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Free Amino Acids and Fatty Acids Composition of *Kejeik*, A Dry Fish Product of the Sudan

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Abstract *Kejeik* product samples were collected from two different locations in Sudan including Singah city (Blue Nile) and Kusti city (White Nile). The aims of the present study were to investigate the contents of free amino acids and fatty acids in *Kejeik* product. The chemical analysis indicated that *Kejeik* product is a good source of protein, amino acids, and fatty acids. The production area has a non-significant effect in most of the chemical components. The results indicated an increase in glutamic acid, lysine, leucine, alanine, arginine, valine and isoleucine levels, whereas levels of methionine, tyrosine, histidine and serine relatively decreased in *Kejeik* product. It is observed that, the fatty acid content of three types of *Kejeik* was significantly affected by the production area. The study concluded that *Kejeik* has a highly nutritive value which is recommended to be utilized in Sudanese meals especially during shortage of protein and other nutrients sources.

Keywords Fish, Amino acids, Fatty acids, Drying

1. Introduction

Fish is highly nutritious, tasty with easily digested animal protein. About 60 percent of many developing countries population consume fish as a source of animal protein, while almost 80 percent in most developed countries obtain less than 20 percent of their animal protein from fish. However, with the increased awareness of the health benefits of eating fish and the ensuing rise in fish prices, these figures are rapidly changing [1].

Fish products have high nutritional quality which depends on the methods used in preservation and preparation. The protein content of most fish averages 15 to 20 percent. Fish also contains significant amounts of all essential amino acids.

Drying is a method of food preservation that works by removing water from the food, which inhibits the growth of microorganisms. Open air drying using sun and wind has been practiced since ancient times to preserve food. Bacteria, yeasts and molds need the water in the food to grow, and drying effectively prevents them from surviving in the food [2]. Fish are preserved through such traditional methods as drying, smoking and salting. The oldest traditional way of preserving fish was to let the wind and sun dry it [3].

Sudan, has immense fisheries resources within its inland waters especially along the River Nile and in the marine sub-sector along the Red Sea coast. Currently most fish is consumed fresh and there is no formal export of fish or fishery products. Sudan is, however, a significant importer of fish from other areas of the Great Lakes region of Africa. Most experience with aquaculture has been along the coast with pearl culture but increasingly investors are establishing catfish and tilapia pond farms in the southern parts of the country to supply the developing population in this area [4].

Sudan as one of the developing tropical countries is no exception in practicing the traditionally sun drying of fish all over the country. Sudanese people practice a simple fish drying process to produce a dried fish product locally known as “Kejeik”; this method can be considered as a cheaper one and least labor costing. Kejeik is extensively produced in the southern regions of the Sudan by the Nilotic tribes, and to a less extent in some parts of northern Sudan, along the Main Nile, Blue Nile, White Nile and Atbara River [5]. The product has a definite proteolytic flavor. There is ample time, during processing, for fermentation to take place before the water activity of the product finally drops to levels prohibitive to microbial growth. *Kejeik* is used to make mullah (sauce) for dressing aceda porridge, usually made from unfermented *feterita* sorghum flour. The data about the fish industry is scanty in Sudan, and most of the products are produced by traditional methods, as for *Kejeik* product, the nutritive value has not been researched satisfactorily, this initiate the researchers to study nutritive value of *kejeik* product collected from two production areas in Sudan focusing on amino acid composition and fatty acid

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composition.

2. Materials and Methods

2.1. *Kejeik* Preparation

Eighteen processed samples of *Kejeik* product initially made from Garmut (Eelcat fish), Ijl (Nile perch) and Nawk (Armoured fish) fish types were collected from local markets in Singah and Kusti (Central Sudan) during the period January-April 2010. The fish samples had been produced by natural fermentation. In this process the fisher men usually prepare *Kejeik* from fresh water Nile fish, the fish were split longitudinally, gutted and beheaded, the split fish were then hung on ropes or spread on rock or tree branches, out in the open air, under the direct sun. When the drying process was over, the large pieces of fish are stacked together on mats, covered with another set of mats and trodden check on by fishermen, to flatten and pack the dry fish more compactly, further shade drying them follows after which the fish production were ready to be transported by the local markets.

The *Kejeik* samples were collected in clean, dry containers, and were ground, homogenized and packed in sterilized plastic vacuum bags, stored in carton and transported to to the Central Food Laboratory in Qatar pending chemical analyses.

