, oxygen absorbers have been widely applied in packaging various food products, including bread, biscuits, fruits, vegetables, nut products, fish, and meat.[17] The application significantly reduces rancidity in cheese spreads, causing a delay in the degradation of vitamin C and extending shelf life.[18] However, there are no research on the combined use of vacuum packaging, oxygen absorbers, and sterilization pretreatments to extend shelf life of semi-moist products

such as fish cake. Therefore, this research aimed to analyze and optimize the effects of sterilization and oxygen absorbers on the physicochemical and microbial changes of fish cake during storage.

## Materials and Methods

*Preparation of fish cake (Pempek)*
Fish cake used in this research was of the cylindrical type (4 cm in diameter) and was cut into slices with a thickness of 2 cm. The samples were purchased from a branded retailer in Kayu Agung, South Sumatra Province. The initial physicochemical and microbiological characteristics of samples are presented in Table 1.

**Table 1:** Initial characteristics of fish cake (Pempek)

| Parameter | Value |
|---|---|
| *L** | 59.94±0.27 |
| *a** | -4.02±0.20 |
| *b** | 5.94±0.46 |
| Texture (hardness) (N) | 17.8±0.20 |
| pH | 6.90±0.00 |
| TVB-N (mg-N/100 g) | 0.00±0.00 |
| TPC (log CFU/g) | 3.24±0.03 |

*L** – lightness (0-50 black, 51-100 white); *a** – red to green (+a = redder, -a = greener); *b** – yellow to blue (+b = yellower, -b = bluer)

*Experimental design*
The experimental design applied was a split-plot design. Specifically, the main plot was the type of sterilization, consisting of semi-aseptic fish cake (SACP) and STERILE. The subplot was the type of oxygen absorbers (with or without oxygen absorbers). SACP was sterilized using a laboratory-scale sterilizer (HVE-50, Hirayama) at 121°C for 15 minutes. During sterilization, the samples were placed inside stainless-steel containers. Subsequently, the samples consisting of five pieces per bag were packed in sterile plastic bags with or without oxygen absorbers, and vacuum-sealed (-0.1 MPa for 30 seconds) using a vacuum sealer (Double Leopard DZ-280).
For STERILE treatment, fish cake samples comprising five pieces per bag were packed in sterile plastic bags with or without oxygen absorbers, vacuum-sealed under the same conditions as SACP samples, and hot-sealed. The sealed packages were sterilized in a laboratory-scale sterilizer (HVE-50, Hirayama) at 121°C for 15 minutes. All samples were stored in a laboratory incubator at 25 ± 0.3°C for 30 days to simulate the typical display conditions used by fish cake (Pempek) vendors.
Physicochemical and microbiological analyses were conducted on days 0 (initial), 3, 6, 9, 12, 24, and 30. The number of prepared sample bags corresponded to the combination of treatments and storage periods. Several parameters were measured, including pH, TVB-N, TPC, hardness (texture), texture profile analysis (cohesiveness, gumminess, and chewiness), and colour changes. All measurements were conducted in triplicate, except for TPC, TVB-N, and texture profile analysis, which were performed in duplicate.

*pH*
A 10 g fish cake was randomly sampled, ground in a mortar until homogeneous, and mixed with 10 mL of distilled water. The slurry was homogenized by stirring with a glass rod.[26] Subsequently, pH was determined using a calibrated pH meter (Waterproof pH Testr 10, Eutech Instruments, USA) with standard buffer solutions at pH 4, 7, and 10.

*TVB-N*
TVB-N was determined using National Indonesian Standard (SNI 2354.8:2009) method for fish products.[27] In this research, TVB was determined using the microdiffusion method. The sample used was ground and weighed to 5 grams, added with 10 mL of 7% TCA solution, and left to stand for 30 minutes before being filtered using Whatman No. 2 paper. This was followed by adding 1 mL of 2% boric acid solution to the chamber in the Conway unit. On the left side, a 50% $K_2CO_3$ solution and sample filtrate (1 mL) were added. The Conway dish was covered and shaken in a figure-eight motion, which was incubated at 37°C for 2 hours. Following incubation, 3 drops of Tashiro indicator were added, and the solution was titrated with 0.02 N HCl until a pink colour was achieved.

