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Chemical and Microbiological Characteristics of Fermented Fish Product, Fassiekh

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Abstract The indigenous fermented fish, 'fasiekh', is the major fermented product from fish in the Sudan. It is made mainly from two common Nile fish, namely, Kawara (*Alestes spp*) and kass (*Hydrocyonus spp.*). In the present study, The chemical and microbiological characteristics of fassiekh were determined. The samples have shown slight differences in most of their proximate components, mineral contents and microbial characteristics. However, there was a significant difference ($p<0.05$) between dry salted fasiekh samples (B), paste fasiekh sample (C) and fasiekh in salted water sample (D) in most of the proximate chemical composition when compared with those of the control fish (A), these included contents of proteins, fats, ash. On the other hand, there was non-significant differences ($p<0.05$) between the different types of fasiekh compared with the control fish (A) in pH value. The content of calcium, magnesium, sodium, potassium and nitrogen they were significantly different ($p<0.05$) when compared with the control (A), but there was no- significant difference in phosphorus contents when compared with the control (A). The microbiological analyses revealed that all samples contained high microbial load, and there were significant differences ($p<0.05$) between the most of the samples when compared with the control sample (A).

Keywords Fish, Fassiekh, Microbiological Analysis, Minerals

1. Introduction

Fermented fish products are important sources of nourishment, they contain great amount of a high quality protein. These products are used sometimes as seasoning, and at other times as the only source of animal protein in various dishes to replace meat and fresh fish[1][2].

In the Sudan, special types of traditional fermented fish products have long been made. Dirar[3] 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 (ferment ed 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.

Neither modern industries nor improvement of classical rationale concerning fish processing has been established in the Sudan. Therefore curing in a primeval way is still the principal method of fish processing , i.e. sun-drying without salt , dry as well as wet-salting and fermenting are the common method of soft fish curing.

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 Egypti on descent, particularly the ethnic group called Nagada. Other Sudanese dominate in the fishing and manufacturing stages of the business[4].

The objectives of the present study were to evaluate the chemical constituents and microbiological characteristics of fasiekh samples obtained from Eldueim local market, one of the famous markets of fish and fish products in Sudan, to assess sanitary measures with regard to producers personal hygiene, handling and packaging of fermented fish product, fasiekh in retail shops and to evaluate the nutritive value according to the different types of fasiekh processing.

2. Materials and Methods

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2.1. Collection of Samples

Samples of fresh fish, namely, Kass (*Hydrocyonus spp*) which were previously prepared traditionally by small-scale producers in Eldueim town (Central Sudan), on White Nile during the period July 2008. The fasiekh samples included dry salted fasiekh sample (B), paste fasiekh sample (C), fasiekh sample in salted water (D), and fasiekh packed in tin gallon container sample (F). Also fresh fish samples control (A) was collected from anglers immediately after landing.

These samples were collected in sterile glass containers and polyethylene bags. The samples were then transported (early in the morning) to the laboratory where microbiological and chemical analyses were immediately carried out.

2.2. Microbiological Analysis

The microbiological analyses were conducted on samples consisting of fresh unsalted fish sample (A), dry salted fasiekh sample (B), paste fasiekh sample (C), fasiekh in salted water sample (D) and fasiekh packed in tin gallon container sample (F). The appropriate dilutions of the various samples were spread on pre-poured plates of Plate count agar, Mac Conkey agar, Baird-Parker agar and Potato Dextrose Agar for counting of total viable count (aerobic and anaerobic), coliforms, staphylococci *spp*. and for yeasts and moulds, respectively. All plates were inoculated at 37℃ for 24-48 h except Potato dextrose agar palates which were incubated for 72 h at 25℃. Characteristic colonies appearing on the respective selective agar media were counted, multiplied by the dilution factor and expressed as colony forming units per ml c.f.u/g. For coliform determination, one ml sample was plated onto MacConky Agar medium. The plates were incubated at 37℃ for 48 hours and the counts were presented as colony forming units per gram (cfu/g). Plates showing positive coliform were subjected to the confirmed test using Brilliant green bile lactose broth in test tubes with Durham tubes. The test tubes were then incubated at 44℃ for 48 hours. Each confirmed positive tube was subcultured into E.C. broth medium and then incubated at 44.5℃ for 24 hours for *E.coli*. Tubes showing any amount of gas production were considered to be positive.

