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CHARACTERISATION OF FISH OILS OF MUKENE (RASTRINEOBOLA ARGENTAE) OF NILE BASIN WATERS – LAKE VICTORIA, LAKE KYOGA AND THE VICTORIA NILE RIVER.

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ABSTRACT

*Mukene (**Rastrineobola argentea**) is a freshwater tiny cyprinid endemic to Lake Victoria, Lake Kyoga and the Victoria Nile River. It is the third most important commercial fishery of Uganda after **Lates niloticus** and **Oreochromis niloticus**. The study investigated the fatty acid content of the fishmeal of whole fish of **R. argentea** and for differences in the fatty acid profile of the samples from the different water bodies. Fatty acid composition of fishmeal obtained from samples collected from the two lakes and the Victoria Nile River was determined using chemometric method for five fish samples from each location and subjected to multivariate analysis. In each fish the fatty acids detected ranged from 14 to 24 carbons. Palmitic (16:00), stearic (18:00), oleic (18:1n9), arachidonic (20:4n6) and docosahexaenoic (22:6n3) were the principal fatty acids in all sampled fish. The fatty acid composition showed that **R. argentea** contain essential unsaturated fatty acids, Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA). A 2-way ANOVA showed that there were no significant differences at 95% level between the three samples from the three connected water bodies, therefore fatty acid profiles are probably not suitable for use as a chemotaxonomic tool for differentiating mukene stocks. This probably due to lack of physical barriers between the three connected water bodies, that allows for unlimited genetic exchange among the populations of **R. argentea** in the three water bodies.*

Keywords: Mukene, fatty acids profiles, Nile basin waters, chemotaxonomy

INTRODUCTION

Rastrineobola argentae locally known as 'mukene' are freshwater tiny cyprinid fish endemic to Lake Victoria, Lake Kyoga and Victoria Nile River, mostly found in the littoral and sub-littoral waters (Greenwood, 1966). Adults stay at the bottom during day and move to the surface at night to feed (Wanink, 1988). *R. argentae* feed on zooplankton, surface insects, chironomids, and the prawns *Caridina nilotica* (Corbet, 1961; Greenwood, 1966; Wanink, 1988). Mukene grow to a standard length ranging between 35mm to 55mm at 100% maturity (Okedi, 1974; Wanink, 1988; Wandera, 1990). Together with Nile perch (*Lates niloticus)* and Nile tilapia (*Oreochromis niloticus*), mukene form the three major commercial fisheries of Lake Victoria and Lake Kyoga. Mukene is used for both human and animal nutrition, in either case it is crushed whole and eaten or mixed in animal feed as the source of proteins and rarely considered as a source of fatty acids.

Fatty acids (FAs) are long straight-chain carboxylic acids, some are saturated (do not contain double bonds) whereas others are unsaturated (contain one or more double bonds). Fatty acids with more than one double bond are referred to as polyunsaturated fatty acids (PUFA). There are more than 20 fatty acids found in food including fish (Paul, 2004). Fatty acids play two major roles in biological systems, that is, acting as fuel molecules and provision of building blocks for phospholipids and glycolipids both very important components of biological membranes, but they have also been strongly associated with health development, fight and prevention against a number of ailments, both physical and mental diseases (Paul, 2004; Sjurour and Secher, 2002; Gustafsson *et al.,* 1992; Leaf and Weber, 1988). Similarly it has been established that docosahexaenoic acid (DHA) a fatty acid associated with fish oils is vital for the proper development of an infant's brain and retina (Natasa *et al.,* 2000). Human infants require an adequate supply of omega-3 and omega-6 long-chain polyunsaturated fatty acids for optimal growth and neural development (Carlson, 1999). FAs eicosapentanoic acid (EPA) [20:5(n-3)] and DHA [22:6(n-3)] offer extraordinary health benefits in treating chronic diseases, improving athletic performance and enhancing emotional well being in humans by helping in reduction of inflammation and increasing blood flow throughout the all body. Arachidonic acid [20:4(n-6)] and docosahexaenoic acid [22:6(n-3)] are important to normal neurodevelopment and visual function (Innis *et al.,* 1995). But while Omega-3 can be found in some foods like flaxseed and walnuts, the most beneficial form of Omega-3 containing 2 FAs - DHA and EPA that are essential in fighting both physical and mental diseases are mostly found in fish. The misconception has been that freshwater fish do not contain these essential fatty acids and that they are only found in marine products or saltwater fish. The fact is that various fats from both saltwater and freshwater fish contain Omega-3 fatty acids, Omega-6 fatty acids, monounsaturated fatty acids, all of which are required in our diets (Wang *et al.,* 1990); it's only the amounts that vary from one fish species to another. *R. argentea* are warm freshwater fish, which have been taken to lack essential fatty acids, establishing their fatty acids profile will not only dispel this myth but also determine their nutrition value.

