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Microbial Status of Sun-Dried Fish (Wazef) Sold in Different Yemeni Markets

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Abstract
Heading from the fact that Yemenis consumers bother more about quantity and less about quality as a result they buy the product without considering health implications, this study concerned with the microbial analysis of 25 samples of sun-dried fish (Wazef) collected randomly from three different Yemeni cities. The bacterial count ranged from 1.4×10^{2} - 4.6×10^{5} cfu/g, while fungal counts ranged from 1.1×10^{1} - 7.2×10^{3} cfu/g. Ten bacterial species were obtained that belonged to 8 genera; *Klebsiella, Bacillus, Staphylococcus, Pseudomonas, Serratia, E. coli, Streotococcus,* and *Lactobacillus*, from which *Klebsiella* was the most dominant with a frequency of 28% of the samples. The mycological analysis revealed the presence of 26 fungal species in samples which belonged to 11 genera; *Alternaria, Aspergillus, Eurotium, Hemicola, Mucor, Penicillium, Rhizopus, Scopulariopsis, Trichophyton, Trichoderma* and yeasts, from which *M. racemosus* and *Rhizopus stolonifer* were the most dominant with an equivalent presence (84%) of samples. Findings of this study will increase the knowledge base towards adoption of improved handling and drying methods hence minimize microbial growth and possible contamination in the dried fish sector.
Keywords: Wazef**,** Sun-dried fish, Microbial status, Total count, Clupeiformes, Yemen.

INTRODUCTION

Fish is widely accepted on the menu card and form a much cherished delicacy that cuts across socio-economic, age, religious and educational barriers. It enjoys a very special consideration in human civilization from times immemorial (Adeleye, 1992). It is the major source of protein and its harvesting, handling, and processing provide livehood for millions of hungry people where livestock is relatively scarce (Aljufaili and Opara, 2006). Fish supplies a good balance of vitamins, minerals and protein which represents about 14% of all animal protein on a global basis (Adeleye, 1992; Albolagba and Melle, 2008), this ideal nutrients make fish an extremely perishable food and susceptible to microbial attack after catch or even before taken out from water (Carruthers, 1986).

Immediately fish dies, it remains in first class quality only for a short while (Cluscas and Ward, 1996). However, spoilage soon sets in which is occasioned by an increase in the ambient temperature triggers favourable conditions for microorganisms to thrive (Microbe wiki, 2009). For this reason, fish must undergo preservation just after capture. Food processing and preservation, including refrigeration, canning, drying and smoking (Ayers *et al*., 1980), are used to inhibit microbial growth, improve acceptability and above all extend the shelf-life of the products (Yakubu and Ngueku, 2015).

Sun drying of fish is a simple, oldest, the least expensive and traditional known method of fish preservation. Drying method is considered as the least expensive method of fish preservation followed especially in rural areas (Balachandran, 2001). When the humidity is high, during the monsoon, drying cannot be achieved by traditional methods. In such cases, the dried fish can reabsorb the moisture from air, and serves as a habitat for microbial population such as bacteria, fungi, viruses and insect attack (Azam, 2002). The growth of bacteria and fungi in food and food products is costly as well as sometimes hazardous (Anderson and Thrane, 2006).

The traditional sun-drying technique of fish processing is mostly practiced in Yemen. It conducted traditionally on the seacoast especially for a fish commonly named Wazef (a dried fish that believed to be belonging to the order Clupeiformes (un published data). This small dried fish is considered as a chief plate on the table, particularly in poor and rural communities of Taiz Governorate. Traditional drying is often rudimentary and good hygiene is rarely practiced. Some people bother more about quantity and less about quality; as a result they buy the product without considering health implications. Hence, they are more susceptible to health hazard. This study was, therefore, conducted to investigate possible presence of microbial contamination and provide a list of microbes commonly involved in infestation and deterioration of usually available sun-dried fish in Yemen.