*TPC*
TPC for the number of microbes was determined using SNI (7388:2009),[28] with pour plate method. A 25 g sample was measured and transferred to a sterile container, combined with 225 mL of 0.85% NaCl solution, and thoroughly homogenized to achieve a $10^{-1}$ dilution. Serial dilutions were prepared by transferring 1 mL of this initial dilution into 9 mL of NaCl solution to create a $10^{-2}$ dilution. This process was continued to generate $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions from the original homogenate. From each dilution level, 1 mL of suspension was dispensed into sterile Petri dishes, followed by adding 15 mL of plate count agar (PCA) maintained at 45±1°C. The inoculated plates were incubated in an inverted orientation at 35-37°C for 48 hours.

*Hardness (Texture)*
Texture was determined using a Digital Force Gauge Dynamometer (HF-1000N, NANBEI, China). Samples were placed under a cylindrical probe (8 mm diameter), and hardness was measured by pressing the lever on the instrument stand. The applied force was recorded and displayed on LCD screen in Newton (N) units.

*Texture Profile Analysis (TPA)*
Textural characteristics were evaluated using Texture Analyzer (TA.XT Express, Texture Technologies Corp, USA). Samples were prepared as 1 × 1 × 1 cm³ cubes and subjected to dual compression to 60% of the initial height through a 75 mm diameter compression plate. Force–time profiles were generated at a crosshead velocity of 5 mm/s with matching data acquisition rates. The instrument accompanying software was used to process force-time profiles to calculate chewiness (g), gumminess (g), and cohesiveness parameters.

*Colour changes*
Colour changes were measured using a colour difference meter (Colorimeter CS-10, CHNSpec Technology (Zhejiang), China) based on three colour coordinates, namely *L** (lightness), *a** (redness), and *b** (yellowness). Measurements were performed in triplicate, and the mean values were used for analysis. The total colour difference ($\Delta E$) between the control and treated fish cake was calculated as the square root of the sum of squared differences in *L**, *a**, and *b** values (Equation 1).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \text{……Eq. 1}$$

*Statistical analysis*
Data preparation was conducted using Microsoft Excel 2016 and Prism 10, and statistical analysis was performed with SPSS version 25 (IBM, NY, USA). The experiment followed Split-Plot Randomized Design (SPRD), with sterilization type (SACP and STERILE) as the main plot and type of oxygen absorbers as the subplot. Subsequently, treatment means were compared using Tukey's HSD (Honestly Significant Difference) test, and differences were considered statistically significant at $P < 0.05$.

## Results and Discussion

*pH*
pH level is a critical quality metric for food products, serving as an indicator of possible microbial contamination from bacteria, fungi, and other spoilage-causing organisms.[29] As shown in Figure 1, pH measurements obtained for fish cake samples were stored under room temperature conditions. Initial readings ranged between 6.1 and 7.2, obtaining a mean of 6.7 ± 0.06, which corresponded to the report by Moulia et al. (2019).[12] After 30 days of storage, mean pH levels across all experimental groups decreased to 4.5–5.7.

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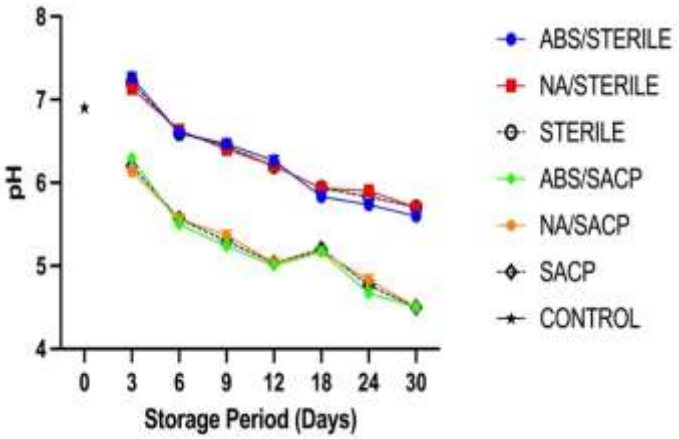


**Figure 1:** Changes in the pH of fish cake during storage at 25±0.3°C for 30 days
The values represent mean ± standard deviation (n = 3). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake; CONTROL – initial fish cake.

