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# The Effect of Type of Fish and the Levels of Salt on Quality Properties of Fessiekh during Storage Period

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# Abstract

Chemical, microbiological and sensory properties of fresh fish and final products of Fessiekh were investigated. Three types of fish (Kass, Kawara and Debs), were salted with 20, 25 and 30% salt, stored for six months and cooked. The chemical analysis revealed that, the contents of moisture, protein, fat, ash and salt were 74.10-76.50%, 15.90-18.90%, 3.10-4.60%, 1.32-1.51% and 0.29-0.37% in fresh fish, while in salted fish were 34.73-38.84%, 29.86-33.01, 17.36-23.03%, 5.22-6.37% and 4.35-4.58% respectively. The pH values of the three samples had recorded values that are around neutrality. Types of fish, level of salt and storage have significantly ( $P \leq 0.05$ ) affected the chemical properties and acceptability of cooked fessiekh. Microbiological examination revealed that, the total viable count and aerobic halophilic bacterial count of fresh fish ranged between 2.1x107 to 2.2x107 (cfu) and 8.3×10<sup>2</sup> to 9.2×10<sup>2</sup> (cfu), while of salted fish were  $3.7 \times 10^4$  to  $4.1 \times 10^4$  (cfu) and  $4.0 \times 10^2$  to  $4.5 \times 10^2$  (cfu) respectively. Other bacterial groups such as sporeforming bacteria, anaerobes and coliform bacteria were also detected. Moulds and yeast were absent during salting of fish. The initial microbial load increased in the first day, arrived at a maximum, and then decreased during storage. Regarding coliform bacteria, the count after an initial increase, decrease to nil in all treatments. The level of salt affected the bacterial viable count during fessiekh storage. The dominant microfloras of fessiekh were found to be Bacillus species, staphylococcus species and Micrococcus species. Sensory evaluation of fish and cooked fessiekh revealed that the samples prepared with Kawara fish having, 25% level of salt and stored for three months were the best in quality.

Keywords: Type of fish, Salt, Storage Period, quality properties, Fessiekh

#### **INTRODUCTION:**

Fermented food products, especially those from fish, are important sources of essential protein; sometimes they are used as seasoning, and as the only source of animal protein to replace meat and fresh fish, particularly in South-East Asia. Sudan being one of the developing nations is not an exception to the problem of malnutrition and malnourishment, although endowed with numerous rivers and Red sea coast. The fresh water fishery resources are distributed in an area of about 100.000 km<sup>2</sup>. The Red Sea represents the marine fisheries along a coastline extending for more than seven hundred kilometers.

In the Sudan, special types of traditional fermented fish products have long been made. Dirar, (1989) described the most significant fermented fish items, namely, "Fasiekh " (wet salted fish), which was introduced into Sudan from Egypt during the 19th century, "Tarkeen" (fermented fish sauce), which is a true Sudanese fermented food, "kejiek" (dried- fish), an African product; "Mindeshi" (fermented fish paste) possibly another true Sudanese food, and Batarikh(fermented roe fish), is a household fermentation, of Mediterranean origin. For the three latter products, it is difficult to say when or in which country they were started (Osman *et al.*, 2012).

Fasiekh is not a truly indigenous Sudanese food product. The technique of its making entered the Sudan during the Turko-Egyptian rule (1821-1885) but its production on large scale was only well established during the Anglo-Egyption condominium rule (1898-1956). Therefore, it is acceptable to assume that fasiekh production in the Sudan is about a century old. During this period, the Sudanese have brought about some changes in the preparation method. Nevertheless, both local consumption and export trade of the product are today almost completely monopolized by families of Egyption descent, particularly the ethnic group called Nagada. Other Sudanese dominate in the fishing and manufacturing stages of the business (Dirar, 1993).

The technique of fessiekh-making entered the Sudan during the Turkish- Egyptian rule 1821-1885, but its production on a large scale was only well established during the Anglo-Egyptian condominium rule 1898-1956. The bulk of the product is made from the fish locally named kawara (pebbly fish; *Alestes spp.*). The other kinds of fish are called kass (tiger fish; *hydrocynus spp.*), debs (Nile crab; *labeo niloticus*), shilbaye (buffer fish; *schilbe spp.*) and Ras-El Hagar (Nile prom fret; *petrocephalus bane*).

Whereas fermented foods produced in Europe, North America, Australia, and New Zealand usually depend on defined starter cultures, those made in Asia and Africa often rely on spontaneous fermentation. Likewise, in developing countries, fermented foods are not often commercially produced on

an industrial scale (Tamang *et al.*, 2020). Salting as a method of preserving fish has been used for centuries and in many places around the world such as Asia, Europe and Latin America. The simplicity of the salting process encounters in the low cost of production and the ease with which it coincides with other preservation methods, such as drying or smoking, the merit that has led to its popularity and extensive use. Neither modern industries nor improvement of classical rationale concerning fish processing have been established in the Sudan. Therefore, curing in a primeval way is still the principal method of fish processing, i.e. un-drying without salt, dry as well as wet, salting and fermenting are the common methods of fish curing (Osman *et al.*, 2012). The aim of this work is to: study the effect of types of fish (Kass, Kawara and Debs), level of salt (20, 25 and 30%) and storage on quality of Sudanese Fessiesh, and to evaluate the role of microorganisms on fermentation of fish to ensure a popular wholesome food, and shelf life of Sudanese Fessiekh.

