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A Feast or a Threat? Multi-Mycotoxin Contamination in Sun-Dried Fish, Collected from Al Mukha Markets, Taiz, Yemen

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Abstract:

In the bustling fish markets of Yemen, where sun-dried Wazef has nourished generations, our study uncovered a troubling paradox beneath its protein-rich surface. Analysis of 20 samples revealed excellent nutritional quality (47.07% protein, 22.48% lipids, 9.34% moisture), yet all carried an alarming burden of toxic fungi. Among 142 fungal isolates tested, 66 (46.5%) produced mycotoxins, with *Aspergillus* (67%) and *Penicillium* (24.7%) species predominating. Alarmingly, we detected multiple mycotoxins in 95% of samples, including co-occurrence of aflatoxins (B1, B2, G1, G2) in 40% of samples, often alongside Citrinin (30%), Ochratoxin A (20%), and Sterigmatocystin (30%). The most prolific producers were *A. flavus* (55.6% toxigenic) and *P. citrinum* (76.9% toxigenic), which also exhibited potent enzymatic activity (protease: 78%; lipase: 89.4%). This widespread multi-mycotoxin contamination, combined with the spoilage potential of fungi, creates a perfect storm of nutritional degradation and health risks. Our findings expose a critical paradox: a food designed to sustain coastal communities may instead jeopardize their health through chronic exposure to carcinogenic compounds. The study sounds an urgent alarm for implementing controlled drying technologies and stricter food safety measures to preserve both the nutritional benefits and safety of this culturally vital food source.



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INTRODUCTION

Sun-dried fish represents a traditional and economically crucial food product in numerous coastal regions, including Yemen, where it serves as a critical source of nourishment and livelihood for local populations (FAO, 2021). This preservation technique, relying on natural sunlight and open-air drying, is extensively employed due to its low cost and simplicity. However, the sun-drying process often lacks regulated environmental conditions, rendering the product vulnerable to contamination by diverse microorganisms, including fungi (Adebayo-Tayo *et al.*, 2020). The fungal colonization of sun-dried fish is a significant concern, as many fungi produce harmful secondary metabolites known as mycotoxins, which pose serious health hazards to consumers (Eskola *et al.*, 2022).

Mycotoxins, such as aflatoxins, ochratoxins, and fumonisins, are highly toxic compounds that can induce a range of deleterious health effects, including carcinogenicity, liver damage, kidney toxicity, and impairment of the immune system (Alshannaq and Yu, 2021; Iqbal *et al.*, 2021). These harmful toxins pose a particularly grave concern in developing nations, where food safety regulations and monitoring systems are frequently inadequate, and populations may already grapple with nutritional and health challenges (Wu *et al.*, 2022). The extensive literature has well-documented the global burden of mycotoxin contamination in food products, with estimates suggesting that up to a quarter of the world's food supply may be tainted by these toxins (Eskola *et al.*, 2022). Nonetheless, no research has been conducted on the prevalence of mycotoxin contamination in sun-dried fish, especially in regions like Yemen, where this food item serves as a dietary staple.

Fungi associated with sun-dried fish not only pose a concern due to mycotoxin production but also through their enzymatic activities, which can contribute to food spoilage and nutritional degradation. Many fungal species secrete extracellular enzymes, such as proteases, lipases, and amylases, that can degrade proteins, fats, and carbohydrates, respectively

(Frisvad *et al.*, 2019). These enzymatic processes can alter the texture, flavor, and nutritional value of sun-dried fish, thereby reducing its quality and marketability. Furthermore, certain fungal genera, particularly *Aspergillus*, *Penicillium*, and *Fusarium*, are well-documented producers of mycotoxins and are frequently isolated from food products processed under suboptimal conditions (Iqbal *et al.*, 2018; Pitt and Hocking, 2021). The lack of controlled drying conditions, coupled with inadequate storage facilities, exacerbates the risk of fungal contamination and mycotoxin production in sun-dried fish, especially in resource-limited settings (Adebayo-Tayo *et al.*, 2020).