This reduction shows substantial biochemical and microbiological processes occurring in the storage duration. Statistical evaluation through Analysis of Variance (ANOVA) showed that sterilization type significantly influenced ($P < 0.05$) fish cake pH values. Among all samples, STERILE showed a substantial difference from SACP.
These results show that negative pressure packaging before sterilization (STERILE) is more effective in preventing bacteria contamination from the environment compared to SACP. The decrease in pH during storage is mainly attributed to the accumulation of organic acids from protein degradation, bacteria respiration, and anaerobic glycolysis, producing carboxylic acids that contaminate fish cake.[30,31] Similar trends have been observed in other food products. For example, pH of meatballs decreased with storage time due to myofibrillar protein denaturation and microbial growth,[32] while the metabolites produced by microorganisms during wet noodle storage caused a significant reduction.[33] Microbial metabolites can cause the decomposition of starch molecules.

*TVB-N*
TVB-N serves as a standard parameter for evaluating quality degradation in fish-based products with high protein content.[34] Generally, quality deterioration occurs through the production of volatile basic compounds, including ammonia, hydrogen sulfide, dimethylamine, and TMA, generated during protein breakdown. TVB-N has gained widespread acceptance as a spoilage marker due to the ability to quantify the buildup of ammonia alongside other volatile nitrogenous bases from microbial degradation of nitrogen-bearing components.[35] As shown in Figure 2, TVB-N measurements were recorded for fish cake samples maintained under room temperature conditions in the storage period.
At the beginning of this research, samples showed TVB-N levels between 0.00 and 0.19 mg-N/100 g, with mean of 0.07 ± 0.09 mg-N/100 g. After 30 days of storage, mean concentrations across experimental groups increased to 5.04–11.67 mg-N/100 g. These measurements were below the acceptable threshold established by SNI (2354.8:2009), which defined <30 mg-N/100 g as the maximum permissible level for fish products intended for human consumption.[27,36] Statistical analysis using ANOVA indicated that sterilization type significantly influenced ($P < 0.05$) TVB-N concentrations, with SACP showing substantial differences compared to STERILE. The high levels observed in SACP samples showed reduced efficacy in controlling microbial proliferation, increasing the generation of volatile nitrogenous compounds from proteolytic enzyme-mediated protein degradation.
The increase is mainly attributed to microbial metabolism and the oxidation of protein and non-protein components.[37] Similar trends in increasing TVB-N levels during storage have also been reported in rainbow trout fillets packaged with oxygen absorbers,[38] sardines stored under vacuum at 4°C,[16] and bulgogi sterilized in an autoclave at 121°C for 15 minutes.[31] A faster increase in TVB-N values in association with higher microbial counts shows a more advanced stage of spoilage.[39] Proteolytic bacteria play a major role by degrading proteins and derivatives into volatile bases, including ammonia, TMA, histamine, indole, hydrogen sulfide, and skatole, as well as reducing TMA oxide (TMAO) into TMA.[40]

*TPC*
TPC is often used to determine the number of microbial colonies in food products.[41] Generally, microbial growth is defined as an increase in cell components or biomass, followed by proliferation that leads to a rise in cell numbers. The microbial load in food products largely depends on the effectiveness of processing and handling during production. TPC results of fish cake during storage at room temperature are shown in Figure 3.

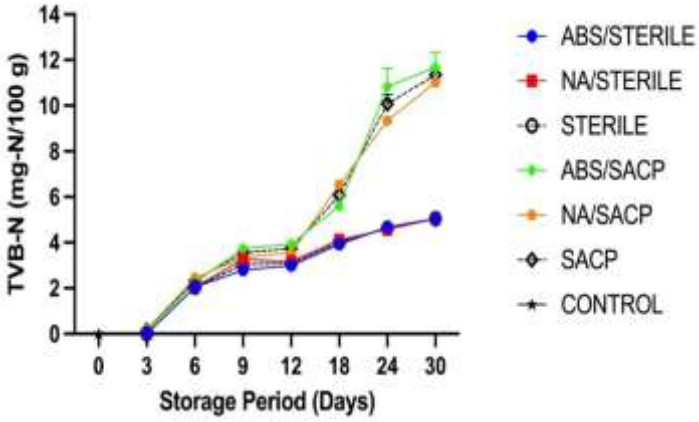


**Figure 2:** Changes in the TVB-N of fish cake during storage at 25±0.3°C for 30 days
The values represent mean ± standard deviation (n = 2). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake; CONTROL – initial fish cake.

