مجلة
كلية التربية
جامعة الخرطوم
العدد السادس عشر
السنة الثانية عشرة
سبتمبر 2020م

Determination of protein structures, content, moisture content and lead amount in eight commercial Nile fish in Khartoum state - Sudan.

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Abstract:

Eight species of commercial Nile fish were studied to determine the protein, secondary protein, lead amount and moisture content. The study employed ordinary laboratory equipments together with Fourier Transformation Infrared (FTIR) and Atomic Absorption Spectroscopy (AAS). The results obtained showed that the protein content was in the range 19.7 to 51.3 %, lead amount in the range of 0.08 to 0.8 ppm, and the moisture content 16.3 to 24.5 %. The structures showed that the

eight samples of α-helix, β- parallel, β- antiparallel and unordered forms all exist in the Nile fish. It is concluded that the Nile fish is good nutritive, in a safe aquatic biota.

Key words: Nile fish, protein, secondary protein, lead and moisture.

مستخلص

شملت الدراسة ثماني عينات من أسماك النيل لتحديد محتوى البروتين ، البروتين الثانوي، الرصاص و محتوى الرطوبة. إن هيكلة البروتين و كمياته مهمة للغذاء كما امتدت الدراسة لتشمل توقع المخاطر التي تنتج من السلاسل المتراكبة لتنظيم البروتين و التي تختص بالمشكلات الصحية للإنسان و الحيوان و الدواجن .استخدمت الدراسة الأجهزة المختبرية الزجاجية كما استخدمت تقنية جهاز الأشعة تحت الحمراء على نظام فورير و جهاز المطيافية الإمتصاصية الذرية. و قد توصلت الدراسة للنتائج التالية: المحتوى البروتيني للعينات في المدى 19.7– 51.3%، الرصاص في المدى0.08 - 0.8 جزء من المليون (و هو نسبة صغيرة جداً لا تكاد تذكر) و محتوى الرطوبة16.3– 24.5% . أثبتت الدراسة أن العينات بها حلزون ألفا و متوازي بيتا و متوازي عكسي بيتا و النوع غير المنتظم و أن الأسماك النيلية هذه جيدة للغذاء و في بيئة مائية إحيائية آمنة

الكلمات المفتاحية: أسماك النيل، البروتين، البروتين الثانوي، الرصاص، و الرطوبة.

1. Introduction:

Proteins are the most versatile macromolecules in living systems and serve crucial functions in all essential biological processes (Jeremy, 2002).They also play many roles in foods, the most important one being nutritional (Pieter, 2003).Some soluble proteins can stabilize foams by forming insoluble, rigid layers at the gas/liquid interface (Laurier, 2005).

With the ever increasing need for cheap sources of protein to meet the world's overpopulation problem, more attention is focused on fish farming. In the developing countries where the problem is acute (Balarin, 1979). Fish is one of the most important animal protein sources that are widely consumed by all races and classes of people (Abdullahi, 2005). It compares favorably with milk, meat, pork and poultry (James, 1984). It has an important role in food security and poverty alleviation in both rural and urban areas of Sudan, but little is known about the nutritional value of the Nile fish that are normally utilized either fresh or preserved dried, salted or smoked. Better knowledge of their nutritional value, which is expected to be closely associated with fish species, could contribute to the understanding of variability in meat quality of different Nile fish species (Mohamed et al., 2010). Moreover, the measurement of some proximate profiles such as protein content, and moisture content is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Waterman, 2000). Besides being used as food, fish is also increasingly demanded for use as feed. High consumption of fish has a beneficial role on human health as it minimizes the appearance of cardiovascular diseases, moderates the inflammatory response as well as improves the carbohydrate metabolism. However, information concerning the chemical composition of fresh water fish in general is of great significance to nutritionists concerned with readily available fish

sources of low fat and high protein (Deka et al. 2012).To understand protein structures and function in detail, they often need to be separated from other cellular components (lipids, nucleic acids, sugars, etc.) and isolated to homogeneity. Many cytosolic proteins are water soluble and their solubility is a function of the ionic strength and pH of the solution (Aaron and Fernandis , 2007).

