Nutritive Value and Microflora of Salted Kawara (*Alestes sp.*) During Storage

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Abstract: The present study was performed to investgate the influence of salt (25%) and storage time under temperature between 36 to 37± 1˚C on the nutritive value and microbiological analysis of salted *Alestes sp.* Protein, crude fat, ether extract, ash content, dray matter, moisture content, pH, and some minerals content were analyzed. Chemical composition was reduced during storage period and the reduction was statistically significant (P<0.05) during 4-12 days of storag time. Total viable bacteria, total *Staphylococcus sp.*, *Micrococcus sp.* and yeast-mould were also measured to examine the microbial quality during storage time. *Staphylococcus sp.* was the dominant species, no yeast and mould were detected during the storage period. The total viable count of bacteria reduced duringe storage time. The result of this study indicated that, salted fish stored for three months had the best quality and shelf-life for *Alestes* species.

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Key words: Salted fish, *Alestes* species, Nutritive Value, Microbiological changes, Storage.

1. Introduction

Fish is one of the main sources for the provision of animal protein for a growing demand in a world of ever-growing population and increasing consumption. In this respect, the Sudan is no exception and the various aquatic resources (marine, freshwater, brackish, groundwater and others) are tapped in order to fulfill the needs in this direction.

Fish in general usually spoil more rapidly than other muscle foods, particularly when mishandled and such spoilage is primarily bacterial in nature, about 30% of landed fish are lost through microbial activity alone (Ghaly *et al*., 2010). With the over growing world population and need to store and transport food, fish preservation becomes necessary to supply the distant market, to produce a range of products with different flavours and textures and creation of conditions unfavorable to the growth or survival of spoilage organisms (Gracey, 1986, Yohama *et al*., 2011).

Fish processing remains the predominant and most important method of fish preservation in Africa (Gumisiriza *et a*l., 2009, Foline, *et a*l., 2011, Kiin-Kabari *et al*, 2011). The principal methods are salting, fermentation, drying and smoking. These processes may either be used alone or combined in order to achieve the desired product (Turan *et al*., 2007). Preservation by salting, smoking, drying is called curing of fish. This term is defined as fish preserved without the need for refrigeration or freezing, but excluding sterilized products in air-tight containers. Drying, smoking, salting and combination of these treatments are the basic means whereby, such product is prepared. Cured fish are consumed mainly in tropical countries (FAO, 1981).

It is unfortunate that the prevalent traditional preservation methods employed throughout Sudan are defective and need efforts pertaining to their improvement and development. In this connection, reference is made to drying, salting, fermentation, smoking, chilling and freezing, which are practised. However, this study is designed to concentrate on salting of fish and attempts to carry out exhaustive investigation on Kawara species (*Alestes sp.*) leading hopefully to achieve a promoted status to be placed at the disposal of practitioners who enter competition on quality. *Alestes species* (pebbly fish) locally known as kawara, belonging to the family Characidae, is the one of the common species used in salted fish in Sudan. The main objectives of this study was to carry out the chemical and microbiological analysis of the salted *Alestes sp.* during storage time.

2. Materials and Methods

This study was conducted at the Fisheries Research Center, Ministry of Science and Technology, University of Khartoum and Veterinary Research Center, Soba.

Sample Collection

Sample of fresh fish, namely, Kawara (*Alestes spp.*) were obtained from El Khartoum market (Mawrada). The samples were transported immediately (early in the morning) to the laboratory

at Elshagra Fisheries Research Center. The microbiological and chemical analysis were immediately carried out for fresh sample. The salt used in the processing of the experimental methods was obtained from Khartoum market.

During processing of salted fish in the laboratory, samples were withdrawn at random for salt concentrations (25%) from different containers of replication for microbiological and chemical analyses. Sampling was carried out every four days for the first 2 weeks and then was taken monthly until 6 month.

Treatment

Fresh fishes were weighed, washed, eviscerated, washed again and transferred to baskets to dry up. Then weighed again to obtain the actual weight, which will be treated with salt. Fishes were then divided into 3 groups each one divided into containers (small plastic barrels with lids) each has an equal weight of 3 kg. Each group was thoroughly treated with commercial salt, each group were treated with 25% salt (w/w) (3kg of fish +0.75kg of salt). In each group, salt was applied by brushing and rubbing of the fish surface, the gill chamber and the inner lining of eviscerated abdominal cavity.