For detection of salmonella, a sample of 10 grams was weighed aseptically and mixed well with 100 ml sterile nutrient broth. This was incubated at 37℃ for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml selenite broth and incubated at 37℃ for 24 hours. Then with a loopful streaking was done on dried Bismuth sulphite agar plates. The plates were then incubated at 37℃ for 72 hours. Black metallic sheen discrete colonies indicated the presence of salmonella. A confirmatory test was carried out by taking a discrete black sheen colony and subculturing it in a Triple sugar iron agar tubes. Production of a black colour at the bottom of the tube confirms the presence of *Salmonella*.

2.3. Proximate Analysis of Fasiekh

The proximate analyses were carried out in triplicates in all fasiekh collected samples according to AOAC[6]. These analyses included determination contents of moisture, protein, fat, ash and value of pH.

2.4. Minerals Determination

The minerals contents were determined using atomic absorption spectrophotometer in all fasiekh samples according to Perkin Elmer[7]. For determination of sodium and calcium, 2 gm sample were maintained in a muffle furnace at 550℃ for 4 hrs. Samples were cooled and 10 ml of 3N HCl were added, covered with watch glass and boiled gently for 10 minutes, then cooled, filtered, diluted to volume (100 ml) with distilled water, The delution was taken for determination of sodium and calcuim contents, for determination of calcium, 1 ml of 1% lanthanum chloride was added to final dilution[7].

For determination of magnisum, potassium and phosphorus, 2 gm sample were maintained in a muffle furnace at 550℃ for 4 hrs, samples were cooled and 10 ml of 3N HCl were added, covered with watch glass and boiled gently for 10 minutes, then cooled, filtered, diluted to volume (100 ml) with distilled water, and taken for determination of magnisum, potassium and phosphorus contents.

3. Results and Discussion

3.1. Proximate Chemical Composition Fasiekh

Data presented in Table (1) present the proximate chemical composition of fasiekh samples. The moisture content of dry salted fasiekh (B), paste fasiekh sample (C), fasiekh sample in salted water (D), and fasiekh packed in tin gallon container (F) were 46.34%, 39.59%, 50.15%, and 39.62, respectively. These value were lower than that of the fresh control sample (A) which was 77.03%. Generally, the moisture content of the various fasiekh samples are in agreement with that of Mahmoud[8] who reported a value for dry salted *Hydrocyonus ssp*. fish. In addition, these results almost fall in the range 1.72 – 45.5% of moisture content given by Agab and Shafi[9] for dry salted (fasiekh). However, Omer[10] reported a slightly high moisture content (51.79 ± 6.76%) in dry salted *Hydrocyonus. ssp*. Salama *et al*,[11] found that the moisture content of salted sardine ranged from 45 -53% (a product made in Egypt). It seems that moisture content highly reduced in fasiekh samples compared to fresh sample. This can be attributed to the plasmolysis occurring as an action of salt applied to the fish. However, the moisture content preference and limits of microorganisms causing spoilage of cured fish are not known. Nevertheless, in the present study the levels of moisture content in all "fasiekh" samples, except sample (B and D) were lower than 44%, the recommended safe moisture level given by Hennessey[12] who worked on *Gadus Morhua*.

