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RESEARCH ARTICLE

Chemical and Microbiological Quality of Smoked Halfbeak (*Hemiramphus* sp.) under Different Storage Conditions

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Abstract. Smoked halfbeak (*Hemiramphus* sp.) is an economically important traditional product in Boalemo Regency, Gorontalo, but it is commonly stored without adequate packaging, which may compromise its quality. This study evaluated the effect of storage conditions on the chemical and microbiological quality of traditionally smoked halfbeak. A completely randomized design was used with three treatments (freshly smoked product as the control, and open and closed storage for 3 months) and three replications. Total plate count (TPC), yeast and mold count, and moisture, ash, fat, and protein contents were analyzed by ANOVA followed by Duncan's multiple range test ($\alpha = 0.05$). Storage conditions significantly affected all parameters ($p < 0.01$). TPC ranged from 1.90×10^4 CFU/g (open storage) to 3.94×10^4 CFU/g (closed storage), below the maximum of 5.0×10^4 CFU/g set by the Indonesian National Standard (SNI 2725:2013). In contrast, yeast and mold counts (3.55×10^3–6.75×10^3 CFU/g) exceeded the limit of 1.0×10^2 CFU/g in all treatments, including the control. In the control, open storage, and closed storage treatments, respectively, moisture content was 54.04%, 13.22%, and 15.53%, and fat content was 13.27%, 8.56%, and 8.57% both complied with the SNI limits (≤60% and ≤20%). Ash (3.22%, 1.23%, and 1.32%) and protein (35.48%, 24.74%, and 26.74%) contents, which are not regulated by the standard, were lower after storage. Overall, the product met the TPC, moisture, and fat requirements, whereas the yeast and mold contamination already present after smoking suggests that hygiene during smoking and post-smoking handling needs improvement.

Keywords: smoked halfbeak, storage conditions, total plate count, yeast and mold count, proximate composition.

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INTRODUCTION

Halfbeak (*Hemiramphus* sp.) is a schooling fish that typically reaches a body length of approximately 45 cm, although some individuals measure around 30 cm, and it is characterized by a lower jaw that is longer than the upper jaw. It is one of the important fishery commodities of Tomini Bay (Putri & Madduppa, 2020). Known locally as roa, halfbeak plays a significant role in the economy of Gorontalo communities, particularly in Boalemo Regency, where it is generally caught with purse seines known locally as pukat tambai or pukat roa (Olii, 2022).

Indonesian capture fisheries production reached 7.99 million tons in 2022, approximately 96.03% of the national target (KKP, 2022). Current data on the share of this catch that is processed into traditional products, such as salted and smoked fish, are not available, the most recent figures, from 2006, indicate that approximately 49.99% of marine fish utilization went to traditional products (Syariah & Asruddin, 2019). Traditional processing remains widespread because modern processing imposes requirements that small-scale fisheries enterprises find difficult to meet, including uniform fish size, quality that complies with industry standards, and food safety assurance (Putra *et al.*, 2024).

Halfbeak has a relatively high moisture content of approximately 73.69%, with protein and fat contents of 23.19% and 1.77%, respectively (Siahaya, 2020). Because protein is the main constituent of fish after water, fish is highly perishable, and spoilage-related changes begin within eight hours of capture and landing if the fish is not handled promptly (Sirait & Saputra, 2020). High moisture content creates an environment that supports the growth of spoilage microorganisms, while the degradation of fish protein by autolytic enzymes and microbial activity produces volatile basic nitrogen compounds and biogenic amines that contribute to quality loss (Nie *et al.*, 2022). Smoking is one preservation method that can be applied to control spoilage.

Smoking is the process of flavoring, cooking, or preserving food by exposing it to smoke from burning or smoldering material, typically wood (Kwaghvihi *et al.*, 2020). Carbonyl and phenolic compounds in the smoke give fish its characteristic color and flavor (Alçiçek *et al.*, 2010), and smoking extends shelf life through the dehydrating, antimicrobial, and antioxidant effects of smoke constituents (Pagu *et al.*, 2013). Smoking also alters product texture (Wang *et al.*, 2021). Smoking methods comprise cold smoking, hot smoking, and the use of liquid smoke (Susanto, 2014) traditional smoking in Indonesia is predominantly hot smoking at 60–120°C.

The quality of traditionally smoked fish is nevertheless variable. Smoking temperature and the quality of the smoking material vary, which affects product quality and, in turn, consumer acceptance (Litaay *et al.*, 2022). In addition, traditional smoking equipment is used inefficiently because much of the smoke disperses into the air (Wahab *et al.*, 2019), which is presumed to contribute to quality deterioration during subsequent storage, particularly under different storage conditions. Evaluating the quality changes of smoked halfbeak during storage is therefore essential for determining its shelf life and maintaining product safety and quality. However, information on how storage conditions affect the quality of traditionally smoked halfbeak from Boalemo remains limited. Accordingly, this study evaluated the effect of storage conditions (open and closed storage for 3 months) on the chemical and microbiological quality of traditionally smoked halfbeak from Boalemo Regency, in order to inform appropriate storage practices and improve product quality.