This investigation aims to address the critical gap in knowledge by examining the fungal diversity associated with Yemen's sun-dried fish, evaluating the enzymatic potential of these fungi, and qualifying the presence of mycotoxins. By integrating microbiological, biochemical, and toxicological analyses, this research endeavors to provide a comprehensive understanding of the risks posed by fungal contamination in sun-dried fish and to inform strategies for enhancing the safety and quality of this vital food resource. The findings of this study hold significant implications for public health, food security, and the development of sustainable preservation techniques in resource-limited settings.

MATERIALS AND METHODS

Sample collection

Sun-dried fish samples were collected from Al Mukha district, located in the Taiz governorate of Yemen, a region renowned for its traditional fish-drying practices. A total of 20 samples were randomly collected from local markets and drying sites during the dry season (March to May 2024) to ensure consistency in environmental conditions. Samples included commonly consumed fish Sardine (Clupeidae) (Wazef). Sampling was performed according to (ISO 2859-1 Standards). Each sample (100~150 g) was kept in a sterile polyethylene bag, labeled, and transported to the laboratory under refrigerated conditions (4°C) to prevent further

microbial growth, till chemical, mycological, mycotoxins and enzymatical investigations. All the analysis was performed in the laboratories of Faculty of Agriculture, Sana'a University.

Determination of the proximate composition

The proximate composition includes moisture by oven drying method (method no. 925.09), crude protein by Kjeldahl method (method no. 978.04), crude lipid by Soxhlet method (method no. 930.09), ash content by muffle furnace method (method no. 930.05), and carbohydrate content was determined using the subtraction method. This was done by adding the moisture content, crude protein, crude fat, ash, and crude fiber and subtracting from 100.

$$\% \text{ Carbohydrate} = [100 - \% (\text{moisture} + \text{crude protein} + \text{crude fat} + \text{ash} + \text{crude fiber})]$$

All the determinations were made in triplicates and were analyzed using standard procedures outlined in the Association of Official Analytical Chemists method (AOAC, 2005).

Determination of pH

The fish samples were weighed in triplicates, weighing two grams each. Fish slurry was made by adding water and thoroughly mixing. The Beckman pH meter was used to take the readings. After the electrode had been cleaned and submerged in the fish slurry, the pH values were noted.

Determination of mycoflora

Fungal isolation and identification were conducted using the dilution-plate method described by Johnson and Curl (1972). Potato-dextrose agar was employed to isolate fungal species. Chloramphenicol was added to the media as bacteriostatic agents. For each sample, three plates were used. The plates were incubated for five days at $25 \pm 2^\circ\text{C}$. After that, the fungi that were growing were recognized, counted, and measured per gram of fresh weight fish. Numerous studies were employed for the identification of the isolated fungal species (Ellis, 1971 & 1976; Gilman, 1975; Raper and Fennel, 1977; Pitt, 1979 & 1991; Domsch *et al.*, 1980; Ramirez, 1982; Sivanesan, 1984; Kozakiewicz,

1989; Moubasher, 1993; Echevarría and Iqbal, 2021; Samson *et al.*, 2023).

Mycotoxin analysis

For qualitative mycotoxin detection, thin layer chromatography technique was performed using precoated silica gel 60 plates (E, Merck, Germany). Aflatoxins B1, B2, G1 & G2, Ochratoxin, Sterigmatocystin, Citrinin, T-2 toxin, Penicillic acid and Patulin were applied as standard references. Ethyl acetate-hexane (v/v, 30:70) was the developing solvent system, and the developed plates were examined under a UV lamp (AOAC, 1980; Dorner, 1998). Confirmation of mycotoxins was achieved by comparing the Rf values with those of standard mycotoxin solutions.

Screening for extracellular enzymes production by isolated fungi

Fungal proteolytic activity was tested using a solid casein hydrolysis media (Paterson and Bridge, 1994). Casein hydrolysis is indicated by the formation of a clear zone surrounding the fungal colony. The lipolytic activity of Wazef fungi was demonstrated in cultures grown on Ultman and Blasins (1974) agar medium which contained Tween 80. Production of lipolytic enzymes by a colony was observed either as a readily apparent precipitate due to the development of calcium salt crystals of oleic acid or as opaque zone surrounding the colony.

