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Analysis of the Microbiological Quality of Processed *Engraulis encrasicolus* and *Sardinella aurita* Obtained from Processing Houses and Retail Markets in Accra and Tema, Ghana

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Abstract: Microbiological quality analyses were conducted on processed anchovy (*Engraulis encrasicolus*) and round sardinella (*Sardinella aurita*) collected from processing houses and local retail markets in Accra and Tema to assess their quality. A total of approximately 500 g each of smoked and sun-dried *E. encrasicolus* and smoked *S. aurita* were randomly collected from randomly selected processing houses and retail markets from Accra and Tema for analysis. The serial dilution, pour plate and spread plate methods were used to enumerate levels of total heterotrophic bacteria, total coliform bacteria, yeast and moulds and *Bacillus cereus* colonies in the samples. The results showed that samples obtained from the retail markets recorded total heterotrophic bacteria counts ranging from $1.9 \times 10^4 - 5.9 \times 10^5$ cfu/g, while those obtained from the processing houses ranged from $1.2 \times 10^3 - 6.5 \times 10^4$ cfu/g, which were within accepted limits (1×10^6 cfu/g) for fish and fish products. There were counts of total coliform bacteria, yeast and moulds and *B. cereus* for the samples, but they were all within accepted limits, except for *B. cereus*, which recorded counts higher than accepted limits (1×10^4 cfu/g) for some samples obtained from retail markets in both Tema and Accra. The contaminated samples were attributed to poor processing, packaging, transporting and storage conditions used by the fish traders. Continuous education of fish traders to use general good management practices and regular hygiene inspections by the standards authority is however required to improve the microbial quality of processed fish in local retail markets.

Key words: Microbiological · *Engraulis encrasicolus* · *Sardinella aurita* · Smoked · Sun-Dried · Processed Fish

INTRODUCTION

Fishing is an extremely important economic activity in Ghana. It has been estimated that the fish resources in Ghana's water bodies support the livelihoods of a total of about 2 million people which includes fishers, fish processors (including fish canneries and cold stores), traders and boat builders. These people, together with their dependents, account for about 10% of the population [1].

Because fresh fish is generally soft, it easily gets damaged; therefore, rough handling and bruising can result in contamination of its flesh. In high ambient temperatures of the tropics, fresh fish usually spoils very quickly. Unless it is subjected to some form of processing or preservation, fresh fish becomes unfit for human consumption normally within about a day after capture. Even after it has been processed, particularly, if traditional methods such as smoking and sun-drying are used, the fish is still subject to many forms of loss and spoilage [2].

The common traditional preservative methods used in Ghana for fish are; depuration, freezing, smoking, sun-drying and salting [3]. Fishes have been preserved by smoking and sun-drying before the dawn of recorded history and people in all cultures of the world have relied on smoking for long-term storage of fish and fish products [4]. Clucas and Ward [5] reported that up to 70% of the total fish catch in developing countries is preserved by smoking.

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Smoking is one of the traditional processing methods used to prevent or reduce postharvest losses in the fishing industry. It involves application of heat to remove water which inhibits both bacterial and enzymatic actions [6] and ends up giving the product a desirable taste and odour, providing a longer shelf-life, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage [7, 8]. Levels and distributions of microbial flora in smoked fish products varies largely, depending on the quality of fish at the time of smoking, the smoking temperature and duration, the salt content and the drying time [9].

Sun-drying of fish also removes water which inhibits bacterial and enzymatic actions in fish but does not add any desirable taste and odour to the end product. The length of drying depends on the type of fish, its size and the weather [4]. Traditionally, whole small fish or split large fish are simply spread in the sun often laid directly on the ground, or on mats, nets, roofs and sometimes on raised racks. Sun drying usually does not allow control over drying times, exposes the fish to attack by insect and animal pests and allows contamination by sand, dirt, etc. [10].

The quality of preserved fish is therefore linked to the handling, processing and post processing procedures of the fish during which periods fish is susceptible to microbial attack. Microorganisms are the major cause of spoilage of most seafood products. Some microorganisms that contaminate fish are *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Shigella spp., Clostridium botulinium* etc. [3]. This study was conducted to analyse the microbiological quality of smoked and sun-dried anchovy (*Engraulis encrasicolus*) and smoked round sardinella (*Sardinella aurita*) collected from processing houses and local retail markets in Accra and Tema.