MATERIALS AND METHODS

The primary material was smoked halfbeak obtained from fishermen. The chemicals used for analysis were chloroform (Merck), sulfuric acid (Merck), K_2SO_4 and $CuSO_4$ catalysts (Merck), methyl red or methylene blue indicator (Merck), and distilled water. The materials used for the total plate count (TPC) and yeast and mold count (YMC) analyses were nutrient agar (HiMedia), potato dextrose agar (PDA; HiMedia), plate count agar (PCA; Merck), sterile distilled water (OneMed), 70% alcohol (OneMed), peptone (Merck), and 0.9% NaCl physiological solution (Merck).

Sampling Technique

Samples were collected by purposive sampling, in which sampling points are selected on the basis of specific criteria so that the data represent conditions at the study site. Smoked fish samples were taken from three points in the production process using the grab sampling method. Sterile containers were prepared and labeled with the sample identity and the date and time of collection. The samples were collected aseptically with sterile tongs or gloves, placed in sterile plastic bags or containers, and transported to the laboratory in a cool box with ice packs. Parameters that may change rapidly, such as temperature, were measured directly in the field.

Sample Preparation

1. Preparation of Halfbeak

Fresh halfbeak obtained from fishermen in Bangga Village, Paguyaman Pantai District, were sorted by size. The gills, fins, and viscera were removed, and the fish were washed thoroughly under running water and drained.

2. Smoking and Storage

The prepared fish were clamped, tied, and arranged on bamboo racks over the smoking furnace. Smoking was carried out in two stages: the first for 8–10 hours at 40–80°C and the second for 5–6 hours at approximately 40°C. The smoked fish were then stored for 3 months under open storage conditions or under closed storage conditions in containers. The experiment followed a completely randomized design with three treatments (control, open storage, and closed storage) and three replications.

Parameters and Test Methods

1. Total Plate Count (TPC)

TPC was determined by the spread plate method, which is based on the growth of aerobic and anaerobic microorganisms into countable colonies after incubation on agar medium at 35 ± 1°C for 48 hours. A 25 g sample was weighed aseptically and homogenized for 2 minutes with 225 mL of Butterfield's phosphate-buffered solution to give the 10^{-1} dilution. One milliliter of this dilution was transferred with a sterile pipette into 9 mL of the same buffer to obtain the 10^{-2} dilution, and the procedure was repeated for the 10^{-3}, 10^{-4}, and subsequent dilutions as required. Each dilution was vortexed. PCA medium (12–15 mL) was poured into sterile Petri dishes, swirled gently, and allowed to solidify. An aliquot of each dilution (10^{-2}, 10^{-3}, and so on) was pipetted onto the medium and spread evenly over the surface with an L-shaped glass rod. After the inoculum had been absorbed for approximately 1 hour, the dishes were inverted and incubated at 35 ± 1°C for 48 hours. Plates containing 25–250 colonies were counted, the dilution factor was recorded, and the count was calculated as follows:

$$N = \frac{\sum C}{[\,(1 \times n_1)+(0{,}1 \times n_2)\,] \times (d)} \qquad (1)$$

where N is the number of colonies per mL or per g of product, ΣC is the total number of colonies on all counted plates, n_1 and n_2 are the numbers of plates counted at the first and second dilutions, respectively, and d is the first dilution counted.

2. Yeast and Mold Count (YMC)

YMC was determined by the pour plate method (BSN, 2009), which is based on the growth of aerobic microorganisms after incubation on agar medium at 22–25°C for 5 days. Serial dilutions were prepared as described for TPC, and an aliquot of each dilution (10^{-2}, 10^{-3}, and subsequent dilutions) was transferred to a sterile Petri dish. Approximately 15–20 mL of PDA medium was poured into each dish, mixed with the sample by gentle swirling, and allowed to solidify. After approximately 1 hour, the dishes were inverted and incubated at 22–25°C for 5 days. Plates containing 10–150 colonies were counted, the dilution factor was recorded, and the count was calculated with the same formula as for TPC.

3. Moisture Content

Moisture content was determined by the oven-drying method (AOAC, 2005). A 2 g homogenized sample was weighed on aluminum foil of known weight, dried in an oven at 150°C for 4 hours, cooled in a desiccator for 15 minutes, and weighed. The sample was then reheated for 30 minutes, cooled in the desiccator, and reweighed, and this step was repeated until a constant weight was reached. The weight loss, which represents the water evaporated from the sample, was used to calculate moisture content as follows:

$$\text{Moisture (\%)} = \frac{(W\text{-}W_1)}{W_2} \text{ x } 100 \quad (2)$$

where W is the weight of the sample before drying (g), W_1 is the weight of the sample after drying (g), and W_2 is the initial weight of the sample (g).

