

NUTRITIONAL AND MICROBIAL EVALUATION OF FRESH, SOLAR, SMOKED, AND FRY DRIED *SIGANUS* (RABBIT) FISH SPECIES OFF KENYAN MARINE COAST

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Abstract

The study investigated nutritional and microbial load status of fresh, solar, smoked, and fry dried *Siganus* (Rabbit) fish species. Nutritive quality parameters such as moisture, protein, fat, ash, carbohydrates, and microorganism loads were evaluated from the fish samples and highest protein content (58.3±2.6%) recorded in the smoked fish samples while the least (17.6±0.3%) was in fresh samples. Smoked and solar dried samples proximate composition were not significantly different ($P>0.05$) but differed with fresh and fried samples. The study suggests that fresh and traditionally dried fish products marketed in the Kenyan open air markets and processing sites are less hygienic in their current states as detected microorganism levels were higher than is normally recommended. Fish vending communities processing safety enhancement capacity building training programs is therefore needed and usage of the hybrid tunnel solar dryer could be the best available option for relatively hygienic and nutritive dried fish products production.

Key words: Hygienic fish processing, preservation, nutritional and microbial evaluations

1.0 Introduction

Fishes constitute very important diet components for many people and often provide the much needed nutrients not provided in cereal based diets (Abdullahi *et al.*, 2001; Woodland, 1983; Blanchet *et al.*, 2000; Lie, 2004; Innis and Elias, 2003; Din *et al.*, 2004). They are rich in proteins with amino acid compositions that are very well suited to human dietary requirements that compares favourably with egg, milk and meat in nutritional value. Fresh, smoked or dried fishes are therefore a traditional part of the diet of a large section of the world's population. Fish drying is a traditional and widely used preservation method in tropical countries where refrigerated storage facilities lack.

Fish is usually eaten in Kenya fresh and the surplus is locally preserved or processed to be delivered to the consumers by fisher and other communities settled nearby the Kenyan coastline and wetland areas (rivers, lakes, etc). Sun (solar) drying, kiln smoke drying, and deep frying are therefore common traditional practices of fish processing widely used in different parts of Kenya. However, fish being a quickly perishable commodity due to its susceptibility to damage as soon as it is harvested (Abolagba and Uwagbai, 2011), chemical breakdown of proteins, fats, and water contribute to its quick spoilage even at ambient temperatures in the tropics (Igene, 1983). Spoilage factors responsible for the breakdowns include the prevailing higher temperatures; unhygienic processing; poor storage; unsafe distribution facilities and longer distribution channels (Coulter and Disney, 1987).

Siganus (Rabbit) fishes which are locally known in Kenya as "Tafi" (Anam and Mostarda, 2012) are herbivorous marine and brackish water fishes that belong to the *Siganus* genus within the Siganidae family [Woodland, 1990]. The fishes are found throughout the Indo-West Pacific Region (Woodland, 1983) and in Kenya, the fishes fetch medium to high prices and are most demanded objects of traditional subsistence and commercial fisheries. In view of the above factors, nutritive and microbial quality assessments are necessary to ensure safety of the processed products. A number of biochemical and microbiological tests are respectively used by authorities elsewhere to check against food spoilage and contaminations by microorganisms. However, in Kenya, such nutritional and microbial food safety test value information is scanty for both fresh and preserved fish products.

Therefore, since fish is a much-cherished delicacy in Kenya that cuts across socio-economic, age, religious, and educational barriers; and is eaten fresh; preserved; or processed; the study aimed to assess nutritional and microbial values between fresh and the differently preserved fish products marketed in the country as a guide to their public health safety, appropriate handling, and recommendation for possible future large scale mass production adoption of cured fish and fishery products.

2.0 Materials and Methods

Sample Collection, Preparation, and Analysis

The commercially valued indigenous *Siganus* fish species was selected for the study. The traditional smoked, and fry dried Rabbit (*Siganus*) fish samples used for the study were purchased from the small scale subsistence fish traders along the Kenyan coastline and aseptically handled during the seven months of study beginning from December, 2013 to June, 2014. The samples were aseptically packed into sterile zip locked polythene bags immediately after purchase, placed in labelled dry separate cooler boxes, and transported back to Kenya Marine and Fisheries Research Institute's (KMFRI) Natural Product and Post-Harvest Technology Laboratory for nutritional (proximate), percent rancidity, and microbial analyses. Similarly, fresh fish samples were also purchased from the Kenyan south coast artisanal fishermen landings at Shimoni landing beach (S 04°44'54.2", E 039°21'58.8") and splashed with clean portable tap water for removal of attached sand particles before gutting.

During gutting, a bilateral incision was made to expose the stomach contents which were removed and the stomach cavity washed with clean portable tap water. The samples were then pooled together and randomly divided into two portions. One of the portions was then aseptically packed fresh into zip locked bags and packed into a labelled cooler box with ice and conveyed to KMFRI laboratory for analysis within 4 hours. The other portion was experimentally dried using a hybrid solar tunnel dryer installed by KMFRI at the site until the products attained 30% mass loss. The samples were then aseptically cooled after drying, packed into sterile zip locked bags and transported to the laboratory for analysis.