MATERIALS AND METHODS

Study Locations: The study was conducted in Accra and Tema in the Greater Accra Region of Ghana. Accra, located at 5.55°N 0.2°W is the capital and largest city in Ghana with a population of 2,291,352. Also, Tema is located at 5.667°N 0°E ?and it is a city on the Atlantic Ocean coast, 25 km east of Accra. It has a population of 161,612 [11].

Collection of Processed *E. encrasicolus* and *S. aurita* Samples: Samples for the study were collected from randomly selected smoke-houses, sun-dry-houses and retail markets in the surroundings of the Jamestown fish landing site in Accra and the fish market at the canoe basin in Tema. Two smoke-houses, two sun-dry-houses and two retailers at the retail market were randomly selected in both cities for the study. A total of about 500 g each of smoked and sun-dry samples of *E. encrasicolus* and smoked samples of *S. aurita* were randomly collected from the selected smoke-houses, sun-dry-houses and retail markets. All collected samples were placed in well labeled sterile plastic bags (ziplock bags) and immediately delivered to the laboratory on ice in an ice-chest under hygienic conditions for analysis. Three separate analyses were conducted at monthly intervals from March to May.

Processing of Samples for Microbiological Analysis: Twenty-five grams (25 g) of each sample was aseptically weighed on an electronic balance and macerated in sterile laboratory mortar and pestle. Each macerated sample was then kept in a 250 ml conical flask containing 225 ml sterile 0.1% peptone water. The content was vortexed for 60 seconds to homogenize the mixture to obtain a 1:10 (10^{-1}) dilution. Aseptically, 1 ml of the 1:10 dilution sample was transferred into a 25 ml universal bottle containing 9 ml of sterile 0.1% peptone water with a sterile microtitre tip to make a 1:100 or 10^{-2} dilution. This procedure was continued until 10^{-8} dilution was obtained.

Microbiological Analysis of Samples: Microbiological analysis was performed according to the standard procedure for the enumeration and identification of microorganisms [12].

Preparation and Sterilization of Media: All media were prepared and sterilized according to manufacturer's instructions. The media used for this study were obtained from the Oxoid Limited, England. Sterility control plates of each media and diluents were made by incubating them overnight at 37 °C [13].

Enumeration of Total Heterotrophic Bacteria: For enumeration of total heterotrophic bacteria, 1 ml of each dilution of the samples was pour-plated on Standard Plate Count Agar in duplicates. Plates were aerobically incubated in inverted positions at 37°C. The colonies were counted after 24 hours and 48 hours of incubation using the colony counter (Stuart colony counter-SC6+). Colonies were counted as colony forming units per gram of fish sample (cfu/g) according to the microbiology of food and animal feeding stuffs [14].

Enumeration of Total Coliforms: For enumeration of total coliforms, 1 ml of each dilution of the samples was pour-plated on MacConkey Agar in duplicates. Plates were aerobically incubated in inverted positions at 37°C. The colonies were counted after 24 hours and 48 hours of incubation using the colony counter (Stuart colony counter-SC6+). Colonies were counted as colony forming units per gram of fish sample (cfu/g) according to the microbiology of food and animal feeding stuffs [14].

Enumeration of Yeast and Moulds: For enumeration of yeast and moulds, 1 ml of each dilution of the samples was pour-plated on Oxytetracycline-Glucose-Yeast Extract Agar in duplicates. Plates were aerobically incubated at 26°C. Counting of microbial colonies were done after 24 hours and repeated after 48 hours and 72 hours of incubation using the colony counter (Stuart colony counter-SC6+). Colonies were counted as colony forming units per gram of fish sample (cfu/g) according to the microbiology of food and animal feeding stuffs [14].

Enumeration of Bacillus Cereus: For enumeration of *Bacillus cereus*, 0.1 ml of each dilution spread- plated on the surface of well dried *Bacillus cereus* Selective Agar in duplicates. Plates were aerobically incubated in inverted positions at 37°C. The colonies were counted after 24 hours and 48 hours of incubation using the colony counter (Stuart colony counter-SC6+). Colonies were counted as colony forming units per gram of fish sample (cfu/g) according to the microbiology of food and animal feeding stuffs [14].