4. Ash Content

Ash content was determined by dry ashing (AOAC, 2019), in which organic compounds are decomposed into water and CO_2, leaving the inorganic residue. A 2–3 g sample was placed in a porcelain crucible of known weight, charred on a hot plate, and ashed in a muffle furnace at 550°C until ashing was complete. The crucible was cooled and weighed to constant weight, and ash content was calculated as follows:

$$\text{Ash (\%)} = \frac{(W_1\text{-}W_2)}{W} \text{ x } 100 \quad (3)$$

where W_1 is the weight of the crucible and ash after ashing (g), W_2 is the weight of the empty crucible (g), and W is the weight of the sample (g).

5. Fat Content

Fat content was determined by the Soxhlet method (AOAC, 2019), in which free fat is extracted with a nonpolar solvent. A 1–2 g sample was weighed into a paper thimble lined with fat-free cotton and dried in an oven at approximately 80°C for 1 hour. The thimble was placed in a Soxhlet apparatus connected to a fat flask that had been dried and weighed, and the fat was extracted for approximately 5 hours with a nonpolar solvent such as chloroform or hexane. The extracted fat was dried in an oven at 105°C, cooled, and weighed, and drying was repeated until a constant weight was obtained. Fat content was calculated as follows:

$$\text{Fat (\%)} = \frac{(W\text{-}W_1)}{W_2} \text{ x } 100 \quad (3)$$

where W is the weight of the fat flask and extracted fat after drying (g), W_1 is the weight of the empty fat flask (g), and W_2 is the weight of the sample (g).

6. Protein Content

Protein content was determined by the Kjeldahl method (AOAC, 2019), which measures the nitrogen content of the sample in three stages: digestion, distillation, and titration. For digestion, a 0.5 g sample was weighed accurately into a 100 mL Kjeldahl flask, 2 g of selenium mixture and 25 mL of concentrated sulfuric acid were added, and the sample was digested for approximately 2 hours or until the solution turned clear green. After cooling, the digest was transferred to a 100 mL volumetric flask and made up to the mark with distilled water. For distillation, a 5 mL aliquot of the digest was placed in a distillation flask with 5 mL of 30% NaOH solution and a few drops of phenolphthalein indicator and distilled for approximately 10 minutes. The distillate was collected in 10 mL of 2% boric acid solution containing indicator, and the condenser tip was rinsed with distilled water. For titration, the distillate was titrated with 0.01 N HCl

until the color changed to faint gray, and the volume of HCl used was recorded. A blank was run in the same way. Protein content was calculated as follows:

$$\text{Protein (\%)} = \frac{(V_1\text{-}V_2) \text{ x N HCl x } 14 \text{ x FP x FKJ}}{\text{sampel weight (g)}} \text{ x } 100 \quad (4)$$

where V_1 and V_2 are the titration volumes for the sample and the blank, respectively, FP is the dilution factor, and FK is the protein conversion factor (6.25 for general foods, 6.38 for milk and its products, and 5.46 for peanut oil).

Data Analysis

The data (n = 3 replications) were analyzed by analysis of variance (ANOVA). Before ANOVA, normality was tested with the Shapiro–Wilk test and homogeneity of variance with Levene's test. Where a significant effect was found, means were compared with Duncan's multiple range test (DMRT) at α = 0.05 using the Statistical Tool for Agricultural Research (STAR) application. F-values, degrees of freedom, and p-values are reported in the respective figures.

RESULTS AND DISCUSSION

Total Plate Count (TPC)

Smoked fish products must be safe for consumption, and microbiological quality is therefore one of the attributes that requires particular attention. It can be evaluated through several parameters, including the total plate count (TPC), which represents the number of aerobic mesophilic bacteria per gram or milliliter of sample. Because TPC reflects the total bacterial load of smoked fish, it serves as a primary indicator of product hygiene and microbiological safety (Joesidawati *et al.*, 2022). Measuring TPC is particularly relevant for halfbeak, which has a protein content of 23.19% in its fresh form (Siahaya, 2020).

The TPC of smoked halfbeak ranged from 1.90 × 10^4 to 3.94 × 10^4 CFU/g, with the lowest value in the open storage treatment and the highest in the closed storage treatment (Figure 1). Analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on TPC (p = 0.005). According to Duncan's multiple range test, the control (2.06 × 10^4 CFU/g) did not differ significantly from open storage, whereas closed storage differed significantly from open storage.

