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Research article

Microbial Quality of Salted Dried Fish Sold Near Caspian Sea, Iran

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Abstract

Moisture, pH, NaCl and microbial counts of salted dried fish at Golestan Province near Caspian Sea, Iran, were studied.Results showed that moisture, pH and NaClof samples were ranged from %12.8-44.4, 5.9-6.07 and %4.16-5.36 respectively. Total count, Yeast and molds, Staphylococcus aureus, Enterobacteriaceae, Aerobic spores, Anaerobic spores (clostridia spp), Psychrophilic bacteria, Lactic acid bacteria, Halophilic microorganisms, Salmonalla and Shigella count were ranged from 2.2×10^3-3.2×10^4, 0 -6×10^2 , 0- 9.8×10^2 , 0- 6.7×10^1 , 3.8×10^1 - 6×10^3 , 2×10^1 - 5×10^2 , 1×10^1 - 4×10^1 , 0- 4×10^1 , 4×10^2 -3×10^3 , 0- 2×10^1 and 0.4×10^1-1.2×10^2 respectively. Decrease the moisture and pH of samples leaded to decrease the microbial counts butthe high content of NaCl in salted fishs inducedhalophillic bacteria, hence halophillic bacteria count were high. Assessment of the microbiological quality of salted dried fish in the Goestan Province, Iran, showed that the conditions for their production and sale were poor and suggested that producers and vendors follow good hygiene practices. Some pathogens such as Staphylococcus aureus and Salmonella spp. in the samplesare harmful, also anaerobic spores could be spores of pathogenic Clostridium botulinum type E or Clostridium perfringens. It is therefore important that actions be taken to improve the sanitation.

Key Words: Salted Dried Fish, Moisture, Microbial count.Staphylococcus aureus, sanitation.

1- Introduction

Fish is one of the most important sources of animal protein and has been widely accepted as a good protein source and other elements for the maintenance of healthy body (Ravichandran et al., 2012). It also provides a good source of high quality protein and contains many vitamins and minerals. It is an extremely perishable commodity and quality loss can occur very rapidly after catch (Khan and Khan, 2001; Musa et al., 2010; Dewi et al., 2011). Curing of fish is an ancient method of preservation in the worldthat primarily involves two stages viz, salting anddrying (Sanjeev and Surendran, 1996; Anon, 2001). Salted fish products have ben shown to be safe for consumption. It decreases the water activity and is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes (Turan et al., 2007). Sun drying of fishes is a simple and the oldest known method of fish preservation. Sun drying method is considered as the least expensive method of fish preservation (Balachandran , 2001). This traditional method is followed for the preservation of fish especially in rural areas (Chakrabarti and Varma, 1999). Traditional drying is often rudimentary and good hygiene is rarely practiced. During the monsoon, when the humidity is high, drying cannot be achieved by traditional methods. By this time, the fish can absorb the moisture and it serves as a habitat for microbial population (Azam, 2002).Salted dried was a major source of anima protein available at cheaper price for the economically weaker sections areas (Prasad et al. 1999). Fish is harvested from relatively cleaner environments but during subsequent handling, bacteria of spoilage type and of public health significance type come in contact with the fish (Chichester and Graham 1973). Immediate cooling or salting of the catch is more important in tropical conditions because the ambient temperature is high and it leads to rapid spoilage (Jeyasakila et al. 2003).
The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. The presence of the pathogenic loads in dried fishes is acquiring importance in view of the safety and quality of the seafood (Patterson and Ranjitha, 2009). The spoilage indicators and visible fungal attacks caused by microorganisms are known to adversely affect the quality of cured fishes. Growth of fungus caused off flavors, soften the flesh and some can produce potentially dangerous mycotoxins under certain circumstances (FAO, 1982). This causes considerable decrease in the consumption of dried fish. Apart from contaminated salted and dries fish, other common sources of contamination are air and dust in and around fish processing place, contaminated coastal water and soil and unhygienic handling (FAO,1982, Prabhakaran and Gupta 1990). Many of the bacteria capable of causing disease are considered to be saprophytic in nature but only become pathogenic when fishes are physiologicaly unbalanced nutritionally deficient, or as a result of other stressors such as poor water quality, overstocking, which allowe opportunistic bacterial infections to human beings (Akinjogunla et al. 2011). Curing is a simple and cheap method of processing requiring least technical expertise. Golestan Province is well known for local consumption of salted and dried fishes in Iran.
The objective of the present study was to determine themicrobial quality of dried fishesconsumpted in cities near the Caspian sea in Golestan Province, Iran.

2- Materials and methods

2-1 Sampling

The Sun dried fishes were aseptically collected from the Gomishan and Bandar Torkman shore market on the Caspian Sea, Iran. Then the samples transferred to the Department of Food Science and Technology at Baharan Institute of higher education lab for analysis.

2-2 Chemical analysis

The sun dried samples were analyzed for Moisture, pH and salt (sodium chloride) content (AOAC, 1990).

