

STUDIES ON MICROBIOLOGICAL QUALITY OF SMOKED FISH IN SOME MARKETS IN ACCRA, GHANA

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Abstract

The microbiological quality (MQ) of smoked mackerel and tuna in some Accra markets (Mallamatta, Madina, Kaneshie and Dome) were assessed and related to other retail outlets. Microbiological load and profile were determined using the methods of pour plate and identification by morphological characteristics and biochemical tests. Wide variations and significant differences ($P < 0.05$) were recorded in the counts of microorganisms in both smoked mackerel (SM) and tuna (ST) within and between the markets. Madina market had a higher MQ of smoked fish compared to those of Dome, Mallamatta and Kaneshie markets. Total viable counts (TVC) ranged from 2.45 to 9.09 (\log_{10} cfu/g), coliform counts (CC) ranged from 0 to 8.13 (\log_{10} cfu/g) and moulds and yeasts counts (MYC) ranged from 0 to 5.87 (\log_{10} cfu/g) for both SM and ST. Based on microbiological counts and profile, SM had a more satisfactory MQ compared to ST. The TVC, CC and MYC were all significantly ($P < 0.05$) higher at the markets compared to smokehouses. No significant differences ($P < 0.05$) were recorded between TVC, CC and MYC smokehouses and the canteens. *Klebsiella pneumoniae*, *Enterobacter sakazakii*, *Aspergillus niger* and yeasts were the predominant microorganisms isolated from the SM and ST. In a comparative profile analysis, market samples gave seven isolates while the canteen and smokehouse gave four and two isolates, respectively. The market environment is more important than the processing technology in determining the MQ of smoked fish. The MQ of smoked fish in markets is unsatisfactory, probably due to the mode of storage, handling, exposure, and poor environmental and sanitary conditions.

Résumé

ADU-GYAMFI A.: Etude sur la qualité microbiologique de poisson fumé en quatre marchés d'Accra, Ghana. La qualité microbiologique (QM) de maquereau et de thon fumés en quatre marchés d'Accra (Mallamatta, Madina, Kaneshie and Dome) était évaluée et liée aux autres débouchés de détail. La charge et le profil microbiologique étaient déterminés employant les méthodes de la lamelle de verre et l'identification par les caractéristiques morphologiques et les essais biochimiques. Des grandes variations et des différences considérables ($P < 0.05$) étaient enregistrées après les comptes des micro-organismes en maquereau fumé (MF) et en thon fumé (TF) sur les marchés et entre les marchés. Le marché de Madina avait une QM plus élevée de poisson fumé comparée avec celles des marchés de Dome, Mallamatta et Kaneshie. Les comptes viables totaux (CVT) variaient entre 2.45 et 9.09 [\log_{10} cfu/g] et les comptes de coliforme (CC) variaient entre 0 et 8.13 [\log_{10} cfu/g] et les comptes des moisissures et des levures (CML) variaient entre 0 et 5.87 [\log_{10} cfu/g] pour MF et TF. Fondée sur les comptes et les profils microbiologiques, MF avait plus de QM satisfaisante comparée à TF. Les CVT, CC et CML étaient tous considérablement ($P < 0.05$) plus élevés sur les marchés comparés aux maisons de fumage de poisson. Aucune différence considérable n'était enregistrée de CVT, CC et CML entre les maisons de fumage de poisson et les cantines. *Klebsiella pneumoniae*, *Enterobacter sakazakii*, *Aspergillus niger* et les levures étaient les micro-organismes prédominants isolés de MF et TF. En analyse de profil comparative, les échantillons de marché rapportaient 7 isolats alors que la cantine et la maison de fumage de poisson rapportaient respectivement 4 et 2 isolats. L'environnement du marché est plus important que la technologie de traitement en déterminant la QM de poisson fumé. La QM de poisson fumé sur les marchés est peu satisfaisante, probablement en raison de mode de stockage, de manutention d'exposition et de mauvaises conditions environnementales et sanitaires. Il est nécessaire d'appliquer les règlements et usages ainsi que les niveaux de sécurité aux marchés surtout par les autorités responsables au niveau national et municipal pour améliorer la qualité de poisson fumé pour sauvegarder la santé publique du pays.

