

Isolation and Identification of Staphylococci Species from Fermented Salt Fish (Fassiekh)

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Abstract: Fassiekh is one of the fermented fish produced in different parts of Sudan, especially in coastal cities. It is made from the two common Nile fish, locally named Kawara (*Alestes spp.*) and Kass (*Hydrocyonus spp.*) which was most preferred by Sudanese consumers. The aim of this study was to isolate and identify the Staphylococcus species present in the salted fassiekh produced in Ed Dueim city. Three types of samples (Dry salted fassiekh, Paste fassiekh and Wet salted fassiekh) were collected from different retailers in sterile polyethylene bags and stored in ice containers. The laboratory analysis began immediately after arrival using pour plate methods and biochemical tests to evaluate total viable bacterial load and *Staphylococcus spp.* (*Staph. spp.*) existence in fassiekh. Total viable counts were ranged from (4.0×10^3 - 1.8×10^6 cfu/g); Staphylococci counts (1.1×10^3 - 1.9×10^4 cfu/g). Seven isolates belonging to five species were isolated and identified: *Staph. saccharolyticus* (43.0%) predominate in all samples, and then followed by *Staph. epidermidis*, *Staph. caprac*, *Staph. carnosus* and *Staph. schleiferi* with the frequency (14.3%) respectively. The results from this study revealed that the unhygienic practices of fassiekh making, and the higher number of bacterial load and staphylococci count may pose hazards to human health.

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1. Introduction

Fermentation is the traditional methods used in Africa countries in different ways according to their indigenous cultures (Oyewole, 1997, Caplice and Fitzgerald, 1999, Euziclei, *et al.*, 2007). It is well known that fermented food characterized with high nutritive value, edible, safe, easy digestible and a short cooking time (Padonou *et al.*, 2009). kass (*Hydrocyonus spp.*) is one of the Nile fish available along the White Nile river, Sudan. The anglers are hunting these fishes and processed to produce "Fassiekh" (wet salted fermented fish) using traditional methods based upon their experiences. The fermented fassiekh has a strong odor and normally produced in the houses or in small-scale sectors by covering fish with the salt in alternate layers for up to 7 days or less depending on temperature, then transferred to fermenter with additional salt and left to ferment up to 10 days (Dirar, 1993, Anihouvi *et al.*, 2012). It is consumed after cooking together with additives such as peanut paste and some spices, which make it more acceptable and delicious. According to Osman *et al.*, (2012) the process of fassiekh making has about a century old, introduced to the Sudan from Egypt during the Turko-Egyptian rule (1821-1885), and then transferred and established tradition within a family or through non-formal training (Suliman and Khamis, 2011). However, El Hag *et al.* (2012) reported that the prevalence of traditional preservation methods employed throughout Sudan

are defective and need efforts pertaining to their improvement and development.

Ed Dueim town is one of the famous market's cities of fish and fish products in Sudan, located on the west of the White Nile River (200km South of Khartoum City). It is producing different forms of fassiekh such as paste fassiekh (soft in texture), dry salted and wet salted fassiekh through small-scale producer who are unauthorized. Therefore, the objective of the present study was to examine the microbial load and staphylococci species present in salted fassiekh in order to evaluate the hygienic practices of fassiekh making.

2. Materials and Methods

2.1. Sample collection:

Fassiekh samples (Dry salted, Paste and Wet salted) were collected from different retailers of Ed Dueim markets, White Nile State, Sudan. These samples were placed in ice containers and transferred immediately to the microbiological lab, Faculty of agriculture, University of Bakht Alruda, where analyses were carried out.

2.2. Microbiological Analysis

2.2.1. Total bacterial counts

Appropriate serial dilution was made by using a desired amount of samples (30g) and transferred to a sterile bottle containing 270 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^{-2} dilution; then further dilutions were made in a similar manner. Total viable count and staphylococci count were enumerated by pouring plate method using Plate Count Agar and Mannitol Salt Agar (MSA), respectively as described in Harrigan (1998).

2.2.2. Isolation and purification of *Staphylococcus spp.*

Nutrient Agar, Blood agar medium and Glucose phosphate medium were used as general and enriched media, while a Mannitol salt agar was used as a selective and differential characteristic medium. All media were prepared according to the manufacturer's specification and sterilized at 121°C 1 bar for 15 min. More representative staphylococcus colonies were picked from selective media (MSA) and subjected to a gram staining and catalase test. Purification was done by several sub-culturing on corresponding media. The pure cultures were inoculated on nutrient agar slant medium and incubated at 37°C for 24-4 hrs, and then stored at 4°C in refrigerator.