2-3 Microbial analysis

The microbiological media and incubation conditions used for enumeration of microorganisms were Plate Count Agar (PCA) for Total Viable Count (at 37ºC, 24 h). Biard Parker agar (BP) was used for counting Staphylococcus aureus(37 ºC, 24-48 h) (BAM, 1995). Yeasts and molds were enumerated using acidified potato dextrose agar (Merck, Germany) after incubating at 30 ºC for 3days (El ziney and El turkey, 2006).Total psychrophilic bacteria were medium and incubation at 30 enumerated on plate count agar medium after incubation at 5ºC for 7 days as recommended by APHA (1992).Lactic acid bacteria were counted by the pour plate over layer method on MRS medium (Harrigan and Mccane 1976). Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation for 20–24 h at 37 ºC(Roberts et al., 1995). For aerobic spore forming bacteria, the dilutions were pasteurized at 80°C for 20 min in water bath, then the were plated on nutrient agar medium and incubated at 30°C for 3 days (Kilinc and Cakli, 2004). Dilution frequency technique was adopted to determine the densities of anaerobic spore forming clostridia, using Cooked Meat Medium (CMM), in 5 tubes for each dilution. The inoculated tubes were sealed with sterile mixture of Vaseline and Paraffin oil in 1:1 ratio and incubated at 35±2ºC for up to 7 days. The presence of clostridia was detected at the end of the incubation period by accumulation of gases pushing the vaspar layer up (Difco, 1974). Total halophilus microorganisms count was also performed using plate count agar (PCA) supplemented with 5% NaCl. The plates were incubated under aerobic condition at 35 ºC for 2 days (Thongthai and Suntinanalert, 1991). Salmonella spp was carried out using the most probable number technique (M.P.N) according to (ISO, 1982), after enrichment at 37 C for 24 h, in Silent broth, the cultures were streaked on Brilliant green agar and incubated at 37ºC for 24 h, then colonies were biochemical examined in Triple sugar iron agar (TSI) and Lysine carbonate broth. For shigella a loopful Silent broth was streaking onto salmonella and shigella agar (SSA) and incubated at 37ºC for 18-24 h (APHA,1992).

2-4 Statistical analysis

Data were analyzed using SPSS version 10.05-computer program.

3- Results and discussion

The FAO/WHO Codex Alimentarius Commission (1983) adopted the RecommendedInternational Code of Practice for Salted Fish in December 1979. The Code covers thetechnological and essential hygienic requirements for the preparation of high-quality saltedfish products, but the drying of salted fish is not covered. Pirimiphos methyl has been cleared by FAO/WHO for use on salted dried fish to protect against blowfly infestation during processing and against further insect infestation during storage (Esser et aL., 1990). This can increase microbial load in some unclean areas. Results of chemical analysis of samples shown in table 1.

Table 1. Results of chemical properties of dried fish samples

properties	Aghghala	Bandar torkman	Gomishan
pH	5.7 [b]	5.49 [a]	6.07 [c]
Moisture (%)	26 [b]	12.8 [a]	44.4 [c]
NaCl (%)	4.16 [a]	5.36 [c]	4.66 [b]

Table 2. Results of microbial counts of dried fish samples

Properties	Aghghala	Bandar torkman	Gomishan
Total count	3.2×10^{4} [b]	2.2×10^{3} [a]	2.3×10^{3} [a]

Yeast and molds	3×10^{1b}	0^{a}	6×10^{2c}
Staphylococcus aureus	9.8×10^{2c}	0.7×10^{1b}	0^{a}
Enterobacteriaceae	6.7×10^{1b}	0^{a}	0^{a}
Aerobic spores	3.8×10^{1a}	6×10^{3c}	1.2×10^{3b}
Anaerobic spores	2×10^{1a}	5×10^{2c}	8×10^{1b}
Psychrophilic bacteria	3×10^{1b}	1×10^{1a}	4×10^{1c}
Lactic acid bacteria	2×10^{1b}	0^{a}	4×10^{1c}
Halophilic microorganisms	2×10^{3b}	4×10^{2a}	1.5×10^{3b}
Salmonalla	0.8×10^{1b}	0^{a}	2×10^{1c}
Shigella	7×10^{1b}	0.4×10^{1a}	1.2×10^{2c}

Values of microbiological examinations of salted dried fish samples are presented in Table 2. Resuts showed that Samples collected from Bandar torkman had lower pH and moisture and higher NaCl in comparison to others, hence, yeasts and mold, Enterobacteriaceae and lactic acid bacteria counts were zero.Halophilus microorganisms and Psychrophilic bacteria were significantly ($p<0.05$) lower than others and only decrease the total count were not significant ($p<0.05$). This is agree with Zaki et al. (1976) that reported the total bacterial count decreased after drying, owing to the high salt content and the lack of free water in fish tissues and Coliforms were not present after drying.Also Elmossalami and Sedik (1972) reported total bacterial counts were lower in sand-salted fish than in tin-salted fish. Microbial counts in Gomishan and Aghghala samples were significant difference ($p<0.05$) due to difference in pH, moisture and NaCl content (except about Halophilus microorganisms that was not significant ($p<0.05$)).Total bacteria count in this study lower than those reported by El- Dengawy et al (2012) in salted fish samples.

