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Bacteriological and physicochemical qualities of traditionally dry-salted Pebbly fish (*Alestes baremoze*) sold in different markets of West Nile Region, Uganda

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The present study aimed at estimating the microbiological and chemical characteristics of traditionally dry-salted fish product, *Alestes baremoze*. A total of 40 random dry fish samples were collected from Arua, Nebbi, Packwach and Panyimur markets. Moisture content, pH, crude protein, crude fat and sodium chloride were analysed to determine chemical quality while *Escherichia coli, fecal streptococci, Staphylococcus aureus, Salmonella, Vibrio parahaemolyticus, Bacillus cereus and Pseudomonas* spp. were determined to estimate the microbial quality. The moisture content of dry-salted fish collected from different markets was in the range of 37 to 41%. Mean values of sodium chloride obtained in the fish muscle were in the range of 13 to 14% and significantly differed across fish markets. Results from microbial analysis expressed as colony-forming units per gram of sample indicated that *S. aureus* was the most dominant bacteria identified in dry-salted fish sold in all markets with Nebbi market having the highest counts (9.4×10^6), Panyimur (2.2×10^6), Packwach (2.3×10^5) and Arua (9.6×10^4). *Salmonella* was absent in fish samples collected from three markets of Arua, Packwach and Panyimur apart from Nebbi market. *E. coli* counts were found to be < 10^1 and *fecal streptococci* counts were relatively high in fish from Panyimur market (1.1×10^3). There was presence of *B. cereus* in all the samples ranging from 8×10^1 in Arua market to <20 in Nebbi and Panyimur markets. The present study has revealed that most of the fish products sold in these markets had bacterial counts beyond the maximum tolerable limits recommended by Uganda National Bureau of Standards (UNBS). There is need to control storage temperature and also ensure proper cooking procedures in order to eliminate or reduce the microorganisms to acceptable levels.

Key words: *Alestes baremoze*, salted fish, microbial quality, fish preservation.

INTRODUCTION

Dry salting has been traditionally used as a method of fish preservation, since it lowers the water activity of fish flesh (Horner, 1997). The salt mainly contains chloride ions that are toxic to some microorganisms (Leroi et al., 2000; Goulas and Kontominas, 2005). This technique is hence used to preserve fish from spoilage owing to tissue

autolysis and microbial action (Chaijan, 2011). Bacterial spoilage is for example characterized by softening of the muscle tissue, which can however be prevented by salt, because it forms a more membranous surface that inhibits the growth of microorganisms (Horner, 1997; Rorvik, 2000). Although salting reduces the rate of autolysis, it does not completely stop enzymatic action that increases with increasing temperature.

The presence of foodborne pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FDA, 2001). The handling of fish products during processing involves a risk of contamination by pathogenic bacteria such as *Vibrio parahaemolyticus* and *Staphylococcus aureus* causing foodborne human intoxication (Huss et al., 1998; Shena and Sanjeev, 2007). There is substantial evidence that fish and seafood are high on the list of foods associated with outbreaks of food borne diseases around the world (Kaysner and DePaola, 2000; Huss et al., 2003). The safety of foodstuffs should be ensured through preventive approaches, such as implementation of good hygiene practices and application of procedures based on hazard analysis and critical control point (HACCP) principles.

Alestes baremoze commonly known as *Angara* in Uganda is highly marketable and valued fish in Northern Uganda, South Sudan, Sudan and in the Democratic Republic of Congo (Kasozi et al., 2014). In Sudan, *A. baremoze* is normally prepared by wet salting. After several methods of salting, fermentation and storage, the final product is called *fassiekh* (Yousif, 1989; Adam and Mohammed 2012). *Angara* is prepared by dry salting which involves stacking the fish in salt and the formed brine is allowed to drain away while allowing it to dry under natural sunlight for two to three days. Many consumers, especially in the West Nile region appreciate the taste, special flavour and texture characteristics of this fish. Salting is not only a method to prolong shelf life, but a method to produce fish products that meet demand of consumers. Almost 90% of the total catches of *Angara* around Lake Albert are dry-salted. However, the available traditional fish processing practices expose the fish to different kinds of microbial and chemical degradation. The current wide spread practice of drying the fish directly on the ground and use of old fishing nets results in microbial contaminated fish products. There are currently no published work on the microbiological changes during production and storage of salted *Angara* yet the quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. The need for determination of microbiological quality of dry-salted fish products is important to prevent risk of bacterial infection to the consumers. This study therefore evaluates the bacteriological and physicochemical qualities of dry-salted *Angara* sold in different markets to serve as a guide to consumers and regulatory bodies.

