American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Food Technology

ISSN 1557-4571
DOI: 10.3923/ajft.2020.69.74

Research Article
Nutritional Value and Microflora of Salted *Schilbe mystus* (LINNE 1758) During the Storage at Ambient Temperatures (37 and 27°C)

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Abstract

Background and Objective: In attempts to the preservation of the fishes and their products for consumption at subsequent times and places far away from the source, salted preservation methods were used. Such type of methods has to give good coverage for various types of fish. The present study was carried out to evaluate the changes in nutritional quality and microbial content of salted (20% of the fish weight) *Schilbe mystus*, kept under two ambient temperatures 37 and 27°C for one month. **Materials and Method:** Moisture, dry matter, ash, crude protein, crude fat, crude fiber, pH and mineral contents (Phosphorus, Iron, Copper, Calcium, Sodium and Potassium) of salted *Schilbe mystus* were analyzed. Total viable counts, isolation and identification of bacteria and mold were measured for the microbial quality. **Results:** The temperature had a direct effect on the microbial quality of salted *Schilbe mystus*. It was observed that the total bacterial count was increased at 27°C till the tenth day, followed by a remarkable decrease. Two species Staphylococcus genera (*Staphylococcus aureus* and *Staphylococcus lentus*) were isolated from samples of salted fish and appreciate about 57.14% of total isolates. Also, two species of Micrococcus genera were isolated (*Micrococcus leuteus* and *Micrococcus roseus*) and they represented 14.29%. *Aerococcus viridans* and *Pseudomonas aeroginosa* isolated also from samples. No yeast-mold were detected during the storage time. **Conclusion:** The storage period and the temperatures were effective significantly ($p \leq 0.05$) to the chemical composition of *Schilbe mystus*, but (27°C) showed a better quality of the product compared to higher degrees (37°C).

Key words: Salting, storage, temperature, nutritive value, *Schilbe mystus*

Citation: Ahmed, E.O., M.E. Ali, A.A. Aziz, A.H. Mukhtar and R.H. Ahmed, 2020. Nutritional value and microflora of salted *Schilbe mystus* (LINNE 1758) during the storage at ambient temperatures (37 and 27°C). Am. J. Food Technol., 15: 69-74.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish is considered an important source of protein which has a great nutritional value and contains all the essential amino acids, which are very important for human health. Fish are highly perishable food items that start to spoil as soon as they are harvested[1]. The causes of fish spoilage are bacterial activity, autolysis, oxidation and, most commonly by a combination of these which bring about very noticeable changes in the texture, flavor, odor and general appearance of the product[2]. To enhance the shelf life of fish different fish curing has been practiced. Cured fish comprise that portion of the product, which is consumed fresh, neither refrigerated nor frozen. 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[3]. Salting is one of the oldest methods of fish preservation. Traditionally; salting is performed either by dry salting, pickling or brining. The main purpose of salting is to separate water from the fish and replace it with salt. Thus, the water concentration in fish decreases. Chlorine and sodium ions are carried from brine to fish and water dipoles are carried from fish to the environment. The rate during this process is high and it slows during ripening[4]. The process of salting fish is influenced by many factors such as weather, size and species of fish and the quality of salt used[5]. In Sudan, nearly 70% of the total fish landings are consumed fresh; the rest is cured either by salting, fermentation or sun drying. Salted fish is always made from *Schilbe* sp., "Shilbaia" which belongs to the Family Schilbeidae. The freshwater Nile silver Schilbeid catfish, Order: Siluriformes, constituted one of the dominant fish species in Sudan. These carnivorous species are good quality food fish of white and very tasty flesh serving as the delicacy for many riverine communities. In addition, they are important both in ecological and economic terms, playing a salient role in determining the dynamics and structure of the aquatic ecosystem[6]. Yet in Sudan with its large fisheries resources, fish is not well utilized for human benefit. Despite the large potential yield of the Sudanese water resources, fish does not comprise an important part of the people's diet. This work aimed to investigate the chemical and microbiological of salted *Schilbe mystus*. And to contribute to the development of salted fish through the improvement of the product and the skill of the practitioners.

MATERIALS AND METHODS

Study area: This study was conducted at the department of fish sciences and the department of microbiology, El Neelain University, Khartoum. The study took place in January and May, 2017 at temperatures 27 and 37°C respectively.

Materials

Collection of samples: A total of 60 kg of assorted fresh fish samples, *Schilbe mystus* (16-22 cm in total length) were brought from the local fisherman's market. The samples were kept in polyethylene bags with crushed ice and transported to the farm of the Fish Sciences department for processing. Salted samples for chemical and microbiological analysis were carried out at the faculty of agriculture and the faculty of Sciences, El Neelain university. Sampling was carried out every ten days after the matured of the product.