Also for good fishery management, it is important that stocks are identified so as to have a sustainable harvest of a wild species like mukene, because fish abundances defer from place to place (Sinclair, 1988). The stocks of mukene in their natural habitats are not defined, this investigation attempted to use chemotaxonomy to identify these different stocks like Grahl-Nielsen and Ulvund (1990) used fatty acid profile of the heart tissue to identify different stocks of Atlantic herring in north European waters.

Therefore the aim of this study was to establish the fatty acid profile of whole crushed mukene fishmeal so as to determine its nutrition value and to determine spatial differences in the fatty acid profiles of *R. argentea* from the different hitherto connected water bodies.

In this investigation we used the chemometric method of determining fatty acid composition of animal tissue (Barnung and Grahl-Nielsen, 1987; Ulvund and Grahl-Nielsen, 1988), which involves direct methanolysis of the tissue, yielding methyl esters of the total fatty acids present. The methyl esters are then gas chromatographed and the outcome analyzed using a multivariate statistical method by which a combined influence of all the fatty acids on similarities can be expressed quantitatively. Multivariate analysis of the data assists investigation of all the variables under play to bring out their relationships (Esbensen, 1994).

STUDY AREA

Samples were picked from landed catches got from Lake Victoria, Lake Kyoga, and the Victoria Nile River, all waters in which *R.argentea* is endemic. Lake Victoria is located south of Uganda whereas Lake Kyoga is located in central Uganda; the Victoria Nile River connects the two lakes Victoria and Kyoga. In lake Victoria the samples were caught offshore Ssanga Island, Kibalama landing site of Mukono district, in lake Kyoga samples were caught offshore Bukungu landing site in Kamuli district, whereas in the Victoria Nile river samples were caught offshore Mbulamuti landing site in Kamuli district.

MATERIALS AND METHODS

Sampling: Samples were bought fresh as catch were landed ashore in the early morning and immediately placed in a cooler on ice chips and then immediately covered by ice and transported to the laboratory. Only mature fish were picked, estimated at 40mm and above in standard length. In the laboratory before analysis samples were kept under refrigeration at -20°C.

Laboratory analysis: Whole fish sample were crushed and mixed thoroughly to obtain a homogenous mixture using pestle and motor. A portion (40mg) of the obtained fishmeal was then placed into a test tube fitted with a Teflon lined lid. 1000µl of acidified methanol (2M HCl) was immediately added to the test tube and its lid tightened. This procedure was done for five fish picked randomly from each of the three samples of mukene from the three different water bodies or sample sites.



Fig. 1: Map of Uganda showing the water bodies - L. Victoria, L. Kyoga and the Victoria Nile from where the analysed mukene fish samples were collected.

The samples in the test tubes were then placed into an oven at 100 ^{o}C, after 20 minutes the lids were further tightened and esterification allowed to proceed for 2 hours. Samples were then removed from the oven and allowed to cool in the open at room temperature. Dry nitrogen gas was then blown into the samples until volume of each test tube reduced to about half the original volume. 500µl of distilled deionised water was then added to each sample followed by 1000µl of hexane. The samples were then shaken using the wrist shaker for 2 minutes and there after centrifuged at high speed for 2 minutes. The upper organic layer was picked from each test tube and a second extraction done by repeating part of the above procedure starting with the addition of the deionised water. 1µl of the methyl esters so formed was run through a Parkin Elmer gas chromatograph (9700 series) with a flame ionization detector (FID) with pure hydrogen gas as the carrier gas. A carboxywax packed column, 25mm, 50m long was used. The defined operation parameters used were - injection temperature was 90°C, kept