MATERIALS AND METHODS

Sample collection

The study was conducted in four selected markets in West Nile region of Uganda. The process of dry salting (Figure 1) is normally carried out at Panyimur landing site and it's from this site that the dry- salted fish products are obtained and transported to other markets within the region. A total of 40 dry- salted fish samples were purchased from the markets of Arua, Nebbi, Packwach where they had been on stall ready for sale for five days and from Panyimur market where they had been dried for one day (Figure 2). At least 10 samples were collected from each market. These were labeled, sealed in airtight polythene bags and later transported to the laboratory for analysis.

Physicochemical analysis

Fish samples were analysed to determine the moisture content, fat, protein, sodium chloride and pH. Moisture content was determined by oven drying of 5 g of fish fillet at 105ºC until a constant weight was obtained (AOAC, 1995). Measurement of salt content was carried out using the Volhard method according to AOAC (1985). Crude protein was determined by the Kjeldahl method using sulphuric acid for sample digestion. Crude fat was obtained by exhaustively extracting 2.0 g of each sample in a Soxhlet apparatus using petroleum ether (b.p. 40 - 60°C) as the extractant (AOAC, 2000). pH was determined after homogenizing 10 g of fish sample into 100 ml of distilled water. The pH of filtrate was then measured using pH meter (HI 8014, USA).

Enumeration and isolation of bacteria

Serial dilutions from each sample were prepared before subsequent culturing according to the microbiological techniques of AOAC (1995). The total viable count of *Angara* samples were carried out using plate count agar according to the standard methods of AOAC (1995). The microbiological parameters were conducted in duplicate, the means and standard deviations were also calculated. Plate count number was presented as colony-forming units per gram of sample (cfu/g).

Pseudomonas

Pseudomonas was determined by spread plate method where 0.5 ml of decimal dilution was spread on the surface of Pseudomonas CN Selective Agar and incubated at 37°C for 48 h. The plates containing 15 to 150 colonies were counted under florescence under UV lamp. Confirmation for the presence of *Pseudomonas* was prepared with oxidase test and fermentation of glucose on purple glucose agar.

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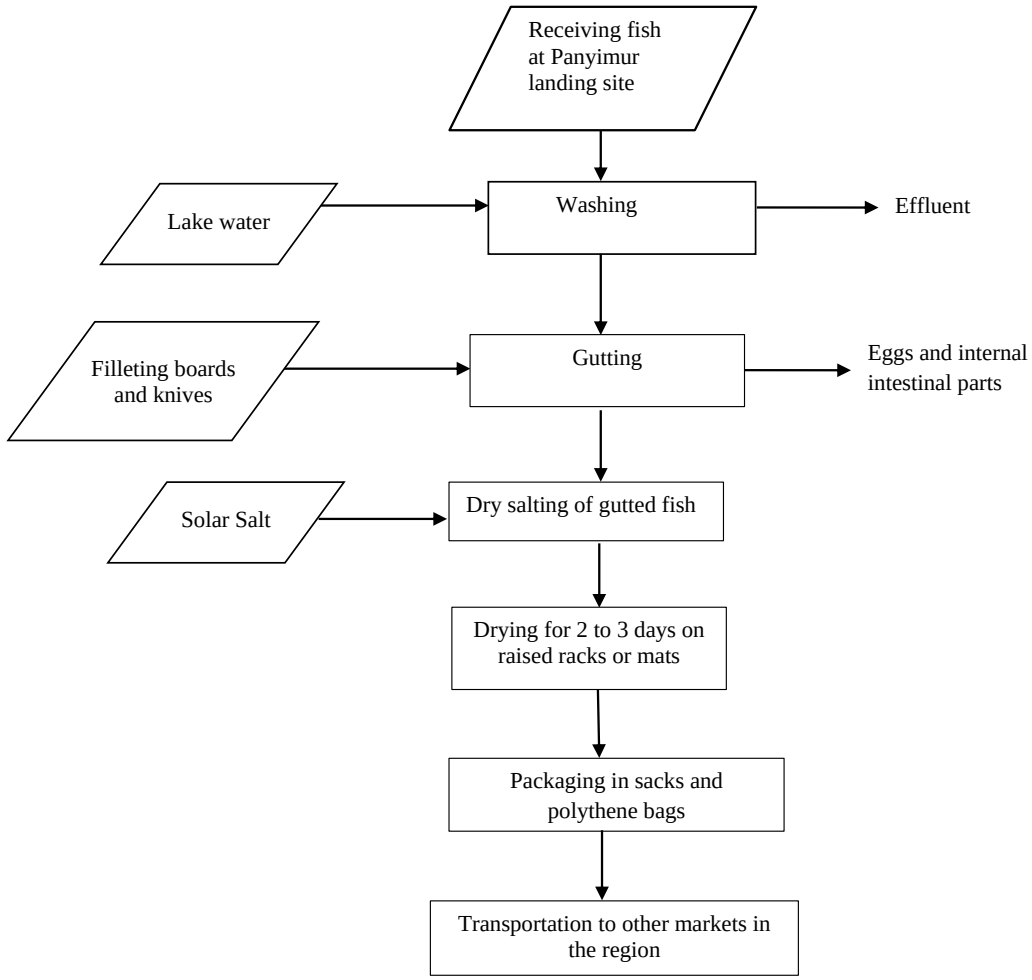

