Microbial contamination of smoked *Anchovis*

C. T. ARTHUR & A. OSEI-SOMUAH
Animal Research Institute, CSIR, P. O. Box AH 20, Achimota, Ghana

ABSTRACT

Smoked *Anchovis* spp. (*Engraulis encrasicholus* Linndeus) obtained from Senya Beraku (a major fishing centre), Kasoa (a marketing centre), and the Animal Research Institute Feedmill (feed-processing centre) was assessed for microbial contamination, using total viable count technique (pour plate method) and culture techniques. Sixty samples were aseptically and randomly selected into sterile universal bottles from the three study areas, with 20 samples from each study area. Total viable count ranged from 1×10^5 to 4.9×10^7, 3.4×10^5 to 2.0×10^8, and 6.5×10^5 to 3.1×10^8 for Kasoa, Senya Beraku and Feedmill, respectively. A total of 139 isolates made up of 84 bacteria and 55 fungi were isolated. The 84 bacteria isolates belonged to 11 genera: *Streptococcus, Staphylococcus, Micrococcus, Salmonella, Enterobacter, Escherichia, Pasteurella, Pseudomonas, Bacillus, Listeria,* and *Aeromonas*. The 55 fungi isolates belonged to five genera: *Aspergillus, Trichoderma, Rhizopus, Penicillium,* and *Mucor*. Since most of the organisms isolated may cause disease in man and animal, it is suggested that processing, handling, and storage of fish be improved to reduce contamination.

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RÉSUMÉ

ARTHUR, C. T. & OSEI-SOMUAH, A.: *Contamination microbienne d'anchois fumé.* L'espèce d'anchois fumé *(Engraulis encrasicholus* Linndeus*)* obtenu d'une centre majeur de pêche, centre de vente, et centre de fabrication d'aliment (moulin d'aliment) était étudié pour la contamination microbienne en utilisant la technique de compte viable total (méthode verser plat) et les techniques de culture. Les zones d'étude étaient Senya Beraku (centre de pêche), Kasoa (centre de vente) et Recherche d'Alimentation Animale (centre de préparation). Soixante échantillons étaient sélectionné d'une manière aseptique et au hasard en bouteilles stériles universelles de trois zones d'étude avec 20 échantillons de chaque zone d'étude. Compte viable total variait de 1×10^5 à 4.9×10^7, 3.4×10^5 à 2.0×10^8 et 6.5×10^5 à 3.1×10^8 respectivement pour Kasoa, Senya Beraku, et Moulin d'aliment. Un total de 139 isolats apartenait à 11 genres, à savoir *Streptococcus, Staphylococcus, Micrococcus, Salmonella, Enterobacter, Escherichia, Pasteurella, Pseudomonas, Bacillus, Listeria,* et *Aeromonas*. Les 55 moisissures isolées apartenaient à 5 espèces à savoir *Aspergillus, Trichoderma, Rhizopus, Penicillium,* et *Mucor.* Puisque la plupart d'organismes isolés pourraient causer des maladies pour l'homme et l'animal, il est suggéré que le traitement, manutention et conservation doivent être amélioré pour réduire la contamination.

Introduction

Fish and fish products are sources of animal protein for the growth and development of man and animal. In Ghana, animal protein constitutes over 5 per cent of average daily diet (FAO, 1993), and it is estimated that about 64 per cent of animal protein is from fish (Heinbuck, 1994). This is mainly due to its affordability as compared to meat. The choice of fish as protein source in Ghana is due to its lack of cultural, ethnic, and religious barriers. The other advantage is that it is not associated with zoonotic diseases such as *Brucellosis, Listeriosis,* and *Anthrax* (Cruishank *et al.*, 1975).

Anchovis spp. one of the cheapest fishes in Ghana, is considered as a household fish. Its popularity has given it the nickname, "Keta School Boys", derived from a popular fishing town, Keta. Most livestock and poultry farmers who prepare their own feed also use *Anchovis* as their animal protein source. However, poor hygienic conditions during handling, processing, and storage result in microbial contamination and spoilage

(Mulder, 1996; Lu, Pace & Plahar, 1991; Wit & Kampelmacher, 1981). Though the microflora of smoked and sun-dried *Anchovis* had been studied (Osei-Somuah & Nartey, 1999), work on tracing the sources of contamination in smoked *Anchovis* is yet to be done.

