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Assessing diversity index of contaminant fungi in traditional home-made salted fish: Implications for public health in a tropical setting

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ABSTRACT

Introduction: Fungal contamination poses a significant threat to the quality of salted fish products, leading to both economic losses and health concerns. The aim of this study is to elucidate the profile of contaminant fungi in traditional home-made salted fishes and assess their diversity index.

Methods: Samples of salted fish were collected from Lhok Seudu, Aceh Besar, Indonesia, following approximately three months of storage. The samples were salted barred queenfish (*Scomberoides tala*), red snapper (*Lutjanus compchanus*), and blackfin snapper (*Lutjanus buncanella*). After diluted to a 10^{-6} concentration, the sample was inoculated onto potato dextrose agar media for 5-7 days of incubation. Grown fungal colonies were enumerated, and distinct macroscopic variants were isolated for further analysis, with subsequent incubation periods of 5-7 days. Observations encompassed both macroscopic and microscopic characteristics of the fungi.

Results: The findings suggested that the appearance of Aspergillus species, such as *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus*, was prevalence. The diversity index of contaminant fungi in the salted fish products was 1.15, categorized as having moderate diversity.

Conclusion: Indonesian public health authorities should maintain a heightened awareness of the potential threat posed by mycotoxins in traditional home-made salted fish. The moderate diversity of fungal contamination highlights the probability of exposure to a range of fungal contaminants, each presenting distinct health risks.

KEYWORDS

Fungal contamination; salted fish; *Aspergillus* sp; diversity index; food safety.

INTRODUCTION

The presence of various contaminants in food arises from factors such as contaminated ingredients, manufacturing techniques, and storage methods, among others. Notably, mycotoxins produced by fungi represent a significant concern due to their adverse effects on the shelf life and safety of food items. Indonesia's tropical climate, characterized by high humidity, temperatures, and rainfall, creates an environment conducive to fungal growth and mycotoxin production[1]. This poses serious risks to food and feed safety, leading to economic losses and health hazards for both humans and animals[2]. Despite the traditional sun-drying methods used in salted fish production, variations in weather conditions can impact drying efficiency, leaving the product vulnerable to contamination by Aspergillus fungi, notably *Aspergillus flavus*[3,4]. Additionally, issues such as inadequate raw materials and processing techniques, as well as formalin contamination, contribute to the degradation of salted fish quality[5,6].

Given the potential health risks associated with high levels of fungal contamination, understanding the diversity of contaminant fungi is paramount[4]. Fungal contamination, characterized by factors such as colony morphology and hyphal structure, presents significant challenges in various industries,

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including food preservation, where salted fish products are particularly vulnerable[3]. Despite salted fish being a traditional preservation method relying on high salt content to inhibit microbial growth, fungal contamination remains a persistent concern, especially under suboptimal storage conditions[7]. The presence of fungi such as Aspergillus species underscores the necessity for stringent quality control measures to mitigate health hazards effectively. This paper investigates the factors influencing fungal contamination in salted fish products, emphasizing the urgent need for comprehensive strategies to safeguard food safety and public health.

Coastal areas like Lhok Seudu in Aceh Besar, known for their culinary specialties like salted fish, present an ideal setting for studying fungal contamination in salted fish products. Lhok Seudu has high fish production, traditional fish processing methods, and reliance on manual techniques. Traditional sun-drying methods, such as drying fish on bamboo racks in open yards, may expose the fish to environmental factors, potentially leading to fungal contamination. Mitigating public health risks associated with fungal contamination in foods manufactured by small-scale enterprises employing traditional processing methods is a critical priority, notably in Indonesia, where such practices are commonplace[8]. Therefore, this research aims to investigate the characteristics and diversity of contaminant fungi in salted fish products, emphasizing the implications for food safety and public health. By elucidating the fungal contamination profile and evaluating its impact on salted fish quality, this study seeks to contribute to the development of strategies for ensuring food safety and enhancing the quality of food products in coastal communities and beyond.

METHODS

Specimen and materials

The materials used in this study included analytical grade potato dextrose agar (PDA) medium, distilled water, ethanol 70%, and methylene blue dye procured from Merck (Selangor, Malaysia).