Figure 1. Traditional method for the production of salted *Alestes baremoze (Angara)* at the landing site of Panyimur.

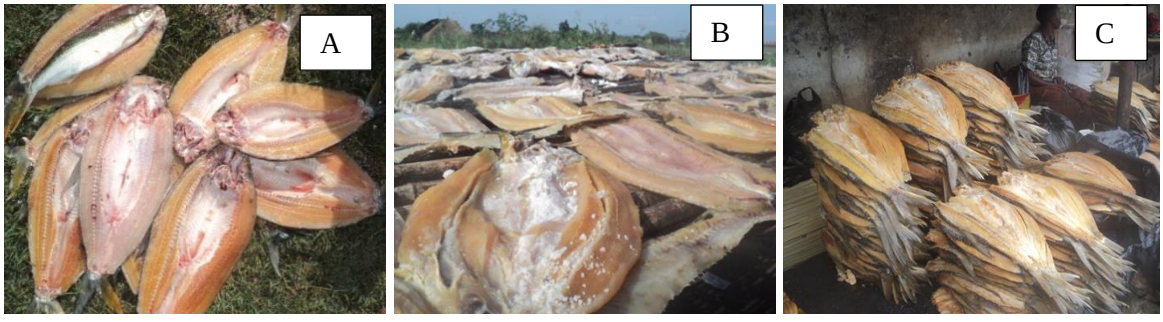

Figure 2. *Angara* fish samples: A: Gutted *Angara* samples on ground at the landing site; B: Sun drying of split fish on raised platforms and C: *Angara* fish on stalls in different markets.

V. parahaemolyticus

V. parahaemolyticus was detected according to the General guidance for the detection of *V. parahaemolyticus* (ISO 8914:1990). Twenty five grams of each sample were weighed into sterile stomacher bag containing 225 ml alkaline peptone water and then blended for 60 s. Serial dilutions were prepared to get 10^2 and10^3 diluents, and 1 ml aliquot of samples were transferred into 3% NaCl dilution tubes, and incubated at 35˚C for 24 h. The turbid tubes were streaked on Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS)

Table 1. Physicochemical analysis of dry–salted *A. baremoze* samples collected from different markets after a storage period of five days.

Parameter	Markets				*P*-value
	Arua	Nebbi	Packwach	Panyimur	
Moisture content (%)	37.0±2.97	36.0±0.83	39.5±0.37	41.6±0.47	0.0014*
pH	6.3±0.01	6.9±0.045	6.6±0.02	6.8±0.01	0.0001*
Fat content (%)	12.9±0.66	16.4±0.17	14.9±0.67	16.6±0.17	0.0001*
Protein content (%)	32.0±0.81	33.0±0.69	31.7±0.61	35.1±0.88	0.0002*
Sodium chloride (%)	14.9±0.01	13.8±0.76	13.8±0.14	13.1±0.10	0.0003*

*,Significant differences across markets; Fish samples at Panyimur market had been stored for one day.

plates and incubated at 37˚C for 24 h. Distinct colonies with blue green color were presumed as *V. parahaemolyticus* and yellow colonies were presumed as *Vibrio cholera*. To facilitate identification of suspect *Vibrio* isolates, the isolated colonies were further identified by API 20E system.