RESULTS AND DISCUSSION

The proximate analysis of sun-dried Wazef fish from Al Mukha markets reveals a nutritionally rich product with optimal preservation characteristics (Table 1), exhibiting protein (47.07 ± 0.18), lipid (22.48 ± 0.43), ash (17.35 ± 0.11) and carbohydrates (3.76 ± 0.02) contents that compare favorably with similar dried fish products across different regions.

Table 1. Proximate composition (%) of sun-dried fish samples (Wazef) collected from Al Mukha markets*

Parameters	Average \pm SDa
Moisture%	9.34 \pm 0.16
pH	6.95 \pm 0.43
Ash%	17.35 \pm 0.11
Lipids%	22.48 \pm 0.43
Protein%	47.07 \pm 0.18
Carbohydrates%	3.76 \pm 0.02

*Average of 20 samples. a: Standard deviation using New Duncan's Multiple Range Test.

The moisture content (9.34%) is notably lower than values reported in several studies (Flowra *et al.*, 2012: 14.06-24.58%; Al Mamun *et al.*, 2024: 10.30-17.50%; Adebami *et al.*, 2024: 12.99-17.82%), indicating superior drying efficiency and storage stability, aligning closely with the ideal 9.27% reported by Akinneye *et al.* (2010) for well-preserved dried fish. The protein content exceeds that of Banda *et al.* (2023) (12.81%) and falls within the range observed by Shamsan and Al Maqtari (2018) (32.63-55.92%) for Yemeni dried fish, and Kalita *et al.* (2024) (16.45-54.11%), though lower than the 59.13-72.02% reported by Al Mamun *et al.* (2024) for experimentally dried Bangladeshi fish and 60.61% mentioned by Siddiky *et al.* (2017). The lipid content is higher than values from Adebami *et al.* (2024) (0.49-6.00%) and Paul *et al.* (2018) (2.74-15.44%), but comparable to Shamsan and Al Maqtari (2018) (30.86-38.73%), suggesting species-specific variations. The ash content is consistent with Flowra *et al.* (2012) (9.63-22.73%) and Al Banna *et al.* (2022) (13.89-20.07%), though higher than some reports (Majumdar *et al.*, 2023: 9.96-11.98%; Kalita *et al.*, 2024: 3.79-11.34%). Despite its nutritional quality, the Wazef fish's fungal contamination mirrors findings in other studies (Baniga *et al.*, 2019; Adebami *et al.*, 2024), emphasizing the need for improved hygiene in traditional drying processes to mitigate health risks while preserving its dietary benefits.

Dried fish products continue to dominate the processed seafood market in developing nations globally (Junaid *et al.*, 2010). Their compact and economical packaging, storage, and shipping capabilities contribute to their widespread

availability, particularly in less developed regions like Yemen. However, microbial analysis in this study reveals that although sun-dried fish may appear safe for consumption, it is heavily contaminated as all the samples analyzed were contaminated with fungi. This aligns with the observations reported by Junaid *et al.* (2010) who found that all the stockfish samples they examined were contaminated. Additionally, the results are consistent with Ekundayo (1984) who stated that molds possess the capacity to survive harsh environmental conditions and low moisture levels. The investigation identified 35 fungal species across 11 genera (Table 2).

This fungal presence is likely attributed to post-harvest delays, unsanitary handling and processing during traditional sun-drying, contaminated work surfaces, and improper transportation. After processing, the products are placed in locally constructed baskets or jute sacks and transported to various domestic markets. Frequently, these products are not adequately packaged or stored, leading to moisture reabsorption and further post-processing contamination (Oku and Amakoromo, 2013).

