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THE CHEMICAL COMPOSITION MICROBIOLOGICAL DETECTION AND SENSORY EVALUATION OF FRESH FISH SAUSAGE MADE FROM *CLARIAS LAZERA* AND *TETRADON FAHAKA*

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Abstract- The aim of this study to improve and upgrade the nutritive values of two Nile fishes (*Clarias lazera Tetradon fahaka*). Distinct formulations of fresh fish sausages were developed from commercially cheap price and unfavorable fish caught from Jebel Aulia reservoir. Three types of fresh fish sausage were prepared, one from *Clarias lazera*, the other from *Tetradon fahaka* while the third from a mixture of the two species. Nutritional composition, sensory evaluation and microbiological detection of fish sausage made from mince of the two species and a mix were determined. Outcome of mincing composition was 76.26±0.008%, 65.73±0.003% mince; 14.20±0.003%, 24.0±0.004% residue and 9.54±0.008%, 10.27±0.008% loss for *Clarias lazera* and *Tetradon fahaka* respectively. Moisture, ash, protein, fat and pH were analyzed to determine chemical quality of mince and the fish sausage types as well as effect of storage. The chemical quality was found to be statistically significant ($p<0.05$) with respect to the fish sausage types and storage time. Microbiological analysis showed that the total viable counts (TVC) in fish sausage samples least $4x10^3$,highest $6x10^5$ and it decreased with increased in freezing period. It was found that the samples contained coliform group bacteria, *Staphylococcus aureus, Escherichia coli* and *Klebsiella* spp but *Salmonella* spp were not detected. The results obtained were within the acceptable limit of Sudanese Standards and Metrology Organization for fish sausage products. The organoleptic properties of the fish sausage samples indicated that the fish sausage made from a mix recorded high score when compared to the two other types. The study showed that the fish sausage from *Clarias lazera*, *Tetradon fahaka* and a mix (*Clarias lazera+Tetradon fahaka*) are microbiologically safe and nutritionally high.
Key words: Fish sausage, yield, mince, chemical quality and storage

INTRODUCTION

Fish received increased attention as potential source of animal protein and essential nutrients for human diets [10]. Fish meat contains significantly low lipids and higher water than beef or chicken and is favored over other white or red meats [19]. The nutritional value of fish meats comprise the contents of moisture, protein, vitamins and minerals plus the caloric value of the fish [23]. Fish as a rich source of protein needs to be introduced locally into Sudanese diet, so as to reduce the burden of high cost of red meat pertaining in this country's market. Yet in Sudan with its large fisheries resources, little is known about nutritional value of the fishes that are normally utilized either fresh or preserved dried, salted or smoked [7]. Sudan strategic planning programs 1999 for the increase of fish annual average per capita consumption (pcc) from 1.4 kg to 3.2 kg as being championed by the Ministry of Agriculture and Animal Production. Such increasing program is still less when compared to some neighboring countries like Egypt which has pcc 4 kg and Uganda 8.8 kg [8]. Increasingly, seafood is being used as the dish of choice owing to its healthy image and delicious taste. In particular, the fish industry has been developing processed or minced fish products such as fish burgers, fingers and sausages, which add cooking convenience to nutritional benefits [18]. Fish flesh can be used as raw material for sausage production because muscle protein can form gel and act as an emulsifying agent [24]. Fish sausage is a product in which fish flesh is mixed with additives, stuffed into suitable casings and heat processed. The sausage butter has decisive influence on quality factors of the finals products, such as texture, flavour, appearance and nutritive value. A sausage butter of constants composition also guarantees a predetermined uniformity of the final products throughout the production. This in turn, provides economic advantage to the processor and continued satisfaction to customer [16]. The objective of this work was to improve the taste of the fish to meet the expectation of consumer and accordingly increase the chances of getting better returns.