Protein structures prediction is the prediction of its folding and its secondary, tertiary, and quaternary structures from its primary structure. It is one of the most important goals pursued by bioinformatics and theoretical chemistry; it is highly important in medicine and biotechnology(de Jongh, et. al. 1996).Current research efforts are focused primarily on the design of drugs that can potentially inhibit protein misfolding. It is now understood that the body utilizes a variety of proteins called chemical chaperones to guide the folding of other proteins. By studying how these chaperones function, scientists believe that they will be able to synthesize chaperones that will prevent formation of the misfolded proteins characteristic of prion diseases (prions are believed to be normal proteins that have become misfolded). Prion diseases are an extreme example of the relationship between structure and function. Examples of prion diseases include *Creutzfeldt–Jakob disease* in humans, *scrapie*in sheep's and bovine spongiform encephalopathy (cow disease) (Klein, 2012).Secondary structure compositions (α-helix, β-sheet, turns, etc.) from the peptide

bond region can be studied by:Circular Dichroism (CD), X-raycrystallography, NMR and FTIR (Sharon, et.al. 2005, John and Larry, 2000, and Graham..and Craig. 2011).

Elemental lead (Pb) occurs naturally in the environment as well as being produced by mining and manufacturing activities (Health AG., 1987). Lead and its compounds are serious pollutants of the aquatic environment. Moreover, several authors agree that toxic and non-biodegradable metals such as lead accumulate in many fish species, causing various diseases such as renal (Lliopoulou, et. al 2001 and Gordon, et . al. 2002), hepatic lesions (Banoy and Hasan, 1990), endocrine impairment (Veena, et. al. 1997) and effect of cell membrane lipids in cells of the central nervous system. With regards to the above concepts and observations an attempt is made in this study to determine the moisture content, protein content (by pH shift method), lead amount (for this purpose samples prepared by dry-ashing for subsequent determination of lead by atomic absorption spectroscopy) and the protein secondary structures by FTIR; in eight commercial Nile fish in Sudan.

2. Experimental

2.1. Samples collection:

Fish samples used for the study include *L. niloticus*, *Tilapia SP ,Bagrus bagrus,, Labeo , Schilbe , Synodontis , Clarias and Bagrus docma*; purchased from the fish local markets. All the samples were collected

fresh, transferred directly to the laboratory, washed, cutoff, dried at 105^0C to avoid protein denaturation and ground (thus they were ready for use in subsequent analysis).

Reagents: all reagents are of analytical grade (KBr, NaOH and HCl.), Deionized water (used in all experiments).

2.2. Apparatus and equipments:

Ordinary laboratory tools. Infrared spectra were recorded on A **Shimadzu 8400S FTIR** spectrophotometer calibrated with polystyrene film. A **Shimadzu** analyst 6800 model **atomic absorption** spectrophotometer.

2.3. Determination of moisture content (general procedure):

Moisture content was carried out by weighting the sample before and after drying at 105◦C for 2 hr. till constant weight.

2.4. Determination of lead as toxic metal (general procedure):

3g of fish sample was dried and ashed in furnace at a temperature of 400˚C for 3hr. The ashed sample was treated with 1 cm^3 concentrated hydrochloric acid for ash whitening. Then the residue was dissolved in 3cm^3 concentrated hydrochloric acid (25% v/v) and filtered through filter paper. The filtrate was diluted to 30cm^3 with deionized water. The concentration of lead was determined by Atomic Absorption

Spectrophotometer (AAS) and calculated in ppm.

2.5. Determination of the total protein (general procedure):

To 1g of dried sample, 30cm^3 of 0.01 M NaOH solution was added, and the mixture was left for 30 min. Then filtered, HCl with pH 4was added drop wise to the filtrate till a precipitate was formed; the precipitate was filtered, dried and weighed. Protein secondary structures were determined by FTIR without further purification.

3. Results and Discussion:

Moisture content represents the freshness of the fish samples and the amount of aqueous material surround the fish and protein.

It is very important to study the protein content because of its dietary importance out of cheap source of fish and to obtained food to the growing population and solve the problems of limited resources nowadays. The type of protein, its structures and function are now representing the main directives of the studies conducted by prominent scientists.

The study of pollutant material e.g. Lead amount in the aquatic fish and the environmental biota is essential study to evaluate the danger out of a very toxic material that has accumulative behavior in the living organism. Fish is one of the most important and cheap animal protein sources that are available for consumers in the Sudan; either from Nile river or cultivated; so determination of protein content in Nile fish is very important, when someone talks about fish as substituent of meat,

or other animal protein sources. Protein is an important component in the nutritional value of fish; protein requirement is defined as the minimum amount needed to meet requirements for amino acids and to achieve maximum growth (National Research Council, 1993). The results from this study agree with this fact. Jameset al. 1984, explained the production and storage of dried fish to meet the demand of far-living people. While Waterman, 2000, studied the composition and quality of fish as good protein source in the United Kingdom. Deka, et. al. 2012, studied the variation of proteins and amino acid content of certain types of fish. The best protein is obtained from fish to be used as food for the growing population besides the prevention of cardiovascular diseases by minimizing cholesterol precipitation in the veins, especially if taken with mono unsaturated vegetable oils.