The study revealed interesting patterns in fungal contamination of dried fish. While the current study found *Aspergillus* as the dominant genus (67%), followed by *Penicillium* (24.7%), other researchers reported varying distributions: Shamsan and Al Jobory (2018) reported *Mucor* and *Rhizopus* (84% both), Deng *et al.* (2021) found *Fusarium* (80.4%) as the predominant genus, followed by *Penicillium* (70.7%) and *Aspergillus* (63.9%); while Chukwurah *et al.* (2024) consistently identified *Aspergillus*, *Penicillium*, and *Fusarium* as dominant genera. The current study's total fungal count of 11,742 colonies/g aligns with findings from Sulieman *et al.* (2014). The diversity of species (35 species across 11 genera) in the current study exceeds most other reports, such as Shamsan and Al Jobory (2018) who identified 26 species in 11 genera, suggesting potentially higher contamination levels or more thorough detection methods.

Table 2. Total counts (TC, calculated per g sample), number of cases of isolation (NCI, out of 20 samples) and occurrence remarks (OR) of fungal genera and species isolated from sun-dried fish (Wazef) obtained from Al Mukha district, Taiz Governorate, Yemen.

Genera & Species	TC	TC%	NCI	OR
<i>Aspergillus</i> (total count)	7,903	67	20	H
<i>A. flavus</i> Link	1,786	15.2	18	H
<i>A. niger</i> Van Tieghem	1,513	12.9	15	H
<i>A. fumigatus</i> Fresenius	1,029	8.8	14	H
<i>A. parasiticus</i> Speare	987	8.4	12	H
<i>A. sydowi</i> (Bain. & Sart.) Thom & Church	734	6.3	10	M
<i>A. ochraceus</i> Wilhelm	376	3.2	8	M
<i>A. terreus</i> Thom	391	3.3	5	L
<i>A. candidus</i> Link	312	2.7	4	L
<i>A. sulphureus</i> (Fres.) Thom & Church	285	2.4	3	R
<i>A. oryzae</i> (Ahlb.) Cohn	198	1.7	3	R
<i>A. versicolor</i> (Vuillemin) Tiraboschi	165	1.4	2	R
<i>A. clavatus</i> Desm.	81	0.7	1	R
<i>A. ustus</i> (Bainier) Thom & Church	46	0.4	1	R
<i>Cladosporium cladosporioides</i> (Fr.) de Vries	40	0.34	2	R
<i>Eurotium</i> (total count)	246	2.1	6	L
<i>E. chevalieri</i> Mangin	135	1.2	3	R
<i>E. herbariorum</i> Mangin	63	0.5	2	R
<i>E. amstelodami</i> Mangin	34	0.3	2	R
<i>E. verruculosum</i> Vuillemin	12	0.1	1	R
<i>Fusarium verticillioides</i> (Sacc.) Nirenberg	13	0.11	2	R
<i>Mucor</i> (total count)	21	0.18	4	L
<i>M. circinelloides</i> Van Tieghem	16	0.1	3	R
<i>M. hiemalis</i> Wehmer	5	0.04	1	R
<i>Penicillium</i> (total count)	2,902	24.7	18	H
<i>P. citrinum</i> Thom	1,112	9.5	13	H
<i>P. puberulum</i> Bainier	894	7.6	9	M
<i>P. brevicompactum</i> Dierckx	532	4.5	8	M
<i>P. chrysogenum</i> Thom	210	1.8	6	L
<i>P. expansum</i> Link ex Gray	56	0.5	4	L
<i>P. italicum</i> Wehmer	32	0.3	2	R
<i>P. roquefortii</i> Thom	26	0.2	1	R
<i>P. stekii</i> Zalessky	23	0.2	1	R
<i>P. variotii</i> Bainier	17	0.1	1	R
<i>Phoma herbarum</i> Westend	3	0.03	1	R
<i>Rhizopus stolonifer</i> (Ehrenberg) Lind	264	2.3	7	M
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	165	1.4	5	L
<i>Trichoderma viride</i> Pers. Ex S. F. Gray	46	0.39	4	L
Yeast	139	1.18	3	R
Total count	11,742			
Number of genera	11			
Number of species	35			

OR: Occurrence remarks. H: High occurrence; more than 10 cases out of 20 tested. M: Moderate occurrence; between 7~10 cases. L: Low occurrence; between 4~6 cases. R: Rare occurrence; less than 4 cases.