S. aureus

S. aureus was determined according to the method for the enumeration of coagulase-positive staphylococci (*S. aureus* and other species) using Baird-Parker agar medium (ISO 6888-1: 1999). Twenty five grams of each sample were weighed into stomacher bag containing 225 ml peptone water and then blended for 60 s. The resultant stock solution was then serially diluted and 0.5 ml diluents were spread on Baird-Parker agar plate. All inoculated plates were dried and incubated at 37˚C for 48 h. Then clear zone with typical gray-black colonies was taken as presumptive evidence of *S. aureus*. Confirmation of *Staphylococcus* spp. was done using *Staphylococcus* latex test.

Salmonella spp.

Salmonella spp. was determined according to the horizontal method for the enumeration of *Salmonella* spp. (ISO 6579: 2002). Pre-enrichment was conducted with 25 g of sample diluted in 225 ml sterile buffered peptone water incubated at 37°C for 24 h. Secondary selective enrichment was performed in Rappaport-Vassiliadis peptone broth (41°C for 24 h) and Muller-Kaufmann tetrathionate broth with Novobiocin (37°C for 24 h), and streaking on Xylose Lysine Desoxycholate (XLD) agar and incubated at 37°C for 24 h. Typical *Salmonella* spp. exhibited pink colonies with black centers.

Escherichia coli

E. coli was determined by pour plate method using Rapid' *E.coli* 2 Agar (AFNOR BRD 07/1 - 07/93). Using a sterile pipette, 1 ml of each decimal dilution was inoculated to a sterile Petri dish and then 15 ml of Rapid *Ecoli* Agar was dispensed, mixed thoroughly and after setting, a thin overlay of 5 ml of Rapid *Ecoli* agar and later incubated at 44ºC for 24 h. Plates with purple colonies were counted and confirmed with Kovac's reagent and all positive colonies showed a purple layer.

Bacillus cereus

B. cereus was determined according horizontal method for the enumeration of presumptive *B. cereus* (ISO 7932:2004). Twenty five grams of each sample were homogenized in 225 ml sterile peptone water for 60 s. Serial dilution was carried out and 0.1 ml diluents were spread on *B. cereus* Selective Agar. The inoculated plates were then incubated at 30˚C for 24 h; large pink colonies with egg yolk precipitate were presumed as *B. cereus*. Confirmation was done with haemolysis test.

Fecal streptococci

Fecal streptococci was determined by spread plate method where 25 g of fish sample was taken aseptically and homogenized with 225 ml sterile peptone water for 60 s. 0.5 ml of each of decimal dilutions of the samples was spread on Typhon Soya Broth Agar and overlay with Kanamycin Esculin Azide Agar added and later incubated at 42°C for 24 h. The characteristic black colonies were counted after incubation confirmatory tests.

Statistical analysis

Data was analysed using Graph pad version 6 statistical software. Comparisons between means for physicochemical parameters were carried out using a One Way Analysis of Variance (ANOVA) and results with p values < 0.05 were considered statistically significant. Comparisons between mean values of physicochemical parameters across fish markets were done using Tukey`s multiple comparison test. Data are represented as means ± standard deviation. Results of physicochemical analysis and mean microbial counts of the dry - salted fish samples were compared with the set standards established by UNBS.

RESULTS AND DISCUSSION

Chemical analysis

Results from the chemical analysis (Table 1) revealed that moisture content significantly varied across fish markets ($F_{3, 12}$ = 4.0, p = 0.0014). The results showed that moisture content was significantly (p> 0.05) higher in fish collected from Panyimur market (41.6±0.47%) as compared to Nebbi (36.0±0.83%) and Arua (37.0±2.97%) fish markets. The relative higher moisture content in fish samples from Panyimur might be due to a shorter storage period since it is from this site that fish is distributed to other markets. Findings of this study show that values of37 to 41% of dry- salted fish collected from different markets are in accordance to 35 to 40% standard range for moisture content of dry-salted fish and fish products(UNBS, 2012). Accordingly, moisture content of

A. baremoze flesh without any processing ranged between 72 and 75% (Kasozi et al., 2014). Therefore dry salting method employed by fisher folk results in considerable loss of water due to heavy uptake of salt. The moisture content is an indicator of the susceptibility of a product to undergo microbial spoilage. It has a potential effect on the chemical reaction rate and microbial growth rate of the food product. Since moisture content is an indicator of the susceptibility of food products to undergo microbial and chemical spoilage (Horner, 1997; Chaijan, 2011; Goulas and Kontominas, 2005), traditional dry-salting of fish can result in storage stability.