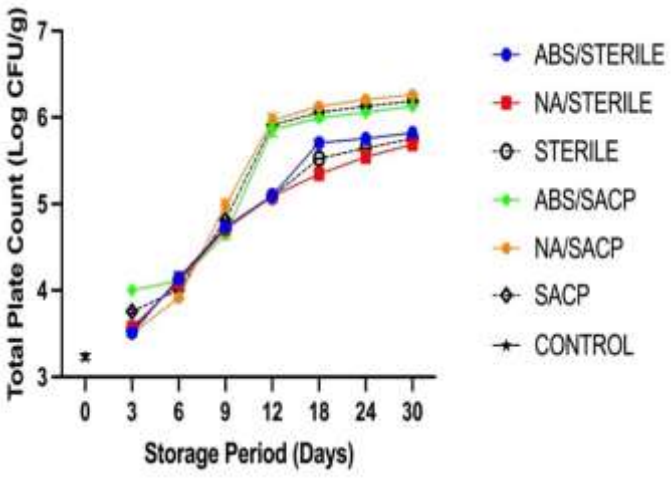


**Figure 3:** Changes in the TPC of fish cake during storage at 25±0.3°C for 30 days
The values represent mean ± standard deviation (n = 2). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake; CONTROL – initial fish cake.

Baseline TPC for fish cake samples in this research ranged between 3.5 and 4.0 log CFU/g, with a mean of 3.64 ± 0.18 log CFU/g. At the 30-

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day mark, mean TPC measurements across experimental groups spanned 5.69 to 6.25 log CFU/g. STERILE samples and related variations, with or without oxygen absorbers inclusion, maintained bacteria counts below safety thresholds at a mean of 5.75 ± 0.05 log CFU/g. International Commission on Microbiological Specifications for Foods establishes 6 log CFU/g as the acceptable upper limit for fish-based products.[42] However, SACP exceeded the recommended threshold by storage day 24. ANOVA results showed that sterilization type significantly affected ($P < 0.05$) TPC outcomes, with SACP treatment having a significant difference from STERILE processing. The progressive rise in TPC across all experimental groups can be attributed to continued bacterial multiplication.

High TPC levels in the storage period show increased bacterial metabolism and replication. Microbial expansion is facilitated by nutrient-rich components present in fish cake, including carbohydrates, proteins, and moisture content. [40] Although vacuum sealing shows considerable effectiveness in restricting bacteria proliferation, modest TPC increases persist during extended storage due to surviving microorganisms. [14,16,43] The combination of sealed packaging with thermal sterilization (autoclave treatment) has also shown effectiveness in controlling microbial development across diverse food matrices, such as bulgogi sauce stored at 35°C,[24] cheese,[44] and wet instant noodles.[33]

*Hardness (Texture)*

One of the most crucial factors in evaluating a product acceptance in the food industry is texture, which includes essential qualities such as hardness, softness, chewiness, and crispness.[45] The hardness of fish cake during storage at room temperature is presented in Figure 4. Based on the results, the initial hardness ranged from 21.9 to 23.2 N, with a mean value of 22.7 ± 1.48 N. After 30 days of storage, mean hardness across all treatments increased to 29.7–36.9 N. ANOVA results showed that the type of sterilization had a significant effect ($P < 0.05$) on hardness. In comparison, STERILE was significantly different from SACP. This result suggests that packaging before sterilization can act as a barrier that minimizes protein denaturation.

The increase in hardness during storage is influenced by several factors, including starch retrogradation, water migration, and redistribution.[46,47] Retrogradation refers to the recrystallization of starch that has previously experienced gelatinization. It also occurs due to the reformation of hydrogen bonds between amylose and amylopectin molecules.[48] Additionally, STERILE experienced protein denaturation, which alongside aggregation, led to collagen shrinkage, reducing the water-binding capacity and causing a harder texture.[19,35,49,50] Similar results have been reported in surimi sausage, where hardness gradually increased during storage.[51]

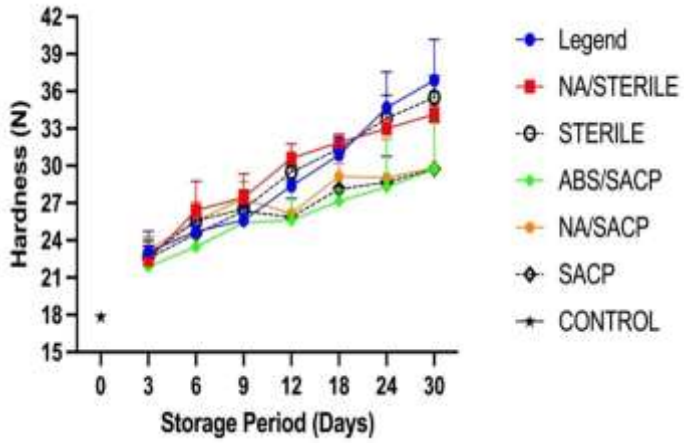


**Figure 4:** Changes in the hardness of fish cake during storage at 25±0.3°C for 30 days

The values represent mean ± standard deviation (n = 3). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake; CONTROL – initial fish cake.