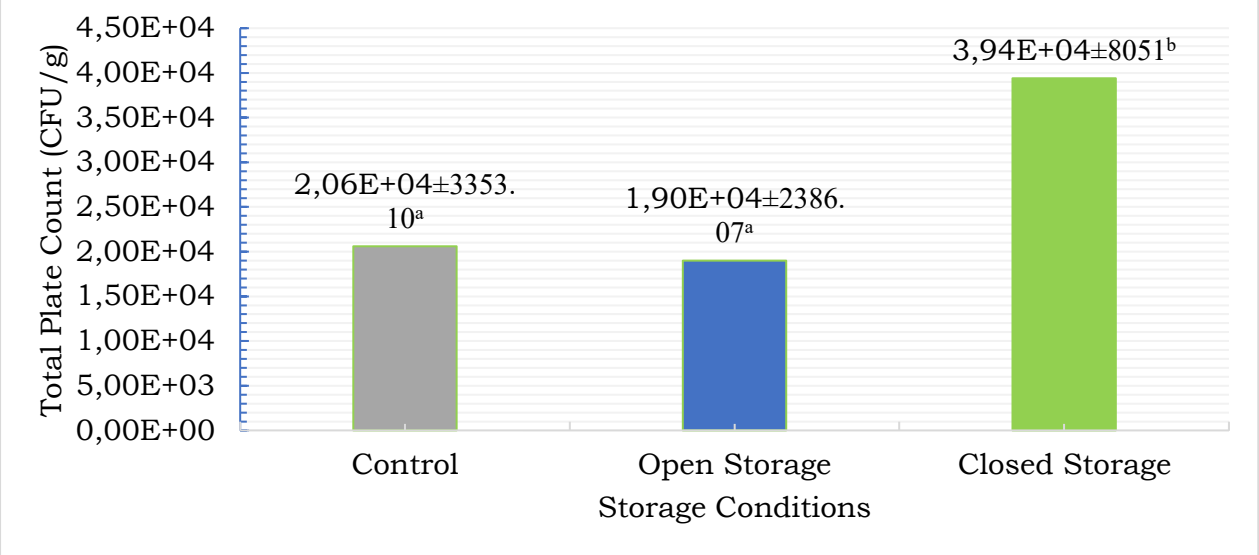


Figure 1. Total Plate Count of Smoked Halfbeak under Different Storage Conditions

After 3 months of storage under different conditions, the TPC of smoked halfbeak approached the maximum limit of 5.0 × 10^4 CFU/g stipulated by the Indonesian National Standard (BSN, 2013). Although the product had a relatively long shelf life, additional measures are needed to improve its durability. The bacterial count of smoked fish can

be controlled through the choice of smoking technique, the hygiene of equipment and materials during production, and packaging and storage that comply with the standard (Sriwulan *et al.*, 2022).

Halfbeak smoking in Boalemo is still carried out traditionally, without attention to the hygiene of equipment and materials. The risk of exposure to microorganisms from the air and other sources is therefore considerable, even though smoke components can inhibit microbial growth. The food safety standard also specifies several bacteria that must be tested for in smoked fish products, namely Escherichia coli, Salmonella, and Staphylococcus aureus (BSN, 2013), which grow well at 30–37°C. A higher TPC indicates greater microbiological deterioration, and the value can serve as a reference for judging whether a product is suitable and safe for consumption. This matters because microbial contamination of food can cause foodborne diseases, which arise through two mechanisms: infection, which occurs when the number of bacteria in a product exceeds the permitted threshold, and intoxication, which is caused by toxins produced by certain microorganisms in the product (Rajkovic *et al.*, 2020).

Yeast and Mold Count (YMC)

The yeast and mold count (YMC) is another microbiological quality attribute of smoked fish products and is regulated by the Indonesian National Standard for hot-smoked fish, which sets a maximum limit of 1×10^2 CFU/g (BSN, 2013). In this study, the YMC of smoked halfbeak ranged from 3.55×10^3 CFU/g in the open storage treatment to 6.75×10^3 CFU/g in the control, with 6.14×10^3 CFU/g in the closed storage treatment (Figure 2). Analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on YMC ($p < 0.001$), and Duncan's multiple range test indicated that all treatments differed significantly from one another.

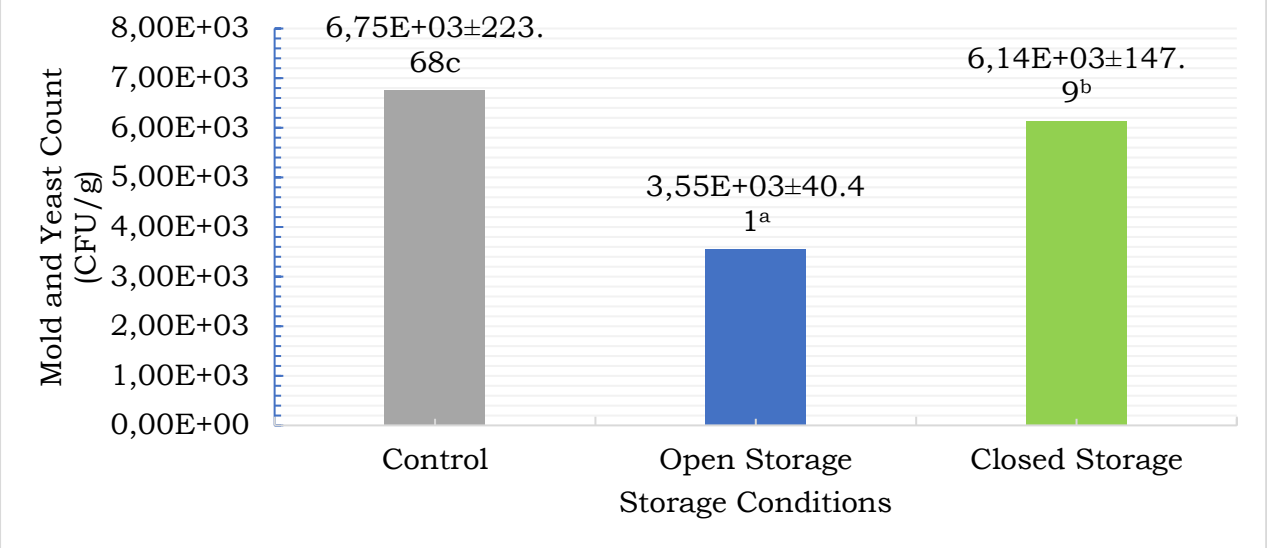


Figure 2. Yeast and Mold Count of Smoked Halfbeak under Different Storage Conditions