Introduction

Fish is one of the most important and cheapest sources of animal protein in the Ghanaian diet. Annual total fish production in Ghana is 400,000 tonnes (FASDEP, 2002). Traditionally, fish is processed through smoking, salting and/or drying. Estimates show that 70 - 80 per cent of fish landed in the country are preserved by smoking in the fish-processing areas of Volta, Greater Accra, Central and Western regions. Traditional smoking generally cooks and partially dries the fish as well as imparting a smoky flavour. This process, which has both pasteurizing and inhibitory effects due to heat and wood smoke, also effectively reduces the water activity and allows long-term storage of smoked fish. (Kagan, 1970; Okraku-Offei, 1970; Reusse, 1968; Plahar, Pace & Lu, 1991; Neequaye-Tetteh, Dassah & Quarshie-Sam, 2002).

Smoked fish is distributed through a marketing web created by the activities of fishermen, fish processors, traders and consumers at various retail outlets. Rising urban demand, variable supplies, extensive distribution chains and unfavourable climatic conditions cause considerable changes in product quality during retailing. Traditional methods of storage result in high post-harvest losses and adversely affect the quality of fish due to frequent insect infestation, microbial decomposition and rodent attack (Cauri *et al.*, 1979; Nequaye-Tetteh, 1979). Physical disintegration of smoked fish, which results from poor handling, packaging and distribution practices aggravates losses and further exposes products to the action of bacteria and moulds. Marketing of fish using uncovered baskets, jute sacks and mats involves little or no organized attempt at controlling product quality (Meeren, vein der, 1980). Though water activity determines the shelf-life of smoked fish, it is crudely controlled due to inherent limitations of traditional processing methods (FAO, 1981). The quality of smoked fish is essentially linked to the processing and post-processing procedures and, therefore,

depends on the skill and expertise of the fish processors and retailers.

The smoking process, storage conditions, handling and distribution methods are important quality determining factors. Studies show that while bacteria are responsible for spoilage in most intermediate moisture fish products with high water activity, moulds and insects are responsible for spoilage in those with low water activity [Hussain, Hax & Chandry, 1989]. Concern has been expressed over the occurrence of pathogens such as *Clostridium botulinum*, *C. perfringens*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aspergillus* spp., and *Penicillium* spp. in fish and other seafoods (Garrett, Jahncke & Tennyson, 1997; Hussain, Haz & Chaudry, 1989). In Ghana 17 genera of bacteria, including food pathogens, have been isolated from smoked and sun-dried anchovies obtained from markets and feedmills (Osei-Somuah & Nartey, 1999).

Studies by Nketsia-Tabiri *et al.* (2003) also show the presence of *Staphylococcus* spp., *Enterobacter sakazaki*, *Klebsiella pneumoniae ozaenae*, *Bacillus* spp., *Aspergillus* spp. and *Penicillium* spp. in smoked sardines. Although no toxicological problems have yet been reported, dangerously high levels of aflatoxin (600-700 p.p.m.) have been found in dried fish (Okonkwo, Umerah & Nwokolo, 1977). Contaminations with pathogenic microorganisms and microbial toxins result in severe chronic or fatal health consequences for the population, in addition to reduced economic productivity for the country.

There is great demand for high quality processed fish for the domestic and export markets. As the most important non-traditional export commodity of Ghana (Osei & Koranteng, 2000), it is necessary to meet international demands for quality and safety to enhance the competitiveness of the fish industry. There is, therefore, the urgent need to maintain quality standards at all places where fish is processed, stored, sold and consumed. Locally, it is essential

that the various microbiological hazards associated with market retailing of smoked fish are identified and quantified. This will help in developing effective food safety strategies to prevent exposure of fish products to unacceptable levels of microbiological agents. The objectives of this study were, therefore, to (i) investigate the microbiological quality of smoked tuna and smoked mackerel in four markets in Accra, and ii) compare the microbiological quality of smoked tuna in markets and other retail outlets (smokehouses and canteens) in Accra.