This study, therefore, seeks to determine the microflora of smoked *Anchovis* and the levels of bacterial and fungal contamination, and to isolate and identify microbial contaminants.

Materials and methods

Sampling areas

Three sample sites were chosen as the study areas for the collection of the smoked *Anchovis*.

Senya Beraku. A coastal town situated about 60 km west of Accra, prominently known for its fishing industry. The major processing methods are sun-drying, smoking, and salting. Samples were collected from Chorkor Smoker, open grills, and store rooms.

Kasoa. A marketing centre 30 km west of Accra, was chosen to assess the effect of the environment and human activity on contamination of smoked *Anchovis* displayed for sale.

Animal Research Institute Feedmill. This feed processing centre was selected to determine the level of contamination of *Anchovis* before it is processed into poultry and livestock feed. The feedmill is used for preparing feed for private as well as the Institute's poultry and livestock. Fish used at the mill are from many sources such as fishing towns and other marketing centres.

Sampling methods and laboratory techniques

Sixty smoked fish samples *(Anchovis)* were randomly and aseptically collected into sterile universal bottles (20 samples from each study area), using sterile forcep. Each fish sample was cut into smaller pieces, using sterile scapel blade. From each sample, 1g was aseptically weighed into 9 ml of sterile 0.1 per cent peptone water in screw cap universal bottles and incubated for 30 min at 37 °C (Collins & Lyne, 1983).

Both total viable count technique for microbial enumeration and culture techniques for isolation and identification of organism were used. For the total viable count technique, the pour plate method was used. One millilitre of the neat sample was serially diluted by 10-fold dilution into five other MacCartney bottles each containing 9 ml of sterile 0.1 per cent blank peptone water. Different pipettes were used for different dilutions. One millilitre of each dilution was aseptically transferred into its own universal bottle, each containing 9 ml of molten plate count agar kept in water bath at 45 °C (Collins & Lyne, 1983). This was mixed by rotation and poured into sterile Petri dishes aseptically and allowed to cool and set.

Cultures

Using the plate-out technique, cultures were prepared from the neat dilutions onto blood agar, MacConkey agar, and malt extract dextrose agar as described by Heritage, Evans & Killington (1996). The cultures were incubated for 18 - 24 h at 37 °C for those in/on plate count agar, blood agar, and MacConkey agar plates. The cultures on malt extract dextrose agar were incubated for 3 - 7 days at 30 °C. After incubation, plates showing viable colonies between 30 and 300 were selected and counted, using colony counter.

Isolation and identification

The colonial morphology of organisms were studied for size, shape and colour on various media. Bacteria were stained, using Gram stains, and examined for Gram reaction by the light microscope at × 100 with oil immersion. They were identified, using the Analytical Profile Index (API) 20E and 20 NE identification system, to the species level. Fungi were stained with lactophenol cotton blue stain and examined by the light microscope at × 10 magnification. Since the API system for fungi was unavailable, the organisms were identified, using colonial morphology and microscopy.

Results

Ten out of the 60 samples showed no growth. Of

the remaining 50 samples, a total of 139 microorganisms made up of 84 bacteria and 55 fungi were isolated. The 84 bacteria isolates belonged to 11 genera, with the 55 fungi isolates belonging to five different genera (Table 1). Twenty four percent of the samples had two or more bacteria isolates.

The most frequently occurring bacterium in all the three study areas was *Staphylococcus,* that is 20.2 per cent; with *Listeria* and *Aeromonas* being the least, that is 1.2 per cent. For the fungi, *Mucor* had the highest isolate of 69.1 per cent while