Statistical Analysis: Data collected from this study were analyzed using the XLSTAT (2012) computer software. A one-way analysis of variance (ANOVA) was used to test the significance of difference in levels of retrieved bacteria from processed fish samples obtained from processing houses and at point of sale in local retail markets. The Tukey's post-hoc test (HSD) was used if the means of two different groups under comparison were significantly different in the normally distributed population from which the samples were drawn with $p < 0.05$ regarded as statistically significant.

RESULTS

Tables 1 – 4 shows the results obtained from three separate microbiological quality analyses of smoked and sun-dried anchovy (*Engraulis encrasicolus*) and round sardinella (*Sardinella aurita*) obtained from processing houses and local retail markets in Accra and Tema. The microbiological analyses carried out on the samples were counts of total heterotrophic bacteria, total coliforms, yeast and moulds and *Bacillus cereus*.

Table 1 shows mean monthly microbial counts enumerated in smoked and sun-dried *E. encrasicolus* obtained from both processing houses and retail markets in Accra expressed in colony forming units per gram (cfu/g). Table 2 shows mean monthly microbial counts enumerated in smoked *S. aurita* obtained from both processing houses and retail markets in Accra expressed in colony forming units per gram (cfu/g). Table 3 shows mean monthly microbial counts enumerated in smoked

Table 1: Mean monthly microbial counts of smoked and sun-dried *E. encrasicolus* obtained from Accra expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
SMOKE HOUSE (SMOKED)	March	1.2×10^3	0	4.0×10^1	7.0×10^2
	April	5.6×10^3	4.4×10^1	3.5×10^1	2.5×10^2
	May	3.7×10^3	0	2.7×10^1	1.5×10^2
	Mean	$3.5 \times 10^{3\,a}$	$1.5 \times 10^{1\,a}$	$3.4 \times 10^{1\,a}$	$3.7 \times 10^{2\,a}$
MARKET (SMOKED)	March	6.8×10^4	4.7×10^2	8.0×10^1	2.2×10^4
	April	6.9×10^4	4.2×10^2	7.5×10^1	3.5×10^2
	May	8.7×10^4	5.3×10^2	3.5×10^1	4.6×10^2
	Mean	$7.5 \times 10^{4\,a}$	$4.7 \times 10^{2\,a}$	$6.3 \times 10^{1\,a}$	$7.6 \times 10^{3\,a}$
DRY HOUSE (DRIED)	March	6.5×10^4	2.5×10^2	1.0×10^1	5.0×10^1
	April	3.0×10^4	4.0×10^2	6.5×10^1	2.5×10^2
	May	4.3×10^4	5.0×10^2	5.4×10^1	3.5×10^3
	Mean	$4.6 \times 10^{4\,a}$	$3.8 \times 10^{2\,a}$	$4.3 \times 10^{1\,a}$	$1.2 \times 10^{3\,a}$
MARKET (DRIED)	March	5.9×10^4	1.2×10^3	6.0×10^1	7.0×10^2
	April	4.9×10^4	1.1×10^3	1.1×10^2	4.0×10^2
	May	3.6×10^5	3.5×10^3	2.6×10^2	4.3×10^4
	Mean	$1.6 \times 10^{5\,a}$	$1.9 \times 10^{3\,a}$	$1.4 \times 10^{2\,a}$	$1.5 \times 10^{4\,a}$

Ghana Standard Authority/ICMSF standards: (Total heterotrophic bacteria count: 1×10^6 cfu/g; Total coliform count: 1×10^4 cfu/g; *Bacillus cereus* count: 1×10^4 cfu/g; Yeast and moulds count: 1×10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

Table 2: Mean monthly microbial counts of smoked *S. aurita* obtained from Accra expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
SMOKE HOUSE (SMOKED)	March	4.1×10^4	0	1.8×10^3	3.5×10^2
	April	1.2×10^4	1.5×10^2	1.2×10^2	0
	May	2.4×10^4	2.7×10^2	3.2×10^2	2.3×10^3
	Mean	$2.7 \times 10^{4\,ab}$	$1.4 \times 10^{2\,a}$	$7.5 \times 10^{2\,a}$	$8.8 \times 10^{2\,a}$
MARKET (SMOKED)	March	7.2×10^4	3.5×10^2	2.2×10^2	4.5×10^2
	April	1.9×10^5	7.0×10^2	4.8×10^2	1.2×10^3
	May	2.9×10^5	4.0×10^2	3.8×10^3	4.2×10^4
	Mean	$1.8 \times 10^{5\,a}$	$4.8 \times 10^{2\,a}$	$1.5 \times 10^{3\,a}$	$1.5 \times 10^{4\,a}$