Processing: Fresh fishes were weighed, washed, eviscerated, washed again and transferred to baskets to dry up while covered by a thin cloth to prevent insects invade. Then the fishes were weighed to the nearest gram using a dial balance (KRUPS type 875), for salting, a total weight of salt estimated 20% of the fish's weight (fish weight: salt weight 5:1). The procedure used is called dry salting. In this method, salt was applied by brushing of the fish surface, the gill chamber and the inner lining of the eviscerated abdominal cavity. The stack of the fish and salt is left about 7 to 10 days according to the temperatures. When the salt has penetrated the fish, it extracts the fish fluids through plasmolysis. The extracts fluid (pickle) was allowed to drain continuously. Salted product was packed and stored at ambient temperatures (37 and 27°C) for one month.

Methods

Chemical analysis

Preparation of the sample: The samples of salted fish were minced through a meat mincer and then mixed several times to be homogenized before analysis. The previously used methods[7] were used to determine the moisture, dry matter, ash content, crude protein, crude fiber and crude fat of the samples.

pH measurement: Hydrogen ion concentration (pH) was determined with a glass-electrode of a newly calibrated Digital pH-meter (JENWAY-3015 pH-meter).

Determinations of minerals: According to the another methods[7], the mineral studies (Phosphorus, Iron, Copper, Calcium, Sodium and Potassium) in fish samples were measured by the Atomic Absorption spectrophotometer (8625, UNI cam-UV/VIS, Germany).

Microbiological examination

Total viable counts (TVC): Appropriate serial dilution was made by using a desired amount of samples (20 g) and transferred to a sterile bottle containing 180 mL of Peptone water (0.1% w/v) to give 10^{-1} dilution, then 1 mL from the bottle was transferred to a tube containing 9 ml of Peptone water to give 10^{-6} dilutions; then further dilutions were made in a similar manner. A total viable count was enumerated by pouring plate method using Plate Count Agar (PCA) at (37±1°C, 48 hrs).

Isolation and identification of the colonies cultures: Colonies to be identified were picked from the Nutrient Agar (N.A) and Blood Agar (B.A) medium was used as general and enriched media, while a Mannitol salt agar was used as a selective and differential characteristic medium. Pure colonies isolate were differentiated by conducting coagulase test as well as biochemical tests such as Urea test, Voges-Proskauer (VP) test and Sugar fermentation[8]. Potato dextrose agar was used for counting mold and yeast (22±1°C, 5 days). Statistical analysis: The data obtained were analyzed computerized using Statistical Package for Social Science (SPSS) Software (version 21), the means were tested for significance using (ANOVA, two way) and the post Hock test used the Least Significant Difference (LSD) test for the mean separation and the significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Total viable bacterial counts of salted fish: Fish, because of their soft tissues and aquatic environments are extremely susceptible to microbial contamination. Although the flesh itself is normally sterile, bacterial growth and invasion on the fish are prevented by the body's natural defense system while they are alive, but after death, the defense system breaks down and the bacteria multiply and invade the flesh[9]. Total Viable Bacterial (TVC) of salted fish muscle is dependent on many factors; including the thickness of the fish, osmotic pressure, temperature, purity of the salt, the freshness of the fish and the fat content of the fish. Viable counts of bacteria of salted *Schilbe mystus* with two temperatures varied during the storage time varying from 6.5×10^3 to 1.0×10^3 cfu g^{-1} Table 1. It is noted that the number viable counts of bacteria during storage increased on the tenth day temperature (27°C), compared to the zero-day, then it decreased as the salting proceeded. These might be due to water activity, still higher during these periods and reduction occurred with salt penetration inside the muscle. Also, the early increase occurred while fishes were wet, as the fish became drier, there was a decrease in the water activity and this, to gather with the accumulated salt in the flesh, resulting in suppression of bacterial growth. In this work 10^6 cfu g^{-1} of the total viable counts of bacteria was used as the limit for the evaluation of microbial spoilage. When aerobic plate counts reach 10^6 cfu g^{-1}, the food product was assumed to be at or near spoilage[10,11]. In this study, the microbial growth was lower in salted samples and hasn't reached 10^6 cfu g^{-1} and was of the acceptable limit (6.5×10^3 cfu g^{-1}) according to the other study[12]. The TVC in this study was in agreement with the findings of other studiess[13,14].