MATERIALS AND METHODS

Collection of samples

A total of 25 random samples of ready to eat sun-dried fish (Wazef) were collected from different markets in three Yemeni cities namely: Sana'a, Taiz and Al-Hodida. To avoid contamination during sampling, transportation and storage, each sample (200g; consisting of a large number of small fishes) was kept in labeled polythene bags and taken immediately to the laboratory for analysis, and kept at 4°C, till microbiological investigation.

Preparation of samples for culture/ serial dilution:

A ten-folded serial dilution was made according to the method of Johnson and curl (1972). For each sample; which were blended with a high– speed blender (Phillips model 2012) and mixed thoroughly; four (4) test tubes were used.

Culturing, incubation and colony count:

The pour plate method was used for each sample, 1ml aliquots from the first three dilutions were plated in replicates, on Nutrient agar (for bacteria) and on potato dextrose agar (for mould growth), and then incubated for 24h and 4 days at 37°C and 28°C, for bacteria and fungi, respectively. After 24h, the bacterial count was done according to (FDA BAM, 2001; Iqbal *et al.,* 2012), same for fungi after 4 days (Surendran *et al.,* 2006), the count was expressed as cfu/g.

Preparation of pure culture:

The pure culture was prepared using the method of Blackwell (2011), then inoculated into sterile NA and PDA slants and stored as stock cultures in the refrigerator at 4°C prior to slide preparation.

Identification of the isolates:

Identification of the isolated bacteria was based on cultural characteristics, cell morphology and biochemical tests (Holt *et al*., 1994; Iqbal *et al.,* 2016). Fungal isolates were stained with lactophenol cotton blue and examined microscopically. The isolates were identified based on cultural characteristics, morphology of hyphae, diffusible pigmentation, cells and spores and kind of fruiting bodies and compared with the standard colour atlas as described by Ochei and Kolhatkar (2000).

RESULTS

Microbial analysis in the present study showed that although sun-dried fish seems healthy to eat, it is microbes loaded, ten bacterial species belonging to 8 genera were isolated from sun-dried fish samples (Table 1).

The study showed that 21 samples out of 25 (84%) were contaminated with bacteria, and among all bacterial

species obtained, *Klebiella* sp. ranked first (28% of samples) with a total count of 6.6×10^4 cfu/g, followed by *Staphyllococcus aureus* and *Bacillus* sp. with a total count of 4.6×10^5 and 3.3×10^5 cfu/g, respectively.

Pseudomonas sp., *Bacillus cereus, Serratia fanticola* and *E. coli*., were of low incidence in samples tested, recording a total count of 5.3×10^4, 2.9×10^4, 1.1×10^4 and 2.1×10^2 cfu/g, respectively (Table 2).

Other bacterial species which were detected during this study with rare occurrence were *Streptococcus* sp., *Lactobacillus* sp. and *B. subtilis* subsp. *spizizmii.,* with a total count oscillated between 1.4×10^2–2.4×10^3 cfu/g.

On the other hand, the mycological analysis of tested samples based on dilution-plating method revealed that 26 fungal species belonging to 11 genera were identified (Table 3). *Aspergillus* (9 species), *Penicillium* (5), *Eurotium* (3), *Mucor* (2), and one species for each of the other genera.

Table 1. Biochemical characters of Bacteria isolated from sun-dried fish (Wazef) samples collected from different Yemeni cities.