Chemical Analysis

Preparation of the Sample

The samples of fresh and treated fish were minced through a meat mincer, and then mixed several times to be homogenized before analysis. The methods of A.O.A.C. (1990) were used to determine the crude protein (C.P), ether extract (E.E), ash content, dry matter (D.M), moisture and the crude fat of the sample.

pH Measurement

The pH was read using digital pH-meter (model Jenway 3015). The pH-meter was calibrated using standerd buffer solutions of pH = 4 and pH = 7. Two grams of sample was minced with 9 ml distilled water and was transferred to test tub. The pH was taken as a mean of 3 readings.

Determinations of Minerals

All minerals (Phosphorus, Iron, Copper, Calcium, Sodium and Potassium) studies of fresh and salted fish were determined according to the methods of A.O.A.C. (1990).

Total Viable Bacterial Count

Cruckshank, (1975) methods is used to count the total viable bacteria (TVB). The inoculum is deposited as drops from a calibrated dropping pipette. Each drop, 0.02 ml in volume, is allowed to fall from a height of 2.5 cm onto the medium, where it spread over an area of 1.5-2 cm diameter. Each of six plates receives one drop of each dilution in separate numbered sectors. Counts are made in the drop areas showing the largest number of colonies without confluence (up to 20 or more), the mean of the six counts gives the viable count per 0.02 ml of the dilution.

Isolation and Identification of the Colonies Culture

Colonies to be identified were picked from the Nutrient Agar (N.A), Mannitol salt agar, potato dextrose agar, and Blood Agar (B.A). plated and purified. Standard methods were used for microscopic examination according to Cruckshank, (1975).

Statistical Analysis

The data obtained were analyzed as a completely rando,mized design (ANOVA one and two way) and the means were tested for significance using Duncan Multiple range test as described by Statistical Package for Social Science (SPSS Softword (Vearsion 11).

3. Results

Biochemical composition

The chemical composition tests were carried out to determine protein, crude fat, ether extract, ash, dry matter, moisture content, and some minerals (P, Ca, Fe, Na, K, and Cu) values for fresh and treated samples of Kawara (*Alestes sp.*) during storage.

The biochemical composition of the fresh samples of Kawara species during study are comparable in both contents and values (Table 1). The values obtained in Table 1 change in varying degrees when subjected to salting during short and prolonged times and seasons (Tables 2, 3).

Two-way analysis of variance (Table 1-3) was performed to verify the significance of the effect of salt, days and their interaction on crude protein, crude fiber, ether extract, ash, dry matter, moisture content and some minerals during this study. The varying degree of significance (P 0.05 – P0.00) were reported with respect to correlated models, salt and days (Table 2). pH measurement during this study ranged between 7.o7.9± 2.94 to 6.31± 1.66 during study period of time(Table 7).

General magnitude change between fresh and treated samples

The general magnitude of change between fresh and treated material can be illustrated by a summary table (Table 4) for Kawara species. Through correlation of the status of level of the parameter as

attached with the salt concetration (25% of weight) and storage time.

Effect of the quality of the treated samples

The given tables 1-4 can be illustrated by a number of observations such as:

The findings obtained during the first 4, 8, and 12 days show a significant variations in the enlisted parameters in terms of time, but in general, the quality is kept within comparable levels.

During the first levels before gradual deterioration starts toward the end of the sixth month which marks the onset for further losses in quality.

This fact can be further illustrated by the fate of crude protein of Kawara species during storage time Fig. 1.

Total viable bacterial counts

The total viable bacterial counts of fresh sample throughout selected structures and whole fish indicate differences over storage time (Table 7). In fresh samples, some species of bacteria were found during the storage (Table 8). In the treated material the occurrence of bacteria is closely correlated with the salt penetration and both preliminary treatment during the first 12 days as well as the prolonged phase lasting six month (Tables 6, 7,8). *Staphylococcus xylosus* was the dominant bacteria found in *Alestes* sp. during the first days of treatment at 25% salt concentration (Table 8).

Total viable bacterial count was subjected to two-way analysis of variance for statistical significance. The effect of salt and storage time and their interaction was found significantly ($P<0.05$) during study period (Table 6, 7,8). No yeast and mould were detected for the period of storage.

Table No. (1): Chemical composition of fresh sample of *Alestes* species

Kawara sp.	Parameters											
	C.P	C.F	E.E	Ash	D.M	Moist	P	Ca	Fe	Na	k	Cu
Kw	17.45b	1.05	1.63	1.05	29.60	70.41b	1.35	8.20b	56.50[a]	81.00[a]	5.53	5.03
±SE	0.46	0.716	0.833	0.19	0.392	0.374	0.10	0.18	0.58	0.221	0.21	0.2
Sig.	*	NS	NS	NS	NS	*	NS	*	*	*	NS	NS

In this and subsequent tables means within the same column followed by different superscript are significantly different ($p<0.05$). * Significant at 5% level NS not significant.