# MATERIALS AND METHODS:

# Materials

**Sample collection:** The samples (5 kg) of fresh fish, namely, Kawara (*Alestes* Spp.), Kass (*Hydrocyonus* Spp.) and Debs (*Labio niloticus*) were obtained from fisherman immediately after landing from Jabel Awlia reservoir on White Nile (47 km. south of Khartoum), early in the morning. All samples were wrapped in sterile polyethylene bags, kept in an icebox and transported immediately to the laboratory, where chemical and microbiological analyses were immediately carried out.

Salt: The salt used in the processing of the Fessiekh (consisted of coarse particles, diameters up to 4 mm and average 1.1 mm), was obtained from Khartoum market.

**Polyethylene bags:** The polyethylene bags used for keeping after processing of the experimental fessiekh were obtained from Afra Mall, made by Gulf Pazarlama Company.

# Methods

**Processing:** The procedure used was dry salting (20, 25 and 30% w/w salt). This procedure was conduced by algning the fish in layers separated by coarse salt layers inside the 'Guffa' (indigenous container made from palm leaves). The pickle was allowed to drain away during the fermentation process, and wrapped in polyethylene bags. Each type of fish was treated separately.

**Cooking:** Fifty gram of each fish product (before cooking) was heated to 180°C for 10 min. they were further pretreated by adding 20 gram of tomato paste, 4-gram garle, 10-ml oil, and mixed carefully.

**Proximate analysis:** Moisture content, protein content, fat content, ash content, salt content and pH-values were determined using the method of AOAC (1990).

# The microbiological analysis

**Preparation of sample dilution**: A representative sample of 25 gm salted fish was minced through a meat mincer (meat grinder MK-G30NR- Japan), was weighed aseptically and then blended in sterile mixer both with 225ml sterile distilled water by using electric blender (Homogenizer MS-Japan). To get a dilution of 10<sup>-1</sup>, serial dilutions of 10<sup>-1</sup>-10<sup>-5</sup>, were prepared as described by Harrigan and McCance (1976).

#### **Microbial parameters**

**Detection of total bacterial count**: Total viable count for bacteria was carried out using the standard plates count method as described by Harrigan and McCance (1976). One ml from respective dilution was transferred aseptically into sterile Petri-dishes. To each dilution 10-15 ml of melted and cooled to 45°C plate count agar were added. The inoculums was mixed with media and allowed to solidify. The plates were then incubated at 37°C for 48 hours. A colony counter (Quebec Colony Counter and Hand Tally, Model (1994) - Germany) was used to count the viable bacteria.

**Detection of halophilic bacteria:** Appropriate dilutions of samples were plotted in nutrient agar plus 10% Nacl, and incubated at 37°C for 48 hours (Harrigan and McCance, 1976).

**Detection of aerobic spore-forming bacteria:** Ten milliliters of 10<sup>-1</sup>dilution of the sample was heated in a water bath at 80°C for 15 minutes as described by Harrigan and McCance (1976), then further dilutions were made, plated in nutrient agar and

**Detection of anaerobic bacteria**: Respective dilutions of the samples were plated in nutrient agar and incubated anaerobically at 37°C for 48 hours. All anaerobic incubations were carried out in jars equipped with a BBL (H<sub>2</sub>+CO<sub>2</sub>) generating gas pack systems as described by the manufacturer (Harrigan, 1998).

#### **Coliform bacteria**

**Presumptive coliform test**: Three tubes each containing nine ml of MacConkey broth (enrichment medium), fitted with Durham tubes, were inoculated with 1 ml from suitable dilutions  $(10^{-1}, 10^{-2} \text{ and } 10^{-3})$  of fish samples and incubated at 37°C for 48 hours. Growth and gas production after 24 and 28 hours were recorded. Gas production constituted a positive test (Harrigan and McCance, 1976).

**Confirmatory coliform test:** All fermentation tubes from the presumptive test showing gas within 48 hours at 37°C were utilized in the confirmation test. The medium used in this test was Brilliant Green Bile Broth (BGB). Each tube contained 10 ml of media fitted with Durham tubes. Presumptive test tubes were transformed to each BGB tubes, and then incubated at 37°C for 48 hours. Faecal coliforms were calculated as most probable number (MPN) via (MPN) tables (FAO, 1992).

**Isolation of** *Escherichia coli (EC)*: For further confirmation of faecal coliform in tubes giving positive reaction on *E. coli* media EC at 44.5 °C for 28 hours were streaked on Eosin Methylene Blue (EMB). Colonies with green metallic shine gave a positive test (Harrigan and McCance, 1976).

**Detection of Mould and yeast count:** From suitable sample dilution, was 0.1 ml surface plated on malt extract agar. Sterile bent glass rod was used to spread the culture, and incubated at 25°C for 48-72 hours as described by Harrigan and McCance (1976).

# **Organoleptic Testing**

The sensory evaluation of fish and cooked fessiekh were evaluated by scoring procedure described by Ihekoryne and Ngoddy (1985). Ten of the semi – trained staff from the section of food science and technology, faculty of agriculture Al-Zaeim Al-Azhary University, were asked to examine and evaluated the fessiekh samples.

**Statistical analysis:** Statistical analyses were done using the Statistical Analysis System (SAS, 1997).

# **RESULTS AND DISCUSSION**

#### **Chemical composition**

# Effect of type of fish on chemical composition of Sudanese fish Fesseikh

Table 1 shows the effect of type of fish on chemical composition of raw fish, namely, Kass (sample, A), Kawara (sample, B) and Debs sample, C) were used.