isothermal for 4 minutes. I t was then increased at rate of 30°C per minute to165 ^{o}C. The rate was then changed to 3 ^{o}C per minute to 225 ^{o}C and held isothermal for 3.5 minutes. The signals obtained by the FID were passed onto an interface connecting to a computer and the results were analysed using a Turbochrom program. This program gives the results of the principal components as a percentage of the total available fatty acids. The fatty acid principal components were identified from a chromatogram using peaks of a standard as the reference. This method though it reduces the work-up procedure before gas chromatography it has one draw back that it is only the total fatty acid composition that can be estimated (Otto and Olav, 1992)

RESULTS

A total of 20 fatty acids were found in all the fish samples from all 3 sampling sites. The methyl esters derived from the fatty acids are summarized in Table 1 and 2. In each fish the fatty acids detected ranged from 14 to 24 carbons. Palmitic (16:00), stearic (18:00), oleic (18:1n9), arachidonic (20:4n6) and docosahexaenoic (22:6n3) were the principal fatty acids in all sampled fish, comprising 74% of the total peak areas of the 15 fish sampled from the three different locations. In all the three samples that is Victoria Nile, L. Victoria, and L. Kyoga samples the total unsaturated fatty acids exceeded the total saturated fatty acids (Table 3) and the quantities for the two types of fatty acids did not vary much in the three different samples. Omega-3, Omega-6, and the monounsaturated fatty acids, which are important in our diets, were found in all the samples in significant amounts (Table 3), that is, an overall average of about 15% and 22% of the fish oil in mukene, although the ratio of N-3 (Omega-3) to N-6 (Omega-6) was quite small, less than 1.0 in all three samples, far from the recommended 5:1 (Paul, 2004). The two fatty acids EPA and DHA, which offer extraordinary health benefits, were detected in the fish samples, with DHA being one of the five principal fatty acids (Table 1 and **2**). The $p>0.05$, 2-way ANOVA computations on the pattern of fatty acids showed that there were no significant differences among the three samples VNS, VS, and KS. At $F\alpha=0.05$ (df = 38,240), there was no interaction between location from which sample was gotten, type of fatty acid and the quantities of the fatty acids. Any differences in the quantities in the fatty acids could be due to other factors especially environmental rather than genetic. The patterns of fatty acids in all the fish were quite similar.

TABLE 1

Fatty acid profiles of sampled mukene fish showing Methyl Fatty Acid Esters (FAME) composition in percentages, VNS stands for Victoria Nile sample, VS Lake Victoria sample and KS Lake Kyoga sample.
1-5 stand for the fish number:

sample	14:00	14:1n5	16:00	16:1n7	18:00	18:1n9	18:1n7	18:2n6	18:3n3	20:00
VNS1	1.95	0.25	19.58	1.62	17.4	9.14	5.58	5.43	2.02	0.51
VNS2	1.29	0.16	27.29	1.28	11.77	6.64	2.65	3.17	1.66	1.61
VNS3	1.86	0.16	33.7	1.74	13.73	5.74	3.27	3.7	1.98	0.31
VNS4	3.39	0.29	24.78	3.23	15.38	9.19	6.42	5.85	2.44	0.17
VNS5	1.92	0.14	33.21	2.21	13.9	6.96	3.06	4.89	2.88	0.13
VS1	1.95	0.2	32.49	2.18	14.43	7.08	3.16	4.91	2.82	0.16
VS2	2.11	0.21	22.33	1.96	17.16	8.28	6.71	4.69	2.9	0.11
VS3	1.24	0.41	32.12	1.91	14	7.37	4.29	4.84	3.83	0.27
VS4	2.91	0.12	37.88	3.79	12.43	8.36	4.11	4.94	3.22	0.29
VS5	2.66	0.09	23.18	2.39	15.95	9.94	6.22	7.77	2.01	0.06
KS1	4.97	0.3	31.83	6.75	9.81	11.58	4.46	6.28	3.15	0.47
KS2	2.65	0.12	27.59	4.9	11.97	12.46	4.87	6.86	3.17	0.6
KS3	1.47	0.16	21.4	1.79	17.38	8.26	6.23	5.76	2.15	0.26
KS4	1.41	0.14	33.11	2.38	13.87	7.18	4.61	5.6	3.11	0.3
KS5	1	0.13	28.81	1.78	14.11	6.44	4.39	4.76	2.6	0.36
	32.78	2.88	429.30	39.91	213.29	124.62	70.03	79.45	39.94	5.61