Table1. Moisture content, protein content and lead amount in eight commercial Nile fish in Sudan.

No	species	Parameter		
		Moisture content, % w/w	Protein content, % w/w	Lead amount, (ppm)
1	*L-niloticus*	24.5	51.3	0.62
2	*Tilapia SP*	22.3	49.4	0.23
3	*Labeo*	20.1	28.7	0.29

4	*Schilb*e	16.25	31.9	0.52
5	*Synodontis*	20.6	21.5	0.79
6	*Bagrus docm*a	17.6	26.1	0.083
7	*Clarias*	19.6	22.9	0.08
8	*Bagrus bagrus*	16.6	19.7	0.14

Table (1) represents the percentage of moisture content, protein content and lead amount of the species under study. The moisture content showed high values ranged from 16 to 24.4 % due to the nature of the fish living in waters but the high percentages of moisture is associated with high value of protein content as showed from the results obtained, regardless of lead amount. The amounts of lead varied from 0.08 ppm in *Clarias* to 0.8 ppm in *Synodontis* so it considered as trace amounts, no danger and the Nile water is healthy. The protein as shown from the results in the range from 19.7 to 51.3% is different from that obtained by (Mohamed et al . 2010) that was in the range 77 to 79 % and that may be attributed to the types of fish under study, protein isolation methods, season and other factors. Fish protein extract can be used in soups, sauces, snacks etc. The fishing industry is using fish protein extract for production of baits (JUK Flyer, 2010).

The protein structures prediction is one of the most important goals

pursued by bioinformatics and theoretical chemistry; it is highly important in medicine (for example, in drug design) and biotechnology (for example, in the design of novel enzymes) this was reported by Jongh, 1996. The recent studies of nanobiotechnology could be utilized to enhance the effort in this important field of study. The **secondary structure** of a protein refers to the three dimensional conformations of localized regions of the protein. Depending on the sequence of amino acid residues, localized regions of peptides often adopt particular shapes. Two particularly stable arrangements are **α-helix** and **β- pleated sheet**. As reported by Jongh, 1996, Klein, 2012 and Sharon, 2005, who studied α- helix and β pleated sheets of different absorption of proteins and secondary proteins.

The Nile fish showed accordance of α- helix of all samples in close ranges of 1650 to 1655cm^{-1} in good agreement of the value reported, while the secondary proteins of parallel type (1535-1544cm^{-1}), and anti-parallel β sheets (1510-1527cm^{-1}) and in ordered types of (1536-1540cm^{-1}).Every chain may run in the same direction to form a parallel sheet (1535- 1544 cm^{-1}), every other chain may run in the reverse chemical direction to form an anti-parallel sheet (1514 – 1527 cm^{-1}), or the chains may be parallel and anti-parallel to form a mixed sheet; this showed according to John.et al. 2000, which support their results by Circular Dichroism Spectroscopy, Raman Spectroscopy and Nuclear Magnetic Resonance Spectroscopy.

The careful study of the protein structures indicates that our Nile fish protein structures is far away from prion that causes misfolded proteins responsible for diseases of creutzfeldt -Jokob or scapie or even encephalopathy.

Table2. FTIR spectra analysis for protein isolated from eight commercial Nile fish in Sudan.+

No.	Species	Conformation			
		α-helix (wavenumber cm^{-1})	Parallel β-sheet (wavenumber cm^{-1})	Anti-Parallel β-sheet (wavenumber cm^{-1})	Unordered (wavenumber cm^{-1})
1	*L-niloticus*	1654	--------	1527	1234
2	*Tilapia SP*	1650	1539	--------	1236
3	*Labeo*	1652	1539	--------	1236
4	*Schilbe*	1650	1541	--------	1236
5	*Synodontis*	1649	--------	1514	1240
6	*Bagrus docma*	1650	--------	1517	1236
7	*Clarias*	1647	1535	1517	1257
8	*Bagrus bagrus*	1649	1544	1519	1236
Reference band		1650 -	1530 -	1510 -	Near