#### **Moisture content**

The type of fish seemed to have a significant effect ( $P \le 0.05$ ) on moisture content of fish fesseikh. Sample (C) gave the highest moisture content (38.84%) and sample (B) the lowest (34.73%) with the sample (A) in an intermediate position (36.81%).

These results fall in the range 10.72 to 45.5% of moisture content given by Agab and Bashier (1987) for dry salted fesseikh. Sarojnalini and Vishwanath (1994) found the moisture content of the two fermented fish products 'Hentak' and 'Ngari' 36.30 and 36.03 respectively. Omer (1984) reported the moisture content from 51.79% in salted *Hydrocyonus* spp.

Ahmed (2006) stated that the moisture content ranged from 81.92 to 34.5% in salted fish of Kass (*Hydrocyonus* spp) and Shelbaya (*Schilbe* spp). El-Tom (1998) found that the moisture content ranged from 30 to 35 % in salted fish of Kawara (*Alestes* spp.), Kass (*Hydrocyonus* spp) and Shelba (*Schilbe* spp).

Moledina *et al.*, (2008) reported that the moisture content of final product of salted fish 36 to 38%. It seems that, the moisture contents were highly reduced in fessiekh samples than fresh state. This can be attributed to the plasmolysis occurring as an action of salt applied to the fish.

#### **Protein content**

The protein content of both sample (A) and sample (C) however, were similar ( $P \ge 0.05$ ) and significantly higher ( $P \le 0.05$ ) than sample (B).These results are in full agreement with those reported by Mohamed (1977) who found that the average protein content of 28.8 % (w/w) from *Hydrocyonus* spp. Omer (1984). Said that the protein content ranged from 18.12 to 28.52 (w/w). Sarojnalini and Vishwanath (1994) studied the protein content of fermented fish products 'Hentak' and 'Ngari' 33.33 and 38.38% respectively. Agab and Babiker (1987) found that for dry salted fish Fessiekh the protein content ranged from 18.5 to 23.5 (w/w). Moledina *et al.* (2008) reported that the protein content of final product of salted fish 30 to 32%.

Ahmed (2006) described that the protein content ranged from 19.57 to 16.58 % in salted fish of Kass (*Hydrocyonus* spp) and Shelbaya (*Schilbe* spp). El-Tom (1998) conducted that the protein content ranged from 32 to 34 % in fresh fish of Kawara (*Alestes* spp.). On comparing the protein content of fish before and after salting significant differences were observed, especially in the case of boned kass. The salt-fermented fish is good source of protein. It contains relatively high amount of moisture ranging from 44.8 to 53.46% with a mean of 47.96%, Results obtained in this study are in agreement with those reported for fermented cassava fish (Lanhouin) and tigger fish (Tirkin) (Anihouvi *et al.*, 2009, Mohammed, 2010), but lower than those reported for fermented fish

products. The protein content of Hout-kasef ranged from 23.44 to 29.56 with a mean value 25.71%. Similar results were reported for Momone, lanhouin and Tirkin fermented fish products (Abbey *et al.*, 1994, Anihouvi *et al.*, 2009, Mohammed, 2010).

#### Fat content

The type of fish seemed to have a significant effect ( $P \le 0.05$ ) on moisture content of fish fesseikh. Sample (C) gave the highest fat content (23.03%) and samples (A) the lowest (17.36%) with the sample (B) in an intermediate position (19.54%).

Item	Type of fish					
	Α	В	С			
Moisture content (%)	$36.81 \pm 0.07^{b}$	34.73±0.05°	$38.84{\pm}0.03^{a}$			
Protein content (%)	$29.87 \pm 0.03^{b}$	33.01±0.04ª	$29.86 \pm 0.04^{b}$			
Fats content (%)	17.36±0.08°	$19.54 \pm 0.02^{b}$	$23.03\pm0.05^{a}$			
Ash content (%)	$5.22 \pm 0.05^{b}$	$6.37 \pm 0.05^{a}$	$6.03 \pm 0.02^{ab}$			
Salt content (%)	$4.35 \pm 0.02^{b}$	4.58±0.03 <sup>a</sup>	4.38±0.01 <sup>b</sup>			
pH-value	$6.79 \pm 0.01^{b}$	$6.79 \pm 0.05^{b}$	$6.81 \pm 0.06^{a}$			

Table 1. Effect of type of fish on chemical\* feature of Sudanese fish Fessiekh

\*Mean $\pm$  SD bearing different superscript letters in each row differ significantly (P $\leq$ 0.05). A = Kass; B = Kawara; C = Debs

Moledina *et al.*, (2008) reported that the fat content of final product of salted fish ranged from 15 to 20%. Ahmed (2006) found that the Fat content ranged from 1.62 to 0.88% in salted fish of Kass (*Hydrocyonus* spp). El-Tom (1998) stated that the Fat content ranged from 20 to 22 % in salted fish Kawara (*Alestes* spp.).

The fat content varied between 5.46 and 9.82 with a mean of 7.42%. The fat content of salted fermented fish reported here was in agreement with that reported for the Fassiekh fermented fish product in Sudan (Osman *et al.*, 2012), but higher than that of Wadi Betok, a traditional fermented fish from South Kalimantan Indonesia (Petrus *et al.*, 2013).

