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EFFECT OF DRYING ON MICROBIAL LOAD OF *CLARIAS SP.* MEAT

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ABSTRACT

This study was conducted at Department of Fisheries and Wildlife Science, College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology, to study and evaluate the effect of drying on microbial load and some spoilage organisms of *Clarias sp.* fish meat. 18 fish sample of garmout (*Clarias sp.*) at the period from March to July 2010 samples were collected from Elmourda fish market. Samples were divided into two group 9 fresh and 9 dried samples. Fresh *Clarias sp* fish were treated with open air sun-drying. The bacterial total viable count was conducted before and after treatment, which decreased after the treatment and showed high significant differences (p<0.001) between fresh and cured fish, 7.6 x l05± 4.7 x 105 ,5.6 x105 ± 3x l05 respectively. The *Staphylococcus aureus* and *E. coli* test showed negative result for all studied samples. The *Salmonella paratyphi* A. test was positive for fresh fish and negative for treated cured fish samples.

Keywords: *Clarias sp.*Fish Meat, Micrcobial Load, *Staphylococcus aureus, E. coli*

INTRODUCTION

During the last decades healthy eating habits received increased attention, and it is widely recognized that regular fish consumption is a possible practice for health improvement **[1, 2]**. Fish in the Sudan have been a major source of protein and energy for many

communities especially among the Nilotic tribes of the south and some of Nubian ethnic groups of the far north especially in the lean months of the year. Sudanese people use fish sometimes as the only source of animal protein throughout the years as substitute for meat, particularly in the central Nile valley. Cured fish comprise that portion of the product, which is not consumed fresh, neither refrigerated nor frozen. The principal methods are smoking, sun drying, salting and fermentation. These processes may either be used alone or combined in order to achieve the desired product [3].

The choice of a particular processing method is greatly influenced by the area, geographical location, socio-economic factors and eating habits of the local people. Furthermore, due to the lack of good infrastructure for the transportation of fresh fish to remote towns and villages, cured fish is the most convenient form in which fish can be sent to such areas [4].

In recent years the annual world production of dried fish products was 350 000 tones, and the biggest production came from Asia and Africa [5]. Not all types of fish are used in drying processes. It is generally accepted that personal preference of consumers may be prejudiced by color ,taste or less spines [6].

Clarias sp. (Garmout) which is investigated in this study is capable of existing in muddy ponds and haffirs throughout the dry season, so it is relatively available all the year round, and is more abundant after the rainy season. The specimen of Clarias sp. may exceed one hundred centimeters in length and may approximately weigh 5-7 pounds [7].

The African catfish, Clarias sp., is generally considered to be one of the most important tropical catfish species for aquaculture, and easily cultured in Nigeria and is of great economic interest. It has an almost Pan African distribution, ranging from the Nile to West Africa and from Algeria to South Africa [8]. Sudan as one of the developing tropical countries is not an exception in practicing totally sun drying of fish all over country. Sudanese people practice a simple fish drying process to produce a dried fish product locally known as "Kejeik". Kejeik is generally composed of large flat, long pieces of dry fish. Raw and processed fish product have normal bacterial flora from their environment in addition to the contaminant picked up during harvesting, handling and processing. Coliforms must be absent or present in low number, Salmonella sp and other enteric pathogenmust not occur since these organisms are not part of the normal flora of fish or their environment. The presence thus indicates

contamination. The presence of *Staphylococcus aureus* and *Salmonella* is also considered as hazard. The main objectives of this study were;

1. To determine the microbial load on fresh and dried *Clarias sp.*
2. To identify some contaminant bacteria

MATERIALS AND METHODS

Locality

This study was conducted at Department of Fisheries and Wildlife Science lab and Microbiology lab of the Faculty of Medical Laboratories, College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology.

Experimental Trial

18 fish of Garmut (African catfish) *Clarias sp.* samples were purchased from Elmourda Fish Market, Omdderman, Sudan and 9 samples were dried under sun-open air. The microbial investigation was done for fresh and dried fish samples.

Bacteriological Examination

Preparation of Sample
The sample were homogenized in sterile mortar and put in sterile tubes.

Preparation of Serial Dilutions

Separate sterile pipettes were used; decimal dilution of 10^{-2}, 10^{-3}, and 10^{-4}, 10^{-5} and others were prepared, and sample was homogenized by transferring 1 ml of previous dilutions to 9 ml of diluents. Samples foam avoided all dilution were sacked 25 times within 7 seconds. 1ml of each dilution was pipetted into separate duplicate, appropriately marked Petri dishes. Two plates were inoculated per dilution 15-20 ml plate count agar were add (after cooled to 45 ± 1 C°) to each plate within 15 min. of original dilution.

Test for Total Viable Count (TVC)
Twenty to three hundreds colonies were counted. Two plates were inoculated per dilution. The total colony count per milliliter was calculated by multiplication of the number of colonies counted by dilution level.

Culture Methods

I.   Primary Isolation
Isolates were plated out on blood agar supplemented plates and were incubated at 37 °C for 24 hours depending on the appearance of bacterial growth on the surface of the media.