Ghana Standard Authority/ICMSF standards: (Total heterotrophic bacteria count: 1×10^6 cfu/g; Total coliform count: 1×10^4cfu/g; *Bacillus cereus count*: 1×10^4 cfu/g; Yeast and moulds count: 1×10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

Table 3: Mean monthly microbial counts in smoked and sun-dried *E. encrasicolus* obtained from Tema expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
SMOKE HOUSE (SMOKED)	March	6.0×10^3	0	4.0×10^1	1.5×10^3
	April	1.2×10^3	0	1.0×10^1	1.0×10^2
	May	2.4×10^3	1.7×10^1	2.1×10^1	2.0×10^2
	Mean	$3.2 \times 10^{3\,a}$	$0.6 \times 10^{1\,a}$	$2.4 \times 10^{1\,a}$	$6.0 \times 10^{2\,a}$
MARKET (SMOKED)	March	4.1×10^4	0	5.0×10^1	2.5×10^4
	April	1.9×10^4	0	4.5×10^1	3.0×10^2
	May	5.9×10^5	2.7×10^2	2.3×10^2	4.3×10^3
	Mean	$2.2 \times 10^{5\,a}$	$9.0 \times 10^{1\,a}$	$1.1 \times 10^{2\,a}$	$9.9 \times 10^{3\,a}$
DRY HOUSE (DRIED)	March	2.5×10^4	2.3×10^2	5.0×10^1	6.0×10^2
	April	5.2×10^3	0	1.0×10^1	0
	May	4.6×10^3	2.5×10^2	3.0×10^1	0
	Mean	$1.2 \times 10^{4\,a}$	$1.6 \times 10^{2\,a}$	$2.3 \times 10^{1\,a}$	$2.0 \times 10^{2\,a}$
MARKET (DRIED)	March	4.1×10^4	0	4.5×10^1	3.0×10^4
	April	6.3×10^3	1.2×10^3	2.5×10^1	0
	May	6.4×10^4	3.8×10^3	3.1×10^2	3.4×10^2
	Mean	$3.7 \times 10^{4\,a}$	$1.7 \times 10^{3\,a}$	$1.3 \times 10^{2\,a}$	$1.0 \times 10^{4\,a}$

Ghana Standard Authority/ICMSF standards: (Total heterotrophic bacteria count: 1×10^6 cfu/g; Total coliform count: 1×10^4 cfu/g; *Bacillus cereus* count: 1×10^4 cfu/g; Yeast and moulds count: 1×10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

Table 4: Mean monthly microbial counts of smoked *S. aurita* obtained from Tema expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
SMOKE HOUSE (SMOKED)	March	2.5×10^3	0	3.0×10^1	1.5×10^2
	April	4.9×10^3	1.4×10^2	2.5×10^2	3.5×10^3
	May	3.3×10^3	0	1.2×10^2	2.7×10^3
	Mean	$3.6 \times 10^{3\,a}$	$4.7 \times 10^{1\,a}$	$1.3 \times 10^{2\,a}$	$2.1 \times 10^{3\,a}$
MARKET (SMOKED)	March	3.6×10^4	6.5×10^2	1.1×10^1	2.0×10^3
	April	2.4×10^3	7.6×10^2	2.9×10^2	1.5×10^5
	May	4.4×10^4	2.5×10^2	3.9×10^2	3.6×10^4
	Mean	$3.5 \times 10^{4\,a}$	$5.5 \times 10^{2\,a}$	$2.3 \times 10^{2\,a}$	$1.8 \times 10^{4\,a}$

Ghana Standard Authority/ICMSF standards: (Total heterotrophic bacteria count: 1×10^6 cfu/g; Total coliform count: 1×10^4 cfu/g; *Bacillus cereus* count: 1×10^4 cfu/g; Yeast and moulds count: 1×10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

and sun-dried *E. encrasicolus* obtained from both processing houses and retail markets in Tema expressed in colony forming units per gram (cfu/g). Table 4 shows mean monthly microbial counts enumerated in smoked *S. aurita* obtained from both processing houses and retail markets in Tema expressed in colony forming units per gram (cfu/g).