*TPA*

TPA is used to evaluate the rheological and sensory properties of food.[52] The cohesiveness, gumminess, and chewiness of fish cake treated with preservation methods during storage at 25 ± 0.3°C for 30 days are presented in Figure 5. Generally, cohesiveness shows the strength of internal bonds maintaining the product structure.[53] It is also defined as the degree of deformation occurring before fracture during biting.[54] In this research, the initial cohesiveness values of fish cake ranged from 0.93 to 0.95, with mean of 0.94 ± 0.01. On day 30, mean across treatments decreased to a range of 0.82–0.93.

ANOVA analysis indicated that the type of sterilization had a significant effect ($P < 0.05$) on cohesiveness, with STERILE showing a significant difference from SACP. These results suggested that STERILE had stronger intermolecular attractive forces. The cohesiveness decreased during storage in all treatments (Figure 5A) due to the loss of intramolecular interactions among components.[55] Recrystallization of starch and structural reorganization of starch-protein matrix during storage can cause a thermodynamic mismatch between protein and starch phase. A cohesiveness value of 1 shows that the food remains stable and returns to the original structure between compressions. In comparison, a value below 1 indicates that deformation after the first compression is irreversible.[54]

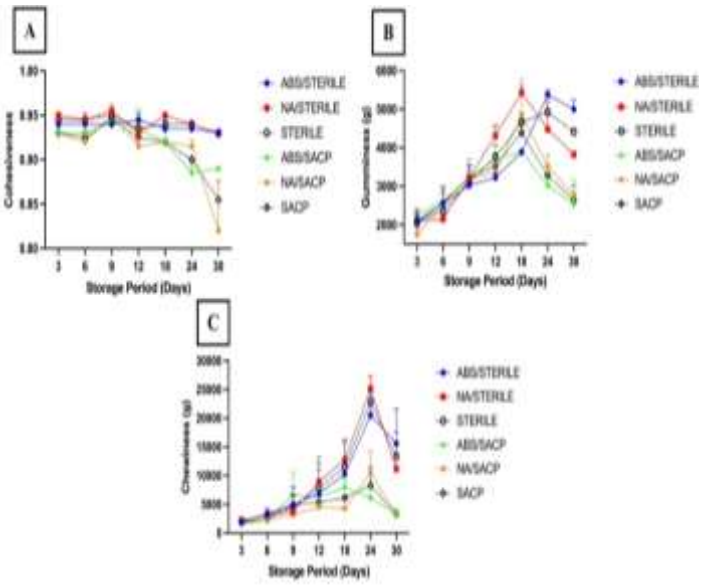


**Figure 5:** Changes in the cohesiveness (A), gumminess (B), and chewiness (C) of fish cake during storage at 25±0.3°C for 30 days

The values represent mean ± standard deviation (n = 2). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake.

Gumminess is defined as the force required to disintegrate a sample to the point of swallowing.[56] In this research, the initial gumminess values of fish cake ranged from 1745 to 2279 g, with mean of 2058 g. By day 30, the mean across all treatments increased to 2532–5000 g (Figure 5B). Gumminess parameter is a secondary textural attribute calculated as the product of hardness and cohesiveness. It is specifically applied to semi-solid foods with low hardness and high cohesiveness. ANOVA results showed that the type of sterilization had a significant effect ($P < 0.05$) on gumminess, with STERILE being significantly different from SACP. This difference is attributed to the higher hardness values observed in STERILE.[57] The gumminess increased during storage across all samples, which can be directly associated with the progressive increase in hardness. Progressive moisture loss during storage leads to higher protein concentrations per unit volume, increasing matrix density and reducing the intermolecular space available for deformation, thereby increasing resistance to compression.