2.2. Determination of Protein and Fat Contents

Protein and fat contents of the various *Kejeik* samples was determined according to AOAC [6] methods using Kejeldahl and soxhlet apparatus, respectively.

2.3. Amino Acid Analysis

Amino acids composition of samples was measured on hydrolysates using amino acids analyzer (Sykam-S433) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein [7]. Two hundred milligrams of sample were taken in hydrolysis tube. Then 5 mL 6N HCl were added to sample into the tube, tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered and 200 µl of the filtrate were evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one milliliter of 0.12 N, pH 2.2 citrate buffer, the same as the amino acid standards (amino acid standards H, Pierce. Inc., Bockford). Aliquot of 150 µL of sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrin solution and an eluent buffer (The buffer system contained solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 mL/min. The buffer/ninhydrin mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as percentages.

2.4. Fatty Acid Content

The preparation of the sample and methylation of the fatty acid were done using a method similar to that of Punstinen *et. al*. [8]. the oil was extracted from samples with petroleum ether (boiling range (40℃, –60℃), the glyceride esters of fatty acids are saponified under mild conditions, esterifies with methanol and the relative percentage of methyl ester was determined by gas chromatography. The c**onditions of instruments were as follows:**

Instrument:	Gas chromatography
Detector:	Flame ionization detector (FID)
Column:	Agilnt-122-2362-DB-23.
Column rate:	0.8ml/mint
Column temperature:	150℃ for 3 mints then rose to 300℃ at 5 ℃/min
Detector temperature:	250℃
Injector temperature:	250℃
Carrier gas:	Nitrogen flow rate 30ml/min
Flow rate of H2:	35 ml/min
Flow rate of air:	350ml/min
Run time:	38 mint
Column capillary:	60.0 meter x 250 µm x0.25µm.nominal

Fatty acid methyl ester standards from Sigma, St.Louis USA were used for comparison. The results were expressed as g/100g of oil.

3. Results and Discussion

3.1. Amino Acid Composition

The present study aimed at the determination of nutritive value of *Kejeik* Samples (dried fish product) collected from two different areas of the Sudan known with their high productivity of this product, with emphasis on the amino acid composition and fatty acid composition.

The protein and amino acids (essential and non – essential amino acid) content in *Kejeik* samples collected from Singah city (Blue Nile) are summarized in Table (1). The content of protein of *Kejeik* samples prepared from Ijl, Garmout and Nawk fish was 78.06%, 67.01% and 74.96%, respectively. However, the contents of essential amino acids (mg/100g) threonine, histidine, valine, methionine, isoleucine, leucine, phenylanine and lysine, ranged in (mg/100g) between 1803.09-1919.1, 1168.76-1416.44, 2968.15-3272.47, 1290.89-1481.79, 2817.14-2948.77, 4215.69-4491.669, 2070.18-2506.53, 4431.29-4587.03. On the other hand, the non-essential amino acids (mg/100g) aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, tyrosine, and arginine, varied considerably and ranged between, 3142.89-3718.16, 480.62-581.67, 4997.69-6255.25, 2717.10-3222.39, 3406.24-3769.69, 577.52-676.76, 1279.51-1435.42 and 3271.67-3543.52, respectively. Then proline ranged between 2392.82-3078 (mg/100g).

Table 1. Protein content (%) and amino acid composition (mg/100g) of *Kejeik* samples Collected from Singah city (Blue Nile)

Amino acid Content	*Kejeik* samples		
	Ijl	Garmut	Nawk
Protein (%)	78.06	67.01	74.96
Aspartic acid	3718.165	3526.910	3142.898
Threonine	1919.408	1828.549	1803.090
Serine	581.672	560.831	480.616
Glutamic Acid	6255.252	5656.379	4997.694
Proline	3078.976	2392.820	2652.107
Glycine	3222.393	2717.104	2903.279
Alanine	3722.664	3406.241	3769.690
Cystine	585.379	577.523	676.762
Valine	3272.478	2968.157	3223.600
Methionine	1481.792	1365.010	1290.893
Isolucine	2948.777	2817.140	2909.489
Leucine	4491.669	4215.698	4294.728
Tyrosine	1414.431	1279.515	1435.420
Phenylalanine	2506.535	2070.183	2401.310
Histidine	1416.443	1168.767	1320.203
Lysine	4587.038	4431.297	4371.414
Ammonia	2716.670	2903.484	2797.584
Arginine	3390.519	3271.671	3543.523
Total	51310.261	47157.279	48014.300