2.2.3. Identification of staphylococci isolates

Pure colonies of staphylococci isolates were identified based on Coagulase test according to Prof. Elsanousi scheme for identification of staphylococci species as well as biochemical tests such as Urea test, Voges-Proskauer (VP) test and Sugar fermentation as described in (Harrigan, 1998) and (Barrow and Gelthan, 1993).

3. Results

Twenty-one samples of salted fassiekh comprised: Dry salted fassiekh (7), Paste fassiekh (8) and Wet salted fassiekh (6) were collected from different retailers in Ed Dueim market. These samples were examined for the total viable bacterial count and staph. count. The total viable counts of bacteria were in the range of (2.2×10^4 - 3.1×10^4), (3.1×10^4 - 1.8×10^6); (4.0×10^3 - 1.6×10^4) and Staphylococci count ranged between (1.1×10^2 - 2.4×10^3), (2.1×10^3 - 1.9×10^4); (4.2×10^2 - 4.0×10^3), respectively as shown in Table 1. In this study seven staphylococci isolate belonging to five *Staphylococcus spp.* were isolated. These isolates were appearing at different percentage in different types of fassiekh's examined as shown in Table 2.

Table 1. Total viable count (TVC) and Staphylococci count (Staph. count) of Fassiekh samples collected from Ed Dueim retailers

Samples	No. of samples	TVC cfu/g	Staph. count cfu/g
A	7	2.2×10^4 - 3.1×10^4	1.1×10^2 - 2.4×10^3
B	8	3.1×10^4 - 1.8×10^6	2.1×10^3 - 1.9×10^4
C	6	4.0×10^3 - 1.6×10^4	4.2×10^2 - 4.0×10^3

A \equiv Dry salted fassiekh, B \equiv Paste fassiekh, C \equiv Wet salted fassiekh

Table 2. Percentage of *Staphylococcus spp.* isolated from fassiekh samples

<i>Staph. spp.</i>	Total No. of isolates	Individual% of Staph. spp. to the total No. of isolates	Percentage (%)		
			A	B	C
<i>Stah.saccharolyticus</i>	3	42.8	33.30	33.30	33.30
<i>Staph.epidermidis</i>	1	14.3	100.0	00.00	00.00
<i>Staph.caprac</i>	1	14.3	00.00	100.0	00.00
<i>Staph.carnosus</i>	1	14.3	00.00	100.0	00.00
<i>Staph.schleiferi</i>	1	14.3	00.00	100.0	00.00

A \equiv Dry salted fassiekh, B \equiv Paste fassiekh, C \equiv Wet salted fassiekh

In the present study, the staphylococci isolates were divided into two groups according to the coagulase test, as shown in Table 3. The results shown all staphylococci isolates were coagulase-negative, and there was no coagulase-positive isolate. The prevalence and the rate of Staphylococci isolates to fassiekh samples were shown in Table 4.

Table 3. Coagulase-positive and coagulase-negative *Staphylococcus* spp. isolated from Fassiekh

Samples	Coagulase- negative <i>Staph. spp.</i>	Coagulase- positive <i>Staph. spp.</i>
A	<i>Staph.epidermidis</i> <i>Site. saccharolyticus</i>	Nis
B	<i>Stah.saccharolyticus</i> <i>Staph.schleiferi</i> <i>Staph.carnosus</i> <i>Staph.caprac</i>	Nis
C	<i>Staph.saccharolyticus</i>	Nis

Nis \equiv No isolate A \equiv Dry salted fassiekh, B \equiv Paste fassiekh, C \equiv Wet salted fassiekh

Table 4. Prevalence and rate of staphylococci isolates to Fassiekh types (A.B and C)

Sample	No.(%)	<i>Staph.saccharolyticus</i>	<i>Staph.caprac</i>	<i>Staph.schleiferi</i>	<i>Staph.epidermidis</i>	<i>Staph.carnosus</i>
A	2(29)	+	-	-	+	-
B	4(57)	+	+	+	-	+
C	1(14)	+	-	-	-	-

A \equiv Dry salted fassiekh, B \equiv Paste fassiekh, C \equiv Wet salted fassiekh