2.1 Nutritional (Proximate) Analysis of the Samples

Nutritional (Proximate) analysis of the fish samples were carried out in triplicates using ALVA, 1983 and AOAC, 1995 methods. The analyses involved determination of the samples protein, moisture, fats, total ash and carbohydrate contents.

2.2 Microbial Evaluation

Microbiological investigations were carried out using BDH chemical reagents prepared as specified by Lennette *et al.*, 1974 to determine total bacterial colony forming units (cfu) of the food-spoilage microorganisms, and pathogenic organisms such as molds, *Coliforms*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* in the samples using Total Plate Count (TPC) method. Percentage rancidity of the samples was also determined.

2.3 Preparation of Media

The recipe for preparation of media used followed the methods described by Cheesbrough, 1987 in Biomerieux API, 1989.

2.4 Enumeration of Aerobic Heterotrophic Bacterial Counts (method of Holts, 1982)

Surface spreading technique was used to determine the total number of aerobic heterotrophic bacteria present in the samples. Serial dilution of the samples were prepared from 10^{-1} to 10^{-10} and 0.1 ml of each dilution was plated onto MacConkey and nutrient agar containing 5 ngml^{-1} of Nystatin to inhibit fungal growth. The plates were prepared in duplicates and incubated at 37°C for 24 hours before enumeration (Holts, 1982).

2.5 Enumeration of Aerobic Heterotrophic Fungi (Hunter and Bennette, 1973)

The total numbers of fungi present in the samples were enumerated by viable plate count method using surface spreading techniques. Serial dilutions of 10^{-1} to 10^{-3} of the sample were made. 0.1 ml of each dilution was plated into malt extract agar containing 10% lactic acid per ml to inhibit bacterial growth. The plates were prepared in duplicates and incubated at 28°C for 72 hours before enumeration (Hunter and Bennet, 1973).

2.6 Viable Count Method

All plating and counts were done by the pour plate technique of Harrign and McCance, 1976. Viable count Calculations were done using; - Number of colonies = no. of colonies counted x dilution factor x plating factor.

2.7 Purification and Maintenance of Bacterial and Fungal Isolates (Cowan and Steel, 1974)

The bacterial and fungal isolates were purified by repeated sub-culturing. Isolates were subjected to a series of transfers unto fresh media. The bacterial and fungal isolates were incubated at 37°C for 24 hours and 28°C for 72 hours respectively. Pure colonies of bacteria and fungi were maintained on slope of nutrient agar and malt extract agar slants respectively. The slants were stored in a refrigerator at 8°C until needed (Cowan and Steel, 1974).

2.8 Characterization and Identification of Microbial Isolates

The bacterial isolates were examined for colony morphology as well as cell micro-morphology and biochemical characteristics according to the methods described by Gerhardt *et al.*, 1981. Bacterial identifications to their generic levels followed the scheme of Holt, 1982. Fungi isolates on the other hand were characterized based on their macroscopic and microscopic appearances. Their probable identities were determined according to Hunter and Bennette, 1973 and Biomerieux API, 1989 identification schemes.

2.9 Statistical Analyses

Data and information collected from the study were used to analyze any significant differences on nutritional and microbial values between fresh and among the studied fish preservation methods using a two-way ANOVA Microsoft 2007 excel statistical package. All data were expressed as mean \pm Standard Deviation (Std. Dev) and probability tested at 95% level of significant ($p < 0.05$). The values were also compared with World Health Organization (WHO) limit level norms for food fish.

3.0 Results

Nutritional (proximate) analysis of moisture, protein, fats, total ash and carbohydrates were not significantly different between the experimental hybrid tunnel solar and smoke dried samples (Table 1). There was however significant differences among the fresh, fried, hybrid tunnel solar dried, and smoke dried fish products in their proximate compositions (Table 1). The microbiological analyses of total viable bacterial plate counts (TPC); moulds; *E. coli*; *Salmonella sp.*; and rancidity percentages also showed variations among the samples (Table 2).

4.0 Discussion and Conclusions

Traditionally cured fish are mostly safer for human consumption immediately after processing. However, they deteriorate during storage as a result of moisture absorption, and microbial growth. This causes a rise in rancidity due to the resulting higher lipid oxidation rate that consequently affects the products' flavour and general acceptability. The International Commission on Microbiological Specifications for Foods (ICMSF, 1982) therefore indicates a microbiological count limit levels of acceptability in any food to be considered safe for human consumption.

Nutritional (proximate) composition analysis of moisture, protein, fats, total ash and carbohydrates were not significantly different between the experimental hybrid tunnel solar and smoke dried samples (Table 1). There was however significant differences among the fresh, fried, hybrid tunnel solar dried, and smoke dried fish products (Table 1). The variances were however in favour of the dried fish products where proximate composition was mostly higher than in fresh fish samples (Table 1).