Bacterial species isolated: The bacterial species isolated from salted fish (*Schilbe mystus*) were *Staphylococcus aureus* and *Staphylococcus lentus* and appreciates about 57.14% of total isolates. Also, two species of Micrococcus were isolated *Micrococcus luteus* and *Micrococcus roseus* were represented about 14.29%. *Aerococcus viridans* and *Pseudomonas aeroginosa* isolated also from samples. *Staphylococcus aureus* was the most dominant species isolated as shown in Table1. According to previous study[15,16] *Staphylococcus aureus* is growing well in salted food and in low water activity. Similar results were obtained by another study[17] for traditional salted-fermented fish Hout-kasef and disagreed with other study[18] who found that *Staphylococcus saccharolyticus* was the dominant bacteria isolated from fermented salt fish (Fassiekh). No yeast or mold was detected in salted samples.

Table 1: Total Viable Bacterial Counts (TVC) and isolated bacteria species of salted *Schilbe mystus* storage at ambient temperatures (37°C and 27°C)

	Parameters			
	TVC (cfu g^{-1})		Bacterial species	
Storage period	37°C	37°C	37°C	37°C
0 day	4.5×10^3	5×10^3	*Staphylococcus aureus*	*Staphylococcus aureus+Micrococcus roseus*
10 day	4×10^3	6.5×10^3	*Staphylococcus aureus*	*Staphylococcus aureus+Micrococcus leuteus*
20 day	2×10^3	3×10^3	*Staphylococcus aureus+Micrococcus leuteus+Aerococcus viridans*	*Staphylococus lentus*
30 day	1×10^3	1×10^3	*Staphylococcus aureus+Aerococcus viridans+Pseudomonas aeroginosa*	*Staphylococcus aureus*

Chemical composition

Moisture content: The moisture content changes were determined to be of significant difference ($p<0.05$) and decreased progressively during the storage period at two temperatures Table 2, these may be due to penetration of salt in the muscle. It contains relatively high amounts of moisture ranging from 64.06±0.63 to 51.66±1.04%. The results of the present study are similar to the findings of another study[19] who reported that the moisture content was ranged from 77.03 to 39.62 51.79±6.76 on the fermented fish product (Fassiekh). But lower than those reported for fermented cassava fish (Lanhouin)[20].

Ash and dry matter content: The content of ash and dry matter increased significantly ($p<0.05$) on storage through two temperatures. The ash contents varied between 13.13±0.47 and 10.36±0.08%. The increase in ash content during the salting period may be due to the salt penetration into fish flesh during the curing process. These results agreed with other studies[13,14]. But lower than those reported by other study[17] for traditional salted-fermented fish Hout-kasef.

Crude protein: The protein content of salted *Schilbe mystus* samples decreased significantly ($p<0.05$) during storage relative to two temperatures. The crude protein content of the samples ranged from 19.16±0.15 to 15.16±0.28%, (Table2). It is evident that the protein content of processed fish has decreased over the course of salting. Salting of fish is usually accompanied by protein losses, as water is drawn out and meal brine is formed. These results are in accordance with another study[21].

Crude fat and crude fiber: Crude fat decreased significantly ($p<0.05$), ranged from 1.90±0.05 to 0.89±0.05%. This may be due to oxidative deterioration, thereby affecting lipid extraction[22]. The crude fiber content of salted *Schilbe mystus* samples was found significantly ($p<0.05$) decreased and ranged from 1.56±0.05 to 0.83±0.05%. Results obtained in this study are in agreement with that reported for salted treatment Kawara fish (*Alestes* species) during storage[14].

pH: The pH values of the samples were ranging from 6.86±0.10 to 6.0±0.05%. During storage, this implies that the effect of salt on the pH value of salted fish is relatively small. The pH value of the present study is similar to salted fermented fish known as Pedah siam is processed in Thailand. The standard pH requirement for Pedah siam is 6.0-6.4 with a pH of 6.5 or higher considered as indicative of poor quality[23].

Minerals content: There is very little information on mineral contents of salted fish-products. All minerals studied, were reduced significantly ($p<0.05$), this reduction continued with increasing duration of storage Table 3. The values of the minerals found in this study are higher than obtained by other study[24] for the mineral content of traditionally fermented Nile-fish product in Sudan. These may be due to differences in

Table 2: The chemical composition of salted *Schilbe mystus* (g/100 g) during storage at ambient temperatures (37°C and 27°C)

	Parameters							
	Moisture		Dry matter		Ash		Crude protein	
Storage time	37°C	27°C	37°C	27°C	37°C	27°C	37°C	27°C
0 day	60.31±0.49^a	64.06±0.63^b	39.5±0.42^a	35.94±0.63^b	10.36±0.08^a	110.23±0.20^b	18.83±0.20^b	19.16±0.15^a
10 day	56.58±0.36^b	62.51±0.70^a	43.41±0.36^a	38.18±0.50^b	10.76±0.15^a	11.36±0.15^b	18.13±0.15^b	18.50±0.10^a
20 day	55.07±0.81^b	58.99±0.90^a	44.92±0.81^a	41.01±0.93^b	12.16±0.25^a	12.33±0.30^b	17.66±0.20^b	17.86±0.15^a
30 day	51.66±1.04^b	54.75±0.95^a	48.34±1.04^a	45.24±0.95^b	13.13±0.47^a	12.90±0.10^b	15.23±0.20^b	15.90±0.10^a
Significant	*	*	*	*	*	*	*	*