Table 2. Protein content (%) and amino acid composition (mg/100g) of *Kejeik* samples Collected from Kusti city (White Nile)

Amino acid content	*Kejeik* samples		
	Ijl	Garmut	Nawk
Protein (%)	75.71	63.52	68.28
Aspartic Acid	3107.439	2702.831	3015.108
Threonine	1493.156	1551.013	1652.482
Serine	378.898	441.175	469.402
Glutamic Acid	5587.405	4317.762	5011.308
Proline	3192.141	2080.505	3014.807
Glycine	3321.961	2499.676	3126.571
Alanine	3624.463	3262.249	3470.492
Cystine	520.765	446.643	558.334
Valine	2802.834	2669.718	2799.066
Methionine	1113.151	1072.481	1075.166
Isolucine	2559.726	2481.855	2631.669
Leucine	3660.942	3792.653	3927.328
Tyrosine	1142.668	1076.273	1045.980
Phenylalanine	1895.215	1849.032	2125.889
Histidine	1087.476	999.055	1300.924
Lysine	3821.713	3824.606	3870.895
Ammonia	2755.809	2672.939	2781.271
Arginine	3169.974	2684.536	3269.772
Total	45235.736	40425.002	45146.464

Table (2) summarizes the protein content and amino acids content in *Kejei*k samples collected from Kusti city (White Nile). The protein content of Ijl *Kejei*k, Garmout *Kejeik* and Nawk *Kejei*k was 75.71%, 63.52% and 68.28%, respectively. The highest protein content of *Kejeik* samples was found in Ijl *Kejeik* which collected from Singah city (Blue Nile) (78.06%), and the lowest value (63.52%) was found in Garmut *Kejeik* collected from Kusti city (White Nile). However, the contents of essential amino acids (mg/100g), threonine, histidine, valine, methionine, isoleucine, leucine, phenylanine and lysine, ranged between, 1493.15-1652.48, 999.05-1300.92, 2669.71-2802.83, 1072.48-1113.15, 2481.85-2631.66, 3660.94-3927.32, 1849.03-2125.88 and 3821.71-3870.89, respectively. On the other hand, the non-essential amino acids (mg/100g) aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, tyrosine, and arginine, varied considerably and ranged between, 2702.83-3107.43, 378.89-469.40, 4317.76-5587.40, 2499.67-3321.96, 3262.24-3624.46, 446.64-558.33, 1045.98-1142.66 and 2684.53-3269.77, respectively. Then proline ranged 2080.50-3192.14. The contents of essential amino acids (mg/100g), threonine, histidine, valine, methionine, isoleucine, leucine,phenylanine, lysine, ranged between, 1493.15-1652.48, 999.05-1300.92, 2669.71-2802.83, 1072.48-1113.15, 2481.85-2631.66, 3660.94-3927.32, 1045.98-1142.66, 3821.71-3870.89, respectively. On the other hand, the non-essential amino acids (mg/100g) aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, tyrosine, and arginine, varied considerably and ranged between, 2702.83-3107.43, 378.89-469.40, 4317.76-5587.40, 2499.67-3321.96, 3262.24-3470.49, 446.64-558.33, 1849.03-2125.88 and 2684.53-3269.77, respectively. Then proline ranged 2080.50-3192.14. All Kejeik samples contained high amounts of glutamic acid, lysine, leucine, alanine and aspartic acid. The comparison of amino acid composition of Kejeik samples between different fish species showed that lysine was the most abundant essential amino acid in the study.

The world health organization recommended leucine and isoleucine requirements for adults of 14 and 19 mg amino acid/kg body weight per day [9]. Minimum leucine level was determined in ijl *Kejeik* at 4491.66 mg/100g and isoleucine level was determined in ijl *Kejeik* at 2948.77mg/100g. So for an adult a few amount of ijl kjeik can provide the daily requirement.

The essential amino acid content of the *Kejeik* samples in the present study was above the [10] requirements for essential amino acids for adults met the (FAO, 1985) requirements for threonine, valine, isoleucine, leucine, tryptophan and, more importantly, lysine (a limiting amino acid in cereals and flours) for children [11]. Various studies have reported that, in developing countries, lysine intake is below the requirements for children. Therefore, the development of products that are rich in lysine, such as *Kejeik* might be a successful strategy for providing healthy, low-calorie food products containing high biological value protein.

The comparison of amino acid composition of *Kejeik* samples showed an increase in glutamic acid, lysine, leucine, alanine, arginine, valine and isoleucine levels, whereas levels of methionine, tyrosine, histidine and serine decreased in all products. This reduction might have occurred during ensiling due to chemical reactions between _ amino and aldehyde groups (Sugars) present in amino acids [12].