Samples investigated in this research were three salted fishes of different varieties, namely barred queenfish (*Scomberoides tala*), red snapper (*Lutjanus compchanus*), and blackfin snapper (*Lutjanus buncanella*), procured from a home-based industry in Lhok Seudu, Aceh Besar (Figure 1). Upon procurement, the salted fish samples were placed in sterile plastic bags and labeled accordingly. Subsequently, the samples were transported to the Microbiology Laboratory for the identification and diversity assessment of contaminant fungi[9].

Isolation of fungi

Fungi isolation from salted fish was conducted through dilution method. The samples, each weighing 10 grams, were individually homogenized with 90 mL of sterile distilled water. Subsequently, a series of dilutions was prepared using a graduated dilution technique with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} concentrations[10]. The final dilution was used for further processing. The suspension obtained from the final dilution was then isolated by spread-plating onto PDA medium and incubated at room temperature (27±1°C) until fungal growth was observed. Fungal colonies that emerged from the isolation process were quantified using the total plate count (TPC) method, wherein each viable cell was considered ca-

Figure 1. A variety of traditionally processed salted fishes being displayed for sale in Lhook Seudu (a). A close photograph of salted fishes purchased in Lhok Seudu and used in the research (b)

pable of forming a single colony. Separated fungal colonies were then purified through single colony isolation techniques. The enumeration of fungal colonies (Eq. 1) followed established protocols[11,12].

$$N = n \times \frac{1}{fp} \qquad (1)$$

Where *N* represents the number of cells per mL or gram of the sample, *n* – the number of colonies on the agar plate, and *fp* – the dilution factor.

Characterization of isolated fungi

The observation of fungi was conducted based on macroscopic characteristics, including pigment and colony features (color of the upper surface and underside of the colony). Microscopic examination involved observing fungal colonies on agar plates using a sterile needle, placing them on a microscope slide, and staining with methylene blue. Microscopic observations encompassed the shape and size of conidia, vesicles, conidiophores, hyphae, and the presence or absence of septa[13]. Subsequently, the isolated contaminant fungi were described based on both macroscopic and microscopic appearances to identify their respective species or genus. Morphological observations were conducted to discern the fungal species, following the suggestions of published report[14]. To determine the diversity index of fungal species, the Shannon-Wiener formula was utilized as previously suggested[15]. The Shannon-Wiener diversity index (H') was employed to quantify species diversity using the formula (Eq. 3).

$$Pi = ni/N \qquad (2)$$

$$H' = \sum_i^s Pi\ Ln\ Pi \qquad (3)$$

Where Pi represents the proportion of the overall community comprised of species "i", ni – number of individuals of species "i", N – total number of individuals of all species, and s – number of species.

RESULTS

Macroscopic observations

Macroscopic images of the fungal colonies obtained from salted *S. tala*, *L. compchanus*, and *L. buncanella* are presented in Figure 2. All isolates from the *S. tala* had dark green