Legend: C.P. = Crude protein; Ca = Calcium; k= potassium; C.F. = Crude fat; Fe = iron; Kw= Kawara; E.E. =Ether extract; Na =Sodium; D.M.=Dry matter; Cu= Cobalt; P = phosphorus; SE= Standard error

Table No. (2): The effect of Salt concentration and time on C.P, C.F, E.E, Ash, D.M and Moist. of *Alestes* species during storage.

Days	C.P	C.F	E.E	Ash	D.M	Moisture
	25% ±SE Sig.	25% ±SE Sig	25% ± SE Sig.	25% ±SE Sig.	25% ± SE Sig	25% ± SE Sig.
4D	19.27 0.23 NS	0.60 0.77 NS	1.37 0.44 NS	11.42 0.30 NS	41.61 0.156 NS	58.39 0.145 NS
8D	19.13 0.33 NS	0.57 0.675 NS	1.30 0.14 NS	11.39 0.36 NS	42.59 0.141 NS	57.41 0.76 NS
12D	18.37 0.20 NS	0.63 0.778 NS	1.48 0.13 NS	11.42 0.53 NS	41.79 0.36 NS	58.14 0.37 NS
1M	16.76 0.66 NS	0.50 0.484 NS	1.13 0.15 NS	13.65 0.25 NS	34.97 0.248 NS	67.06 0. 2.91 NS
2M	16.08 0.26 NS	0.40 0. .38 NS	1.23 0.555 NS	14.10 0.65 NS	45.21 0.78 NS	54.78 0.80 NS
3M	15.93 0.23 NS	0.36 0.400 NS	1.00 0.40 NS	3.43 0.62 NS	54.78 0.80 NS	45.21 0.80 NS
4M	16.34 0.57 NS	0.26 0.2.94 NS	0.86 0.484 NS	13.77[a] 0.66 *	53.90 0.40 NS	46.10 0.40 NS
5M	14.62 0.650 NS	0.16 0.166 NS	0.50 0.66 NS	12.39 0. 66 NS	65.73 0.10 NS	34.29 0.142 NS
6M	13.74 0.19 NS	0.20b 0.333a *	0.33 0.10 NS	11.66 0.30 NS	72.34 0.125 NS	27.65 0.125 NS

25% = Concentation of salt

Table No. (3): The effect of Salt on P, Ca, Fe, Na, K, and Cu. of *Alestes* species during storage

Days	P 25% SE Sig.	Ca 25% SE Sig	Fe 25% ± SE Sig.	Na 25% ± SE Sig.	K 25% ± SE Sig	Cu 25% ± SE Sig.
4D	1.47 0. 97 NS	8.20 0.83 NS	49.67 0.198 NS	403.00 0.82 NS	5.17 0.11 NS	4.37 0.11 N
8D	1.33 0.12 NS	8.07 0.20 NS	48.33 0.214 NS	501.67[a]b 0. 84 *	5.10 0.909 NS	4.23 0.13 N
12D	1.20[a]b 0.7.7 *	7.43[a]b 0.1 *	44.67 0.307 NS	506.00b 0.99 *	4.96 0.11 NS	4.13 0.10 N
1M	1.27 0.10 NS	7.37 0.86 NS	59.00 0.34 NS	521.00 0..35 NS	5.90 0.16 NS	4.20 0.14 *
2M	1.36 0.77 NS	7.16 0.11 NS	41.00 0.315 NS	496.00b 0.106 *	4.63 0.33 NS	3.93 0.15 *
3M	1.11 0. 66 NS	6.83 0.10 NS	39.00 0.15 NS	410.00b 0.1.22 *	4.13 0.12 NS	3.60 0.1 N
4M	0.96 0.40 NS	6.83 0.15 NS	36.00 0.132 NS	397.66b 0.133 *	3.90 0.10 NS	3.33 0.15 N
5M	0.66 0.01 NS	6.10 0.66 NS	30.00 0.13 NS	351.33b 0.78. *	3.46 0.16 NS	3.13 0.50 N
6M	0.56 0.50 NS	5.80 0.10 NS	25.00 0.50 NS	334.66 0.79 NS	3.10 0. .33 NS	2.70 0. 33 N