MATERIALS AND METHODS

Fish mince preparation: Samples of fresh fish were brought from Jebel-Aulia reservoir 45 km south of Khartoum city, namely *Clarias lazera* (Grmut) and *Tetradon fahaka* (Tambera). They were collected in polyethylene bags with crushed ice and transported to processing laboratory, faculty of agriculture and fish sciences. The fish samples were headed, gutted and washed with cold water to remove all viscera beside trace of kidney, swim bladder and blood. Skin and bones were manually removed to produce pure fillets. The mince was obtained using Schoole Balance (Globe Brand, 221bsX1 oz.), with screen size 1.5 mm perforation. The microbial and chemical compositions of the mince were determined.

Formulation and preparation of freshwater fish sausage: Three types of fresh fish sausage were prepared. One from *Clarias lazera*, the other from *Tetradon fahaka* while the third was from the mixture of (1:1) of the two species. The sausages were prepared in percentage to contain fish mince, flour, fat, salt, onion, pepper and seasoning (table1). The sausage was prepared in two phases: firstly, the minced meat was mixed with salt, shortening and fat. The blended mixture was allowed to stand at 0°C for 24 hours. Following the setting period the second phase ingredients were added and blended well. The completed sausage filling appeared slightly pinkish and was sticky to the touch, it is then passed through a sausage former into one inch natural casing. The sausages were then steamed for 10 minutes over heated water to the level of 85 -90°C and finally the product was cooled and packed in plastic bags and stored in a refrigerator at -5ºC for one month. Further, sub-samples were taken to follow microbial and chemical composition changes.

Chemical analysis: The chemical contents of mince and fish sausage were determined according to Association of Official Methods of Analysis. Moisture content was determined according to [4] after the water in extract was removed, ash in extract was calculated. Protein content (NX6.25) was calculated using the kjeldahl method while lipid content was determined to Soxhlet method described in [4] pH was measured with a digital electronic pH meter with a glass electrode (WT W Mark 320).

Microbiological analysis: For microbiological analysis preparation of the samples was carried out according to [14]. Total viable counts of bacteria (TVC) were determined using Plate Count Agar (PCA) (37±1°C, 48 hours). The count of coliform group bacteria was determined on Violet Red Bile Agar (VRBA) (30±1 °C 24 hours); presence of *Escherichia coli* was determined by applying IMVIC tests to the typical dark colonies from Violet Bile Agar. *Staphylococcus* spp was determined using Mannitol Salt Agar (MSA) (37±1°C, 48 hours), *Staphylococcus aureus* was determined by applying coagulase test on bright yellow halo colonies on (MSA). *Salmonella* spp was determined using Salamonella and Shigella Agar (SSA) (37±1°C, 72hours). All colonies were counted and the data was reported as colony forming units (CFU) per gram.

Sensory evaluation: Frozen fish sausages were thawed and cooked for 10 minutes. The cooked samples were served immediately to each member of the panel. The subjective evaluation of the product quality was carried out by assessors composed of ten students from the school of fish sciences, section from faculty of agriculture and fish sciences. Quality attributes investigations include palatability, juiciness, appearance, texture, flavours and taste. Panel members scored all factors on a 10 point scale(9 = excellent; 8-9 very good; 6.5-7.9 good; 5-6.4 fair; <5 bad), using the score method as reported by [1].

Statistical analysis: All measurements were performed in triplicate and the value expressed as the mean ± SD. Statistical analyses were performed using SPSS 10.0 for windows. Analysis of variance (ANOVA) was used and statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The yield and compositional data of mince meat are presented in table 2. Fresh *Clarias lazera* mince was found to be 76.26±0.008 whereas *Tetradon fahaka* was 65.73± 0.003%. The mince yield from the two species used in this study is higher than the results obtained by [17] on the total yield of mince from various species of marine and fresh water fish ranged from 37- 60 %. Yield of mince varies with species and is a faction of anatomical features, state of maturity and the quality of the species involved [3]. [12] reported that the yield of 60-80% could generally be obtained from whole fish. The finding of this study was conformed to the finding of [20] who reported that the yield of mince from shrimp bycatch was 80.26%. The results of chemical composition of mince meat used in the fresh fish sausage preparation, were shown in table 3. The moisture contents, ash, protein, lipid and pH of samples were found to be significantly different ($p<0.05$). This might be due to the difference in size, maturity and quality of the two species. The moisture content of the fish sausage samples with respect to types and storage time were significantly different ($p< 0.05$), protein, fat, ash and pH followed the same trend table 4. The moisture content of samples was determined least 68.40 ±0.100% highest 71.5±0.368. The moisture contents was decreased when compared to the mince meat, this reduction after processing may be due to the ingredients which were added to the mince meat. The protein content of samples was determined least 15.53±0.781%, highest 20.67±0.368% table 4. It is evident that the protein contents of fish sausages increased after processing when compared to mince meat. In our results, this increased was probably due to the raw materials used, described in table 1. Storage time, directly had impact in decreasing protein content of sausage, this may be due to the degradation of protein. But the value of the protein content was still with the limit of 11.25% recommended by [22] for fresh fish sausages. The fat contents of the fish sausage types were determined least 0.963±0.207%, highest 2.40±0.100%.