According to Strom and Eggum [13], lysine, cystine, and methionine are the most important amino acids for fish from a nutritional viewpoint. From the results presented, all the *Kejeik* products satisfy amino acid requirements according NRC [14] standards. Furthermore, when considering amino acid limiting level, the 30% minimum requirement in fish diets rather than FAO standards, this study shows that all *Kejeik* products fulfilled this requirement [15].

Finally, results suggest that *Kejeik*, in spite of minor deficiencies in certain essential amino acids, do not lose their nutritional value. This fact is even more important if *Kejeik* are to be considered as an ingredient in balanced diets. The amino acid composition of dried fish *Kejeik* may be nutritionally important especially in regions where dried fish serves as a significant source of dietary protein.

Amino acids, the building blocks of protein molecules, make up the largest reservoir of organic nitrogen in most organisms. Amino acids 'have been one of the more frequently studied classes of organic compounds in the marine environment. These compounds account for about one quarter of the particulate organic carbon and half of the particulate organic nitrogen in surface waters. After sinking to the sea floor, particulate amino acids can provide energy and nitrogen to benthic organisms. However, since they are more labile than other carbon compounds present in particulate matter, they become an increasingly smaller percentage of total particulate organic carbon with depth in the water column. In sediments the depositional environment has a strong influence over the relative contribution of proteinaceous material. Amino acids can comprise from less than 1% to as much as 50% of the organic carbon, and 10to 100% of the organic nitrogen; these proportions depend on the source of the organic matter, the depositional rate, the depth in the sediment and other factors [16].

3.2. Fatty Acid Content

The fat content (%) and fatty acid composition of *Kejeik* samples collected from Singah city (Blue Nile) are summarized in Table (3). The fat content of Ijl *Kejei*k, Garmout *Kejeik* and Nawk *Kejei*k was 9.09%, 16.13% and 11.56%, respectively. The content of fatty acids: myristic (C14:0), palmetic (C16:0), palmpitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) varied considerably and ranged between, 1.35-2.5%, 17.51-23.03%, 4.98-5.56%, 8.47-9.61%, 10.84-18.29%, 7.26-9.71%, 2.04-4.46% and 0.86-1.43%, respectively. On the other hand the chemical composition of *Kejei*k samples collected from Kusti city (White Nile) is summarized in Table (4). The content of myristic (C14:0) Palmetic (C16:0), palmpitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolinic (C18:3) and arachidic (C20:0) varied considerably and ranged between, 1.47-2.93%, 22.42-25.11%, 4.8-7.89%, 7.57-9.14%, 18.37-23.39%, 6.41-8.79%, 1.71-3.20%, and 0.69-0.90%. Palmitic acid (C16:0) was the dominant saturated fatty acid in all *Kejeik* samples. Predominance C16:0, C18:1 C18:2,C16:1,C18:3,C14:0 and C20:0 fatty acids in *Kejeik* samples of maybe attributed to the fish diets, the highest fatty acid content was found in Ijl *Kejeik* which collected from Kusti (White Nile) C16:0-25.11%, C18:1-18.37%, C18:0-9.14%, C16:1-6.91%, C14:0-2.93%, C18:3-2.62% and C20:0-0.69% while the lowest value was found in Nawk Kejeik which collected from Singah city (Blue Nile), C16:0-17.51%, C18:1-10.84%, C18:0-9.61%, C16:1-5.55%, C18:3-2.04%, C14:0-1.98%, and C20:0-0.86%.

Fatty acid compositions of fish lipid was highly dependent on a number of factors, specially fish diets [17] [18] [19]. Major fatty acids of *Kejeik* sample were C16:0, C18:1, C18:2, C18:0, C16:1. C18:3, C14:0 and C20:0.The result shows that palmtic acid was very high. This observation was typical because palmtic acid is the key metabolite in fish.

The results of fatty acid content in the present study were higher with the results reported by Lu *et. al*.) [20]. They reported that the major saturated and unsaturated fatty acids of salted mullet roe were C16:0, C16:1 and C18:1, respectively. Palmitic acid contents of raw and beeswaxed caviar oils were most similar each other, 5.9% and 6.7% respectively. But C16:1 and C16:2were found at significantly lower levels in beeswaxed caviar oil than in raw caviar oil, whereas C18:4 showed the opposite trend. In addition, the results indicated that there were significant differences in fatty acid composition of *Kejeik* products obtained from Blue Nile and White Nile rivers (Singah and Kusti). It is observed that, the fatty acid content of three types of fish was affected by the different areas.