D=Day M=Month

Table No.(4) The magnitude of change between fresh and treated materials during storage period

Samples	Parameters											
	C.P	C.F	E.E	Ash	DM	Moist.	P	Ca	Fe	Na	K	cu
F.K.	17.45	1.05	1.63	1.05	29.60	70.41	1.35	8.20	56.50	81.	5.53	5.03
T.K.	16.53	0.49	1.15	13.51	73.59	26.40	1.24	7.38	51.78	5.1	4.25	3.39

F.K.= Fresh Kawara T.K.= Treated Kawara

Table No. (5): Total Viable Bacteria count of fresh samples during experiments

S	Parameters				
	Gill	Gut	Muscle	Skin	Whole
No.	1900.00	2850.00	70.33	1860.00	1516.67
t	3.98	6.92	1.21	5.15	4.76
Sig.	0.15	0.09	0.44	0.12	0.13

S= sample T=t-test Sig=significant

Table No. (6): Bacteria species found in fresh samples during study

Alestes species				
Gill	Gut	Muscle	Skin	Whole
Stomatococcus variaus + *Staphylococcus. capitis*	*Micrococcus*	*Staphylococcus gallinarum*	*Staphylococcus caprae*	*Stomatococcus variaus* + *Stomatococcus variaus*

Table No. (7): The effect of storage time on pH and total viable Bacteria count of *Alestes* species during storage

Days	pH			B. count		
	Mean	±SE	Sig.	Mean	±SE	Sig.
4D	7.07	0.294	*	35.16	0.15	NS
8D	7.01	0.111	NS	390.06	0.354	NS
12D	6.90	0.333	NS	128.40	0.101	*
1M	6.80	0.192	NS	2.23	0.125	*
2M	6.56	0.59	NS	8.37	0.64	*
3M	6.61	0.50	NS	1.45	0.50	NS
4M	6.61	0.50	NS	1.73	0.76	NS
5M	6.45	0.833	NS	0.26	0.10	NS
6M	6.31	0.166	NS	10.25	0. 408	NS

Table No. (8): Bacterial found in treated samples during storage

Days	Bactrial sp.
4 D	*Staphelococcus xylosus* + *Staphelococcus lentus*
8 D	*Staphelococcus xylosus*
12 D	*Staphelococcus xylosus* + *Staphelococcus saprophyticus*
1 M	*Staphelococcus xylosus* + *Staphelococcus saprophyticus*
2 M	*Staphelococcus xylosus* + *Staphelococcus saprophyticus*
3 M	*Staphelococcus xylosus*
4 M	*Staphelococcus saprophyticus*
5 M	*Staphelococcus saprophyticus*
6 M	*Staphelococcus saprophyticus*

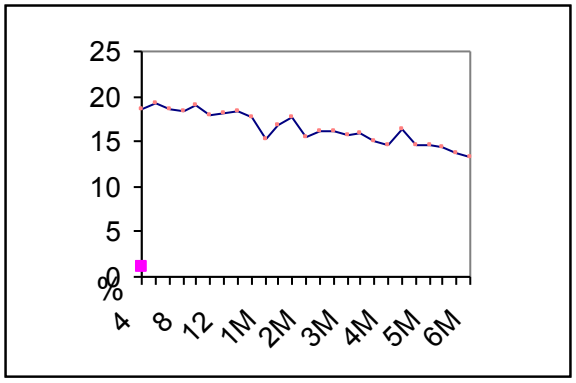

Fig. (1) Crude protein of *Alestes sp.* during storage (during the first 12 days (D) and subsequent 6 month (M)).

4. Discussion

The measurement pH was not found to be statistically significantly ($p<0.05$) especially during early time of processing, and this affected by the addition of sodium chloride during storage time for salted *Alestes sp*. This observation are in keeping with other researchers Gokolue, *et al.* (1994), Duman, *et al*. 2007 and Bahri, *et al*. (2006), although the later author found values reduced depending on storage time for the species of tout, anchovy and mirror carp. The pH ranged between 6.14-6.7 at the starting of expeirment and then changed to 5.34-6.81 during storage, this implies that the effect of salt on the pH value of salted fish is relatively small. Highest level of moiture content (70.41±3.74) was observed in the fresh sample, similar result was observed by Abdullhi (2000) in some fresh fish species, *Alestes nurs, alestus macrolepidotus,* and *Hydrocynus brevis* . the moiture contend was lower during storage time, these may be due to pentration of salt in the muscle. The lower water activity may be important preservative factor, handling, and storage (Samson *et al*., 1987). Products with high moisture content tend to deteriorate faster than dried products especially if the salt level is low (Kofi, 1992).