Materials and methods

Samples for market survey

Samples for the microbiological quality analysis of smoked tuna and smoked mackerel sold in markets were obtained from randomly selected retailers in Mallamatta, Madina, Kaneshie and Dome markets in Accra. Each market was visited twice, at approximately a month interval.

Samples for retail outlet survey

Samples for the microbiological quality analysis of smoked tuna sold at different retail outlets were obtained from the following three retail sources in Accra: (i) smokehouses (at *Chorkor*), (ii) markets (at Kaneshie, Mallamatta, Dome, and Madina), and (iii) canteens (at Dome and Haatso). All samples were kept at 3-5 °C in a refrigerator and analysed within 1-2 h of collection.

Microbiological quality analysis

Five grammes of each sample was added to 45-ml peptone water (1% peptone water + 0.5% NaCl) and homogenised with a blender (Waring Laboratory Blender, Christison, Germany) for 5 min. Microbiological load determination was carried out using standard decimal dilution and plate count methods (APHA, 1976). Enumeration was done using illuminated colony counter (Stuart Scientific, UK). Total viable count was estimated by incubation on plate count agar (Merck, Germany) for 48 h at 36 °C. Moulds and

yeasts counts were estimated by incubation on oxytetracycline glucose yeasts extract agar (Oxoid, UK) for 3 days at room temperature (26-28 °C). Coliform count was estimated by incubation on violet red bile agar (Merck, Germany) for 48 h at 36 °C.

Microbiological profile (coliforms and moulds) of the samples was determined using representative colonies from plates of coliforms and moulds purified by repeated streaking and subculturing, respectively. Identification of coliforms was done using morphological characteristics and various biochemical tests with the API 20E microtube system (BioMerieux, South Africa).

The system performed 22 standardised biochemical tests to generate a profile index number for each coliform which was then identified by consulting the profile recognition system. Moulds were identified using the general fungal flora according to the key of Gilman (Gilman, 1957). Yeasts were counted but types were not identified.

Statistical analysis

Statistical analysis of variance with Fisher's pairwise comparison was carried out on the data using a Minitab computer software.

Results

Total viable counts (TVC) of smoked mackerel (SM) ranged from 2.45 to 8.35 [\log_{10} cfu/g] (Table 1). Coliform counts (CC) were as high as 8.06 [\log_{10} cfu/g] and totally absent in some SM samples whilst moulds and yeasts (MYC) were also absent in some samples and enumerated at 5.32 [\log_{10} cfu/g] in others. With few exceptions, significant differences ($P < 0.05$) occurred in most of the counts among the various SM samples between and within markets. In the case of smoked tuna (ST), TVC varied from 4.89 to 9.09 [\log_{10} cfu/g]. While coliforms were absent in some ST samples, they were counted as high as 8.13 [\log_{10} cfu/g] in others. The MYC ranged from 1.97 to 5.87 [\log_{10} cfu/g]. Except for a few cases, significant

TABLE 1

Microbial counts of smoked tuna and smoked mackerel obtained from markets

Market	Total viable cells		Coliforms		Moulds and yeast	
	Smoked mackerel	Smoked tuna	Smoked mackerel	Smoked tuna	Smoked mackerel	Smoked tuna
Mallamatta ¹	5.44 ^a	6.86 ^a	6.23 ^a	5.77 ^a	1.81 ^a	5.01 ^a
Mallamatta ²	2.45 ^b	7.91 ^b	1.00 ^b	6.08 ^b	1.59 ^b	1.97 ^b
Madina ¹	3.89 ^c	4.89 ^c	0.00 ^c	0.00 ^c	0.00 ^c	2.92 ^c
Madina ²	3.69 ^{cd}	7.83 ^{bd}	2.01 ^d	4.96 ^d	3.72 ^d	5.04 ^{ad}
Kaneshie ¹	3.95 ^{ce}	9.09 ^c	2.82 ^c	8.13 ^c	2.58 ^c	5.87 ^c
Kaneshie ²	4.92 ^{af}	8.15 ^f	4.31 ^f	7.27 ^f	1.78 ^{af}	4.90 ^{af}
Dome ¹	2.47 ^{bd}	4.62 ^{cg}	0.00 ^{bdg}	2.45 ^g	0.00 ^{cg}	3.24 ^{cg}
Dome ²	8.35 ^h	6.41 ^{ah}	8.06 ^h	5.28 ^{ah}	5.32 ^h	4.98 ^{fh}