#### Ash content

The type of fish seemed to have a significant effect ( $P \le 0.05$ ) on ash content of fish fesseikh. Sample (B) gave the highest ash content (6.37%) and sample (A) the lowest (5.22%) with the sample (C) in an intermediate position (6.03%).

Ahmed (2006) described that the Ash content ranged from 10.2 to 13.86 % in salted fish of Kass (*Hydrocyonus* spp) and Shelbaya (*Schilbe* spp). El-Tom (1998) reported that the Ash content ranged from 5 to 7 % in salted fish of Kawara, Kass and Shelba. Sarojnalini and Vishwanath (1994) found the Ash content of the two fermented fish products 'Hentak' and 'Ngari' were 5.49 and

11.43respecttively. Moledina *et al.*, (2008) stated that, the ash content of final product of salted fish ranged from 6 to 7.5%. The salt content in this study is higher than those reported for fermented fish products (Koffi-Nevry *et al.*, 2011 and Anihouvi *et al.*, 2009), but lower than that reported for Wadi Betok, a traditional fermented fish from South Kalimantan, Indonesia, (Petrus *et al.*, 2013).

# Salt content

The type of fish seemed to have a significant effect ( $P \le 0.05$ ) on salt content of fish fesseikh. Sample (B) gave the highest salt content (4.58%) and sample (A) the lowest (4.35%) with the sample (C) in an intermediate position (4.38%).Saisithi *et al.*, (1966) reported that the fish sauce had a salt of 27.9%. Gassem, (2019) stated that the salted-fermented fish also showed high level of total volatile basic nitrogen (78.86 mg/100 gm sample) and thiobarbutric acid number (32.32 mg malonaldehyde/kg) with a pH value of pH 6.3.

# pH-value

The pH-value of both sample (A) and sample (B) however, were similar ( $P \ge 0.05$ ) and significantly higher ( $P \le 0.05$ ) than sample (C).El-Tom (1989) reported that the pH of salted fish fesseikh specimen ranged from 6.69 to 6.81. Saisithi *et al.*, (1966) found that the fish sauce had a pH of 6.4.

Gassem, (2019) stated that the, pH values of the samples were (below 7) it ranging from 6.18 to 6.50 with a mean of 6.39. The pH value of the present study is similar to those reported for *Fesseikh* samples-a traditional fermented fish product in Sudan and *telesech*-a traditional fermented fish product in India (Agab and Shafie, 1989, El-tom, 1989, Roy *et al.*, 2014), but lower than reported by Momone and lanhouin (Anihouvi *et al.*, 2006, Anihouvi *et al.*, 2007). However, in Thailand the standard PH requirement for fermented fish known as Pedah siam is 6.0–6.4 with a pH 6.5 or higher being considered as indicative of poor quality (Anihouvi *et al.*, 2012).

# Microbiological feature

# Effect of type of fish on microbiological feature of Sudanese fish fesseikh

From the results presented in Table 2. it could be noticed that the microbial population of the three various fish common fessiekh –making varieties, namely, Kass (*Hydrocyonus* spp.) sample A, Kawara (*Alestes* spp.) sample B, and Debs (*Labeo niloticus*) sample C. Total viable count, Aerobic halophilic bacterial count and Sporeforming bacterial count, was higher in Kass (sample A) at  $4.1 \times 10^4$ ,  $4.5 \times 10^2$  and  $3.9 \times 10^2$  cfu, respectively. Anaerobic bacterial count and anaerobic halophilic bacterial count was higher in Debs (sample C) at

 $4.2x10^2$  and  $1.2x10^4$  cfu, Kawara (sample B) in an intermediate position. The coliforms bacterial, Mould and yeast counts recorded nil in all samples of Sudanese Fesseikh.

Majumdar *et al.*, (2008) described that the "Lona ilish" is a salt fermented fish product prepared exclusively from fatty Indian shad (*Tenualosa ilisha*). the microbial flora in the product was composed of only two species, tentatively identified as *Bacillus licheniformis* and *Micrococcus kristinae* (2.8x10<sup>3</sup>cfu).Because *Bacillus licheniformis* showed some atypical biochemical reactions

Table 2. Effect of type of fish on microbiological feature (cfu) of Sudanese Fesseikh

Item	Type of fish		
	Α	В	С
Total viable count	$4.1x10^{4}$	$3.9 x 10^4$	$3.7 x 10^4$
Aerobic halophilic bacterial count	$4.5 x 10^2$	$4.0 \mathrm{x} 10^2$	$4.2x10^{2}$
Anaerobic halophilic bacterial count	$9.5 x 10^3$	$9.9 \times 10^3$	$1.2 x 10^4$
Anaerobic bacterial count	$3.6 x 10^2$	$3.7 x 10^2$	$4.2x10^{2}$
Sporeforming bacterial count	$3.9x10^{2}$	$3.8 x 10^2$	$3.5 x 10^2$
Coliforms bacterial count	Nil	Nil	Nil
Mould and yeasts count	Nil	Nil	Nil