Testing of Motility of Isolated Bacteria

The motility of isolate was tested using Graigie 's technique. Small inoculums of the culture was introduced inside a central tube containing semi solid agar place in a test tube

using straight wire. The test tubes incubated at 37 ˚C for 24 hours to 7 days. The tubes were examined for migration the bacteria outside the Graigie's tube.

Biochemical Tests

a)   Catalase Test

According to **[9]** a drop of hydrogen peroxide was placed on a cleaned microscopic slide. Using sterile glass rod small part of isolate colony was taken and emulsified in the hydrogen peroxide drop. The production gas bubbles were considered a positive reaction.

b)   Urease Test

Urea test medium of Christensen was inoculated with the isolate under test, incubated at 37 ˚C, and examined for up to seven days. Urea hydrolysis was indicated by the change of medium to pink colour.

c)   Citrate Utilization Test

The test was performed according to the method described by **[9]**. A single streak was done over the surface of slop of Simmon's citrate medium, incubated at 37°C, and examined daily up to seven days for growth and change in colour to blue that indicated citrate utilization.

d)   Kligler Iron Agar (KIA) Test

For the differentiation and identification of Enterobacteriaceae based upon sugar fermentation and hydrogen sulfide production. Sugar fermentation is indicated by the medium turning yellow. H_2S production results in the medium turning black.

Statistical Analysis

The obtained results were analyzed statistically using SPSS version 10, using paired sample & test.

RESULTS

The result obtained shows that the bacterial count in fresh fish is $7.6 \times 10^5 \pm 4.7 \times 10^5$ while in dry fish is $5.6 \times 10^5 \pm 3 \times 10^5$. Also the study revealed that there is highly significant difference in total bacterial count (P <0.001) between fresh and dried fish as show in **Table 1**. For the investigation of contaminant bacteria the results of this study revealed that the bacteria isolated from fresh sample was *Salmonella pratyphi* A and was not detected after fish was dried, while the other bacterial contaminants *E. coli* and *Staphlycoccus aureus* were not present in fresh or dried forms as shown in **Table 2, 3 & 4**.

Table 1: The Total Bacterial Count for *Clarias sp.* Samples

TREATMENT	TOTAL COUNT MEAN ± S.D (COF/G)
FRESH	$7.6 \pm 4.7 \times 10^5$
DRY	$5.6 \pm 3 \times 10^5$
SIGNIFICANT	**

Table 2: The Chemical Test for Microbial Identification

ORGANISM	GRAM REACTION	SHAPE	MOTILE	L.F	INDOLE	H_2S	UREA	CITRATE
Salmonella paratyphi A	-ve	Rod	+ve	-ve	-ve	-ve	-ve	-ve
E. coli	-ve	Rod	+ve	-ve	-ve	-	-	-

Table 3: Chemical Test for Identification of *Staphylococcus aureus*

ORGANISM	GRAM REACTION	SHAPE	CATALASE	COAGULASE	DNASE	M.F	L.F
Staphylococcus aureus	+ve	Sphere	+ve	-ve	-	-	-

Key: L.F=lactose fermentation; H_2S=Hydrogen sulfide; M.F=Mannitol fermentation

Table 4: Taxonomic Bacteria of *Clarias sp.*

SAMPLE	*Staphylococcus aureus*	*Salmonella paratyphi A*	*E. coli*
Fresh	-ve	+ve	-ve
Dried	-ve	-ve	-ve

DISCUSSION

This study was conducted to determine the microbial load in fresh and dried *Clarias sp.* and to identify some contaminants bacteria. The findings of this research have been presented in **Table 1, 2, 3 & 4**.

Table 1 shows the total count of bacteria for *Clarias sp.* in fresh and drying forms. The total number of bacterial count for fresh (*Clarias sp.)* was 7.6 x $10^5 \pm$ 4.7 x 10^5 and after drying the fish it was 5.6 x105 ±3 x l05. This number is within the accepted limit mentioned by SSMO (Sudanese Standards and Metrology Organization) and this result

agree with [10] who reported a range of 10^2-10^7 c/m/g for the fish meal, this result agree with the finding of [11] who reported a very range of numbers of microorganisms that are found on all outer surfaces, while were slightly more than the range of 3.7 x 10^5 for *Clarias lazera* reported by [12].

The bacterial load of dried fish decreased and this was due to removal of water level below that needed for microbial growth and enzymes activity [13]. [14] reported that bacterial flora on freshly caught fish depends on environment rather than fish species and this reflects the wide range of bacterial count encountered in this study.

Table 2 shows that the *Staphylococcus aureus* and *E. coli* test were negative for all samples, while *Salmonella paratyphi* A. Test was positive for fresh samples and negative for dried samples. These results disagree with [15] and [16] about *E. coli* and *Salmonella sp.* presence.

Also this result agrees with [17] who found that it is moulds rather than bacteria that cause spoilage during the preparation of dehydrated fish. Also drying process remove enough moisture from fish to a limit that greatly decreases these destructive effect.

Salmonella paratyphi A which is considered contaminant bacteria, was countered in this study. This may be due to lack of hygienic and sanitary measures [18].

Differences in the taxonomic composition of microflora of fish are due to regulation by local ecological and physiological condition. Each area including amount and type of different available nutrients pH and nature of adhesion factors for each bacterial groups in the epithelial cells or mucus cell membrane [19].

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