^{1,2} First and second sampling. Counts are expressed as log₁₀ cfu/g. Values in a column bearing the same superscript are not significant at 5%. Values are means of the counts of three independent experiments.

differences ($P < 0.05$) occurred among the various ST samples between and within markets. Smoked fish in Madina market had higher microbiological quality compared to those of Dome, Mallamatta and Kaneshie markets. Beyond these observations, no other patterns could be deduced for the differences in the microbiological indices between and within markets for both SM and ST.

The prevalence of coliforms and fungi on ST and SM in the markets is shown in Table 2. While eight coliforms and six fungi species were isolated from the SM samples, five coliforms and six fungi species were isolated from ST. The most frequently occurring coliforms were *Enterobacter cloacae*, detected in both ST (62%) and SM (9%), and *Klebsiella pneumoniae* also detected in both SM (50%) and ST (13%). *Proteus mirabilis* and *Escherichia coli* were detected in both samples, but at lower prevalence. *Serratia plymuthica*, *Erwinia* spp. and *Enterobacter amnigena* were detected in a few cases but only on SM and *Enterobacter aerogenes* was also detected in a few cases on only ST. Yeasts, *Aspergillus niger*, *A. wentii* and *Rhizopus* spp. were fungal isolates occurring in both ST and SM at prevalence levels of 13-37 per cent. *Geotrichum* spp. and

TABLE 2

Prevalence of coliform and fungal isolates on smoked tuna and smoked mackerel obtained from markets

Isolate	Tuna (%)	Mackerel (%)
<i>Coliforms</i>		
<i>Escherichia coli</i>	9	13 ^a
<i>Enterobacter cloacae</i>	62	9
<i>Enterobacter sakazakii</i>	0	25
<i>Enterobacter amnigena</i>	0	13
<i>Enterobacter aerogenes</i>	9	0
<i>Erwinia</i> spp	0	13
<i>Klebsiella pneumoniae</i>	13	50
<i>Proteus mirabilis</i>	25	13
<i>Serratia plymuthica</i>	0	13
<i>Fungi</i>		
<i>Geotrichum</i> spp.	13	0
<i>Paecilomyces</i> spp.	13	0
<i>Aspergillus niger</i>	19	22
<i>Aspergillus versicolor</i>	0	9
<i>Aspergillus wentii</i>	19	13
<i>Penicillium</i> spp.	0	13
<i>Rhizopus</i> spp.	19	25
Yeasts	28	37

^a Percentage isolation (n=32)

Paecilomyces spp. were isolated only in ST while *A. versicolor* and *Penicillium* spp. were also present only in SM at low prevalence. Considering the prevalence of coliforms in addition to the microbiological counts in markets, SM had a more satisfactory microbiological quality compared to ST.

The microbiological quality of ST in the markets and two other retail outlets is shown in Table 3. The TVC, CC and MYC were all significantly ($P < 0.05$) higher at the market compared to the smokehouses. There were no significant differences ($P < 0.05$) for the counts between the smokehouses and the canteens. However, TVC was significantly lower ($P < 0.05$) for the canteens

Discussion

The fishing industry is one of the most important sub-sectors of the agricultural sector and accounts for about 5 per cent of agricultural GDP (FASDEP, 2002; Anon., 1995). There is growing demand for high quality processed fish for both domestic and export markets. For example, 2,824.61 tonnes of dried/smoked fish valued at US \$ 1,570,431 was exported in 1998 (GEPC, 1998). Despite these potentials, estimates show that poor handling, inadequate storage, distribution and marketing strategies, as well as the absence of improved technologies for preserving and processing fish, result in losses of about 100,000 tonnes annually in Ghana (FAO, 1981; Mensah,