Among the *Aspergillus* species identified, *A. flavus* accounted for (15.2%), *A. niger* (12.9%), *A. fumigatus* (8.8%) and *A. parasiticus* (8.4%), the findings consistent with Adebami et al. (2024) who identified *A. niger* as the most frequently occurring fungus (38%), similarly, Youssef et al. (2003) found *Aspergillus* species, including *A. flavus* and *A. niger*, to be highly prevalent in dried fish samples. While Thiyagarajan and Alruwaili (2021) found lower percentages of *A. parasiticus* compared to our findings. The significant presence of *Penicillium* species (24.7% of total isolates) with *P. citrinum* (9.5%) and *P. puberulm* (7.6%) as dominant species corresponds with research by Chukwurah et al. (2024), who identified *Penicillium* as one of the three dominant fungal genera in dried fish. Most *Aspergillus* species, *Penicillium* spp., *Eurotium* spp., *Mucor* spp., and other species obtained during the study, had been identified before with varied percentages from salted, smoked, sun and oven-dried fish (Youssef et al., 2003; Junaid et al., 2010; Dorostkar and Mabodian, 2011; Saritha et al., 2012; Fafioye and Fafioye, 2013; Oku and Amakoromo, 2013; Sulieman et al., 2014; Yakubu and Ngueku, 2015; Agbabiaka et al., 2017; Nyamwaka et al., 2017; Shamsan and Al Jobory, 2018; John et al., 2020; Daoudou et al., 2020; Thiyagarajan and Alruwaili, 2021; Wambai et al., 2021; Ajimati et al., 2023; Chukwurah et al., 2024; Adebami et al., 2024; Ikeh et al., 2024). The analysis also revealed the presence of mixed fungal growth, with various combinations of two, three, or more fungal species observed across the examined samples. This occurrence could be attributed to the existence of a competitive mycoflora, where the associated growth of other mold species influenced the fungal colonization in the stored products (Bennett and Klich, 2003).

The fungal pathogens rely on potent enzymes as chemical weapons to breach host tissue. It was therefore crucial to investigate the capacity of Wazef fungi to generate these secondary metabolites. During this study, proteolytic,

lipolytic, and urease enzymes were detected in 78%, 89.4%, and 92.3% of the tested fungal isolates, respectively (Table 3). Similarly, Moharram and El-Zayat (1989), investigated the enzymatic capabilities of fungi isolated from the scales of *Tilapia nilotica*, reporting comparable findings regarding the production of protease and lipase enzymes. Youssef et al. (2003), reported similar enzymatic profiles in salted fish samples. The fungal enzymes have the potential to inflict tissue damage, including conditions like invasive aspergillosis and other fish diseases (Mohamed, 1994).

Testing the ability of 142 fungal isolates (represented 16 species appertaining to 5 genera) to produce mycotoxins revealed that 66 isolates (46.5%) were mycotoxin producers (Table 3). The ability of toxin production differs not only among the fungal species but also among the different isolates of the same species, with *Aspergillus* and *Penicillium* being the most prominent genera. The obtained results are in harmony with those obtained by other researchers (Youssef et al., 2003; Manning, 2015; Fredrick et al., 2015; Ounleye and Olaiya, 2015; Job et al., 2016; Adesokan et al., 2016; Rafli et al., 2018; Hassan et al., 2018; Osibona et al., 2018; Kachapulula et al., 2018; Tolosa et al., 2019; Ousman et al., 2019; Namulawa et al., 2020; Walter et al., 2020; Thiyagarajan and Alruwaili, 2021; Deng et al., 2021; Kumar et al., 2021; Wambai et al., 2021; Ajimati et al., 2023; Adebami et al., 2024).