The changes in the pH of dry-salted *A. baremoze* significantly varied across fish markets ($F_{3, 12}$ = 1.5, p < 0.0001). Fish from Arua were associated with significantly lower pH as compared to other fish markets (6.3±0.01). This could be attributed to relatively higher sodium chloride (14.9±0.01%) found in samples collected from this market. Goulas and Kontominas (2005) reported that salt had a highly significant linear decreasing effect on the pH of chub mackerel after day one of storage. Similarly, Chaijan (2011) reported a rapid decrease in the pH of dry salted *Oreochromis niloticus* muscle in the first 10 min of salting. The pH decrease in fish flesh by the addition of salt is explained by the increase of the ionic strength of the solution inside of the cells (Goulas and Kontominas, 2005).

The fat content significantly varied across fish markets ($F_{3, 12}$ = 0.9, p < 0.0001). The lowest fat content reported in fish samples from Arua market (12.9±0.66%) might be due to relatively higher sodium chloride (14.9±0.01%) since increased salt content induces lipid oxidation in muscle tissues and reported to accelerate progressively during dry salting of *Oreochromis niloticus* (Chaijan, 2011).

The protein content of processed fish significantly ($F_{3, 12}$ = 0.1, p = 0.0002) differed across fish markets ranging from 31 to 35% (Table 1). Comparison across fish markets revealed that protein content was significantly higher (35.1±0.88%) in fish from Panyimur as compared to other fish markets. Salting of fish is usually accompanied by protein losses, as water is drawn out and meal brine is formed, with some protein dissolved into the brine (Chaijan, 2011). Since fish was only stored for one day at Panyimur, this might explain the relatively higher protein levels compared to other markets.

Mean values of sodium chloride obtained in the fish muscle were in the range of 13 to 14% and significantly ($F_{3,12}$ = 0.8, p = 0.0003) differed across fish markets. Comparisons across fish markets revealed significantly higher (p> 0.05) sodium chloride levels in fish from Arua market (14.9±0.01%). Although salting effectively prevents the growth of both spoilage and pathogenic bacteria (Leroi et al., 2000); it has been reported that salt content in fish muscle enhances oxidation of the highly unsaturated lipids. Many of the fresh-fish-spoiling bacteria are quite active in salt concentrations up to 6% (Horner, 1997). Above 6 to 8%, they either die or stop growing. As the salt concentration is increased beyond 6%, bacteria of another group, consisting of a much smaller number of species, are still able to grow and spoil the fish. However, the halophiles "salt-loving" can still grow best in salt concentrations that range from 12 or 13% to saturated brine. Therefore, certain halophilic micro-organisms can multiply under the conditions of dry-salting and can also spoil the product.

Bacteriological quality

The quality of salted and sun dried fishes are adversely affected by microbial contamination. Determination of microbiological quality of processed dried fish product is very important for protecting consumer's health (Lilabati et al., 1999). The presence of potentially pathogenic bacteria in dried fishes is critical with regard to safety and quality of seafood. The acceptable microbiological limits set by UNBS for dried and salt-dried fish are indicated in Table 2 and these were compared with the results from the total plate counts of *Angara* from different markets.

Our study showed that *S. aureus* was the most dominant microorganism identified in dry-salted fish sold in all markets of West Nile region (Table 2). *S. aureus* does not appear as a part of the natural microflora of newly caught marine and cultivated fish (Herrero et al., 2003). Therefore, the presence of *S. aureus* is an indicator of poor hygiene and sanitary practices, during processing and storage. In this study, counts of *S. aureus* were above the limit of 2×10^3 cfu /10 g, recommended by the Uganda National Bureau of Standards (2012). However, lower bacterial load in fishery products might not be a serious risk, however, but food poisoning may occur if the product is handled carelessly resulting in high multiplication (>1×10^5 cfu/g) (Varnam and Evans, 1991; Vishwanath et al., 1998).

Although *E. coli* and fecal coliform bacteria can be found in unpolluted warm tropical waters (Huss, 1993; Hazen 1988; Fujioka et al., 1988), they are particularly useful as indicators of fecal contamination and poor handling of seafood. According to UNBS (2012) absence of *E. coli* has been recommended as an upper limit for a very good quality dry salted fish. In this study, *E. coli* counts were found to be <10^1 cfu/g and *fecal streptococci* counts were relatively high (Table 2). Similar results have also been reported by Colakoglu et al. (2006) for *fecal streptococci* counts between <10^1 and 10^3 cfu/g in the fish from wholesale and between <10^1 and 10^5 cfu/g in retail markets. It is reported that unclean boat deck, utensils in the boat, polluted water can certainly add the fecal bacteria load (Sugumar, et al., 1995) and this might explain the high *fecal streptococci* counts of 1.1×10^3 cfu/g (Table 2) of dry- salted fish at Panyimur market situated close to a landing site where fisher folk uses the lake

Table 2. Total viable bacterial count of dry-salted Pebbly fish (*Alestes baremoze*) samples collected from different markets after a storage period of five days.