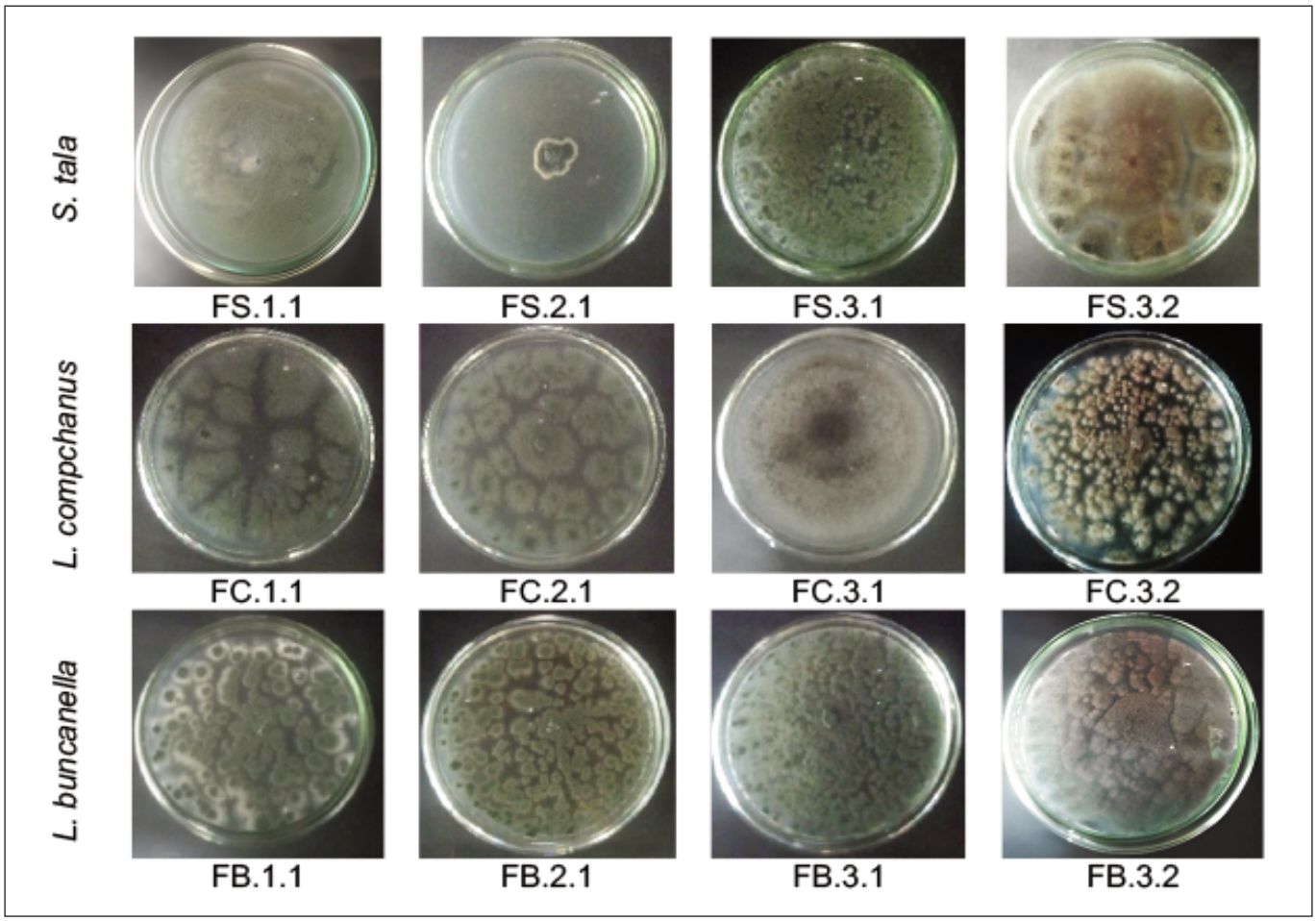

Figure 2. Macroscopic appearance of fungal isolates from salted *S. tala* (FS), *L. compchanus* (FC), and *L. buncanella* (FB)

color on the upper surface, with greyish-green (FS.1.1), whitey-green (FS.2.1), and pale yellowish-white (FS.3.1.) and greyish-yellow colors (FS.3.2) on the underside. The upper surface of two isolates from *L. compchanus* was dark green, but one had dark green (FC.1.1) and the other one had greyish-yellow (FC.2.1) on the underside. The other two of the *L. compchanus* fungal isolates (FC.3.1 and FC.3.2) were whitey-black and light brown on the upper surface, respectively. As for the underside, the colony of FC.3.1 had grey color with pale yellowish white in the center, while the colony of FC.3.2, the colony had only pale yellowish white color. Dark green appearance of the upper surface was observed in three isolates from *L. buncanella* (FB.1.1, FB.2.1, FB.3.1), but the underside appeared with light brown (FB.1.1) and pale green (FB.2.1, FB.3.1). A single isolate from *L. buncanella* (FB.3.2) was observed having violet-brown color on the upper surface and pale grey color on the underside.

Microscopic observations and species assignment

Results of the microscopic observation on the isolates, following the staining using methylene blue, are presented in Figure 3. Isolates FS.3.1, FC.2.1, FC.3.2 were assigned as *Aspergillus* sp. after observing their microscopic and macroscopic appearance. Microscopically, they exhibited septate and branching hyphae, consistent with the characteristics described previously[16], indicating their classification within the genus Aspergillus. Specifically, the presence of septate hyphae with conidiophores emerging from swollen foot cells (mycelium) containing sterigmata and the formation of green, black, and brown conidial chains were noted. Additionally, macroscopically, these isolates displayed filamentous, finely textured, convex colonies with colors ranging from grayish-green, greenish-brown, black, to white, as described in a previous report[17]. The color of the spores could be influenced by the colony color. The erect conidiophores, terminating in well-covered vesicles by layers of phialides or subtending cell layers, further supported their identification as *Aspergillus* sp.