A. flavus was the most prolific mycotoxin producer, with 10 out of 18 isolates producing aflatoxin B1, aflatoxin B2, G1, G2, and sterigmatocystin, consistent with findings by Youssef et al. (2003), who reported *A. flavus* as a major producer of aflatoxins in dried fish. Similarly, *A. parasiticus* produced aflatoxin B1, B2, G1, G2, and sterigmatocystin, aligning with Thiyagarajan and Alruwaili (2021), who identified *A. parasiticus* as a significant mycotoxin producer in dried fish samples. *A. niger* and *A. ochraceus* were found to produce ochratoxin A, a finding corroborated by

Ajimati et al. (2023), who isolated *A. niger* and *A. ochraceus* as producers of ochratoxins.

Within the *Penicillium* genus, *P. citrinum* was the most significant mycotoxin producer, with 10 out of 13 isolates producing citrinin, a finding supported by Youssef et al. (2003), who identified *P. citrinum* as a key producer of citrinin in dried fish. *P. puberulium* produced penicillic acid, while *P. expansum* produced ochratoxin A and citrinin, consistent with Deng et al. (2021), who reported *Penicillium* sp. as a dominant mycotoxin-producing genus in dried fish. Additionally, *Trichoderma viride* produced T-2 toxin, a potent mycotoxin, highlighting the potential for diverse mycotoxin contamination.

The high prevalence of mycotoxin-producing fungi, particularly *Aspergillus* and *Penicillium*,

poses significant health hazards, as these toxins exhibit carcinogenic, hepatotoxic, and acute toxic properties. Furthermore, the enzymatic activity of these fungi, particularly protease and lipase, further exacerbates the risk by degrading the nutritional quality of the fish. These findings align with Chukwurah et al. (2024), who emphasized the health risks associated with fungal contamination in dried fish, and Sulieman et al. (2014), who reported similar mycotoxin profile in dried fish samples. The variability in mycotoxin production across studies may be attributed to differences in fungal strains, environmental conditions, and drying methods.

Table 3. Production of secondary metabolites by dominant fungi isolated from sun-dried fish (Wazef) obtained from Al Mukha district, Taiz Governorate, Yemen.

Genera & Species	TIT	Number of isolates able to produce				Mycotoxins identified
		Protease	Lipase	Urease	Count	
Total	142	111	127	131	66	
%	78	89.4	92.3	46.5		
<i>Aspergillus</i> (total count)	86	72	80	82	41	
<i>A. flavus</i> Link	18	16	16	17	10 5	Aflatoxin B1, Sterigmatocystin Aflatoxin B1, B2, G1, G2
<i>A. niger</i> Van Tieghem	15	12	13	14	5	Ochratoxin A
<i>A. fumigatus</i> Fresenius	14	12	13	13	-	-ve
<i>A. Parasiticus</i> Speare	12	10	12	12	4 3	Aflatoxin B1, B2, G1, G2 Aflatoxin B1, B2, Sterigmatocystin
<i>A. sydowi</i> (Bain. & Sart.) Thom & Church	10	8	9	9	3	Aflatoxin B1, Sterigmatocystin
<i>A. ochraceus</i> Wilhelm	8	7	8	8	4	Ochratoxin A, Citrinin
<i>A. terreus</i> Thom	5	4	5	5	5	Patulin, Citrinin
<i>A. candidus</i> Link	4	3	4	4	2	Citrinin
<i>Penicillium</i> (total count)	40	31	36	37	23	
<i>P. citrinum</i> Thom	13	11	12	13	10	Citrinin
<i>P. puberulium</i> Bainier	9	7	7	8	5	Penicillic acid
<i>P. brevicompactum</i> Dierckx	8	5	7	6	-	-ve
<i>P. chrysogenum</i> Thom	6	4	6	6	5	Ochratoxin A
<i>P. expansum</i> Link ex Gray	4	4	4	4	3	Citrinin
<i>Rhizopus stolonifer</i> (Ehrenberg) Lind	7	3	4	4	-	-ve
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	5	2	3	4	-	-ve
<i>Trichoderma viride</i> Pers. Ex S. F. Gray	4	3	4	4	2	T-2 toxin