TABLE 1

Microorganisms Isolated in Three Study Areas

Organism	*Kasoa (%)*	*Senya Beraku (%)*	*Feedmill (%)*
Bacteria			
Streptococcus	6 (24)	6 (21)	2 (26)
Staphylococcus	5 (20)	7 (25)	4 (13)
Escherichia	1 (4)	2 (7)	3 (10)
Bacillus	4 (16)	3 (11)	8 (26)
Enterobacter	2 (8)	1 (4)	1 (3)
Salmonella	6 (24)	3 (11)	3 (10)
Micrococcus	1 (4)	1 (4)	4 (13)
Pasteurella	-	5 (18)	3 (10)
Listeria	-	-	1 (3)
Pseudomonas	-	-	2 (6)
Aeromonas	-	-	2 (6)
Total bacteria isolated	84		
Fungi			
Trichoderma	2 (18)	-	-
Rhizopus	1 (9)	-	-
Mucor	8 (73)	16 (62)	14 (77.8)
Aspergillus	-	4 (15)	-
Penicillium	-	6 (23)	4(22.2)
Total fungi isolated	55		

Rhizopus had the least, accounting for 1.8 per cent (Table 2).

The total viable counts for the bacteria ranged from 1.0×10^5 to 4.9×10^7 for Kasoa, 3.4×10^5 to 2.0×10^8 for Senya Beraku, and 6.5×10^5 to 3.1×10^8 for Feedmill (Table 3). The difference in the bacteria isolated in all the study areas was not much; with the exception of *Listeria*, *Pseudomonas* and *Aeromonas* which were isolated only at the Feedmill. For the mould isolates, *Mucor* was common to all the study areas, but *Penicillium* was isolated on samples from Senya Beraku and the Feedmill. *Aspergillus* spp., however, were common at Senya Beraku whilst *Trichoderma* and *Rhizopus* were isolated at Kasoa.

TABLE 2

Frequency of Microorganisms Isolated from Smoked Anchovis

Organism	*Frequency*	*Percentage*
Bacteria		
Staphylococcus	17	20.2
Bacillus	15	17.9
Streptococcus	13	15.5
Salmonella	12	14.3
Pasteurella	8	9.5
Micrococcus	6	7.1
Escherichia	5	5.9
Enterobacter	4	4.8
Pseudomonas	2	2.4
Listeria	1	1.2
Aeromonas	1	1.2
Total	84	
Fungi		
Mucor	38	27.3
Penicillium	10	7.2
Aspergillus	4	2.9
Trichoderma	2	1.4
Rhizopus	1	0.7
Total	55	

Discussion

The high total viable count recorded showed that the fish samples were contaminated (Table 3). With the exception of *Listeria*, *Pseudomonas* and *Aeromonas* spp., the rest of the organisms were common to all the study areas (Table 1).

The log of the total viable count determined for the three study areas ranged from 4.6 - 8.2 $\log_{10}$ cfu/ml (Table 3). It was above the range of 5.0 - 6.0 $\log_{10}$ cfu/ml recommended by the Interna-

TABLE 3

Mean Bacteria Count

Organism	*Mean count cfu/ml*		
	Kasoa (log_{10})	*Senya Beraku (log_{10})*	*Feedmill (log_{10})*
Escherichia + Bacillus	2.90×10^6 (6.4)	-	-
Streptococcus + Bacillus	1.33×10^5 (5.1)	-	-
Streptococcus + Staphylococcus	3.83×10^6 (6.5)	5.57×10^6 (6.7)	-
Streptococcus + Salmonella	5.00×10^4 (4.6)	-	-
Staphylococcus + Micrococcus	4.00×10^6 (6.6)	-	4.60×10^7 (7.6)
Enterobacter	1.55×10^7 (7.1)	2.60×10^6 (6.4)	-
Streptococcus	1.70×10^5 (5.2)	2.00×10^6 (6.3)	1.30×10^7 (7.1)
Staphylococcus	2.49×10^7 (7.3)	3.77×10^7 (7.5)	-
Bacillus	1.65×10^6 (6.2)	4.50×10^6 (6.6)	1.11×10^8 (8.0)
Salmonella	5.95×10^5 (5.7)	-	-
Escherichia	-	1.56×10^8 (8.1)	6.50×10^4 (4.8)
Pasteurella	-	9.70×10^5 (5.9)	2.90×10^7 (7.4)
Escherichia + Salmonella	-	2.50×10^5 (5.3)	-
Bacillus + Salmonella	-	5.00×10^5 (5.6)	3.80×10^5 (7.4)
Streptococcus + Pasteurella	-	4.11×10^7 (7.6)	-
Staphylococcus + Salmonella	-	6.75×10^6 (6.8)	2.20×10^7 (7.3)
Staphylococcus + Streptococcus	-	5.57×10^6 (6.7)	-
Micrococcus + Bacillus	-	1.00×10^8 (8.0)	-
Micrococcus	-	-	1.79×10^8 (8.2)
Pseudomonas	-	-	4.50×10^4 (4.6)
Pasteurella + Listeria	-	-	6.00×10^7 (7.7)
Micrococcus + Enterobacter	-	-	7.60×10^7 (7.8)
Bacillus + Aeromonas	-	-	6.30×10^7 (7.7)
Escherichia + Streptococcus	-	-	5.30×10^6 (6.7)
Escherichia + Staphylococcus	-	-	2.87×10^7 (7.4)