Bacterial genera and species	Cell morphology	Gram reaction	Catalase	Oxidase	Citrate	Indore	MR	VP	H_2S	Starch hydrolysis	Spore test	Motility	Sugar fermentation			
													Glucose	Sucrose	Manitole	lactose
***Bacillus* sp.**	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A
Bacillus cereus	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A
Bacillus subtilis* subsp. *spizizmii	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A
E. coli	Rod	-	+	+	-	+	+	-	-	-	-	+	A/G	-	A/G	A/G
***Klebsiella* sp.**	Rod	-	+	-	+	-	+	-	-	+	+	+	A/G	A/G	A/G	G
***Lactobacillus* sp.**	Rod	+	+	-	-	-	-	-	-	-	-	-	A/G	-	-	-
Serratia fonticola	Rod	-	+	-	+	-	+	-	-	-	-	+	A	-	A	A
Staphylococcus aureus	Cocci	+	+	-	+	-	-	-	-	-	-	-	A	-	-	A
***Streotococcus* sp.**	Cocci	+	-	-	+	-	-	-	-	-	-	-	A	-	-	A
***Pseudomonas* sp.**	Rod	-	+	+	+	-	-	-	-	-	-	+	A/G	A	A	A

Table 2. Total counts (cfu/g), number of cases of isolation (NCI, out of 25 samples), and occurrence remarks (OR) of bacterial genera and species isolated from sun-dried fish (Wazef) on NA medium at 37℃.

Bacterial genera and species	TC (cfu/g)	NCI	NCI%	OR
***Klebsiella* sp.**	6.6×10^4	7	28	M
Staphylococcus aureus	4.6×10^5	6	24	M
***Bacillus* sp.**	3.3×10^5	6	24	M
***Pseudomonas* sp.**	5.3×10^4	5	20	L
B. cereus	2.9×10^4	4	16	L
Serratia fonticola	1.1×10^4	4	16	L
E. coli	2.1×10^2	3	12	L
***Streotococcus* sp.**	2.4×10^3	2	8	R
***Lactobacillus* sp.**	1.4×10^2	2	8	R
B.subtilis* subsp. *spizizmii	1.8×10^3	1	4	R

Table 3. Some macro and microscopic characters of fungi isolated from sun-dried fish (Wazef) samples collected from different Yemeni cities.

Fungal genera	Colony morphology	Microscopic appearance
***Alternaria alternata* (Fries) Keissler**	Grey to olivaceous black	Conidiophores branched, straight or curved, pale brown, smooth, conidia ovoid or elliptical, in chains.
***Aspergillus awamori* Nakazawa**	Reddish-brown to black colony with white margins	Conidiophores smooth-walled, thick, light brown towards the apex, conidia hyaline to light brown.
***A. Candidus* Link**	Powdery white colonies changing into orange-white	Conidiophores thick-walled, smooth, colourless, conidial heads globose, radiate; conidia globose, hyaline and smmoth.
***A. flavus* Link**	Greenish yellow mycelium with white margins	Conidiophores aseptate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia
***A. fumigatus* Fresenius**	Greyed-green, fast-growing colonies, with white margins	Conidiophores short, greenish in colour, conidial heads columnar and compact.
***A. niger* Van Tieghem**	Black colonies with white to yellow margin	Conidiophores smooth-walled, hyaline at the base, conidial heads large, conidia brown and globose to subglobose.
***A. oryzae* (Ahlb.) Cohn**	Greyed-brown mycelium with yellow-green margins	Conidiophores long, colourless, rough- walled, conidial heads large; conidia globose, smooth to roughened.
***A. parasiticus* Speare**	Yellow-green colony, with wrinkled white margin, colourless reverse.	Conidiophores long, colourless, smooth, narrow at the base, wide below the vesicle, conidia globose.
***A. sulphureus* (Fres.) Thom and Church**	Yellow orange with whitish margins, reverse yellow-orange	Conidiophores smooth, hyaline to yellowish, conidia globose with smooth –walled.
***A. terreus* Thom**	Greyed-orange colonies with whitish margin	Conidiophores smooth, colourless, conidial heads columnar.
***Eurotium amestelodami* (Mangin)**	Fast-growing-green-yellow colonies with white margin	Cleistothecia abundant, bright yellow, conidiophores pale yellow green, conidia barrel-shaped with flattened ends.
***E. cristatum* (Raper & Fennell) Malloch & Cain**	White- yellow colonies	Cleistothecia abundant, honey yellow to light brownish olive, conidial heads are very rare; conidial heads radiate, pale dull glaucous-blue to greenish glaucous-blue, Conidia ellipsoidal to subglobose, smooth, and pale brown to brown.
***E. rubrum* Koing, Spieckerman and Bremor**	Orange-red to ferruginous colonies	Cleistothecia superficial first bright yellow and gradually turned to light brown, globose to subglobose. Conidia of the fungus were ovate or bacillar, finely roughened to densely spinulose.
***Hemicola* sp. (Tsikl.) Bunce**	Slow -growing cottony golden-brown colonies.	Conidiophores are undistinguished and the spores (aleuriospores) are born directly off the vegetative hyphae or conidiophores. The spores are large, globose or subglobose.
***Mucor hiemalis* Wehmer**	Dark grey mycelium	Sporangiophore slightly branchrd, drak brown at maturity, columella elliptical with truncatebase, Rhizoids are not presents.
***M. racemosus* Fresenius**	Whitish grey mycelium	Sporangiophore branched with spored sporangium, columella well developed. Rhizoids are not presents
***Penicillium brevicompactum* Dierclcx**	Greyed-green colony with white margin, greyed-orange reverse.	Conidiophores long and broad, penicillin asymmetric, compact. Conidia globose, smooth.
***P. citrinum* Thom**	Greyed-green colony with white margin, greyed-yellow reverse.	Conidiophores smooth-walled, penicillin asymmetrical, biverticillate, metulae more or less equal in length, phialides ampulliform, crowded in clusters.
***P. puberulum* Bainier**	Greyed-green colony with white margin, greyed-yellow reverse, with faint reddish colour.	Conidiophores smooth-walled, penicillin asymmetrical, terverticillate, phialides ampulliform with trapped neck.
***P. purpurogenum* Stoll**	Yellow -green colony with yellowish margin, orange-red reverse, with the colour diffusing	Conidiophores smooth-walled, arising from the substratum, short, penicillin symmetrical and biverticillate, compact, metulae bear a compact cluster of 4-6 parallel lanceolate