A = Kass; B = Kawara; C = Debs

# Effect of level of salt on microbiological feature of Sudanese Fish Fessiekh

As shown in Table 3. level of salt (20%) gave the highest counts of total viable count  $(3.9 \times 10^4 \text{cfu})$ , Aerobic halophilic bacterial count  $(4.5 \times 10^4 \text{cfu})$  and sporeforming bacterial count  $(3.8 \times 10^4 \text{ cfu})$ , while the level of salt (30%) gave the highest of anaerobic halophilic bacterial count and anaerobic bacterial count  $(1.1x10^4)$  and  $4.0x10^2$  cfu respectively). The level of salt (25%) in an intermediate position. Coliforms bacterial count, Mould and yeasts count recorded nil in all level of salt. Disenv et al., (1974) stated that the Storing salt in a dry place has been recommended as a means of reducing its bacterial load. Beddows (1985) reported no organism able to graw on patis 20% salt, after one month, but single strains of Bacillus pumilus, Micrococcus copoyenes, M. varians and Condia clausenii were isolated from 10% salt media. Avery (1952) mentioned that the initial bacterial population dropped rapidly as salt fermentation of "Patis and Pagoon" continued, until towards the end of the period. All types of *Clostridium botulinum* are inhibited by 10% to 12% salt and generally by a less than 4.5%. Staphylococcus aureus is inhibited by 15 to 20% salt (Shank, 1972; Okonkwo and Nwokolo, 1978).

# Effect of storage period on microbiological feature of Sudanese fish Fessiekh

As shown in Table 4. the microbial contents were changed during time of fermentation, there was a general increase in the first month and decreased in case of total viable count  $(2.2 \times 10^5$  to  $2.9 \times 10^2$  cfu), Aerobic halophilic bacterial count $(3.7 \times 10^2$  to  $2.6 \times 10^2$  cfu), anaerobic bacterial count  $(3.4 \times 10^2$  to  $1.7 \times 10^2$  cfu) and Sporeforming bacterial count $(3.5 \times 10^2$  to  $1.7 \times 10^2$  cfu). But Anaerobic halophilic bacterial count increase with storage in first three months  $(9.3 \times 10^2$  to  $4.9 \times 10^4$  cfu) and it was decrease or negligible in case of Sporeforming bacterial count. Coliforms bacterial count, Mould and yeasts count recorded nil in all storage period.

The microbial studies of salted-fermented fish revealed a total bacterial count ranging from 2.81 to  $4.72 \text{ Log}_{10} \text{ CFU/g}$ , yeast and mold counts ranging from 0.48 to  $3.14 \text{ Log}_{10} \text{ CFU/g}$ , total *staphylococci* count 2.71– $3.85 \text{ Log}_{10} \text{ CFU/g}$ , halophile bacteria count  $3.26-5.14 \text{ Log}_{10} \text{ CFU/g}$ , and coliforms count <1 Log<sub>10</sub> CFU/g (Gassem, 2019 and Kusmarwati *et al.*, 2021).

El-Tom (1989) reported that, the counts of coliform bacteria after initially increasing dropped to zero in all treatments before end of the assay time. Sayefudin *et al.*, (1991) found the total plate count of fermented fish peda (*Rastrelliger brachysoma*) increased from the first three days to  $2.9 \times 10^5$ cfu and then decreased until 15 days of fermentation.

Table 3. Effect of level of salt on microbiological feature (cfu) of Sudanese Fesseikh

Item	I	Level of salt (%)			
	20	25	30		
Total viable count	$3.9 x 10^4$	$3.5 x 10^4$	$3.7 x 10^4$		
Aerobic halophilic bacterial count	$4.5 x 10^{2}$	$4.3x10^{2}$	$4.0 x 10^2$		
Anaerobic halophilic bacterial count	$8.8 \times 10^{3}$	$7.7 x 10^3$	$1.1 x 10^4$		
Anaerobic bacterial count	$3.6 x 10^2$	$3.9 x 10^2$	$4.0 \mathrm{x} 10^2$		
Sporeforming bacterial count	$3.8 x 10^2$	$3.6 x 10^2$	$3.4x10^{2}$		
Coliforms bacterial count	Nil	Nil	Nil		
Mould and yeasts count	Nil	Nil	Nil		

Crisan and Sands (1975) stated that the microflore of four fermented fish sauces, from nam-pla, they isolated *Bacillus cereus* and a strain of *Bacillus Licheniformis* after 7 months of fermentation, but at the end of the fermentation period they isolated other strains of *Bacillus Licheniforims*. *Bacillus megaterium* and *Bacillus subtilis*; it is not- able that after one month only one strain of *Bacillus licheniformis* was detected. This suggests that microflore is not constant.

The biochemical and microbiological changes were followed at 15 day intervals during the fermentation period of 150 days. From the 45th day of

fermentation onwards, the microbial flora in the product was composed of only two species, tentatively identified as *Bacillus licheniformis* and *Micrococcus kristinae*. Because *Bacillus licheniformis* showed some atypical biochemical reactions, it was tentatively identified as *Bacillus licheniformis* var. (Majumdar et al., 2008).

Fish fermentation involves minimal bacterial conversion of carbohydrates to lactic acid but entails extensive tissue degradation by proteolytic and lipolytic enzymes derived from viscera and muscle tissues. Sauces have a predominantly salty taste and are derived from decanting or pressing fermented fish or shrimp after a 9-month to 1-year fermentation (Ricke *et al.*, 2012). Beddows (1985) found that the storage of pates in one month no organism able to grow on 20% salt, but single strains of *Bacillus pumilus, Micrococcus copoyenes, M. varians* and *Condia clausenii* were isolated from 10% salt medium.