	Parameters					
	Crude fat		Crude fiber		pH	
Storage time	37°C	27°C	37°C	27°C	37°C	27°C
0 day	1.80±0.10^b	1.93±0.06^a	1.23±0.05^b	1.56±0.05^a	6.83±0.05^a	6.76±0.05^b
10 day	1.56±0.05^b	1.65±0.05^a	1.06±0.05^b	1.26±0.05^a	6.56±0.15^a	6.33±0.06^b
20 day	1.36±0.11^b	1.42±0.05^a	0.96±0.05^b	1.06±0.05^a	6.33±0.05^a	6.03±0.15^b
30 day	1.06±0.11^b	1.10±0.10^a	0.83±0.05^b	0.83±0.05^a	6.10±0.17^a	6.00±0.10^b
Significant	*	*	*	*	*	*

Different superscript letters in the same raw indicate significant differences between groups ($p<0.05$), The same column followed by different superscript are significantly different ($p<0.05$), *Significant at 5% level significant

Table 3: The mineral contents of salted Schilbe mystus during storage at ambient temperatures (37°C and 27°C)

	Parameters					
	Phosphorus		Iron		Calcium	
Storage time	37°C	27°C	37°C	27°C	37°C	27°C
0 day	1.66±0.05[a]	1.60±0.10[b]	55.67±0.57[b]	63.3±3.05[a]	8.60±0.10[b]	8.83±0.05[a]
10 day	1.40±0.10[b]	1.43±0.05[a]	49.00±1.00[b]	59.0±1.0[a]	8.10±0.10[b]	8.50±0.10[a]
20 day	1.20±0.10[a]	1.16±0.05[b]	45.00±3.00[b]	52.0±2.0[a]	7.86±0.15[a]	7.63±0.47[b]
30 day	1.06±0.05[a]	1.00±0.01[b]	39.67±0.57[b]	46.33±3.21[a]	7.40±0.10[a]	7.20±0.10[b]
Significant	*	*	*	*	*	*
	Parameters					
	Sodium		Potassium		Copper	
	37°C	27°C	37°C	27°C	37°C	27°C
0 day	363.03±3.33[b]	376.3±3.04[a]	6.53±0.05[b]	6.56±0.05[a]	5.83±0.05[b]	5.93±0.05[a]
10 day	367.90±2.35[b]	383.0±2.64[a]	6.30±0.30[a]	6.16±0.15[b]	5.60±0.10[a]	5.40±0.10[b]
20 day	373.80±3.27[b]	405.0±3.00[a]	6.10±0.10[a]	5.60±0.10[b]	5.30±0.10[a]	4.86±0.11[b]
30 day	389.53±3.70[b]	413.33±1.52[a]	5.90±0.10[a]	5.16±0.15[b]	5.03±0.15[a]	4.26±0.15[b]
Significant	*	*	*	*	*	*

Different superscript letters in the same raw indicate significant differences between groups ($p<0.05$), The same column followed by different superscript are significantly different ($p<0.05$), *Significant at 5% level significant

species, salting process and storage time. These results show that salting and storage period have a negative effect on the mineral concentrations of salted fish species. The negative effect is attributed to loss of water-soluble nutrients which occurs during the exchange of water as it moves out and as salt enters into the fish[25]. The higher value of sodium during storage could be referred to as added amounts of sodium chloride.

CONCLUSIONS

The study showed that temperatures and storage significantly affected the nutritive values of salted *Schilbe mystus* and their effect varied through two temperatures, but 27°C gave the best quality of the product. It was observed that the total viable bacterial counts in salted fish decreased through a storage period in all seasons and are at the acceptable limit of ICMSF. Staphylococcus-Micrococcus were detected, *Staphylococcus aureus* was dominant, but still within the acceptable.

SIGNIFICANCE STATEMENT

In the last years, the production of salted fishes in Sudan has increased, which indicated the progress of this industry. The salted fish which is consumed as a source of protein, also furnished fat, is welcomed addition to the diet in an area where there is a shortage in calories. The variation in the product quality has drawn the attention to the need for further investigation of the nutritive value and correlation with the preparation of the product in the final form in order to satisfy the requirement of the consumer both locally and abroad.

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