FS.1.1, FC.1.1, and FB.1.1 were identified as *A. fumigatus* because they exhibited dark green colonies with granular and compact morphology. Microscopically, they displayed oval chains of conidia attached to one or two rows of regularly arranged sterigmata on the surface of vesicles. Additionally, their conidial heads showed a distinctive columnar shape, with short conidiophores, thin-walled structure, predominantly green coloration (especially at the

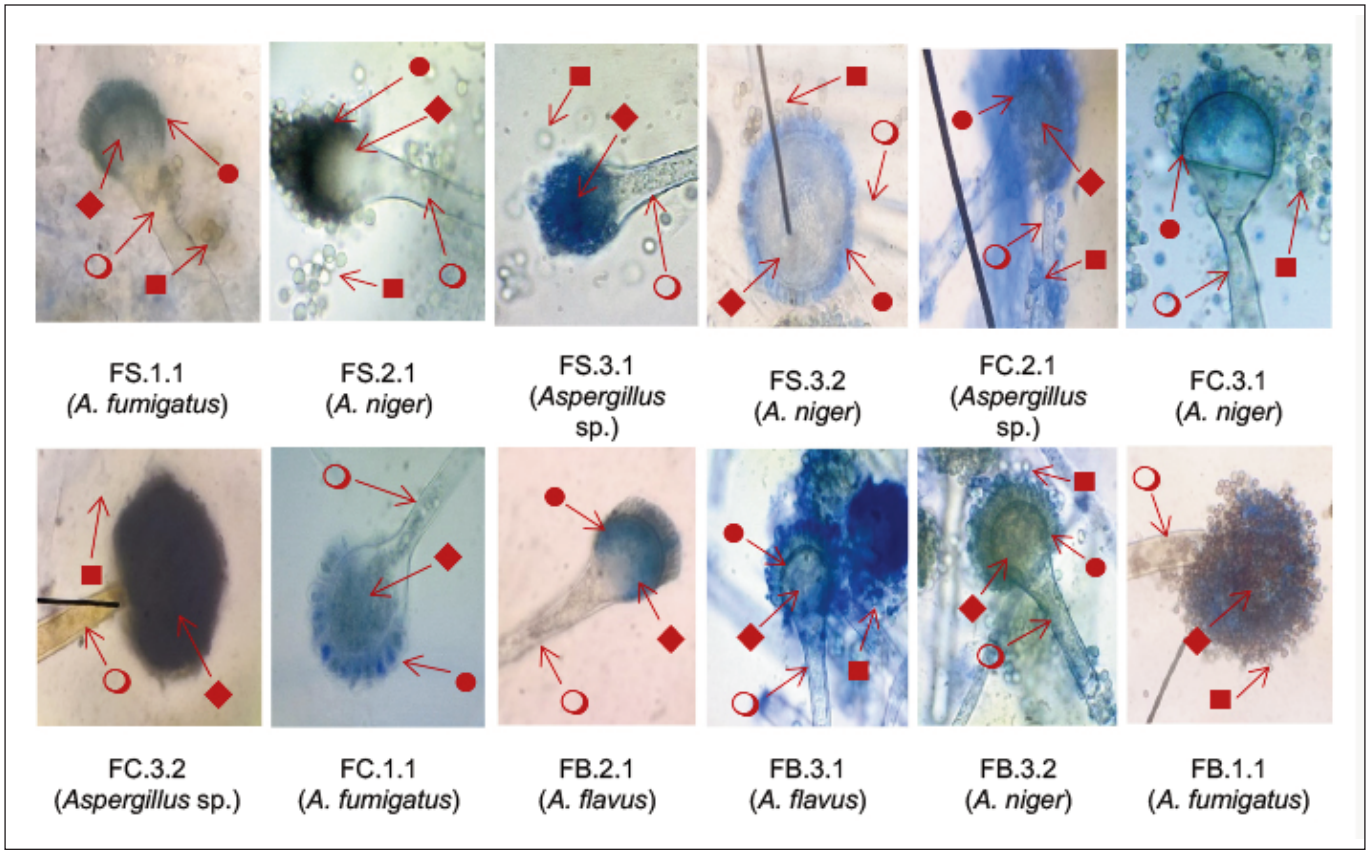

Figure 3. Microscopic appearance of fungal isolates from salted *S. tala* (FS), *L. compchanus* (FC), and *L. buncanella* (FB). (n) Conidia; (l) phialid; (u) vesicle; (m) conidiophore