The high moisture content of the control (54.04%) not only supports bacterial growth but also creates favorable conditions for the proliferation of molds and yeasts, which resulted in a higher YMC than in the other treatments. These microorganisms tolerate low-moisture conditions better than bacteria, and when the moisture content of smoked fish remains high, the environment becomes highly favorable for their growth. Water activity (aw) above 0.90 is highly conducive to yeast development, whereas some mold species can grow at an aw as low as 0.80. Molds and yeasts in smoked fish not only degrade sensory quality, such as taste and aroma, but can also cause surface spoilage and, if the species are toxigenic, mycotoxin production. Foods with a high moisture content that are stored without temperature and relative humidity control are highly susceptible to colonization by molds and yeasts, particularly protein-based products such as smoked fish (Ferreira Júnior *et al.*, 2016).

The high YMC of the freshly smoked control may be attributed to cross-contamination during or after smoking. Although smoking reduces moisture content and inhibits microorganisms, molds and yeasts can persist or can contaminate the product from the air, equipment, unclean smoking surfaces, or the hands of workers with poor hygiene. In addition, if smoking does not reach a sufficiently high temperature or does not last long enough, the more heat-resistant yeasts and molds can survive. A humid and open smoking environment also increases the likelihood that airborne fungal spores settle on the fish surface. Alfaro *et al.* (2013) reported that smoked fish products are susceptible to microbial contamination from the surrounding environment, especially when sanitation during post-smoking handling is inadequate. The microbiological quality of smoked fish therefore depends heavily on environmental hygiene, equipment, and the handling procedures applied after smoking (Wang *et al.*, 2021).

The YMC of smoked halfbeak in all treatments, including the products stored for 3 months, exceeded the maximum limit of 1×10^2 CFU/g stipulated in BSN (2013). Exceeding this limit indicates a decline in food quality, including reduced nutritional value, changes in color, taste, and odor, and the appearance of spoilage. According to Sulistiawati *et al.* (2021), the molds that commonly grow on smoked fish, salted fish, and pindang (boiled salted fish) include *Aspergillus* spp. and *Penicillium* spp., with *Polypaecilum pisce* and *Aspergillus niger* being dominant. Molds readily adapt to a wide range of environmental conditions. Similarly, Akise *et al.* (2013) found that after six weeks of open storage, smoked *Lutjanus agennes*, *Mugil cephalus*, and *Chrysichthys walkeri* were contaminated by various fungi, including *Saccharomyces*, *Penicillium italicum*, *Penicillium oxalicum*, *Mucor* sp., *Rhodotorula* sp., and *Aspergillus* sp. The presence of molds warrants serious attention because some species are pathogenic and produce toxins that can cause serious health disorders and, in certain cases, death (Sulistiawati *et al.*, 2021).

Moisture Content

The moisture content of a food product is the total amount of water it contains, expressed on a dry basis (db) or a wet basis (wb). Keeping moisture content below the critical point for microbial growth can prevent product damage and quality deterioration (Waghmare *et al.*, 2023). On a wet basis, the moisture content of smoked halfbeak was highest in the control (54.04%), followed by the closed storage treatment (15.53%), and lowest in the open storage treatment (13.22%) (Figure 3). Analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on moisture content ($p < 0.001$). According to Duncan's multiple range test, the control differed significantly from both the open and closed storage treatments, whereas the open and closed storage treatments did not differ significantly from each other.