Table 1 cont'd

sample	20:1n9	20:2n6	20:4n6	20:3n3	20:5n3	22:00	22:1n9	24:00	22:6n3	24:1n9
VNS1	0.2	0.73	25.72	0.73	1.01	0.85	0.09	0.26	6.55	0.37
VNS2	3.69	11.15	11.23	1	3.57	0.75	0.28	0.34	12.21	0.25
VNS3	0.28	0.61	12.37	0.83	3.55	0.48	0.22	0.71	14.24	0.51
VNS4	0.47	1.11	21.87	0.61	1.22	0.54	0.06	0.17	2.57	0.25
VNS5	0.61	1.78	13.89	0.84	3.84	0.27	0.17	0.2	8.59	0.51
VS1	0.83	1.38	13.91	0.93	3.58	0.63		0.22	8.68	0.44
VS2	0.32	0.73	23.14	1.03	2.07	0.94	0.08	0.21	4.75	0.27
VS3	1.47	4.17	8.77	1.16	4.2	0.44	0.1	0.3	8.88	0.23
VS4	0.21	0.58	7.5	0.91	3.63	0.27	0.11	0.32	8.19	0.22
VS5	0.04	0.72	23.13	0.45	1.2	0.61	0.05	0.19	3.2	0.15
KS1	0.09	3.78	6.71	0.59	2.27	0.43	0.29	0.2	5.83	0.21
KS2	0.09	1.72	8.91	0.81	3.4	0.38	0.08	0.21	8.91	0.3
KS3	0.2	1.03	23.77	0.83	2.36	0.8	0.12	0.24	5.62	0.16
KS4	0.24	0.86	9.06	0.93	5.9	0.52	0.14	0.23	10.25	0.18
KS5	0.29	0.84	10.94	1.07	6.42	0.43	0.17	0.44	14.82	0.21
	9.03	31.19	220.92	12.72	48.22	8.34	1.96	4.24	123.29	4.26

TABLE 2

Average amounts of FAME composition in percentage of the fish from each location. VNS-A denotes Victoria Nile average, VS-A denotes Lake Victoria average and KS-A the Lake Kyoga average. VNS-T, VS-T, and KS-T the total percentages

Sample	14:00	14:1n5	16:00	16:1n7	18:00	18:1n9	18:1n7	18:2n6	18:3n3	20:00
VNS-T	10.41	1.00	138.56	10.08	72.18	37.67	20.98	23.04	10.98	2.73
VNS-A	2.08	0.20	27.71	2.02	14.44	7.53	4.20	4.61	2.20	0.55
VS-T	10.87	1.03	148.00	12.23	73.97	41.03	24.49	27.15	14.78	0.89
VS-A	2.17	0.21	29.60	2.45	14.79	8.21	4.90	5.43	2.96	0.18
KS-T	11.50	0.85	142.74	17.60	67.14	45.92	24.56	29.26	14.18	1.99
KS-A	2.30	0.17	28.55	3.52	13.43	9.18	4.91	5.85	2.84	0.40

TABLE 2 cont'd

Sample	20:1n9	20:2n6	20:4n6	20:3n3	20:5n3	22:00	22:1n9	24:00	22:6n3	24:1n9
VNS-T	5.25	15.38	85.08	4.01	13.19	2.89	0.82	1.68	44.16	1.89
VNS-A	1.05	3.08	17.02	0.80	2.64	0.58	0.16	0.34	8.83	0.38
VS-T	2.87	7.58	76.45	4.48	14.68	2.89	0.34	1.24	33.70	1.31
VS-A	0.57	1.52	15.29	0.90	2.94	0.58	0.07	0.25	6.74	0.26
KS-T	0.91	8.23	59.39	4.23	20.35	2.56	0.80	1.32	45.43	1.06
KS-A	0.18	1.65	11.88	0.85	4.07	0.51	0.16	0.26	9.09	0.21