Table 1: Nutritional (proximate) composition of the fresh, smoke, fry, and hybrid tunnel solar dried fish samples

Sample Treatments	Moisture (%)	Total Ash (%)	Total Fat (%)	Crude Protein (%)	Carbohydrate (%)
Fresh	78.4 \pm 0.6 c	2.4 \pm 0.3 a	0.4 \pm 0.1 a	17.6 \pm 0.3 a	1.3 \pm 1.0 a
Fried	44.0 \pm 0.0 b	6.1 \pm 0.6 b	21.4 \pm 4.0 c	22.53 \pm 1.0 b	6.0 \pm 3.0 b
Smoked	12.6 \pm 3.0 a	12.5 \pm 0.4 b	15.7 \pm 0.3 b	58.4 \pm 3.0 c	1.0 \pm 0.4 a
Solar dried	11.6 \pm 0.8 a	13.3 \pm 0.6 c	16.83 \pm 0.4 b	57.5 \pm 0.7 c	0.6 \pm 0.1 a

Note: Same letters indicate no significant difference ($p > 0.05$)

The microbial study test sample results were also found to be within the given count limits in the fry and in the experimental hybrid tunnel solar dried fish samples. All fresh fish sample microbial load limits were above the

recommended ICMSF (1978) micro flora guideline limit levels. The smoked fish samples microbial load limit were also above the recommended limits with exception of *Salmonella* counts as evidenced in Table 2. Hence, both smoked and fresh fish samples were poor in microbial quality (Table 2).

Table 2: Microbiological load in the fresh, smoke, fry, and hybrid tunnel solar dried fish samples

Sample Treatments	Moulds g ⁻¹	Coliforms g ⁻¹	<i>Escherichia coli</i> g ⁻¹	<i>Staphylococcus aureus</i> g ⁻¹	<i>Salmonella</i> Species g ⁻¹	Total Plate Count (TPC) g ⁻¹	Rancidity (%)
Fresh	2611 ± 1230	> 2400	> 2400	TNTC	11600	TNTC	6.0 ± 1.8
Fried	3.0 ± 6.0	0.0	0.0	1444 ± 1308	0.0	4200± 1539	1.2 ± 0.3
Smoked	2253 ± 3108	237 ± 225	80 ± 139	TNTC	0.0	2506670± 496622	4.5 ± 1.4
Solar dried	0.0	0.0	0.0	62 ± 9	0.0	2615 ± 124	3.7 ± 0.5

Mould counts were 3.0 ± 6 colony forming units (cfu) g⁻¹ in the fry dried fish samples (Table 2). The ICMSF, (1982) specification recommends a limit of less than 10,000 colony forming units in a gram of food sample. This indicates that the fry dried fish samples met the specified safe food standard requirements. Fresh fish samples however exceeded all the standard requirement limits of acceptability specified by ICMSF, (1982) guidelines for safe foods. Therefore, from the point of consideration that fish caught in unpolluted waters by human and animal wastes should be free from these intrinsic microbiological hazards (Grazyna and Bonnie, 2010), the observed contamination could have only resulted from lack of good commercial practices during handling and processing. Indeed fish and other free-swimming aquatic animals do not usually carry *Escherichia coli*, and the 'faecal *Coliform* organisms' generally considered typical of the mammalian micro flora. Thus, the presence of these human enteric organisms on aquatic food products is a clear evidence of contamination from a terrigenous source. *Coliforms* and *Escherichia coli* (*E. coli*) were not detected in the fry and experimental hybrid tunnel solar dried fish samples. The non-detection of these food poisoning organisms specified by ICMSF, (1982) that should not be more than 10 colony forming units in a gram of food product, indicates that the fish products are safe for human consumption. *Salmonella* species organisms which ICMSF, (1982) recommends zero observe of their presence for food to be considered safe for consumption were also not detected in any of the dried fish samples. Fresh fish samples however had their presence (Table 2).

Fish and shellfish products are minor sources of bacterial food borne diseases in North America, the United Kingdom, and Australia (Todd, 1978); but a high relative incidence of bacterial food-borne diseases result from fish and fishery products in Japan and Southeast Asian countries where fish are commonly eaten raw or with little cooking (Sakazaki, 1979). Raw and unsatisfactorily processed aquatic foods are generally excellent substrates for the growth of most common food-borne disease bacterial agents. This is especially so if the foods are held at temperatures supporting the bacterial disease agents growth. It is therefore important to avoid contamination of these foods during handling, processing, and storage.

In conclusion, since the study reveals that experimental hybrid tunnel solar dried fish products are superior in quality to the traditionally cured fish products, it would be prudent that it is adopted for use in commercial large scale production for satisfaction of the current increasing cured fish and fishery products demand (Ogongo *et al.*, 2015). This is because it produces long lasting hygienic, nutritive, and safer fish products for public consumption in comparison to the traditionally cured fish products.

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