The protein content in wet basis was (17.45± 0.46) near the range founded by other researcher (Ssali, 1988). The crude protein of fresh fish ranges between 14-20% and higher levels are obtained during winter season (Clucas and Ward, 1996).The crude protein was increase during 4-8 days of the storage and then decrease in amount until the end of

experiment, although there is no significant statistical value ($p<0.05$) in crude protein during storage, these may be due to increase the activity of the microorganism at the beging of salted treatment. Lawrie (1990) stated that crude protein decreased with storage of cured meat and this was attributed to some changes during storage that caused by "maillard reaction", where in carbonyl groups of reducing sugars react with amino groups of protein and amino acids non-enzymtically, and might also be due to an attack of myoglobin by bacteria during storage and changes in pH. Salt causes the proteins in fish muscle to swell and salt lead the protein become denatured if increases in the muscle (Hamm, 1994). Some microorganism of treated salted fish enhance nutritive value of the product (Beddows, 1985). In Asia some microorganism used maily to enhance fish product (Zakhia and Cua, 1991).

Crude fat and ether extract were decreases during storage period but no significant different ($p<0.05$) were found, these might be due to the leaching of some substance during processing correlated with pentration of salt in mscule.

The contend of ash and dry matter were increases throught the experiment as similar result reported by Salma *et al*., (1977), but the range of ash in this study was lower than the range mention in their results (14-18%), these may be due to differences in species, salting process and storage time. Chemical composition of fish varies greatly from one species and from one individual to another depending on age, sex, environmental conditions (FAO, 1986). El-Sebahy and Metwali (1988) found a decrease in the level of crude protein, fat and increase in ash content of small and large salted Bouri fish muscle (*Mugil Cephlus*).All of mieral studied were decreased during storge these may be due to lossing of water. The salt penetration is lower water activity in the deepest parts of the flesh (Doe *et al*., 1983), these reduce the water activity and the occurrence of impurities interferes with salt penetration, which lead to low quality product (FAO, 1981).

The total viable bacteria counts were found less in muscle and flesh of fish compared with highest concentration in gut, gill and skin of fresh sample respectively. Gut, gill and skin were the most regions contaminated easily in fish (Evelyn and McDermott, 1961; Olympia *et al*., 1992) because fish is a very good culture media. Fish is low acid food and is therefore very susceptible to the growth of food poisoning bacteria (Yohnna,*et al*. 2011).

The most fish flesh, however, is considered more perishable than meat because of more rapid autolysis by the fish enzymes and because of less acid reaction of fish flesh that favors microbial growth. Fish, in general, usually spoil more rapidly than other muscle foods, particularly when mishandled and such spoilage is primarily bacterial in nature Hess (1950). The primary purpose of food preservation methods is the creation of conditions unfavorable to the growth or survival of spoilage organisms (Gracey, 1986). Abu Gideiri *et al*., (2004) found a significant change in some chemical constituents of salted fish (*Oreocheomis Niloticus*).

Total viable penetration of salt into the fish muscle is dependent on many factors; including the thickness of the fish, osmotic pressure, temperature, purity of the salt, freshness of the fish, and the fat content of the fish (Ingram and Kitchell, 1967). Viable counts of bacteria during storage was significantly ($p<0.05$) increase from 12 days of storage to 2 month and then decreases with time of storage, these might be to activity of water still high during these period, and reduction occure with salt pentration inside the muscle for that sodium chloride has been used as a preservative for a long time. It acts as a bacteriostatic and a bacteriocidal agent when present in sufficient concentration (Beatty and Fougere, 1957). This property of salt has been frequently used in food processing and is the basis for the preservation of salted fish. According to Beatty and Fougere (1957), bacteria which contribute to spoilage in fresh fish cannot survive at salt concentration above 12% (w/w). Micoroflora was changed during storage period of *aji-no-susu* (Kuda, *et al*. 2009).

Staphelococcus xylosus and *Staphelococcus saprophyticus* were the most bacterial species in salted treatment Kawara species and during storage, these species was related to salted fish product Saisithi *et al*. (1966) found that there were an average, 2700 bacteria /g in fermented fish and the total viable count decreased to 2×10^3 cell/cm^3 after 9 months of processing. *Staphelococcus sp.* was detected during stroge period of mirror carp (*Cyprinus carpio* L.) Duman, *et al.* 2007.

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