Table 4. Effect of storage period on microbiological feature (cfu) of Sudanese
fish Fessiekh

Item	Storage period (months)						
	0	1	2	3	4	5	6
Total viable count	$2.2 x 10^{5}$	$3.9x10^{4}$	$3.6 x 10^3$	$3.1 x 10^3$	$7.4x10^{2}$	$3.6 x 10^2$	$2.9x10^{2}$
Aerobic halophilic bacterial count	$3.7 x 10^{2}$	$8.5 x 10^{2}$	$5.3x10^{2}$	$3.5 x 10^2$	$3.3x10^{2}$	$2.9x10^{2}$	$2.6 x 10^2$
Anaerobic halophilic bacterial count	$9.3 x 10^{2}$	8.4x10 <sup>3</sup>	9.1x10 <sup>3</sup>	4.9x104	$5.5 x 10^{2}$	$3.1 x 10^{2}$	$2.7 x 10^{2}$
Anaerobic bacterial count	$3.4x10^{2}$	$7.9x10^{2}$	$4.5 x 10^{2}$	$2.5 x 10^2$	$3.3x10^{2}$	$3.5 x 10^2$	$1.7 x 10^{2}$
Sporeforming bacterial count	$3.5 x 10^2$	8.2x10 <sup>2</sup>	$3.7 x 10^{2}$	$3.1x10^{2}$	$2.9x10^{2}$	$2.6 x 10^2$	$1.7 x 10^{2}$
Coliforms bacterial count	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mould and yeasts count	Nil	Nil	Nil	Nil	Nil	Nil	Nil

#### The rule of microorganisms on fermentation of fish Fessiekh

Table 5 shows the dominant bacterial genera isolated from salted fish. Kawara (Alestes spp.), Kass (Hydrocyonus spp.) and Debs (Labeo niloticus).at three treatments of level of salt (20, 25 and 30%) and storage in six months, were Bacillus species, Staphylococcus species and Micrococcus species. It seems that this group is more salt resistant than the others existing in the fish. El-Tom (1989) found that the dominant microflora of Fessiekh were Bacillus species, Staphylococcus species and Micrococcus species. Ahmed (2006) reported that the dominant microflora of Fessiekh was found to be Staphylococcus species, Micrococcus species, Pseudomonas species and Aerococcus species. The presence of Staphylococcus aureus in dried foods indicates contamination from the skin, mouth or nose of food handlers (FAO, 2000). Ijong et al., (1995) noticed the coliform bacterial were not detected in all samples of Indonesian traditional fermented fish sauce "bakasang", but

the total *Staphylococcus* count was high in all samples ranging from  $4.70 \times 10^3$  to  $5.59 \times 10^3$  cfu.

Staphylococcus Sp. and Lactobacillus Sp. were the predominant bacteria isolated from traditional bakasang samples. Tanasupawat *et al.*, (1992) reported that the new coagulates negative *Staphylococci* were isolated from fermented fish in Thailand. Han Ching *et al.*, (1994) conducted that the lactic acid bacteria play a considerable role in improving the organoleptic, hygienic and nutritional qualities of fish muscle during fermentation. Tanasupawat and Komagata (1998) isolated, forty-seven strains of homofermentative rod-shaped and five hetrofermentative sphere-shaped lactic acid bacteria from four kinks of fermented fish (pla-ra, pla-chom, kungchom and hoi-dong).

Beddows (1985) found that the storage of pates in one month no organism able to grow on 20% salt, but single strains of *Bacillus pumilus*, *Micrococcus copoyenes*, *M. varians* and *Condia clausenii* were isolated from 10% salt medium. Hamm and The presence of *Staphylococcus aureus* in dried foods indicates contamination from the skin, mouth or nose of food handlers (FAO, 2000). Ijong *et al.*, (1995) noticed the coliform bacterial were not detected in all samples of Indonesian traditional fermented fish sauce "bakasang", but the total *Staphylococcus* count was high in all samples ranging from  $4.70 \times 10^3$  to  $5.59 \times 10^3$  cfu.

Gassem, (2019) stated that, The major bacteria species isolated and identified from the salted fermented fish were Bacillus Subtilus, Bacillus mycoides, Bacillus licheniformis, Bacillus pumilus, Staphylococcus aureus Staphylococcus epidermidis, Staphylococcushominis, Staphylococcus xylosus, Staphylococcus saprophticus and Staphylococcus cahnii subsp cahnii. Staphylococcus Sp. and Lactobacillus Sp. were the predominant bacteria isolated from traditional bakasang samples.

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Tanasupawat *et al.*, (1998) isolated forty-seven strains of homofermentative rod-shaped and five hetrofermentative sphere-shaped lactic acid bacteria from four kinks of fermented fish (pla-ra, pla-chom, kungchom and hoi-dong) in Thailand. Al- Jedah *et al.*, (1999) found that, the fermented fish sauce popular in the Gulf States "Mehiawah" could post a health hazard to consumers. An extensive examination of home made and commercial samples were free from vegetative pathogens even offer storage for several month at 20-25°C; *Bacillus cereus* was identified, the inhibitory nature of the product was confirmed, when fresh "Mehiawah" was incubated

with salmonella typhi, staphylococcus aureus, vibrio parahaemolyticus and E. coli. during routine manufacture, that initial processing of the fish eliminated any serious contamination, while the combined effects of salt, acidity, spices and perhaps fatty acids from the fish oil, arrested the growth of any pathogens that might enter the product during bottling.

