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Original Article

Effect of Fermentation Process on the Levels of Bacteria in Terkin (*Altests Dentex*)

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ABSTRACT

The aim of this study to determine the levels of microorganisms in Kawara fish (*Alestes dentex)* fermented by adding salt for a period of time (15 days) and to identify certain contaminant bacteria. This study was conducted in Sudan University of Science and Technology, College of Medical Laboratories. The samples were collected from El-murda Fish Market after that were divided into two groups the first group was transported to Sudan University of Science and Technology, College of Medical Laboratories, Laboratory of microbiology to determine the bacterial load. And the second group was treated by the fermentation process for (15days). Then the samples was tested microbiologically, the results were $3.6x10^{6} \pm 2.3x10^{7}$ CFU/g in fresh samples and $2.1x10^{6} \pm 1.1 \times 10^{6}$ CFU/g in fermented samples (Terkin), from results the data showed significant decrease ($P<0.04$) in the levels of microorganisms in fermented samples. Also from results only *Staphylococcus aureus* were isolated from Terkin samples while *E-coli* and *Salmonella* spp. were not recovered from the Terkin after 15 days of fermentation.

KEY WORDS: Fermentation, Bacterial Load, Contaminant Bacteria, *Alestes dentex*

INTRODUCTION

Fish is an important food, which contributed about one hundred million tones as total world production for 1989, of which fifty five percent (55%) was championed by the developing world (FAO, 1995). Fish in the Sudan have been major source of protein and energy for many communities, especially among the Nilotic tribes. Nuer tribe may become entirely dependent on fish (Jackson, 1923).

In the Sudan, nearly 70% of the total fish landing is cured either by salting, fermentation or sun-drying. Very little of the local fish supply is smoked, except in Southern Sudan where smoked and very dry fermented fish products are very popular among the local community (FAO, 1992a).

There are four major fermented fish products in Sudan (Fessiekh, Kejiek, Mindeshi, and Terkin).The fifth, Batarikh, is prepared in the homes of a few families of relatively recent Egyptian origin. The product is made by fermenting fish roe or milt and is better known in Egypt than in the Sudan (Dirar, 1992).

Traditional methods of fish fermentation are three main techniques that have clearly emerged as methods commonly practiced in many African countries; these are fermentation with salting and drying, fermentation and drying without salting and fermentation with salting but without drying (FAO, 1992a).

Terkin is related to sauces such as Nouc-mam, Nam-pla and Budu as well as to the pastes such Bagoong, pra-hoc and Shikara. The area famous for its production and consumption is centered on Dongla, the ancient town of upper Nubia. The region has a long tradition in fish products and Terkin seems to carry a tag of antiquity (Besyuni, 1979). Historical background of Terkin the word Meluha comes from Arabic word Milh meaning salt, so that the word Meluha was originally memluha (salted), probably. This is an example of where the Arabic name for an African food is still struggling to replace the original name. Eventually, the Arabic name Meluha will replace the Africa name, Terkin, judging by what has happened to many other foods of the Sudan.

The major consumption area for Terkin is Nubia. Today the primary production area is the White Nile, particularly at the centers of Jabel Awlia and Kosti town. It is believed that the Kunuz tribe (of Arabian origin) of the border between Egypt and the Sudan, introduced the art of Terkin or Meluha making from Dongola into Upper Egypt and later, as they migrated south, into the White Nile area (Fisheries Administration, 1986).

This research tend to determine the effect of fermentation on the levels of microorganisms in (Terkin) and to identify some kinds of microorganisms that may be found in the Terkin product.

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MATERIALS AND METHODS

Collection of samples

Fresh fish samples: Fresh Kowara (*Altests dentex*) a total of 18 samples of *Altests dentex* were collected from El-Murda fish market, then taken in iced container and transported to the Sudan University of Science and Technology, College of Medical Laboratories for microbiological analysis.

Fermented fish samples: Fermented fish samples were prepared by washing fish and removing the scales and viscera and washing again, then weighting to determine percentage of salt, after that the fish was heated at low temperature, then salt was added. The mixture was fermented for a period of 15days. In that stage the mixture was stirred once a day to break the bones, until the product became homogeneous after fifteen days, after that the samples were microbiologically tested.

Bacteriological examination

Preparation of sample: The sample were homogenized in sterile morter and put in sterile tubes.