Table 1. Chemical Composition and pH Value of Fasiekh and Control Fresh Fish Samples

Fasiekh Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH
A	77.03a	8.17e	3.47c	11.33e	6.10a
B	46.34c	29.50b	3.63bc	20.53c	6.72a
C	39.59d	33.41a	8.09a	18.91d	6.51a
D	50.15b	25.40d	1.18d	23.27b	6.67a
F	39.62d	28.38c	5.22b	26.78a	6.59a

Means within the same column bearing the same letter(s) are not significantly different ($p<0.05$).
A ≡ Fresh fish samples (unsalted); B ≡ Dry salted Fasiekh samples (fresh)
C ≡ Paste Fasiekh samples; D ≡ Fasiekh samples in salted water (Routo area)
F ≡ Fasiekh samples packed in tin gallon container

There was a significant difference ($p<0.05$) between the samples (B), (C), and (D) with control (A) in moisture content but there was non-significant difference between the samples (C) and (F).

The protein content of samples (B), (C), (D) and (F) were 29.50%, 33.41%, 25.40% and 28.38%, respectively. These values were extremely greater than that of the fresh control sample (A) which was 8.17%. However, these results are in agreement with those reported by Omer[10], Agab and Shafi[9] for dry salted fish fasiekh which ranged between 18.12 – 28.52% and 18.50-23.4 %, respectively. In addition, these values were extremely greater than that of the dry salted *Hydrocyonus vittatus* reported by Clucas[13]. However, VanVeen[15] found that protein content of Pedah Siam (a fasiekh – like product) ranged from 21-22%. And Salama *et al*.[11] reported a protein content ranging from 18-23% for salted sardine product in Egypt.

When comparing the protein content of fish before and after salting, significant differences were observed, especially in the case of boned kass fasiekh. The change of fish state has led to increase in protein content because of moisture removal and concentration of the nutrient materials.

There was a significant difference ($p<0.05$) between the samples (B), (C), (D), and (F) with control sample (A) in protein content.

The fat content of (B), (C), (D), and (F) fasiekh samples were 3.63%, 8.09%, 1.18% and 5.22%, respectively. The values of fat in samples (B) (C) and (F) were significantly greater ($p<0.05$) than that of the fresh control sample (A) which was 3.47%, while the fat content of sample (D) was significantly lower ($p<0.05$) when compared with the fresh control sample (A). VanVeen[14] found that fat content of pedah Siam (fasiekh – like product) ranged between 7-14% w/w. It is clear from the present results that fat content of fasiekh samples is much greater than that of fresh fish except fasiekh sample in salted water sample (D). This is presumably due to the concentration of fat since some protein and other substances leached out during processing. According to Tarr[15] fat losses during salting are usually negligible.

The ash content of B, C, D and F fasiekh samples were 20.53%, 18.91%, 23.27% and 26.78%, respectively. These values were extremely greater than that of the fresh control sample (A) which was 11.33%. There were significant differences ($p<0.05$) between the samples (B), (C), (D) and (F) when compared with that of the control sample (A) in ash content.

From the data in Table (1), it could be also seen that the pH of the various fasiekh samples ranged between (6.5-6.7). Agab and Shafi[9] gave similar results. The relatively neutral pH values could be attributed to the elimination of gut with its acidic secretions. It is interesting to note that this range of pH usually encourages the growth of proteolytic and pathogenic microorganisms, for instance, similar fermented fish products of South – East Asia were reported to contain *Clostridium botulinum* the causative agent of food poisoning[16].

3.2. Minerals Contents

The minerals content of the various fasiekh samples is presented in Table (2). The calcium content of samples: (B), (C), (D) and (F) were 1.5, 2.36, 2.27 and 0.99 mg, respectively. Samples (B) and (F) had higher calcium contents when compared with the control fish sample (A) which was 0.6 mg, while calcium content in samples (C) and (D) were extremely grater than that of the control fish sample (A) . There was a significant difference ($p<0.05$) between samples (B), (C) and (D) when compared with the control fish sample (A) in calcium content, but there is non-significant difference between sample (B) with (D), (C) with (D), (B) with (F), and (A) with (F).