Organism code	Shape	Gram stain	Motility	Aerobiosis	Catalase	Oxidase	O/F test	Spores	Genus
I	Rod	+	-	Facultative anaerobic	+	•	F	+	Bacillus species
п	Rod	+	-	Facultative anaerobic	+	•	F	+	Bacillus species
ш	Cocci	+	-	Aerobic	+	-	O/-	-	Micrococcus species
IV	Cocci	+	-	Facultative anaerobic	+	•	F	-	Staphylococcus species

Table 5. The rule of microorganisms on fermentation of fish Fessiekh

F = Fermentative oxidative organism. O/- = Oxidative organism.

#### Organoleptic quality

# Effect of type of fish on organoleptic quality of Sudanese fish Fessiekh

Table 6 shows the effect of type of fish. Kass (sample A), Kawara (sample B) and Debs (sample C) on organoleptic quality (appearance, odour, texture and acceptability) of Sudanese fish Fessiekh. The organoleptic properties of fish fesseikh were found to be affected by the type of fish. Sample B significantly (P $\leq$  0.05) secured the best appearance (4.00), odour (3.82), texture (4.31) and over all acceptability (3.85), followed by sample A (3.82, 3.82, 3.62 and 3.80 respectively) and the worst appearance (3.55), odour (3.37), texture (3.97) and acceptability (3.55) were recorded by sample C.Van Veen (1965) found that the production of the well- know

ressiekii						
Item	Type of fish					
	Α	В	С			
Appearance	3.82±0.13 <sup>ab</sup>	$4.00{\pm}0.15^{a}$	$3.55 \pm 0.15^{b}$			
Odour	3.82±0.11 <sup>a</sup>	$3.82{\pm}0.12^{a}$	$3.37 \pm 0.14^{b}$			
Texture	$3.62 \pm 0.12^{b}$	4.31±0.11 <sup>a</sup>	$3.97 \pm 0.12^{ab}$			
Over all acceptability	$3.80\pm0.09^{a}$	$3.85 \pm 0.10^{a}$	$3.55 \pm 0.14^{b}$			

Table 6. Effect of type of fish on organoleptic quality\* of Sudanese fish Fessiekh

\*Mean±SD bearing different superscript letters in each row differ significantly  $(P \le 0.05)$ .A = Kass; B = Kawara; C = Debs

Whole salted fish, and Pedah Siam or Pedah Kemburg in South East Asia attributed the brown color to fat oxidation which also produces some of characteristic aroma, which includes methyl ketones. Skierdal *et al.*, (2001)

show that the production of fish sauce from wasted heavy salted cod is possible addition of herring, intestines and halophilic microbes accelerates the process and improves the yield, colour, and flavour and nutrition value of the sauce.

#### CONCLUSIONS

The study concluded the composition of fresh fish and salted fish were significantly different. Different levels of salt (20%, 25and30%) affected the chemical and microbial properties of the three species. Microbiological examination was carried out after salting of fish. The counts of the first month decreased due to the salting. The dominant microfloras of fessiekh were found to be *staphylococcus* species, *micrococcus* species, and *Bacillus* species. Fesseikh prepared using 25% level of salt was found to be more acceptable. Fesseikh obtained after three months storage was judged best.

#### REFERENCES

- 1. El-tom. A.M. (1989). Microbiology and Biochemistry of Fasseikh. M. Sc. Thesis. Faculty of Agriculture. University of Khartoum, Sudan
- 2. Abbey, L. D. l.; Hodari-Okae, M. and Osei-Yaw, A. (1994). Studies on traditional processing and quality of fermented fish momone. Aritisanal Fish Processing and Applied research Report. Ed. Food Research Institute, Accra-Ghana;48.
- Agab. M.A. and Shafie, E.B. (1989). Traditionally salt Fermented Fish (Fessiekh) Sudanese J. Food Sci. Technol., 17, pp. 25-33
- 4. Ahmed, E.O. (2006). Comparison of the nutritive value of Fessiekh in hydrocynusp and schilbe spp. Ph.D. Thesis Al Neelain University. Khartoum, Sudan.
- Al- Jedah, J.H., Ali, M.Z., Robinson, R.K. (1999). Chemical and microbiological properties of Mehiawah-A popular fish sauce in the Gulf. J. Food Sci, Technol., 36:561-564.
- Anihouvi, V.B.; Sakyi-Dawson, E.; Ayernor, G.S. and Hounhouigan, J.D. (2007). (Microbiological changes in naturally fermented cassava fish (Pseudotolithus sp.) for lanhouin production. Int. J., Food Microbiol., 116, pp. 287-291
- Anihouvi, V. B.; Ayernor, G. S.; Hounhouigan, J. D.; Akyi-Dawsoin, E. (2006). Quality Characteristics of Lanhouin: a traditional processed fermented fish product in the Republic of Benin, *African J. Food Agric. Nutrit.* Dev. 6, pp. 1-15
- Anihouvi, V. B.; Sakyi-Dawson, E. G.; Ayenor, S. and Hounhouigan J. B. (2009). Biochemical changes and aronma development during the spontaneous fermentation of cassava fish into Lanhouin and their influence on product acceptability J. Aquat. Food Prod. Technol., 18 pp. 370-384
- Anihouvi, V.B.; Kindossi, J. M. and Hounhouigan, J.D. (2012). Processing and quality characteristics of some major fermented fish products from Africa: A critical review.Int. *Res. J. Biol. Sci.*, 1, pp. 72-84
- 10. AOAC (1990). Official Methods of Analysis of the Association of Official Analytical Chemists (15th ed.), Association of Official Analytical Chemists, Washington, DC
- 11. Beddows, C.G. (1985). Fermented fish and fish products. In microbiology of fermented food, J.B. El Sevier Sci Publishing Comp. New York, 2:31-40.
- 12. Dirar, H.A. (1989). Indigenous fermented foods and beverages of the Sudan. J. Food Sci., 38:52-56
- 13. **Dirar, H.A. (1993).** The indigenous fermented food in the Sudan. A study in African food and Nutrition. CAB International Walling Ford.