4. Discussion

Data presented in Table 1 shows the microbiological load of the different fassiekh samples. The results of bacterial count and Staphylococci count were recorded higher counts. The viable bacterial count ranged from 2.1×10^3 - 1.8×10^6 cfu/g. This high load of viable count could be attributed to the unhygienic practices during fassiekh making, including personal hygiene, handling, packing and packaging of fassiekh products and also contamination may occur from the surrounding environment. Data presented in Table 1 shows the microbiological load of the different fassiekh samples. The results of bacterial count and Staphylococci count were recorded higher counts. The viable bacterial count ranged from 2.1×10^3 - 1.8×10^6 cfu/g. This high load of viable count could be attributed to the unhygienic practices during fassiekh making, including personal hygiene, handling, packing and packaging of fassiekh products and contamination may occur from the surrounding environment. Similar results were obtained by other workers (Osman *et al.*, 2012, Logesh *et al.*, 2012, Abu-Hassan and Adam Sulieman, 2011) they were found the total viable count of salted fish within the range of (1.4×10^3 to 5.3×10^6 cfu/g) However, the maximum number in fassiekh up to 108 were reported by (Ezzeldeen *et al.*, 2011). The TVC in this study was in partially agreeing with findings observed by (Ahmed *et al.*, 2010 and El Hag *et al.*, 2012). Staphylococci count ranged from (1.2×10^2 to 5.6×10^3) obtained by Osman *et al.*, (2012) in fassiekh samples were lower than the value recorded in the present study. Ahmed *et al.*, (2010) studied

staphylococcus-micrococcus count in Salted Kass (*Hydrocynus forskalii*) fish during storage at ambient temperature ($37 \pm 1^\circ\text{C}$) they found the number least <100 cfu/g. The highest count of staphylococci reflects the poor hygiene of food handlers and poor manufacturing practices of fassiekh making. According to Sugumar *et al.*, (2004) unhygienic handling is one of the main factors contributing to poor quality of fish in the retails. Many workers reported that Staphylococcus spp. It was found in a large number of all over human skin and mucous membrane (Allen *et al.*, 1997; Lamb *et al.*, 1990; Duerden *et al.*, 1992). Varnam and Evans (1991) mentioned that *Staphylococcus spp.* can increase up to ($5 \log_{10}$ cfu/g) in food products prepared by hand under bad conditions. Vishwanath *et al.*, (1998) reported that staphylococci grow best in salt and low water activity-containing foods whereas other microorganisms are in lower numbers.

The study also revealed that the staphylococci isolated from fassiekh are belonged to five species Table 2. *Staph.saccharolyticus* (43.0%) was the most dominate species isolated and then followed by *Staph.epidermidis*, *Staph.caprac*, *Staph.carnosus* and *Staph.schleiferi* in the same rate of (14.3%) respectively. These isolates species were appearing at different frequencies in fassiekh samples, as individual it's observed that, the highest prevalence of staphylococci species was that of *Staph. saccharolyticus* (42.8%) Table 2. The obtained result was disagreed with El Hag *et al.*, (2012) who found that *Staphylococcus xylosus* species was the dominant bacteria isolated from

salted treatment Kawara fish (*Alestes spsalted*) during storage.

Table 3 shown that all staphylococci isolates in fassiekh are coagulase-negative and that is may be due to the poor handling of fassiekh between the sellers among the retails. Coagulase-negative staphylococci are normal flora associated with skin, and mucous membranes can be isolated from different sources (Duerden *et al.*, 1992, Mehta *et al.*, 2009, Kools and Bannerman, 1994). Results explained in Table 4 indicated that, out of 7(100%) isolates of *Staphylococcus spp.* 4(57%) isolates were obtained from paste fassiekh samples, meanwhile 2(29%) and 1(14%) isolates were obtained from wet salted fassiekh and dried fassiekh, respectively. This means the paste fassiekh samples were more contaminated than the other's samples and this is could be due to the more frequent contact of hands to the fish during preparation and processing. Moreover, the lack of cleaning of equipment, hands and packaging materials.

Conclusions

From the results, it may conclude that the paste fassiekh is more contaminated than the wet-salted than the dried salted fassiekh and therefore, the consumers should take more consideration to paste fassiekh available in the retails, due to the higher count staphylococci species which are considered as food poisoning organism.

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