TABLE 3

Showing the average total percentages of the different classes of fatty acids - A comparison of the saturated-, monounsaturated-, and the polyunsaturated fatty acids from the different water bodies: VNS, VS and KS

Type of fatty acids	VNS %	VS %	KS %	Overall Average %
Saturated fatty acids	45.70	47.57	45.45	46.24
Unsaturated fatty acids	54.72	52.45	54.56	53.91
Monounsaturated fatty acids (MUFA)	15.54	16.67	18.33	16.85
Polyunsaturated fatty acids(PUFA)	39.18	35.78	36.23	36.85
PUFA N-3	14.47	13.54	16.85	14.95
PUFA N-6	24.71	22.24	19.38	22.11
N-3 to N-6 ratio	0.56	0.61	0.87	0.68

DISCUSSION

The fatty acid profile of mukene (Table 1) showed that this fish contains essential fatty acids, with a higher percentage of the unsaturated than the saturated fatty acids at overall average percentages of 53.91% and 46.24% respectively. The unsaturated fatty acids are both mono- and polyunsaturated (Table 3), with the polyunsaturated fatty acids (36.85%) being more than the monounsaturated fatty acids (16.85%), which makes mukene a nutritious fish. Further more the polyunsaturated fatty acids include the highly regarded EPA and DHA fatty acids (Carlson, 1999; Makrides *et al.,* 2000), though EPA is in smaller quantities, a fact which agrees with earlier studies (Speers-Roesch *et al.,* 2007, Razak *et al.,* 2001, Endinkeau and Tan Kim 1993) that tropical fishes have more arachidonic acid (AA) and DHA than EPA, which is further supported by the fact that AA and DHA, but not EPA, were among the principal fatty acid after the multivariate analysis (Table 1 and 2). The whole fishmeal of *R. argentea* contains not only proteins (Munguti *et al.*, 2006) but also the essential FAs, though the different FAs differ in proportions to each other compared to the temperate and marine fishes. The findings of this study also agree with earlier studies by Razak *et al.* (2001), who studied the fatty acid profiles of another tropical fish – the Monopterus albus endemic to Malaysia, which they found that it also contained a good quantity of essential fatty acids, with the body sponified oil containing AA and DHA at 10.17% and 7.16% respectively and Zenebe *et al.* (1998) who studied the fatty acid profiles of a number of fishes from the lakes of the Ethiopian lift valley, found that tropical fishes were comparable to the temperate fishes as sources of polyunsaturated fatty acids. Mukene a fish of great interest regionally having managed to flourish in the presence of the introduced predatory Nile perch to the point where a large fishery was developed (Witte *et al.,* 1992) and it is now the third most important commercial fishery in Uganda currently used for both human consumption and as an essential ingredient in animal feed manufacture, the findings of this study make it even more important, for it is not only a source of cheap animal protein, but also cheap essential fatty acids.

A 2-way ANOVA showed that there were no significant differences at 95% level between the three samples from the three connected water bodies, therefore fatty acid profiles are probably not suitable for use as a chemotaxonomic tool for differentiating mukene stocks. The lack of significant differences could also probably be due to a number of factors including fish movements although these fish are not known to be migratory, the movement of larvae and floating eggs (Graham, 1929) by water currents may lead to genetic exchange between the fish of the three water bodies.

Our findings indicate that there are 20 different amino acids in the oils of *R. argentea* (mukene); some are saturated, mono- and polyunsaturated fatty acids, including Omega-3 and Omega-6 fatty acids. The source of mukene, that is, whether it is from Lake Victoria, the Victoria Nile River, or Lake Kyoga in East Africa, most probably does not have any influence on the type and quantity of fatty acids in the oils of *R. argentea*. Basing on our findings above, we argue the riparian population around these three water bodies not to under look and despise

this very important and nutritious resource of mukene because of its small size, but instead to strongly rely on it for supply of essential fatty acids. We recommend further studies into how these essential fatty acids could be extracted refined, concentrated and packaged.

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