- 14. FAO (1992). Fermented fish in Africa. Compliance policy guide for FDA. Fish. Rep., Rome, Italy 31:126-132.
- 15. FAO (2000). Assurance of seafood quality. FAO Fish. Tech. Pap, 334:55-56.
- Gassem, M. A. (2019). Microbiological and chemical quality of a traditional salted-fermented fish (Hout-Kasef) product of Jazan Region, Saudi Arabia. Saudi Journal of Biological Sciences, 26(1), 137-140.
- 17. Harrigan, F.W and MacCance, M.E. (1976). Laboratory methods in food microbiology. 3003 Academic Press of London, New York and San Francisco. Pp 127.
- Harrigan, F.W, (1998). Laboratory methods in food microbiology. Academic Press of London. Pp 235.
- 19. **Ijong, F.G., Ohta, Y. (1995).** Microflora and chemical assessment of an Indonesian traditional fermented fish sauce "bakasong". *Applied Biological Sci., 34*(2):95-100.
- 20. Ihekoryne, A. I. and Ngoddy, P. O. (1985). Integrated food science and technology for the tropics. MacMillan pub. London.
- Koffi-Nevry, R.; Ouina, T. S.; Koussememon, M. and Broi, K. (2011). Chemical composition and lactic acid microflora of Adjuevan, traditional Ivorian fermented fish condiment. Pakistan J. Nutrit., 10, pp. 332-337
- Kusmarwati, A., Hizamah, U. and Wibowo, S. (2020). Microbiological and chemical quality of a traditional salted-fermented fish (peda) product of Banten, Indonesia using Leuconostoc mesenteroides ssp. Cremonis BN12 as starter culture. In *IOP Conference Series: Earth and Environmental Science* (Vol. 462, No. 1, p. 012020). IOP Publishing.
- Majumdar, R. K., Nayak, B. B., & Basu, S. (2008). Involvement of Bacillus licheniformis and Micrococcus kristinae during ripening of salt fermented Indian Shad (Tenualosa ilisha). Journal of Aquatic Food Product Technology, 17(4), 423-440.
- 24. **Mohammed, H.M.H., (2010).** Nutritive value of fresh and salted fermented fish(aleste dentex) Terkin. In: Food Science and Technology, Ed. Khartoum. Sudan.
- Moledina. K.H., Regenstein J.M., Baker. R.C., and Steinkraus, K.H. (2008). A process for the preparation of dehydration salted fish-soy cakes. National Oceanic and Atmospheric Administration (NOAA). Food Science. Cornell University.USA.
- Osman, O. A.; Sulieman, A. E.; Elkhalifa, E. A. and Mustafa, W.A. (2012). Chemical and microbiological characteristic of fermented fish product, Fassiekh Food Public Health, 2, pp. 213-218
- Petrus, H.; Suprayitno, E. and Hardoko, L. (2013). Physiochemical characteristics, sensory acceptability and f microbial quality of Wadi Betok a traditional fermented fish from South Kalimantan, Indonesian Int. Food Res. J., 20 pp. 933-939
- Ricke, S. C., Koo, O. K., & Keeton, J. T. (2012). Fermented meat, poultry, and fish products. Food microbiology: fundamentals and frontiers, 857-880.
- Roy, D.; Majumbar, R.K.; Maurya, S.K.; Tripathi, D.B.and Priyadarshini, M.B. (2014). Understanding of traditional knowledge and characterization of Telesech – a fermented fish product of Tripura state. *Indian J. Nat. Prod. Resour.*, 5, pp. 351-358
- Sarojnalini, C.H. and Vishwanath, W. (1994). Nutritional characteristics of the fermented fish products. Hentak and Ngari of manipur. J. Indian Fish Assoc.25:75-81.
- 31. SAS, (1997). SAS/STAT User's, Version 6.03 editon, Cary, NC:SAS Institute Inc., pp. 1028.
- Skierdal, T. and Pedersen, G. (2001). Production of fermented fish sauce from wasted salted fish. Norway J. Process. Fish. Report., 19:13-17.
- Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., ... & Hutkins, R. (2020). Fermented foods in a global age: East meets West. Comprehensive Reviews in Food Science and Food Safety, 19(1), 184-217.
- Tanasupawat, S., Hashimoto, Y., Ezaki, T., Kozaki. M., and Komagata. K. (1992). Staphylococcus piscifermentons spp. From fermented fish in Thailand. International systematic bacteriology, J. Japan. Food. Sci. 42:(4):577-581.
- 35. Tanasupawat, S., Okada, S., and Komagata, K. (1998). Lactic acid bacteria found in fermented fish in Thailand. J. Gen. Appl. Microbio. 44(3):193-200.