It is clear from the present results that fat content was increased; this might be due to the amount of fat added during processing of the sausage. As expected, a significant decrease ($p<0.05$) in fat content was observed in the samples during storage table 4. A comparable pattern of the decrease in fat value has been reported in fish sausage (2.16 – 2.72%) during chilling storage for 6 days [25]. Conversely, a higher fat content (4.20%) was reported by [2], on fresh fish sausage produce from *Clarias lazera* at day 45 under refrigeration at -18°C. Ash content of the fish sausages was increased least 1.6±0.153%, highest 4.70±0.100%. The increase in ash contents was correlated mostly to the amount of salt adding to sausage products and to the storage period. pH of samples was determined least 4.63±0.153, highest 6.30±0.100. The pH of fish sausages was increased when compared to the mince and decreased until the 30 days storage. It might be due to the breaking down of other components of the product, primarily carbohydrate, producing acid compound e.g lactic acid [9]. There was general trend of a decrease in chemical composition of fish sausage in storage time, but this decrease was close to the butter amount as reported by [21]. Who reported that the butter quality characteristics of fresh fish sausage were 68.64%, 16.76%, 2.67% & 6.8% for moisture, protein, ash and pH respectively. But he was reported a higher fat content (5.64%) when compared to the value of the fat obtained in the present study.

Total viable counts of bacteria (TVC) of mince fish was $8.0X10^5$, $1.4X10^5$ and$1.7 X10^5$ cfu/g for *Clarias lazera*, *Tetradon fahaka* and a mix (*Clarias lazera*+*Tetradon fahaka*) respectively. Microbial counts of mince fish lower than that of fish sausage. This could be as the result of fact that the mince fish was still fresh and was just undergoing processing. Microbiological analysis of the fresh fish sausage showed that TVC least $4.0x10^3$, highest $6.0x10^5$ and decreased with increased in frozen period of 30 days storage table 5. When food aerobic plate count reach 10^6 cfu/g, the food product was assumed to be at or near spoilage [5]. [6] stated that the maximum total plate count for the processed food to be consumed safely was 10^7 - 10^8 . In this study the mince and fish sausage didn't reach 10^6 cfu/g. However, by the end of the storage, growth was well over 10^3 cfu/g therefore, the fish sausage has been microbiologically acceptable up to 30 days storage. In present study *Staphylococcus aureus* was determined in two types of fish sausages table 5. The values of *Staphylococcus aureus* was still within the limit of 10^3 cfu/g recommended by [15] in good manufacturing practices. Count of coliform group microorganisms were determined least <50 cfu/g and highest 240 cfu/g in fish sausages during storage table 5. This low counts might have been due to the low of temperature, which could have caused stress to the bacteria resulting in low metabolic rate of the bacteria[11]. This decrease was probably also due to competition to the growth of Psychrotrophic bacteria. Temperature was kept at -5°C and coliform do not grow at low temperature. The presence of the species *Escherichia coli* and *Klebsiella* spp in the formulation of the sausage which can be bactericidal in nature, may also have countered for the low figures of microorganism. This is not problem because the coliforms bacteria are killed during heat processing of sausage products. *Salmonella* spp are regarded as human pathogen, the most sever *Salmonella* infection is Typhoid fever. No *Salmonella* spp was detected in our mince and sausage samples. Still emphasizes the need for better hygienic for mince and sausage processing, although the microbial quality was not a problem within 30 days frozen storage.