top), and vesicles resembling wide clubs. The phialides were directly formed on the vesicles, often greenish in color. Moreover, their conidia were round to semi-round, green in color, and had rough to spiny walls. Furthermore, these strains of *A. fumigatus* were noted to produce secondary metabolites known as mycotoxins, capable of inducing various symptoms and signs depending on the affected organ, dosage, and type of mycotoxin produced. The colony morphology and microscopic characteristics observed align closely with the descriptions provided in previous studies[17,18].

Macroscopic and microscopic observation suggest that FS.2.1, FS.3.2, FC.3.1, and FB3.2 exhibit characteristics consistent with *A. niger*. These isolates displayed black, dark brown, or violet-brown conidia, with an enlarged, globose upper part forming a glucose-like structure. Additionally, their conidiophores appeared smooth and colorless at the top, transitioning to a yellow-brown color toward the base. The vesicles resembled glucose structures, with an enlarged upper part and a small stem-like end, while their conidia exhibited rough surfaces, suggesting ridges or bands and often appeared blackish-brown. Furthermore, *A. niger* is characterized by wide conidiophores ranging from dark brown to black, with black, round conidial heads that tend to crack into columns in older colonies. The conidiophores typically have smooth walls, sometimes with a brownish tint, and the vesicles are spherical to semi-spherical, with a diameter of 50-100 µm. The conidia are round to semi-round, brown in color, with irregular protrusions and irregularly spaced spines. These characteristics of *A. niger* have been described in previous studies[19].

A. flavus was only identified in isolates from *L. buncanella*, namely FB.3.1 and FB.3.2, as suggested by the macroscopic and microscopic observations. Macroscopically, the colonies of *A. flavus* exhibited white margins and green-colored fungal colonies, with a cotton-like appearance on the upper surface and a yellow to brown color on the lower surface. Additionally, microscopic examination revealed long conidiophores, semi-spherical to spherical vesicles, and round to semi-round conidia, all of which were green in color. Moreover, colonies of *A. flavus* displayed pale brown, yellowish-green, gray to black colors, with some colonies appearing dark green and sand-like. The colorless conidiophores had slightly rounded tops, and the conidia were coarse with various colors, approximately 1 mm in size, and located just below the typically rough spherical vesicles. Similar descriptions have been reported previously[17,19].

Diversity index

The diversity index and data underlying the calculation are presented in Table 1. One genus of fungi and three species were found, namely Aspergillus sp, *A. flavus*, *A. niger*, and *A. fumigatus* were identified from 3 salted fishes of different species. For each fungal species, there were 9 individuals of Aspergillus sp, 194 individuals of *A. flavus*, 234 individuals of *A. fumigatus*, and 231 individuals of *A. niger*. The diversity index, according to Shannon-Wiener equation, was found to be 1.15. The number suggests that the diversity of contaminant fungi isolated from the salted fish is moderate.

DISCUSSION

The present study identified several types of fungi isolated from the salted fish, including *Aspergillus* sp, *A. niger*, *A. fumigatus*, and *A. flavus*. The *Aspergillus* sp obtained from the salted fish exhibited macroscopic characteristics such as a dark green or dark brown colony color with irregularly round shape. The diversity of contaminant fungi in the salted fish was moderate, as indicated by a Shannon-Wiener diversity index (H') of 1.15. Ideally, fungi are relatively more prevalent in

Table 1. Biodiversity index of contaminating fungi

Identified fungi	Number	Pi	Ln Pi	Pi x Ln Pi
Family *Trichocomaceae*				
A. fumigatus	234	0.35	-1.05	-0.37
A. niger	231	0.35	-1.06	-0.37
Family *Trichocomaceae*				
Aspergillus sp	9	0.01	-4.31	-0.06
A. flavus	194	0.29	-1.24	-0.36
Total	668			H'= 1.15

Note: Pi, the proportion of the overall community comprised of species "i"; Ln Pi, natural logarithm form of "Pi"; Pi x Ln Pi, "Pi" multiplied by the "Ln Pi".

low salt concentrations (<4%), with some hindered in their growth at 20% salt concentration and unable to survive at 30% [20]. However, incomplete drying processes due to the fish thickness could lead to fungal contamination, particularly by Aspergillus[3].