tional Commission on the Microbiological Specification for Food Standards (ICMFS, 1978). The viable count range for indicator organisms such as *Staphylococcus* spp. (7.3 - 7.5 $\log_{10}$ cfu/ml) was above the ICMFS value of 2.0 - 3.0 $\log_{10}$ cfu/ml. This may indicate sub-optimal smoking, insufficient sanitation and storage (Mossel, Mengerink & Sholts, 1962).

Fish is partially sterile when freshly caught and immediately after smoking, but becomes infected when it comes into contact with fishing boats, when handled by fishermen and fishmongers, and during storage in the fish houses. These contaminants, usually *Achnobacter, Pseudomonas, Flavobacterium, Baccillus, Micrococcus, Clostridium* and *Escherichia,* are transferred to the fish during processing. The high mean viable count of faecal contaminants such as *Escherichia* spp. at Senya Beraku (Table 3) could have been as a result of spreading the fish first on the beaches for partial drying and fermentation. These beaches are contaminated with faecal materials which may be transferred onto the fish through contact. This human activity, as part of processing, contributes

to fish contamination (Mulder, 1996; Frazier, 1958).

The high proportion of *Mucor* at Senya Beraku (Table 1) indicated spoilage during processing and storage. The likely source of *Mucor* might be the sawdust used for smoking, since fungal spores are resistant to heat (Shewan, 1949; Shewan & Hobbs, 1967). Another observation was that the semi-dried samples had higher viable counts because most organisms require moisture for growth and multiplication.

Samples taken from Kasoa Market had low mean viable count compared with Senya Beraku and the Feedmill (Table 3), because fish sent to the market were processed solely for human consumption and, therefore, were clean and dry. The generally high percentage isolate of *Mucor* and *Streptococcus* spp. (Table 1) indicated spoilage and faecal contamination.

The Feedmill samples had the highest levels of mean viable count (Table 3). Most fish samples received were processed and stored for livestock and poultry under conditions akin to Senya Beraku. The high mean count of *Micrococcus* and *Bacillus spp.* (Table 3) indicate human physical and physiological activities that characterize the milling room as well as fish poison (Collins, Harley & Pilsworth, 1977).

The choice of pour plate method for total viable count technique has its own limitations. It gives better discrete colonies compared with other techniques. However, it is unsuitable for heat-sensitive organisms and strict aerobes (Heritage *et al.*, 1996). This was assumed to have affected the total count for the samples. The absence of anaerobic culture techniques restricted the growth of strict anaerobes such as *Cl. botulinum* and *Cl. pefringens* which cause food poisoning.

Most microflora isolated were organisms associated with food contamination, spoilage and poisoning (Claucas, 1990). For instance, the presence of *Bacillus cereus* and *Staphylococcus aureus* (identified using API) indicates food poisoning (Hobbs & Gilbert, 1981). The high level of moulds indicates fish spoilage during processing and storage. The association of these microorganisms with fish render them unwholesome for human and animal consumption because they cause various diseases in man and animal (Blood, Radiostis & Henderson, 1985).

Conclusion

This study shows that the fish *(Anchovis)* sampled were contaminated with moulds and bacteria. As several organisms isolated may cause diseases in man and animal, consumers may be at risk. It is, therefore, suggested that before smoking and during storage, fish should be treated and processed under strict hygienic conditions. There should be no difference in treatment, processing, and storage for fish meant for human and animal consumption to ensure low microbial contamination of processed fish.

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