Preparation of serial dilutions: Separate sterile pipettes were used, decimal dilution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and others were prepared, and sample was homogenized by transferring 1 ml from previous dilutions to 9 ml of diluents. Samples foam avoided, all dilutions were shacked 25 times within 7 seconds. 2 ml of each dilution was pipetted into two Petri dishes, appropriately marked Petri dishes. Two plates were inoculated per dilution 15-20 ml plate count agar were add (after cooled to 45±1C°) to each plate within 15 min. of original dilution. 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: The prepared samples were streaked on blood agar and Mac-Conky agar and incubated at 37C° for 24 hours. A part of a typical and well isolated colony was picked by wire loop and streaked on the surface of afresh plate of the solid medium. All cultures on solid media were examined with naked eye for growth, colonial morphology and changes in medium. The broth cultures were examined for turbidity, color changes, formation of sediments and accumulation of gas.

Purification of culture was made by sub-culturing a part typical well isolated colony on nutrient agar. This process was repeated twice. The resulting growth was checked for purity by examination of smears stained by gram methods. The purified isolated bacteria were identified according to criteria outlined by Barrow and Feltham (1993).

RESULTS

Bacterial load

Result of quantitative estimation of aerobic heterophilic bacteria in fresh Kawara fish (*Altests dentex*) are given in Tables 1 and 2. During the period of study the total viable count of each sample ranged from 4 x 10^5 to 5.8 x 10^6 cells/gram in fresh Kawara (*Altests dentex*) and 2.1x 10^6 to 3.3 x 10^6 cells/gram in Terkin. Each count was the mean viable colonies that appeared in duplicate agar plate made per individual sample. Generally fresh Kowara had high load of bacteria than Terkin in all sample tested.

Taxonomic identification of certain bacterial isolates

Bacteria isolates recovered were identified according to Barrow and Feltham (1993). Only *Staphylococcus aureus* were isolated from fresh kawara and Terkin, while *E-coli* and *Salmonella* ssp. were not recovered from fresh kawara (*Altests dentex*) and Terkin after 15 days of fermentation. The results of bacterial identification was shown in Table 5.

Table 1. The total count ± Standard deviation of bacterial load (CFU/g) on fresh and fermented meat made from *(Alestes dentex)* fish

Sample	Total count ± SD	Range
Fresh Kawara	$3.6\times10^6 \pm 2.3\times10^6$	4×10^5–5.8×10^6
Terkin sample	$2.1\times10^6 \pm 1.1\times10^5$	2.1×10^6–3.3×10^6

CFU/g: colony forming unit\gram

Table 2. Identification of Bacteria

Tests	*Staphylococcus aureus*	*Salmonella spp.*	*Escherichia coli*
Shape	Sphere	-	-
Aerobic G	+	-	-
Catalase	+	-	-
Oxidase	-	-	-
Glucose	+	-	-
O.F	F	-	-
Coagulase	+		

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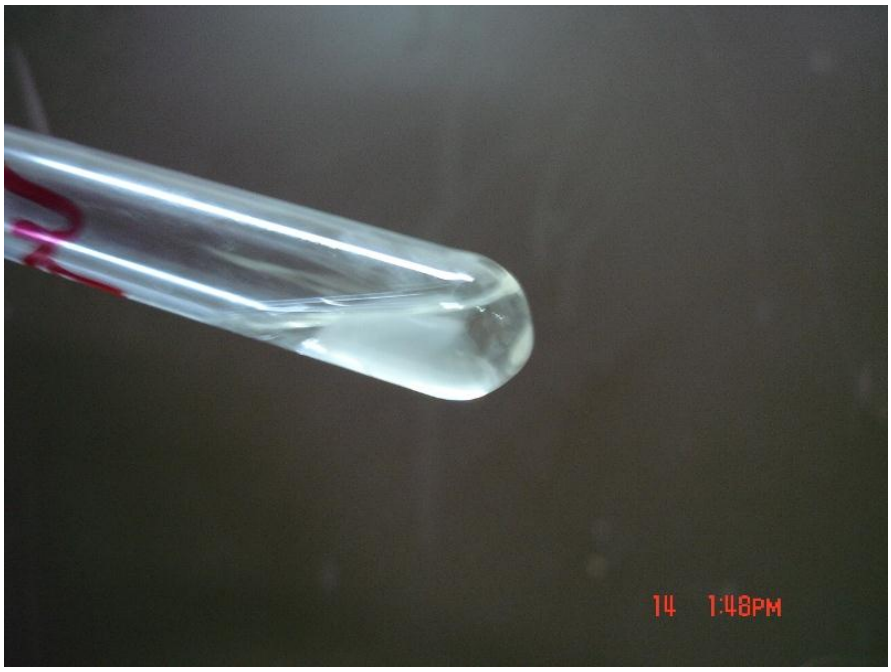