The organoleptic properties of the fish sausages indicated that the products were acceptable according to the panel's evaluation, though statistically there was significant difference ($P < 0.05$) in the sensory evaluation of the different types of the fish sausages based on the panel's score table 6. In the present experiment, scores are the average of 10 panel taste sheets. It could be noticed that the fish sausage made from a mix recorded high score in organoleptic parameters were examined when compared to the two other types. Overall acceptability mean scores indicated that the three types of fish sausages were generally well accepted.

CONCOLUSION

It could be concluded from this study that the meat of *Clarias lazera* and *Tetradon fahaka* can be utilized for preparation of sausage with good nutritive value and taste acceptability. Also it was evident that the sausages could safely be stored in refrigerators up to 30 days. Thus, a better chance could be offered at favorable market times.

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REFERENCES

[1] Afolbi O.A. ,Omosole A. A. and Olusegun L.O. (1984) *J. Food. Techno.*, 19: 333-340.

[2] Agab M. A., Erakha and El Shafie B. (2005) *Annual Report, Food Research Centre: Khartoum, Sudan.* 3-9.

[3] Ali M. T., Babeker S. A. and Tibin I.M. (1996) *In the report and proceeding of sixth*

FAO experts consultation on fish technology in Africa, Kismu, Kenya, FLLU/R. 574(Bi). 211-214.

[4] Associated Official Analytical Chemists (2002) *Official Methods of Analysis 17th edition. Gaithersburg.M D. USA.*

[5] Bahri P., Ayse G., Gulsum O. and Irfan I., (2006) *International Journal of Science & Technology* 2: 91-98.

[6] Connel J.J.(1985) *Fish muscle protein. In Recent Advance in Food Sciences. Vol. 1, ed, J. Hawthorn and J. M. Leitch. Butterworths, London*.

[7] Elagba M.H.A., Rabie A.M. and Mohammed M. (2010) *Afri. J. Food. Sci.,* 10:650-654.

[8] FAO (1991). *Source Book for the Inland Fisheries Resources of Africa. Vol. 3, CIFA Tech*. Pap. 18/3.

[9] Fardias S. (1992) *Guideline of Microbiological Laboratory of Processing Food (In Indonesia). Dept. Pendidikan dan Kebudayaan, Ditjen Pendidikan Tinggi, Pusat Antar University Pangan dan Gizi, Institute Pertanian Bogor.*

[10] Fawole O.O., Ogundiran M.A., Ayandiran T.A., Olagunju O.F. (2007) *J. Food Safety*, 9: 52-55.

[11] Ghorban Z. G. (2002) *Final Project. UNU-Fisheries Training Programme*.

[12] Grantham C. J., (1981) *Minced Fish Technology: A review. FAO Fish Tech. Pap*.212: 72.

[13] Haard N.F. (1995) *In A. Ruiter (ed). Fish and fishery products; composition, nutritive properties and stability* (pp. 78 -115). *Netherland: Cab international*.

[14] Harrigan W.F. (1998) *Laboratory Methods in Food Microbiology. 3rd ed., London: Academic Press*.

[15] ICMSF (1978) *Principle and specific application. International Commission on microbiological specification for Foods. Toronto Canada, University of Toronto Press* pp. 92-104.

[16] Karms E. (1977) Sausage products technology. Partridge, New jersey, U.S.A. Library of Congress, card number: 76-47276.

[17] Miyauchi D. and Steinberg M. (1970) *Machine separation of edible flesh from fish. Fisheries Industries Research* 6: 165-171.

[18] Mohammed S.R. Humaid W. Nujib G. and Stefan K. (2007) *J. Fisheries. Science*., 73: 1166-1176.

[19] Nestel P.J.N. (2000) *Am. J. Cln. Nutr.*, 71:228-231.