Chewiness is defined as the energy necessary to chew solid food until readiness for swallowing.[56]

In this research, the initial chewiness values of fish cake ranged from 1621 to 2200 g, with a mean of 1940 g. By day 30, mean chewiness values across treatments increased to a range of 3263–15,608 g (Figure 5C). ANOVA results indicated that the type of sterilization had a significant effect ($P < 0.05$) on chewiness, with STERILE showing a significant difference from SACP. This difference is related to the elasticity of protein matrix. The increase in chewiness is attributed to protein denaturation and myofibril contraction, reducing actin–myosin interactions.[58] These results have important practical implications for optimizing the commercial production process of fish cake. Understanding the relationship between sterilization methods, protein structural changes, and texture evolution during storage can help manufacturers determine the optimal processing conditions to achieve a balance between microbiological safety, shelf life, and the texture quality desired by consumers.

*Colour Changes*

Colour is an essential quality attribute of fish cake that influences consumer preferences.[3] Additionally, colour can serve as an indicator of chemical changes occurring in food during storage. The results of colour change analysis in fish cake stored at room temperature are presented in Figure 6. The initial colour difference ($\Delta E$) values ranged from 4.21 to 7.20 $\Delta E$, with a mean of 5.74 ± 1.59 $\Delta E$. By day 30, the mean values across treatments increased to 5.21–11.41 $\Delta E$.

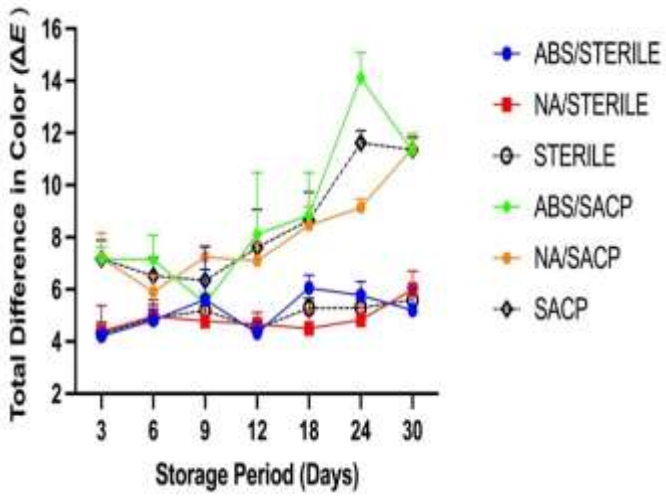


**Figure 6:** Changes in the total difference in colour of fish cake during storage at 25±0.3°C for 30 days

The values represent mean ± standard deviation (n = 3). ABS – with oxygen absorber; NA – without oxygen absorber; STERILE – sterilized fish cake; SACP – semi-aseptic fish cake.

ANOVA results showed a significant effect ($P < 0.05$) of sterilization type on colour change, with SACP having substantial differences from STERILE. This suggested that packaging before sterilization reduced discoloration, preventing the shift from bright to yellowish tones often associated with sterilization. During sterilization, starch degradation into reducing sugars promotes Maillard reactions between sugars and amino acids, thereby contributing to colour changes in fish cake.[59,60] Colour changes during storage are caused by Maillard reaction between proteins and sugars. Similarly, there are browning reactions between proteins and fats, which cause fish cake to develop yellowish to brownish tones.[61] A higher $\Delta E$ value shows a greater intensity of discoloration. Colour changes can occur from moisture loss and structural irregularities induced by microbial decomposition.[62] Sterilization treatment has also been reported to intensify colour differences in fish-based products, including fish cake. The $\Delta E$ value gradually increased 10.46 to 24.50 as sterilization temperature rose at 105-121°C, indicating continuous deviation from the initial point.[63]

## Conclusion

In conclusion, this research shows that preservation technologies, such as sterilization pre-treatment and the use of oxygen absorbers, effectively inhibit physicochemical and microbiological changes in fish cake during storage. The results show that sterilization type has a significant effect on pH (4.5–7.2), TVB-N (0.00–11.67 mg-N/100 g), TPC (3.50–6.25 log CFU/g), hardness (21.9–36.9 N), cohesiveness (0.82–0.95), gumminess (1745–5432 g), chewiness (1621–25,114 g), and colour (4.21–11.41 $\Delta E$). Considering TPC, TVB-N values, and pH, STERILE treatment extends the product shelf life by approximately 30 days at room temperature. Moreover, future research is recommended to investigate the best temperature, pressure, and time combinations for maximizing microbiological safety while keeping texture, flavor, and nutritional value.

## Conflict of Interest

The authors declare no conflict of interest.

## Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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