Factors influencing fungi growth on foodstuffs include humidity, temperature, nutritional needs, and high ambient humidity, facilitating moisture absorption and fungi proliferation[21]. The fungi strains that develop on salted fish, termed halophilic fungi, thrive using NaCl or glucose as substrates, enabling their survival in dried fish. Salted fish is prone to microbial contamination, with deterioration stemming from enzymatic activity and microorganism proliferation[3]. Negligence in sanitation, hygiene, and insufficient knowledge of salted fish quality among sellers exacerbate concerns regarding its quality.

Fungal activity significantly contributes to the deterioration of traditional processed fish product quality, particularly with *Aspergillus* sp. being the predominant fungal species affecting salted fish[4,22]. This leads to chemical and physical alterations, including increased weight or quantity, elevated storage temperature, and the onset of odor. Aspergillus, renowned for its resilience in adverse environments, thrives in high salinity conditions, with *A. niger* exhibiting robust growth at a salinity level of 50% [23]. Temperature plays a crucial role in cellular metabolism, with high temperatures leading to protein denaturation. Studies have previously identified dominant fungal species in salted fish, including *A. fumigatus* and *A. niger*[7,24,25]. *Aspergillus* sp., particularly *A. flavus*, prevails in fermented salted fish, capable of producing aflatoxin[26,27]. This heat-resistant species, *A. flavus*, is prevalent in fermented salted fish, contributing to aflatoxin production[28]. Aflatoxin, prevalent in hot and humid climates, is a natural contaminant produced by Aspergillus species, posing health risks such as Aspergillosis, allergic reactions, and respiratory issues[1]. *A. niger*, capable of producing ochratoxin, induces allergic reactions and respiratory hypersensitivity[29]. Aflatoxin B1, a potent toxin and carcinogen, inhibits growth, swelling, and immune suppression, thereby contributing to human cancer risks[30].

The diversity of contaminant fungi on salted fish is categorized as moderate, indicated by an index (H') of ≤ 1 to ≤ 3. This suggests a relatively stable distribution of fungal species and communities within a given area. Factors contributing to this moderate diversity include stable species dispersal and community presence, as outlined by a previous study[31]. These findings imply that not only salted fish can serve as a habitat for fungi, but it also provides conditions suitable for fungal growth. Nonetheless, while some fungi may thrive in the environment provided by salted fish, others may be inhibited by the absence of sufficient organic material for growth.

To mitigate the risks associated with fungal contamination, several recommendations can be proposed. Firstly, implementing robust sanitation practices during the processing and handling of salted fish can help minimize fungal contamination. This includes maintaining clean processing facilities, proper storage conditions, and regular equipment sanitation. Secondly, monitoring the quality of salted fish products through routine inspections and testing for fungal presence can help identify and address contamination issues promptly.

CONCLUSION

Based on the research findings, it can be concluded that salted fish products from Lhok Seudu are contaminated with characteristic fungi, including Aspergillus species such as *Aspergillus* sp, *A. flavus*, *A. niger*, and *A. fumigatus*. These fungi are commonly associated with food contamination and pose potential health risks if consumed. Moreover, the diversity index of contaminant fungi in these salted fish products falls within the moderate category, as indicated by an H' value of 1.15. This suggests a moderate level of fungal diversity present in the sampled fish products. Consequently, there is a pressing need to develop improved preservation techniques for fish, with reduced salt content, to mitigate the risk of fungal contamination. Standardized processing and storage procedures for salted fish should be implemented to ensure consistent quality and safety. This includes strict adherence to hygiene practices during processing, such as thorough cleaning and sanitization of equipment and facilities, as well as adequate salt concentration and proper storage conditions to inhibit fungal growth. Furthermore, ongoing monitoring and quality control measures should be established to detect and address any potential contamination issues promptly.

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