	in the medium.	phialides.
***P. verrculosum* Peyronel**	Greyed-orange colony with broad white margin, greyed-orange reverse, with large yellowish droplets.	Conidiophores borne from aerial hyphae, smooth-waiied, penicilli symmetrical, biverticillate, phialides lanceolate, with a long narrow neck.
***Rhizopus stolonifer* (Ehrenberg) Lind**	Dark brown mycelium	Rhizoids brownish and unbranched the sporangiophore arising directly of up to five with smooth walls.
***Scopulariopsis brevicaulis* (Sacc.) Bainier**	Fast growing colonies, whitish at first, later orange-white. With a narrow white margin.	Annellophores arising singly on hyphae, cylindrical, conidia globose to ovoid with truncate base with thickened ring.
***Trichophyton verrcosum* E. Bodin**	Very slow-growing compared to other dermatophytes. Flat, white/cream colony, with a glabrous texture.	Macroconidia are rare, and have a rat-tail or string bean shape, while microconidia are tear-shaped and have been only observed in laboratories when grown under enriched conditions. chlamydospores become thick-walled and found in long chains.
***Trichoderma viridae* Pers. Ex s. F. Gray**	Colonies spreading rapidly, yellow green to bright green colonies.	Conidiophores bear branches or phialides irregularly, with flask-shaped, conidia subglobose, green, non septate.
Yeasts	Colonies are moderately fast growing, flat, yellowish-grey to creamy-grey, glabrous.	……..

Table 4. Total counts (cfu/g), number of cases of isolation (NCI, out of 25 samples), and occurrence remarks (OR) of fungal genera and species isolated from sun-dried fish (Wazef) on PDA medium at 28°C.