[20] Osibona A. O., Akinwande A. T., Ozor P. and Akande R. A., (2009) *Afri. J. Food Sci*., 7:177-183.

[21] Raju C.V., Shamasundar B.A. and Udupa, K.S.(2003) *Int. J. Food. Sci. Technol*., 38, 171.

[22] SSMO (2002) *Standard specification for meat, fish and crustaceans products. Sudanese Standards and Metrology Organization.* S. S. 655. Sudan.

[23] Steffens W. (2006) *J. Agric. Sci.,* 12:320-328.

[24] Yada R.Y. and Jackman R. (1994) *Protein structure-function relationships in food. Glasgow: Academic and professional*.

[25] Yusro N.F. Sugiyono Hari E. I. and Suparro (1998) *Use of soybean flour in fish sausage processing. APFIC reports and sessions > Fishery symposia and Forum meeting >* 1998.

Table 1- Formula of fish sausage

Ingredients	Percentage
Minced fish	76.69
Wheat flour	15.38
Thyme	0.46
Salt	2.30
Fat	2.30
Milk powder	0.46
Garlic	0.77
Girfa	0.46
Chili pepper	0.46
Spices	0.46

Table 2- Production yield of the minces from the two Nile fish species

	Parameters	Percentage yield %
Clarias lazera	Mince	76.26±0.008
	Residue	14.20±0.003
	Losses (by difference)	9.54±0.008
Tetradon fahaka	Mince	65.73±0.003
	Residue	24.0±0.004
	Losses (by difference)	10.27±0.008

Table 3- Chemical composition of the mince from the Nile fish species (g/100g)

Parameters	*Clarias lazera*	*Tetradon fahaka*	*Clarias lazera+ Tetradon fahaka*
Moisture	71.91±0.284 ^{b}	72.73±0.191 ^{a}	70.0±0.347c
Ash	2.5±0.186 ^{c}	2.78±0.253 ^{b}	3.23±1.560 ^{a}
Protein	16.93±1.015 ^{c}	18.61±0.941 ^{b}	18.93±1.262 ^{a}
Fat	1.93±0.339 ^{b}	1.31±0.303 ^{c}	2.17±0.262 ^{a}
pH	5.49±0.657 ^{c}	5.72±0.428 ^{a}	5.70±0.584 ^{b}

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same row indicate significant differences between groups ($p<0.05$).

Table 4: Chemical composition of the fish sausages samples during storage times (g/100g)

Fish sausage samples	Parameters	Storage time (day)			
		Zero	10	20	30
Clarias lazera	Moisture	70.2±0.368$^{a\alpha}$	70.1±0.100$^{b\alpha}$	69.80±0.368$^{c\alpha}$	69.53±0.781$^{d\alpha}$
Tetradon fahaka		71.5±0.368$^{a\beta}$	71.2±0.368$^{b\beta}$	70.3±0.100$^{c\beta}$	70.1±0.100$^{d\beta}$
Clarias lazera+ *Tetradon fahaka*		69.23±0.781$^{a\delta}$	69.20±0.100$^{b\delta}$	69.18±0.153$^{c\delta}$	68.4±0.100$^{d\delta}$
Clarias lazera	Ash	2.3±0.368 $^{a\beta}$	2.4±0.100 $^{b\alpha}$	2.6±0.100 $^{c\delta}$	2.7±0.368 $^{d\alpha}$
Tetradon fahaka		2.6±0.100 $^{a\alpha}$	2.730.153 $^{b\beta}$	2.8±0.368 $^{c\alpha}$	3.13±0.781 $^{d\alpha}$
Clarias lazera+ *Tetradon fahaka*		1.6±0.153	1.80±0.100 $^{b\delta}$	3.70±0.781 $^{c\beta}$	4.70±0.100 $^{d\beta}$
Clarias lazera	Protein	18.1±0.368	17.5±0.368 $^{b\delta}$	16.6±0.368 $^{c\delta}$	15.53±0.781 $^{d\alpha}$
Tetradon fahaka		19.6±0.368 $^{a\alpha}$	19.1±0.368 $^{b\alpha}$	18.53±0.153 $^{c\beta}$	17.2±0.368 $^{d\alpha}$
Clarias lazera+ *Tetradon fahaka*		20.67±0.368 $^{a\beta}$	19.23±0.153 $^{b\beta}$	18.50±0.100 $^{c\alpha}$	17.33±0.781 $^{d\beta}$
Clarias lazera	Fat	2.30±0.368 $^{a\alpha}$	2.10±0.100 $^{b\alpha}$	1.86±0.135 $^{c\alpha}$	1.44±0.282 $^{d\alpha}$
Tetradon fahaka		1.75±0.287	1.36±0.488 $^{b\delta}$	1.16±0.153 $^{c\delta}$	0.963±0.207 $^{d\alpha}$
Clarias lazera+ *Tetradon fahaka*		2.4±0.100 $^{a\beta}$	2.35±0.207 $^{b\beta}$	2.13±0.153 $^{c\beta}$	1.8±0.100 $^{d\beta}$
Clarias lazera	pH	6.67±0.153 $^{a\beta}$	6.23±0.126 $^{b\beta}$	5.53±0.153 $^{c\beta}$	5.20±0.100 $^{d\beta}$
Tetradon fahaka		6.30±0.100 $^{a\alpha}$	5.80±0.100 $^{b\alpha}$	5.23±0.781 $^{c\alpha}$	4.63±0.150 $^{d\alpha}$
Clarias lazera+ *Tetradon fahaka*		6.30±0.100 $^{a\alpha}$	5.70±0.100 $^{b\delta}$	5.17±0.115 $^{c\delta}$	4.70±0.100 $^{d\alpha}$