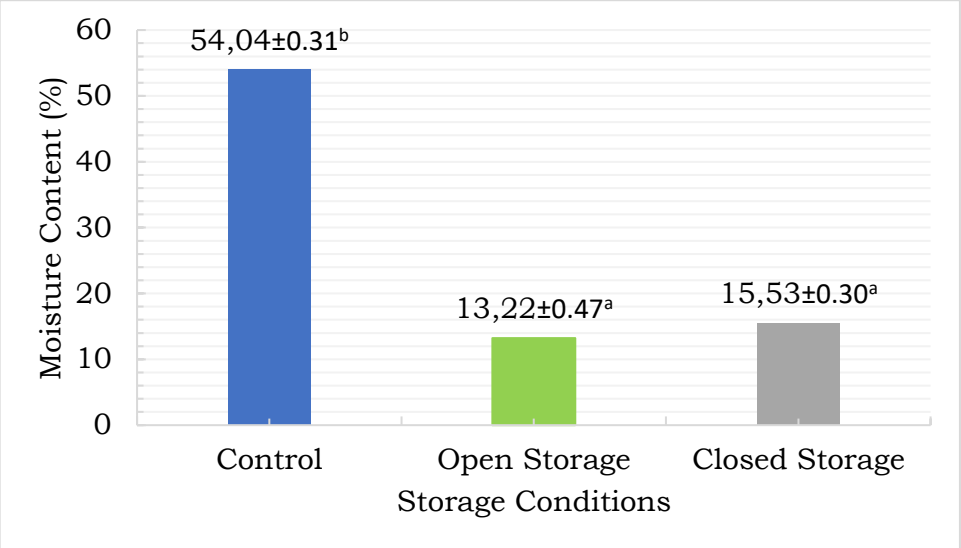


Figure 3. Moisture Content of Smoked Halfbeak under Different Storage Conditions

The longer and hotter the smoking process, the greater the amount of water lost from the fish (Adepoju *et al.*, 2018). The results of this study indicate that smoking for approximately 10 hours by the hot smoking method reduced the moisture content of halfbeak. According to Litaay *et al.* (2022), moisture content differs among fish because a constant smoking temperature is difficult to maintain in traditional processing. After 3 months of storage, the moisture content of smoked halfbeak was approximately 2.3 percentage points higher under closed storage than under open storage, although this difference was not statistically significant. The higher value may be explained by the absorption of water vapor in the closed storage environment: when ambient temperature and humidity are high relative to the humidity of the product, the product absorbs water from its surroundings and its moisture content increases (Kaparang *et al.*, 2013).

The moisture content of smoked halfbeak in the three treatments ranged from 13.22% to 54.04%. The quality standard for hot-smoked fish sets a maximum moisture content of 60% (BSN, 2013), so the smoked halfbeak met the standard established by the National Standardization Agency of Indonesia. These values were lower than the 71.01%–73.52% reported for hot-smoked fish by Andhikawati and Pratiwi (2021). Moisture content is a priority parameter for smoked fish because it affects product quality and durability (Wicaksono *et al.*, 2014). Fish with a moisture content of 10–15% can have a shelf life of 3 to 9 months if stored properly, and the concentration of chemical compounds in smoked fish depends greatly on storage duration and temperature (Usman, 2017).

Ash Content

Ash is an inorganic substance remaining after the combustion of organic matter. Approximately 96% of food materials consist of organic matter and water, while the remainder consists of mineral elements. Minerals in the body serve structural and regulatory functions. Ash content is associated with the mineral composition of a material. Minerals in a material may exist as organic or inorganic salts, and their quantities can be determined by measuring the combustion residue of mineral salts through a process known as ashing (Quirino *et al.*, 2022). The results showed that the ash content of smoked julung-julung fish varied according to the storage treatment. The highest ash content was recorded in the control treatment (3.22%), followed by the closed storage treatment (1.32%), while the lowest was observed in the open storage treatment (1.23%) (Figure 4). The low standard deviation values across all treatments indicate that the measurements were relatively consistent among replicates. These differences suggest that storage, particularly under open and closed conditions, may have caused the dissolution and loss of inorganic minerals through evaporation or chemical degradation reactions, resulting in reduced ash content. This reduction may also be attributed to interactions between moisture and temperature, which accelerate the degradation or leaching of ash components during storage.

The results of the analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on the ash content of smoked julung-julung fish ($p = 0.000 < 0.05$). Duncan's multiple range test further indicated that the control treatment differed significantly from both the open and closed storage treatments. However, no significant difference was observed between the open and closed storage treatments. During smoking, the reduction in moisture content is accompanied by an increase in the relative concentrations of proximate components, including inorganic minerals, in smoked fish products (Aba, 2013). Fresh julung-julung fish before processing had an ash content of 0.01% (Dotulong *et al.*, 2018). In the present study, the ash content of smoked julung-julung fish ranged from 1.23% to 3.22%, indicating that the smoking process increased the ash content of julung-julung fish products. According to Tenyang *et al.* (2020), this increase in ash content is directly associated with moisture reduction during drying, which increases the relative concentration of inorganic minerals in fish tissue.

The quality requirements for smoked julung-julung fish under different storage conditions, particularly regarding ash content, are not specified in the standards established by the National Standardization Agency (BSN, 2013). A systematic review by Andhikawati and Pratiwi (2021) reported that ash content ranged from 0.32% to 0.64%

in hot-smoked fish and from 1.00% to 2.70% in cold-smoked fish. Therefore, the ash content of smoked julung-julung fish stored under different conditions for three months in this study was considered to meet the quality standard.