The findings of mycotoxin contamination in the analyzed samples revealed that all the samples were contaminated (Table 4)—where aflatoxin B1 was detected in one sample, aflatoxins B1 and B2 in six samples, and aflatoxins B1, B2, G1, and G2 in eight samples—are consistent with multiple studies highlighting the prevalence of aflatoxins in dried and smoked fish products. Adebami et al. (2024) reported aflatoxin

contamination in all tested smoked-dried fish samples, with *A. flavus* and *A. niger* as dominant fungi, while Fredrick et al. (2015) found aflatoxin B1 and G1 in dried fish, emphasizing the role of *A. flavus* and *A. niger* in toxin production. Similarly, Namulawa et al. (2020) detected AFB1 in 48–63% of fish feed samples, reinforcing concerns about high contamination levels in improperly stored products. The presence of

citrinin, sterigmatocystin (6 samples), patulin (5 samples), ochratoxin (4 samples), penicillic acid (4 samples) and T-Toxin (1 sample) in the current study further aligns with reports by Deng et al. (2021), who identified ochratoxin A (OTA) in 33.3% of dried seafood, and Osibona et al.,

(2018) who noted OTA and AFB1 in stored smoked fish. The dominance of toxigenic fungi such as *A. flavus*, *A. parasiticus*, *A. ochraceus*, and *Penicillium* spp. mirrors findings by Nyamwaka et al. (2017), and Rafli et al. (2018).

Table 4. Sample number (SN), natural occurring of mycotoxins identified and common mycotoxin-producing fungi associated with the toxic sun-dried fish (Wazef) obtained from Al Mukha district, Taiz Governorate, Yemen.

SN	Mycotoxins identified	Mycotoxin producing-fungi
S1	Patulin Citrinin	<i>A. terreus</i> , <i>P. citrinum</i>
S2	Aflatoxin B1, B2, G1, G2 Sterigmatocystin Patulin Penicillic acid	<i>A. flavus</i> , <i>A. Parasiticus</i> , <i>A. terreus</i> , <i>P. puberulum</i>
S3	Aflatoxin B1, B2, G1, G2	<i>A. flavus</i> , <i>A. Parasiticus</i>
S4	Ochratoxin A Citrinin Penicillic acid	<i>A. niger</i> , <i>A. ochraceus</i> , <i>A. candidus</i> , <i>P. puberulum</i> , <i>P. chrysogenum</i>
S5	Aflatoxin B1, B2, G1, G2 Sterigmatocystin T-2 toxin	<i>A. flavus</i> , <i>A. Parasiticus</i> , <i>A. sydowi</i> , <i>Trichoderma viride</i>
S6	Aflatoxin B1 Ochratoxin A Citrinin	<i>A. flavus</i> , <i>A. niger</i> , <i>P. citrinum</i> , <i>P. chrysogenum</i>
S7	Aflatoxin B1, B2 Sterigmatocystin Penicillic acid	<i>A. flavus</i> , <i>A. Parasiticus</i> , <i>P. puberulum</i>
S8	Patulin	<i>A. terreus</i>
S9	Aflatoxin B1, B2, G1, G2	<i>A. flavus</i> , <i>A. Parasiticus</i>
S10	Aflatoxin B1, B2 Citrinin	<i>A. flavus</i> , <i>A. Parasiticus</i> , <i>P. citrinum</i>
S11	Aflatoxin B1, B2, G1, G2 Citrinin	<i>A. flavus</i> , <i>P. citrinum</i> , <i>P. expansum</i>
S12	Aflatoxin B1, B2, G1, G2 Sterigmatocystin	<i>A. flavus</i> , <i>A. Parasiticus</i> , <i>A. sydowi</i>
S13	Aflatoxin B1, B2, G1, G2 Citrinin	<i>A. flavus</i> , <i>P. citrinum</i>
S14	Citrinin Penicillic acid	<i>P. citrinum</i> , <i>P. puberulum</i> , <i>P. expansum</i>
S15	Aflatoxin B1, B2, G1, G2 Ochratoxin A Patulin	<i>A. flavus</i> , <i>A. niger</i> , <i>A. Parasiticus</i> , <i>A. ochraceus</i> , <i>A. terreus</i> , <i>P. chrysogenum</i>
S16	Aflatoxin B1, B2 Sterigmatocystin	<i>A. flavus</i> , <i>A. Parasiticus</i>
S17	Sterigmatocystin	<i>A. niger</i> , <i>A. Parasiticus</i>
S18	Aflatoxin B1, B2 Patulin	<i>A. flavus</i> , <i>A. terreus</i>
S19	Aflatoxin B1, B2 Ochratoxin A	<i>A. flavus</i> , <i>A. niger</i> , <i>A. Parasiticus</i> , <i>A. ochraceus</i>
S20	Aflatoxin B1, B2 Ochratoxin A Citrinin	<i>A. flavus</i> , <i>A. niger</i> , <i>A. Parasiticus</i> , <i>A. sydowi</i> , <i>A. ochraceus</i> , <i>P. citrinum</i> , <i>P. chrysogenum</i>