The high content of NaCl in salted fish samples in this study may induce halophillic bacteria, where the total bacteria count increased and and reflected on counts of halophilus microorganisms (Zaki et al. (1976).Fong and Walsh (1971) obtained viable cultures on salt-agar of nitrate-reducing halo- bacteria and salt-tolerant S. aureus from aIl samples of Cantonese salt-dried fish that they examined. Onishi et al. (1980) isolated a variety of halophilic bacteria from salted fish, including salmon, salmon roe, codfish, cod roe and guts of cuttlefish. Bacteria isolated from Egytian sand-salted fish included micrococci, gram-positive bacili, Proteus vulgaris, P. mirabils and Aeromonasliquefaciens. All of the same microorganisms except A. liquefaciens were isolated from tin-salted fish; Serratiamarcescens, R rettgeri, P. morganii, Enterobacteraerogenes and Coryebacteriumfreundii were isolated from tin-salted fish only.

Yeast and molds counts in samples collected from Gimishan were high that is due to high moisture content. Species of fungi isolated from lndonesian dry salted fish included Paeczlomycesvariotii, Eurotiumamstelodami, Aspergilluscandidus and A. sydowii(Wheeler and Hocking, 1988). Afatoxin B1, produced by the fungi A. flavus and A. parasiticus, was reported in cured fish.Okonkwo and Nwokolo (1978) found a mean aflatoxin B1 concentration of 650 µg/kg in Nigerian dried fish. Stockfish, a dried imported fish from Scandinavia, contained no aflatoxin. Shank et al. (1972) identified aflatoxins in 5% of 139 samples of dried fish/shrimp purchased in markets in Thailand but in none of 35 samples purchased in Hong Kong. ln the contaminated samples, the mean aflatoxin (B1, B2, G1 and G2) concentration was 166 µg /kg. According to Edema and Agbon (2010), the most common source of fish deterioration is fungal, which have the ability to grow on substrates with low water activity down to 0.6 (Thiam, 1993) and are thus important in determining fish quality.

Aerobic spore forming in current study were lower than those reported by Zaki et al. (1976) in salted fishes. Also samples contained Clostridium spp. (anaerobic spores) and these salted fish may be harmful in human nutrition.This results against to EOS (2005 a, b andc).

Geetha et al (2014) isolated Staphylococcus aureusin all dried fish samples and concluded Staphylococcus was the most predominant organisms. Staphylococcus aureuscan grow in the presence of salt. Staphylococcus aureuscounts in this study was lower than those reported by Goja (2013) about fresh fishes. Staphylococcus aureus has also been

detected during the process dyring and subsequent smoking of eels in Alaska in 1993 (Eklund et al., 2004). Lactic acid bacteria usually sensitive to high concentrations of salt, but in low concentration of NaCl can survive even generated.Villar, Ruiz-Holgado and Sanchez (1985) found that Pediococcushalophilus was a dominant bacterium at the end of the curing process of anchovies, also many microorganisms such as Lactobacillus species were found during fish sauce fermentation (Saisithi et al., 1966; Thongthai et al., 1991; Saisithi, 1994; Thongsanit et al., 2002; Fukami et al., 2004).

Salmonella sp. was not detected in the sample collected from Bandar torkman. But Salmonella contamination was detected in other samples and shigellawas observed in all of the samples. The absence of Salmonella was similar to the results of Oulai et al. (2007) and Dodds et al. (1992). However, Djinou (2001) found that 0.8% of their samples had Salmonella. Contamination of fish and fishery products with Salmonella and Shigella has been reported by many researchers (Bandekar et al., 1995, Lyer and Shrivastava 1989a; Ponda, 2002; Sinduja et al., 2011). Incidence of pathogens in the sample of fish market may be attributed to external contamination and poor handling at ambient temperature (Iyer and shrivastava, 1989b). In some of the cases, the food borne illness such as scombroid poisoning is observed in dry fishes mainly due to chemical agent and histamine and it is also called as histamine poisoning. E. coli is responsible for the production of histamine in the dried fishes (Logesh et al., 2012). In rare cases Salmonella and Staphylococcus species produce histamine residue (Huang et al., 2010). So safety measures should be taken to reduce the contaminations and insect infestations.

4- Conclusion

Assessment of the microbiological quality of salted dried fishes sold in the Goestan Province, Iran, showed that the conditions for their production and sale were poor and suggested that producers and vendors were not following good hygiene practices. Somepathogenic microorganisms such as Staphylococcus aureus and Salmonella sp, were identified in the samples analyzed that could be spores of pathogenic Clostridium botulinum type E or Clostridium perfringens. It is therefore important that actions be taken to improve the situation.

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