DISCUSSION

All processed fish (smoked and sun-dried) samples obtained from processing houses and retail markets in Accra and Tema recorded counts of total heterotrophic bacteria, total coliforms and yeast and moulds below the Ghana Standard Authority (GSA) and the International Commission on Microbiological Specifications for Foods (ICMSF) standards. *Bacillus cereus* also recorded counts for all fish samples collected, but those obtained from the retail markets mostly recorded values higher than the GSA/ICMSF standard of 1 x 10^4 cfu/g.

B. cereus is an important food-borne pathogen, which causes two distinct types of food poisoning- diarrhoea and emesis- which are caused by two different types of toxins [15, 16]. This organism is responsible for spoilage of different food products including fish [17]. Because it is a spore forming organism, there is a risk of its transmission through heat-treated and processed food products since its spore can survive very high temperatures. This organism is known to resist even pasteurization process of milk [18]. In India, the presence of this organism has been reported in various food products including fish [19, 20].

In an earlier study by Kombat *et al*. [21], *B. cereus* was not recorded in fresh *E. encrasicolus* and *S. aurita* samples collected from landing beaches in Accra and Tema, which suggested that the raw materials (in this case the fresh fish) used to produce smoked and sun-dried anchovy and smoked sardines were devoid of *B. cereus* contamination. The result from this study, however, may have been because the raw materials got contaminated with *B. cereus* only after they arrived at the processing points either before or after they had been processed. This suggests that, just smoking or sun-drying fish is not enough to get rid of *B. cereus* or its spores from an already contaminated raw material and that the use of high quality fresh fish and hygienic processing is necessary to produce high quality processed fish. Also good storage methods should be employed in storing processed fish so that its quality will be preserved. The presence of *B. cereus* in the processed samples is a testimony that, the spore-forming character of *B. cereus* enables it to survive high temperatures as reported by Das *et al*. [22] and Nova *et al*. [18]. Its presence in the fish samples also confirms Kamat *et al*. [19] report that, it is a food-borne pathogen in fish.

Kombat *et al*. [21] recorded higher coliform values in fresh *E. encrasicolus* and *S. aurita* than those recorded in the processed samples in this study. The reduction of coliform loads in the processed fish, especially, in those collected at the processing houses is indications that heat/smoke application and eventual withdrawal of water from fish is enough to get rid of coliform bacteria from fresh fish. This result conforms to results reported by Nickelson *et al*. [9], Sengor *et al*. [8], Abolagba and Melle [7] and Kumolu-Johnson *et al*. [6].

Processed fish from local retail markets, however, recorded higher coliform loads than those from the processing houses which were the case in the findings of Obodai *et al*. [3]. Their observation was attributed to poor handling, packaging, transporting and storage conditions of processed fish. These were identified as probable factors for higher counts of coliform bacteria in processed samples obtained from retail markets.

Higher counts of *B. cereus* in the processed *E. encrasicolus* and *S. aurita* samples from retail markets suggests that there are potential human pathogens present in fish sold in local retail markets in Ghana. This implies that fish and fish products from these retail markets could pose serious health threats to humans when they are consumed without adequate hygienic processing such as properly cooking them. Consumption of these potential human pathogens in large quantities could cause serious food-borne illnesses or poisoning [23].

CONCLUSION

The study revealed that, the quality of processed *E. encrasicolus* and *S. aurita* obtained from processing houses in Accra and Tema were microbiologically good, but those obtained from retail markets were comparatively contaminated. This was because they registered higher levels of *B. cereus* counts per national and international standards which meant they were unwholesome for consumption. Caution should therefore be taken in consuming smoked and sun-dried fish which have been displayed openly in markets because such fish could contain pathogenic microorganisms. Such products should be properly cooked before consumption. Continuous education of fish traders to use general good management practices and regular hygiene inspections by the standards authority is therefore required to improve the microbial quality of processed fish. Similarly, consumers should be continuously sensitized to raise awareness of the existence of such microbes in order to encourage adequate cooking of fish prior to consumption.

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