These parallels underscore the global nature of mycotoxin contamination in dried fish, as further evidenced by Ziem et al. (2024) (76% AFB1 contamination in Cameroonian smoked fish) and

Walter et al. (2020) (32.88% prevalence of *A. flavus*, with 25% being aflatoxigenic). The recurring theme is that poor storage conditions, high moisture, and inadequate processing

exacerbate fungal growth and mycotoxin production (Ounleye and Olaiya, 2015; Youssef *et al.*, 2003). Public health risks are well-documented, with Fredrick *et al.* (2015) warning of long-term health hazards from mycotoxin exposure and Deng *et al.* (2021) advocating for improved processing methods. Collectively, these studies, alongside Indriati *et al.* (2017), Ousman *et al.* (2019) and Ikeh *et al.* (2024) highlight the urgent need for stricter regulatory measures, better drying techniques, and enhanced storage practices to mitigate mycotoxin contamination in dried fish products.

In this study, the co-occurrence of multiple mycotoxins in all dried fish samples analyzed (except one sample) was detected. Co-occurrence of mycotoxins is due to at least three different reasons; (i) most fungi are simultaneously able to produce different mycotoxins, (ii) competition among different fungi cause them to secrete fungal toxins to resist invasion by others, and (iii) a self-protective mechanism produced by fungi to survive against adversity (low water activity and high protein) (Alassane-Kpembi *et al.*, 2017). Multi-mycotoxin studies reported foodstuff containing more than one mycotoxin could impact animal and human health at low concentrations (Alassane-Kpembi *et al.*, 2017; Mahdjoubi *et al.*, 2020). Since 1992, the contamination of sun/smoked dried fish products with a single or a combination of mycotoxins has been highlighted (Jonsyn and Lahai, 1992). These findings imply potential health risk to people's health if someone consumes dried fish with multi-fungal contamination. Therefore, it is important for dried fish to be packaged and stored at low temperature, which effectively reduce the probability of air exposure, decrease water activity and inhibit microbial reproduction on dried fish.

CONCLUSION

The study highlights a highly nutritional profile of sun-dried Wazef fish from Al Mukha markets, characterized by elevated protein and lipid contents alongside optimal moisture and pH levels, making it a valuable dietary resource.

However, all samples were contaminated with toxigenic fungi, primarily *Aspergillus* and *Penicillium* species and the widespread occurrence of multiple mycotoxins, including aflatoxins, citrinin, sterigmatocystin, patulin, ochratoxin, and penicillic acid, raise serious food safety concerns. The co-occurrence of multiple mycotoxins and high enzymatic activity in these fungi further compromises food safety and nutritional quality. These findings align with global studies and underscore the urgent need for improved drying techniques, stricter hygiene practices, and better storage conditions to mitigate contamination and ensure the safety of this traditional food source. Enhanced regulatory oversight and consumer awareness are also essential to mitigate the risks associated with mycotoxin exposure from dried fish products.

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CONFLICT OF INTEREST

The author hereby declares no conflict of interest.

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