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same row indicate significant differences between groups in storage time (p<0.05). Different superscript symbol in the same column indicate significant differences between the sausages groups (p<0.05).

Table 5- Microbial quality of the fish sausage samples during storage time (cfu/g)

Fish sausage samples	Storage time (days)	TVC	Coliform	*Staphylococcus aureus*	*Salmonella* spp	*E. coli*	*Klebsiella* Spp
Clarias lazera	Zero day	6.0×10^{5}	240	-ve	-ve	+ve	-ve
	10 day	4.4×10^{5}	220	-ve	-ve	+ve	-ve
	20 day	3.2×10^{4}	200	-ve	-ve	-ve	+ve
	30 day	2.8×10^{3}	<100	-ve	-ve	+ve	+ve
Tetradon fahaka	Zero day	2.2×10^{5}	200	-ve	-ve	-ve	+ve
	10 day	1.1×10^{5}	<100	<100	-ve	-ve	-ve
	20 day	3.0×10^{4}	<100	<50	-ve	-ve	-ve
	30 day	2.7×10^{3}	<50	-ve	-ve	+ve	-ve
Clarias lazera+ *Tetradon fahaka*	Zero day	4.2×10^{5}	220	-ve	-ve	+ve	-ve
	10 day	3.8×10^{5}	180	<100	-ve	-ve	-ve
	20 day	3.8×10^{4}	<100	<50	-ve	-ve	-ve
	30 day	4.0×10^{3}	<50	<50	-ve	+ve	-ve

-ve = Not detected
+ve =Detected

Table 6- sensory evaluation of fish sausage by taste panel

Fish sausage samples	Appearance	Texture	Flavour	Taste	Juiciness	Palatability
Clarias slazera	9.40 ±0.368^{c}	9.4±0.368^{a}	8.0±0.100 ^{b}	9.3±0.100 ^{a}	7.4±0.100 ^{b}	7.8±0.368 ^{b}
Tetradon fahaka	8.40± 0.368^{b}	8.1±0.368^{c}	7.77±0.153 ^{c}	8.8±0.100 ^{b}	7.4±0.100 ^{b}	7.8±0.368 ^{b}
Clarias lazera+ *Tetradon fahaka*	9.60±0.368^{a}	8.7±0.100^{b}	9.6±0.368 ^{a}	9.3±0.100 ^{a}	7.8±0.368 ^{a}	8.2±0.100 ^{a}

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same column indicate significant differences between groups (p<0.05).