The magnesium content of samples: B, C, D and F were 0.97, 0.30, 0.90 and 1.05 mg, respectively. The values of magnesium in samples B, D, and F were high than that the control fish sample (A) which was 0.26 mg, while that of sample (C) was found similar to that of the control fish sample. There was significant difference ($p<0.05$) between the sample (B), (D) and (F) when compared with control (A) in magnesium content, but there was non- significant difference ($p<0.05$) between sample (C) when compared with that of the control fish sample (A), and between (B), (D) and (F).

On the other hand, the sodium contents of fasiekh samples: (B), (C), (D) and (F) were 9.23, 8.33, 10.01 and 10.23 mg, respectively. Values of sodium in samples B, C, D, and F were extremely high than that of the control fish sample (A). There was a significant difference ($p<0.05$) between the sample (B), (C), (D) and (F) and control sample (A) in sodium content, but there was non- significant difference between sample (B) with (C), and between (B), (D) and (F).

The potassium content of fasiekh samples: (B), (C), (D) and (F) were 0.79, 1.15, 0.89 and 1.10 mg, respectively. Potassium content of samples (B) and (D) were greater than that of the control fish sample (A) which was 0.37 mg, while that of sample (C) and (F) were high when compared with that of the control fish sample (A). There was no significant difference ($p<0.05$) when compared potassium content of samples (C) and (F) with that of the control fish sample (A) in potassium content, but there was non- significant difference between samples, (B), (D) when compared with that of the control fish sample (A).

The phosphorus contents of fasiekh samples: (B), (C), (D) and (F) were 0.24, 0.26, 0.33 and 0.27 mg, respectively. Values of phosphorus in samples B, C, D, and F were similar to that of the control fish sample (A). There was non-significant difference ($p>0.5$) between the sample (B), (C), (D) and (F) and control (A) in phosphorus content.

Table 2. The Minerals content of fasiekh samples (mg/g)

Fasiekh samples	Ca	Mg	Na	K	P
A	0.60d	0.26b	0.58c	0.37c	0.28a
B	1.50bc	0.97a	9.23ab	0.80ab	0.24a
C	2.37a	0.30b	8.33b	1.15a	0.26a
D	2.27ab	0.90a	10.01a	0.89ab	0.33a
F	0.99cd	1.05a	10.23a	1.10a	0.27a

Means within the same column bearing the same letter(s) are not significantly different ($p<0.05$).
A ≡ Fresh fish samples (unsalted); B ≡ Dry salted Fasiekh samples (fresh)
C ≡ Paste Fasiekh samples; D ≡ Fasiekh samples in salted water (Routo area)
F ≡ Fasiekh samples packed in tin gallon container

3.3. Microbial Evaluation

Data presented in Table (3) show the microbiological characteristics of the different fasiekh samples, The count of aerobic bacteria in samples: (B), (C), (D) and (F) were 1.40×10^5, 6.7×10^5, 1.50×10^6 and 3.7×10^5 cfu/g, respectively. The count of sample (D) was higher than that of the control fish sample (A) which was 6.8×10^4 cfu/g. While those of samples B, D and F were greater than that of the control fish sample (A). There was a significant difference ($p<0.05$) between the samples (B), (C), (D) and (F) when compared with control (A) in aerobic count, but there were non-significant difference between sample (B) with (F), and (C) with (D). The high Total viable count of aerobic bacterial load of fasiekh samples could be due to improper handling and sanitary conditions during the preparation and moisture content.

The total viable count of anaerobic bacteria of fasiekh samples: (B), (C), (D) and (F) were 7.0×10^4, 2.0×10^3, 7.2×10^4 and 1.60×10^3 cfu/g, respectively. The value in sample (B) was similar to that of sample (D), while those of samples (B) and (D) were greater when compared with that of the control fish sample (A) which was 2.3×10^4. On the other hand, samples (C) and (F) were low in counts of anaerobic bacteria than that of the control fish sample (A). The high anaerobic bacterial load of fasiekh samples could be due to improper handling and sanitary conditions during the processing. There was a significant difference between the samples (B), (C), and (D) with control (A) in anaerobic count, but there was non- significant difference between sample (F) compared with (A).