TABLE 3

Microbial quality of smoked tuna at different retail outlets

Outlet	Total viable counts	Coliforms counts	Moulds and yeasts counts	Coliform and fungal isolates
Smoke-house	4.24 ^a	1.54 ^a	1.39 ^a	<i>Enterobacter cloacae</i> , Yeasts.
Market	7.00 ^b	3.67 ^b	3.49 ^b	<i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i> , <i>Proteus mirabilis</i> , <i>Aspergillus</i> spp., <i>Rhizopus</i> spp., <i>Paecilomyces</i> spp., Yeasts.
Canteen	5.16 ^a	3.19 ^{ab}	2.21 ^{ab}	<i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i> , <i>Paecilomyces</i> spp., Yeasts

Values are means of the counts of four independent experiments. Values in a column bearing the same superscript are not significant at 5%. Counts are expressed as log₁₀ cfu/g.

compared to the markets. No significant differences ($P < 0.05$) were established between the markets and canteens for the CC and MYC. Seven isolates were detected in the ST from markets while four and two were detected in the canteens and smokehouses, respectively. *Enterobacter cloacae* and yeasts were present in the ST at all the retail outlets but *Proteus mirabilis*, *Geotrichum* spp., *Rhizopus* spp. and *Paecilomyces* spp. were isolated in only the market samples. *Aspergillus* spp. was present in the ST of both markets and canteens.

1997).

Although smoked fish in Madina market had a more satisfactory microbiological quality compared to those of Dome, Mallamatta and Kaneshie markets, this study has confirmed the poor and unacceptable microbial quality of some smoked fish in local markets. The microbial counts of both smoked mackerel (SM) and smoked tuna (ST) showed wide variations between markets and also within the same markets over the period surveyed. These differences suggest possible differences in processing, storage and handling of ST and SM resulting in probably significant

differences in shelf-life.

Studies have shown that the shelf-life of smoked fish ranges from 2 to 24 weeks and depends on factors such as dryness of the product, temperature of storage, degree of smoking and the microbial load. For example, in high moisture smoked fish microbial spoilage sets in after 2-3 days after smoking whilst smoke-dried lean sardines can store for more than 3 months without spoilage (FAO, 1981; Nketsia-Tabiri & Sefa-Dedeh, 2000). The high microbial loads reported in this work compare with those reported by Nketsia-Tabiri *et al.* (2003) on smoked sardines (Aerobic Plate Count, 4.8-7.5 \log_{10}) and Osei-Somuah & Nartey (1999) on smoked and sun-dried *Anchovies* spp. (Standard Plate Count $> 10^5$).

The isolation of potential pathogens such as *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Aspergillus niger*, and *A. wentii* from ST and SM shows the ease of contamination and their capacity to cause food-borne illnesses. The presence of *Aspergillus* spp. and *Penicillium* spp. in ST and SM have also been confirmed in other studies (Hussain *et al.*, 1989; Appiah *et al.*, 1990). Although the study covered four markets, and, notwithstanding the fact that SM had a more satisfactory microbiological quality compared to ST, it has shown the poor hygienic quality of smoked fish when counts are rated on accepted national and international standards, i.e. total viable cells $< 10^6$; moulds and yeasts cells $< 10^4$ cfu/g (Ghana Standards, 1997; Solberg *et al.*, 1990; Watson, 1996; FAO/WHO, 1969; FAO, 1977). From the foregoing, it is evident that the quality of smoked fish is unsatisfactory and depends on the skill of fish artisans in handling, as well as storage efficiency of retailers in markets.