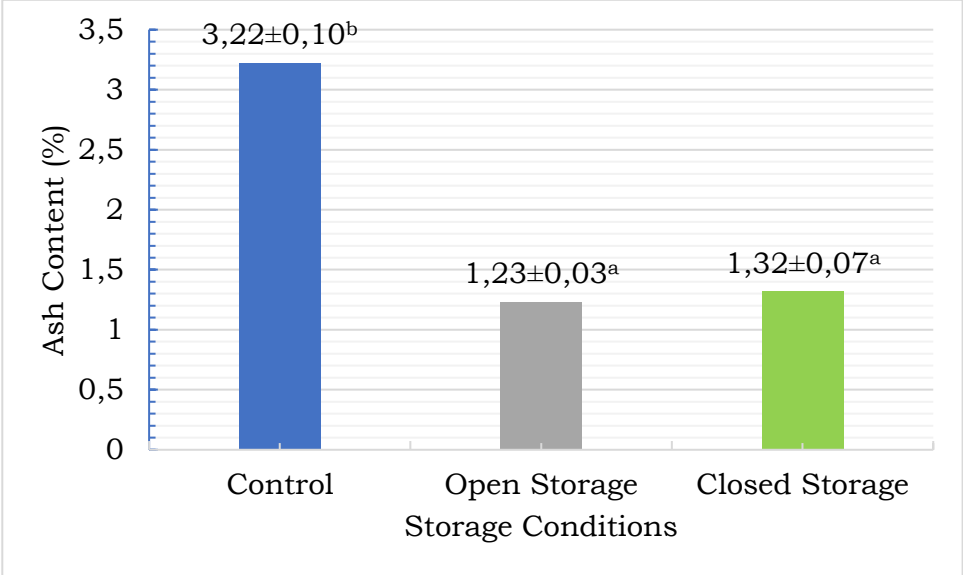


Figure 4. Ash Content of Smoked Halfbeak Fish under Different Storage Conditions

Fat Content

Fat is present in fish at lower levels than protein, but it plays an important role in the development of flavor and aroma in smoked fish, in which its content is generally higher than in fresh fish. Fresh halfbeak has a fat content of 1.77% (Siahaya, 2020), whereas the fat content of smoked halfbeak in this study ranged from 8.56% to 13.27% (Figure 5). During smoking, proximate values increase as moisture content decreases (Aba, 2013). The fat content was highest in the control (13.27%) and lower after 3 months of open storage (8.56%) and closed storage (8.57%). Analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on fat content ($p < 0.001$). According to Duncan's multiple range test, the control differed significantly from both the open and closed storage treatments, whereas the open and closed storage treatments did not differ significantly from each other.

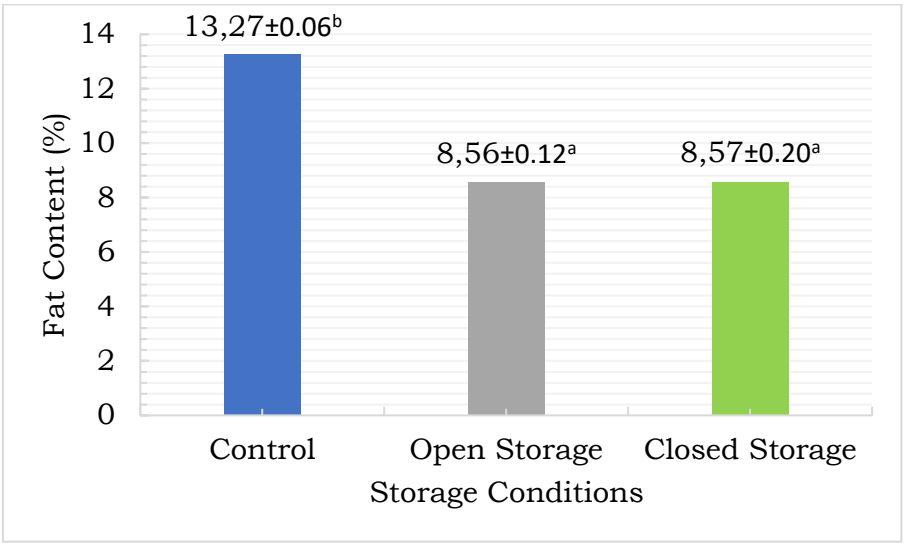


Figure 5. Fat Content of Smoked Halfbeak under Different Storage Conditions

The heating involved in smoking can also reduce fat content. Smoking takes place at a relatively high temperature that can cause fat degradation: heating accelerates the movement of fat molecules and increases the distance between them. The higher the

temperature and the longer the heating time, the more likely fat is to be lost through evaporation or release from the fish tissue, which lowers the fat content of the final product (Sriyani *et al.*, 2019).

Exposure to air during storage also affects fat content. Fat in smoked fish is highly susceptible to oxidation because fish contain high concentrations of polyunsaturated fatty acids (PUFA), which, when exposed to air, are converted into free fatty acids and glycerol (Ahmed *et al.*, 2016). The fat content of smoked halfbeak in all treatments, including the products stored for 3 months under open and closed conditions, complied with the maximum of 20% set by the quality standard of the National Standardization Agency of Indonesia (BSN, 2013).