Fungal genera and species	TC (cfu/g)	NCI	NCI%	OR
Alternaria alternata	3.8×10^1	5	20	L
Aspergillus awamori	5.0×10^2	11	44	H
A. candidus	1.8×10^3	7	28	M
A. flavus	4.5×10^3	16	64	H
A. fumigatus	7.0×10^2	10	40	M
A. niger	3.9×10^3	19	76	H
A.oryzae	7.0×10^2	2	8	R
A .parasiticus	2.0×10^2	4	16	L
A.sulphureus	6.0×10^2	9	36	M
A. terreus	1.4×10^3	13	52	H
Eurotium amestelodami	1.1×10^1	2	8	R
E. cristatum	8.0×10^1	1	4	R
E. rubrum	1.3×10^1	1	4	R
***Hemicola* sp.**	5.0×10^1	1	4	R
Mucor hiemalis	4.0×10^2	1	4	R
M. racemosus	4.7×10^3	21	84	H
Penicillium brevicompactum	3.0×10^2	1	4	R
P. citrinum	2.6×10^3	3	12	L
P. puberulum	1.2×10^3	2	8	R
P. purpurogenum	2.8×10^3	17	68	H
P. verrculosum	1.2×10^3	8	32	M
Rhizopus stolonifer	7.2×10^3	21	84	H
Scopulariopsis brevicaulis	2.0×10^2	5	20	L
Trichophyton verrcosum	2.0×10^1	1	4	R
Trichoderma viridae	4.0×10^1	2	8	R
Yeasts	2.1×10^2	9	36	M

Rhizopus stolonifer and *Mucor racemosus* followed by *A. niger, P. purpurogenum, A. flavus, A. terreus* and *A. awamori* were isolated highly from most of samples with a total count of (7.2×10^3, 4.7×10^3, 2.8×10^3, 4.5×10^3, 1.4×10^3 and 5.0×10^2 cfu/g, respectively), whereas each of *A. fumigatus, A. sulphureus,* yeasts, *P. verrculosum* and *A. candidus* were moderately recovered recording total count of (7.0×10^2, 6.2×10^2, 2.1×10^2 and 1.2×10^3 cfu/g, respectively), on the other hand, *Alternaria alternata, Scopulariopsis brevicaulis, A. parasiticus* and *P. citrinum* were of low frequency (3.8×10^1, 2.0×10^2, 1.8×10^3 and 2.6×10^3 cfu/g, respectively), while the rest of fungal genera and species were rarely isolated with a total count ranged between 1.1×10^1 - 1.2×10^3 cfu/g (Table 4). It is worth to mention that mixed growth of fungi in various combinations of two or three or even more fungal spp. was observed in many analyzed samples.

DISCUSSION

Dried fish products still possess the largest volume on the processed seafood market among developing nations in the world today (Junaid *et al.,* 2010). Their finished forms can be packed, stored and shipped economically which explains their long lasting presence especially in less developed areas of this world including Yemen.

Microbial analysis in the present study showed that although sun-dried fish seems healthy to eat, it is microbes loaded. Ten bacterial species belonging to 8 genera were isolated during this investigation. The presence of microbes may due to the post-harvest delay, unhygienic handling and processing during the traditional sun-drying process, contaminated floor on which the processing was carried out, and improper transportation.

As after processing, the products are placed in locally made baskets or jute sacs ready for transportation to various markets in the country. Often, the products are not properly packed and stored. Consequently, reabsorption of moisture and post processing contamination of fish occur (Oku *et al.,* 2013).

Klebiella sp., *Pseudomonas* sp., *Bacillus* sp., and *Lactobacillus* sp. isolated and identified during this study, can be said to be normal flora which are often beneficial as they safe-guard against invasion of flesh by other microbes as those identified by (Gonzales *et al.,* 2000; Ishikawa *et al.,* 2003; Abolagba *et al.,* 2011; Agbabiaka *et al,* 2017), however, some of the previous species associated with favourable relative humidity, bad handling, poor hygiene and delayed processing cause them to be pathogenic (Yakubu and Ngueku, 2015), the result which were similar to those of Patterson and Ranjitha (2009).