Organisms	Markets				Maximum limit[a]
	Arua	Nebbi	Packwach	*Panyimur	
*Staphylococcus aureus (*cfu*/g)*	$9.6\times10^{4}\pm8.1\times10^{3}$	$9.4\times10^{6}\pm0.1\times10^{1}$	$2.3\times10^{5}\pm1.0\times10^{3}$	$2.2\times10^{6}\pm8.1\times10^{4}$	2×10^{3}
Escherichia coli (cfu/g)	<10	<10	<10	<10	Absent
Feacal streptococci (cfu/g)	<20	<20	$2\times10^{1}\pm0.1\times10^{1}$	$1.1\times10^{3}\pm5.0\times10^{1}$	Absent
Salmonella / 25 g	Absent	Present	Absent	Absent	Absent
Vibrio parahaemolyticus /25 g	Absent	Absent	Absent	Absent	N/A
Bacillus cereus (cfu/g)	$8\times10^{1}\pm0.01\times10^{1}$	<20	$4\times10^{1}\pm0.01\times10^{1}$	<20	N/A
Pseudomonas (cfu/g)	<20	<20	<20	$12\times10^{1}\pm0.01\times10^{1}$	Absent

*Fish samples at Panyimur market had been stored for one day; [a]UNBS, 2012; N/A = Data not available.

water during the salting process.

Salmonella is highly pathogenic and this is the major reason for isolation of such bacteria from sample fishes. *Salmonella* was absent in three markets of Arua, Packwach and Panyimur apart from Nebbi market (Table 2). Incidence of *Salmonella* in the sample of fish from this market may be attributed to external contamination and poor handling at ambient temperature.

V. parahaemolyticus is an indigenous bacterium in the marine environment and can also grow at 1 to 8% salt concentrations (Huss, 1993). It occurs in a variety of fish and shellfish, including clams, shrimp, lobster, crayfish, scallops and crabs, as well as in oysters (Kaysner and DePaola, 2000) It is very heat sensitive and easily destroyed by cooking (Huss et al., 2003).

B. cereus strains are widely distributed in the environment and their spores are resistant to drying and can easily be spread with dust (Huss et al., 2003). There was presence of low density of *B. cereus* in all the samples ranging from 8×10^{1} *B. cereus* (cfu/g) in Arua market to <20 cfu/g in Nebbi and Panyimur (Table 2). A small number of *Bacillus* spp. in foods is not considered significant (Beumer, 2001).

Many species of *Pseudomonas* spp. have a psychrophilic nature and are regarded as part of the natural flora of fish. The species can form aldehydes, ketones, esters and sulphides following food spoilage, causing odours described as fruity and rotten (Tryfinopoulo et al., 2002). The isolation of *Pseudomonas* spp from the collected fish samples is of high importance because this bacterium plays considerable role as a potential pathogenic bacteria for human and as an indicator of food spoilage. According to UNBS (2102), *Pseudomonas* spp. should be absent in dried and salted dried fish however this study reveals that *Pseudomonas* was detected in all samples, < 20 cfu/g in three markets of Arua, Nebbi and Packwach and 12×10^{1} cfu/g for Panyimur (Table 2).

Conclusion

Bacteriological quality of most *Angara* samples analyzed in this study did not meet the standards established by the Uganda National Bureau of Standards (UNBS) for dried and dry-salted fish. The study pointed out that *Angara* obtained from the markets was contaminated with substantial number of *S. aureus. Salmonella* and fecal *streptococci* and were also detected in fish from Panyimur and Nebbi markets, respectively. The substantial number of these microorganisms in *Angara* suggests poor personal hygiene, particularly among fish handlers and improper storage. Hence control measures such as use of good quality raw material, hygienic handling practices, potable water, good quality packaging material, hygienic processing place may be considered to improve the microbial quality of the dried fish product. Proper cooking procedures should be emphasized to eliminate or reduce the microorganisms to an acceptable level.

Conflict of Interests

The authors have not declared any conflict of interests.

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