The coliform bacteria were not detected in all fasiekh samples, but the control sample (A) contained 44 c.f.u/g. The absence of coliforms in the different fasiekh samples could be attributed to the salt added during the processing. In addition, yeasts and moulds were not detected in all of fresh fish and fasiekh samples. Yeast and mould are examples of fungi, responsible for the food spoilage and produce mycotoxin [17].

Table 3. The Microbiological characteristics* of fasiekh samples

Fasiekh samples	Total viable count of aerobic bacteria (cfu/g)	Total viable count of anaerobic bacteria (cfu/g	Coliform MPN per gram		Yeasts and moulds (cfu/g)	Staphylococci	Detection of salmonella
			Total	E. coli			
A	6.8×104 b	2.3×104 a	44	6	N.G	3.6×102 b	-ve
B	1.4×105 a	7.0×104 b	0	0	N.G	1.2×102 b	-ve
C	6.7×105 c	2.0×103 d	0	0	N.G	4.0×102 c	-ve
D	1.5×106 c	7.2×104 c	0	0	N.G	5.6×103 c	+ve
F	3.7×105 a	1.6×103 a	0	0	N.G	3.0×103 a	+ve

*Means within the same column bearing the same letter(s) are not significantly different ($p<0.05$).
A ≡ Fresh fish samples (unsalted); B ≡ Dry salted Fasiekh samples (fresh)
C ≡ Paste Fasiekh samples; D ≡ Fasiekh samples in salted water (Routo area)
F ≡ Fasiekh samples packed in tin gallon container

Table (3) also show that the counts of *Staphylococcus* was higher in (D) and (F) samples which were 5.6×10^3 and 3.0×10^3, respectively. While those of (B) and (C) samples were similar to that of the control sample (A). Fasiekh contaminated with the *staphylococcus* toxin makes people sick, with nausea, vomiting and diarrhea usually appearing from tow to six hours after eating the staphylococci infected food (O'connell, 2002). There was a significant difference between the sample (C), (D), and (F) and control (A) in Staphylococci content, but there was non- significant difference between sample (B) with (A) and (C) with (D). The presence of *Staph. aureus* indicates contamination from the skin, mouth or nose of food handlers also contamination of processed food may also occur when contaminated food is processed on surfaces to which food products are exposed (Andrews, 1992).

The result showed absence of *Salmonella* in (B) (C) and the control (A) samples. Nevertheless, sample (D) and sample (F) showed positive results. This will create health risks to the fasiekh consumers. When consumed, can cause symptoms such as diarrhea, stomach pains, nausea, vomiting and stomach infections or cramps. In very serious cases, it can cause death.

Generally, this close range of microbial content among the various fasiekh samples could be due to the living of fish in the same environment. The using of salt in fish preservation is not limited to dry application. Salt is an important additive in the preparation of fermented, pickled, or processed fish or fish products. In the making of fermented fish, known concentrations of salt are added to promote degradation of proteins and retard the growth of undesirable, putrefactive microorganisms. In addition, this allows desirable, NaCl-tolerant (halotolerant), fermentative species such as lactic acid bacteria to grow[20].

Generally, the contamination of fasiekh occurs from water of unsatisfactory microbiological quality that is used in the processing of fish, unwashed hands and improperly cleaned or sanitized equipments and from environment storage during fermentation.

4. Conclusions

Fassiekh samples have shown slight differences in most of their chemical components, mineral contents and microbial characteristics. However, there significant difference ($p<0.05$) were indicated between some of these samples when compared with the control fish sample (A). However, the microbiological analyses revealed that all samples contained high microbial load, and there were significant differences ($p<0.05$) between the most of the samples when compared with the control sample (A).

It is highly recommended to follow the personal hygiene during processing and handling of fasiekh, use of proper packaging containers . In addition, sanitary measures must be followed during presentation of fasiekh in retail shops. Further studies are needed to produce of fasiekh under controlled and sanitary conditions.

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