The presence of *E. coli* indicates the recent and possibly hazardous fecal pollution (Kakatkar *et al.,* 2010), or might be due to cross contamination from other food contact surfaces or poor handling as noted by Fang *et al.,* (2003). It is worthy to mention that the higher bacterial count which reaches the value of 4.6×10^5 cfu/g recorded by *S. aureus* wasn't above the permissible limits (ICMSF, 1986).

None of the fish samples was contaminated with *Salmonella* spp. during this study. Similarly Yaqub et al. (2017) documented the absence of *Salmonella* spp. from fish samples in Lahore, Pakistan, indicating the safe healthy condition for consumption of fish as food.

Based on the fact that spores produced by fungi which are moderately resistant to drying and therefore easily implicated on the contamination and spoilage of dry and semi-dry materials, it was reasonable to accept the recent study mycological analysis which revealed that all samples tested were fungi-bearing samples (92%) except 2 samples only. It seems that the previous result is a mirror image of those of Junaid *et al.,* (2010) which noted that all the stockfish samples tested were contaminated with fungi. As well as the agreement with the observation of Ekundayo (1984) who stated that moulds have the ability to survive harsh conditions and low moisture content.

Most of *Aspergillus* species, *Penicillium* spp., *Eurotium* spp., *Mucor* spp., and other species obtained during the study, had been identified before from salted, smoked and sun-dried fish (Youssef *et al.,* 2003; Junaid *et al*., 2010; Saritha *et al.,* 2012; Fafioye and Fafioye, 2013; Oku *et al.,* 2013 and Yakubu and Ngueku, 2015) and even oven-dried fish (Agbabiaka *et al,* 2017). *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp. were mentioned as pathogenic to human beings (Shanthini and Paterson, 2003). In this trend, Sharma (1989) reported that *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. are known to cause food spoilage. Thus, the presence of these fungi is of a great significance in view of seafood safety and quality. Most of them on the other hand, are common in air and soil, and since their presence in fish samples which might contain metabolites (toxins) produced by them, make the fish consumption hazardous to health. Similarly, (Mitchell, 2007) mentioned that the potency of these metabolites is not affected by cooking and may cause severe or fatal damage to the liver and kidney.

The presence of *Trichophyton verrucosum,* a dermatophyte in one sample only during this study, could be as a result of acquisition of fungus from the retailers since it is acquired by contact with contamination soil or with infected animals or humans (Mitchell, 2007). Where it affects the beard, neck, wrist and back of hands (Ochei and Kolhatkar, 2000) which are all exposed parts of the body.

In case of yeasts, they appeared moderately with a considerable ratio of 36%, in contrast, in the recent study of Youssef *et al.,* (2003), yeasts represented 18.2% of total fungal count.

Mixed growth of fungi in various combinations of two or three or even more fungal spp. was observed in many analyzed samples, which could be as a result of the presence of competitive mycoflora that is, the associated growth of other moulds which influences fungal growth is stored products (Bennett and Klich, 2003). The highest fungal count of a value of 7.2×10^3 cfu/g recorded by *Rhizopus stolonifer* wasn't above the permissible limits (ICMSF, 1986).

CONCLUSION

Based on informations obtained from this study, it is clearly evident that sun-dried fish (Wazef) collected from different Yemeni markets; is considered a vehicle for numerous fungal and bacterial pathogens contamination. However, even if the higher total count of either bacteria or fungi weren't above the permissible limits, a risk of mycotoxins as carcinogenic and hepatotoxic agents should be taken into consideration.

RECOMMENDATION

Since improper drying of fishes may lead to fungal and bacterial attack, and degradation of the product, it is therefore important that both the artisanal fishermen and the marketers should adapt a better method of processing and preservation, to improve the quality of the sun-dried sea foods for the benefits of the consumer.

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CONFLICT OF INTEREST

There is no conflict of interest.

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