Protein Content

Protein is the largest component of fish after water, which makes fish a highly perishable food. Fresh halfbeak has a protein content of 23.19% (Siahaya, 2020), whereas the protein content of smoked halfbeak in this study ranged from 24.74% to 35.48% (Figure 6). During smoking, proximate values increase as moisture content decreases (Aba, 2013). The protein content was highest in the control (35.48%), followed by the closed storage treatment (26.74%), and lowest in the open storage treatment (24.74%). Analysis of variance (ANOVA) showed that storage conditions had a highly significant effect on protein content ($p < 0.001$), and Duncan's multiple range test indicated that all treatments differed significantly from one another.

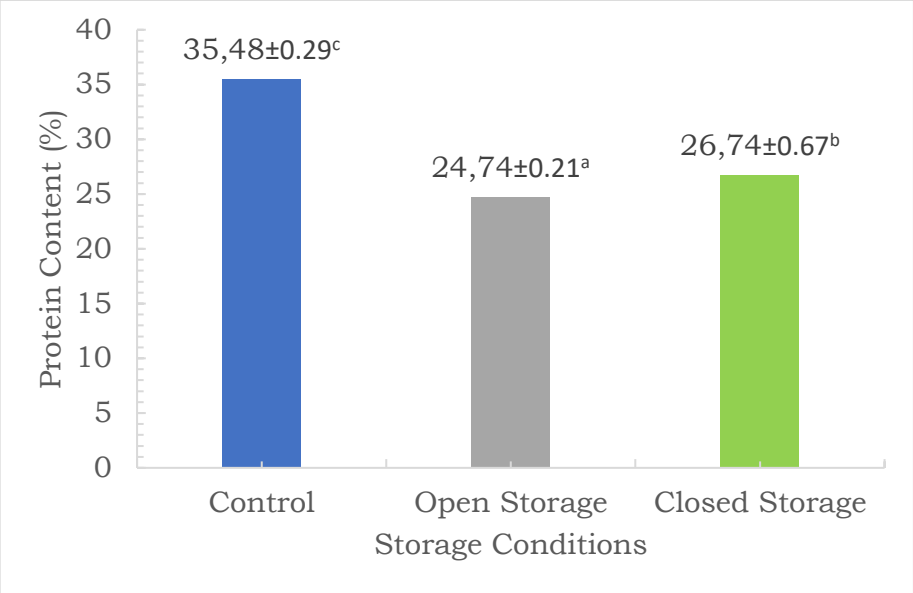


Figure 6. Protein Content of Smoked Halfbeak under Different Storage Conditions

Smoking can also reduce protein. Protein reduction can be caused by the denaturation of nitrogen-containing compounds at high temperatures and during prolonged smoking [citation]. Protein coagulates at 50°C or higher, and the reaction between lysine and carbonyl compounds reduces the protein quality of smoked fish, so smoking should be carried out at the lowest possible smoke density (Hallik *et al.*, 2021).

The lower protein content after storage represents a loss of nutritional value. Protein is degraded during storage, and open storage conditions allow the growth of bacteria that produce proteolytic enzymes, namely aerobic Gram-negative bacteria such as *Pseudomonas* spp. and *Shewanella* spp. Andhikawati and Pratiwi (2021) stated that the threshold for Gram-negative psychrotrophic bacteria in hot-smoked fish products is < 1 log CFU/g. Microbial proteolytic enzymes break proteins down into small peptides and free amino acids, which can then be converted into ammonia and biogenic amines by deaminases and decarboxylases (Yi & Xie, 2021). These changes alter the aroma of the fish and lead to quality deterioration.

Protein oxidation during storage can also reduce the nutritional quality of protein. Husain *et al.* (2017) found that increasing the storage temperature from 0°C to 40°C increased the rate of protein oxidation in red snapper (*Lutjanus* sp.), as indicated by an increase in protein carbonyl content. Protein content is not regulated by the quality standard for hot-smoked fish (SNI 2725, 2013). In a systematic review, Andhikawati and Pratiwi (2021) reported protein contents of 16.11%–52.13% for hot-smoked fish, and the protein content of smoked halfbeak in all treatments of this study fell within that range.

CONCLUSION

Storage conditions significantly affected the microbiological and chemical quality of smoked halfbeak. The total plate count (TPC) remained below the maximum limit of SNI 2725:2013 in all treatments (control, open storage, and closed storage for 3 months), with the lowest value under open storage and the highest under closed storage. In contrast, the yeast and mold count (YMC) exceeded the permissible limit in all treatments, including the control, which indicates that contamination was already present after smoking. Moisture and fat contents complied with the standard in all treatments, whereas ash and protein contents, which the standard does not regulate, were lower after storage than in the control. Smoked halfbeak therefore remained acceptable in terms of TPC, moisture, and fat after 3 months of storage, but improved hygiene during smoking and post-smoking handling is needed to bring yeast and mold contamination within the standard.

AUTHOR CONTRIBUTIONS

The authors declare their contributions to this work as follows: DD, as the lead and corresponding author, contributed to research design, conceptualization, data collection, data analysis, validation, manuscript editing, and additional discussion. IA, AFH, YA, SYN, and YB, as members, contributed to research design, conceptualization, data collection, data analysis, validation, and manuscript editing. The authors have attached the signed Author Declaration Form.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the conduct of the research, data analysis, or preparation and publication of this article.

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