Figure 1. Shows Coagulase test for staphylococcus aureus

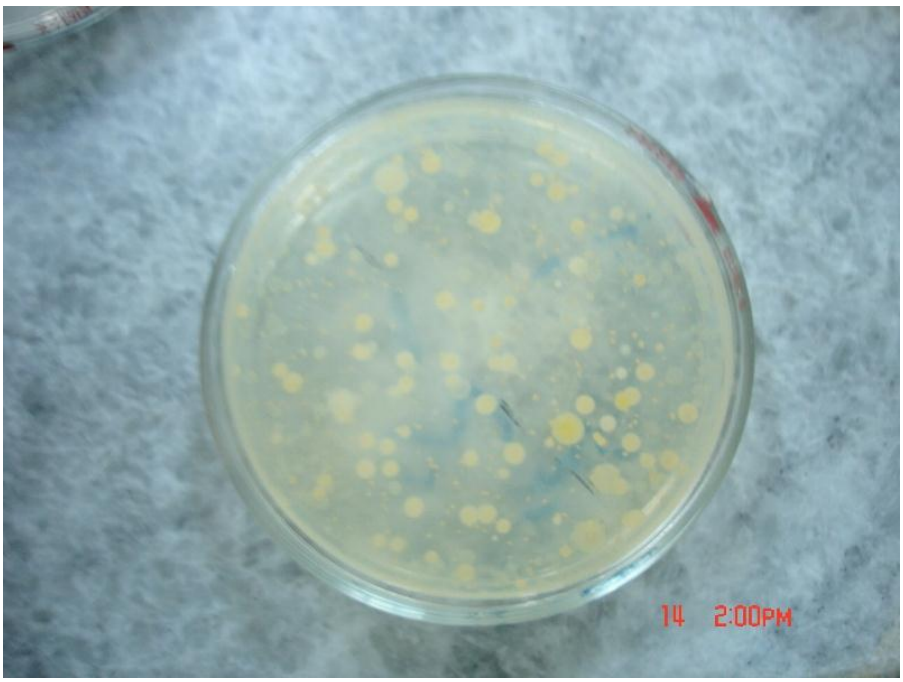

Figure 2. Shows growth of bacteria from samples

DISCSSTION

Micro-organisms are important causes for spoilage because they break down the food into a form that they can utilize. Therefore food quality decrease and spoilage start at this stage and estimation and quality of food product relies on quantification of total number of micro-organisms. In this study the total number of bacterial count for fresh Kawara fish (*Altests dentex*) was ($3.6x10^{6} \pm 2.3\times10^{6}$ c.f.u/gram) of fish meat, and this number was in the accepted limit mentioned by SSMO (Sudanese Standards and Metrology Organization, SDS 357) which was $5\times10^{5} – 10^{6}$ C.F.U/gm for fresh fish products.

From the result bacterial count showed significant ($P<0.04$) decrease in the total count values between fresh Kawara fish (*Altests dentex*) and Terkin. The highest value ($3.6x10^{6} \pm 2.3\times10^{6}$ c.f.u/gram) was reported in fresh Kawara fish, while the lowest value ($2.1x10^{6} \pm 1.1\times10^{5}$ c.f.u/gram) was reported in terkin (15days). This result is in agreement with the findings of El-Tom (1989) and Abu Griddeire (2001) who reported that the count of microorganism increased rapidly during first fermentation days and began to decrease later, and also in agreement with Dirar (1992) who reported that the total viable count of microorganism in whole fresh Kawara (*Altests dentex*) before salting was about 42,000,000 cell/gram. The gills of the fish had account of 530,000,000, the viscera 440,000,000 cells/gram, and the mussels about 2,000,000 cell/gram. Concerning the salt tolerant bacterial count the whole Kawara fish (*Altests dentex*) contained 6100,

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the gills 270,000,000, the mussels 15,000 and viscera 550 cells/gram. Also this result is in agreement with study of Knochel and Huss (1984) on the microbiology of barrel salted herring which recorded that both aerobic and anaerobic viable count in media containing 15 percent chloride were low. The same negative results for microbiological counts were also found in other studies (Frangose et al., 2010 and Fuselli et al., 2003).

Also Shenwan (1977) report that the bacterial flora on freshly caught fish depend on environment rather than fish species, and this reflects the wide range of bacterial count. Also this result agrees with finding of Liston (1980) who mentioned that the normal range was 10^2-10^7.

From result only *Staphylococcus aureus* were isolated from Terkin while *E.coli* spp. and *Slmonella* spp. were not recovered from the Terkin after (15 days). This result is in agreement with ICMSF,(1980) which reported that *Staphylococcus aureus* can survive for many weeks in salted fish, and also differ from ICMSF,(1980) in case of *E.coli* which mentioned the *Escherichia coli* can survive for many weeks in salted fish.

Also our result is in agreement with the study conducted by Nerquaye-Tetteh et al., (1978) who isolated various micro-organisms, no *Salmonella* spp. were isolated from samples of fermented fishery products. The absence of *Salmonella* spp. from fermented fish products could be attributed to the high salt level and low water activity of the products.

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