Using smoked tuna (ST) as an example, it can be observed that considerable changes occur in the microbiological quality of smoked fish during retailing. The microbiological indices (TVC, CC, MYC) of ST in smokehouses were all significantly low ($P < 0.05$) and met the accepted standards when compared to those from markets. The

effectiveness of smoking and sun-drying as traditional processing techniques are well known and the adoption of improved versions of traditional fish-smoking techniques has further enhanced production and preservation (AFPP Project Document, 1988). In spite of these achievement, there still continues to be problems with handling, storage and distribution of smoked fish, especially at the market level. Cultural and traditional perceptions play fundamental roles in marketing such that poor quality fish (badly infested, broken and mouldy) does not lose market value (Disney, 1974; Watanabe & Mensah, 1976; FAO, 1981). These observations, together with the deteriorating state of environmental sanitation, might account for the poor hygienic quise reasons may also account for the higher prevalence of microbes including potential pathogens such as *Enterobacter cloacae* and *Aspergillus* spp. in ST at the markets.

Although TVC was significantly lower ($P < 0.05$) for the canteens, the count of 5.159 \log_{10} cfu/g was quite high and raises concern for catering standards. It can be deduced that a significant proportion of market contaminants of smoked fish are transferred eventually to meals served in canteens. This situation is worsened by the persistence of poor food handling practices such as over-exposure, inappropriate holding temperatures and contamination from personnel and equipment in canteens. Even though the CC of the ST in canteen samples met the national and international acceptable limits, the presence of *K. pneumoniae* and *E. cloacae* is alarming since this indicates ease of contamination of ST in canteens.

The prevalence of *K.a pneumoniae*, *E. cloacae*, *Escherichia coli*, *Aspergillus* spp. and *Penicillium* spp. from ST and SM in the markets raises serious food safety concerns. This is because *K. pneumoniae* which causes about 1 per cent of bacterial pneumonia in man also produces a heat-stable enterotoxin, and has been implicated in histamine poisoning outbreaks. (Stewart & Beswick, 1977; Klipstein, Engert &

Short, 1977; Ababouch *et al.*, 1991; Taylor, 1986). Some strains of *Enterobacter cloacae* and *Escherichia coli* are enterotoxigenic and have been associated with gastroenteritis, especially infantile diarrhoea in developing countries (Anon, 1996). Though *Aspergillus* spp. and *Penicillium* spp. are common moulds, their widespread occurrence on smoked fish is alarming since some species/strains are mycotoxigenic and produce potential carcinogens (Cole & Cox, 1981; Terao, 1983; Pitt & Leistner, 1991). According to WHO (2002), about 2.2 million people died from diarrhoeal diseases in 1998 of which 1.8 million were children.

The study has identified the poor microbiological quality of smoked fish in markets and the implications for hygienic standards of other retail outlets, especially canteens. Efforts should be strengthened to enforce codes of practice and safety standards in market retailing of smoked fish by the relevant national and municipal authorities. Furthermore, effective implementation of the Environmental Sanitation Policy by local government authorities is required to safeguard markets. This, it is hoped, will help meet one of the most important challenges of the fish processing industry, that of quality assurance.

Conclusion

The study has shown the unsatisfactory microbiological quality of smoked tuna and mackerel in some local markets. Smoked fish in Madina market had a higher microbiological quality compared to those of Dome, Mallamatta and Kaneshie markets. Generally, smoked mackerel had a more satisfactory microbiological quality compared to smoked tuna in the markets. Although some microbiological indices of smoked fish in canteens met standards, the results also seem to indicate the ease of contamination of canteen smoked fish, probably from market sources. The market environment rather than the processing technology determines the microbiological quality of smoked fish.

The mode of storage, handling and

environmental sanitation are probable factors responsible for the contamination of smoked fish in markets. Non-enforcement of safety standards during market retailing and occasionally during catering at canteens poses the greatest challenge to maintaining the microbiological quality of smoked fish products. There is the need to critically and continuously examine the processing, post-processing procedures and catering of fish products to allow for systematic identification of potential hazards along the whole retailing chain, and also to enforce codes of practice and safety standards in markets, by the relevant national and municipal authorities to improve quality of smoked fish to safeguard public health in the country. This approach could improve the market quality of smoked fish, enhance food safety, and help safeguard public health in the country in these critical times of food insecurity.

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