Table 3. Fatty acid content of *Kejeik* samples (dried fish product) collected from Singah city (Blue Nile) (Mean + STDEV)

	Type of fatty acid							
Kejeik sample	C14:0*	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Ijl	2.50±0.08	23.03±2.15	5.56±0.12	9.12±0.39	12.86±0.05	9.71±0.36	4.46±0.11	0.9±0.02
Garmut	1.35±0.05	21.98±0.48	4.98±0.20	8.47±0.13	18.29±0.35	7.85±0.25	2.70±0.14	1.43±0.03
Nawk	1.98±0.02	17.51±0.19	5.55±0.02	9.61±0.08	10.84±0.17	7.26±0.11	2.04±0.01	0.86±0.008

* Tetradecdnoic (MYRISTIC) - (C14:0);
Heptadecanoic (Palmetic)-(C16:0)
Heptadecenoic (Palmpitoleic)-(C16:1)
Octa Decanoic (Stearic)-(C18:0)
Octa Decenoic (OLEIC)-(C18:1)
Octa Decenoic (Linoleic)-(C18:2)
Octa Decenoic (Linolinic)-(C18:3)
Arachidic-(C20:0)

Table 4. Fatty acid content of Kejeik samples (dried fish product) collected from Kusti city (White Nile) (Mean + STDEV)

	Type of fatty acid							
Kejeik sample	C14:0*	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Ijl	2.93±0.06	25.11±0.68	6.91±0.17	9.14±0.28	18.37±0.69	6.41±0.08	2.62±0.05	0.69±0.03
Garmut	1.47±0.08	24.41±0.74	4.80±0.17	8.35±0.18	21.50±0.14	8.79±0.20	3.20±0.16	0.79±0.02
Nawk	2.03±0.01	22.42±0.19	7.89±0.02	7.57±0.05	23.39±0.19	8.05±0.12	1.71±0.02	0.90±0.001

* Tetradecdnoic (MYRISTIC) - (C14:0); Octa Decenoic (OLEIC)-(C18:1)
Heptadecanoic (Palmetic)-(C16:0) Octa Decenoic (Linoleic)-(C18:2)
Heptadecenoic (Palmpitoleic)-(C16:1) Octa Decenoic (Linolinic)-(C18:3)
Octa Decanoic (Stearic)-(C18:0) Arachidic-(C20:0)

For the fatty acid comparison between the Blue Nile versus the White Nile, the T-Test indicated a significant difference with P-value 0.000 in favored of the White Nile. On the other hand, the statistical analysis for three fish types in which ANOVA was used, shows that the comparison of fatty acid between (Ijl, Garmut and Nawk) is significantly different with a p-value 0.00. The Post Hock test ordered all *Kejeik* samples as following from higher to lower, C16:1, C18:1, C18:0, C18:2, C14:0 and C20:0.

Essential fatty acids are molecules that cannot be synthesized by the human body but are vital for normal metabolism. The lipid in fish muscle can influence product quality through interaction with other components and through degradative changes after death. These changes are lipolysis and auto-oxidative deterioration of unsaturated fatty acids, resulting in product deterioration and undesirable aroma and flavors [21]. The effect produced by these changes is a major problem in the frozen storage of some fish species. Therefore, lipid composition of fish and fish products is of practical importance, particularly in relation to the effects of lipid components on deterioration during frozen storage and consumer acceptance. Detailed information about lipid components and their fatty acids constituents is needed to understand how to diminish oxidative or hydrolytic factors which affect quality of fish. Also, fatty acids composition is the surest method of determining the selectivity of a hydrogenation reaction since fatty acids profile will aid in determining oils suitable for the production of solid fats for industrial.

4. Conclusions

The present study aimed to determine the contents of amino acids and fatty acids of Kejeik (a traditionally dried fish product) prepared in rural areas in many parts of Blue Nile and White Nile basins, and consumed by many people in these areas and other parts of Sudan. Kejeik samples contained of good quality proteins which contained both essential and non-essential amino acids in appreciable amounts. The product also contained saturated and non-saturated fatty acids in good amounts. It is highly recommended to utilize *Kejeik* in Sudanese meals especially during shortage of protein and other nutrients sources Effort from governments and applied research institutions could save some of the fish losses inflicted by such biological agents on *Kejei*k as a vital protein source.

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