	1655	1550	1530	1300

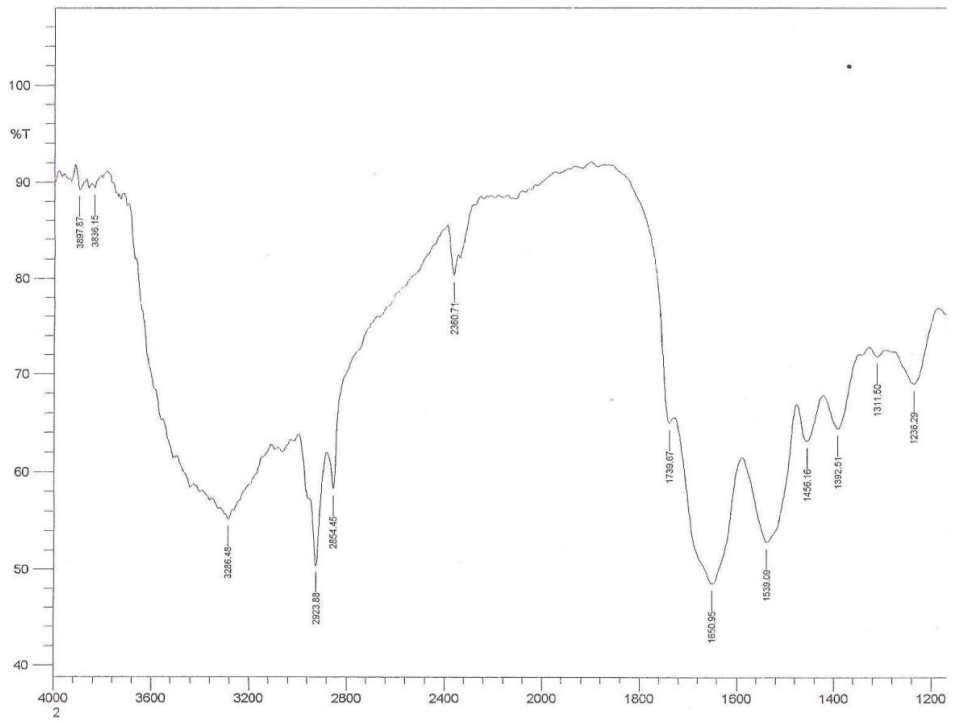

Figure1.**FTIR** spectrum represents secondary protein of *Tilapia SP*

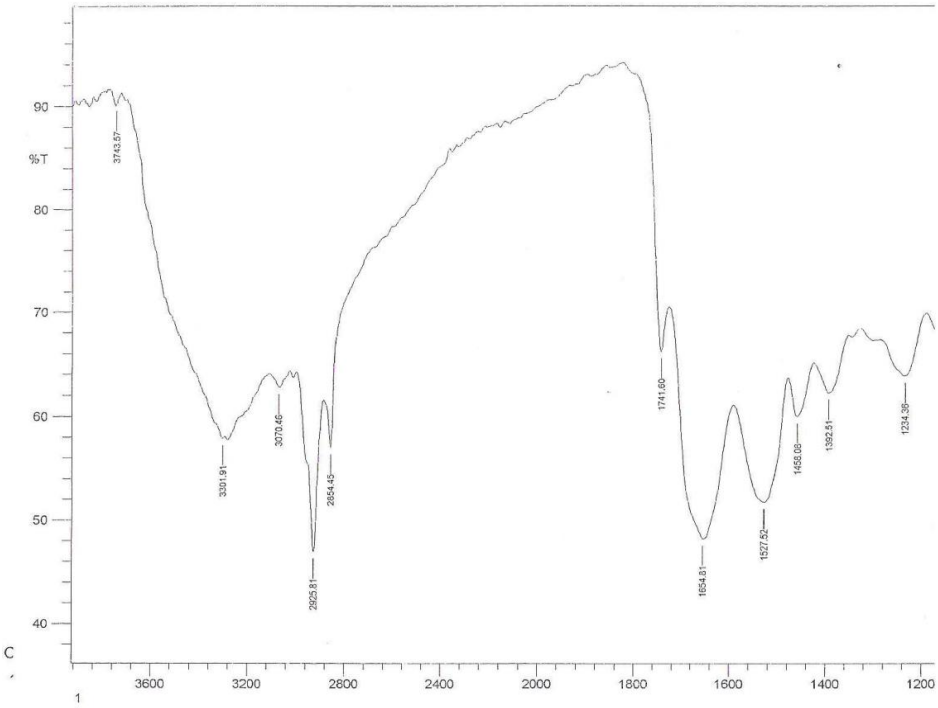

Figure2. **FTIR** spectrum represents secondary protein of *L. niloticus*

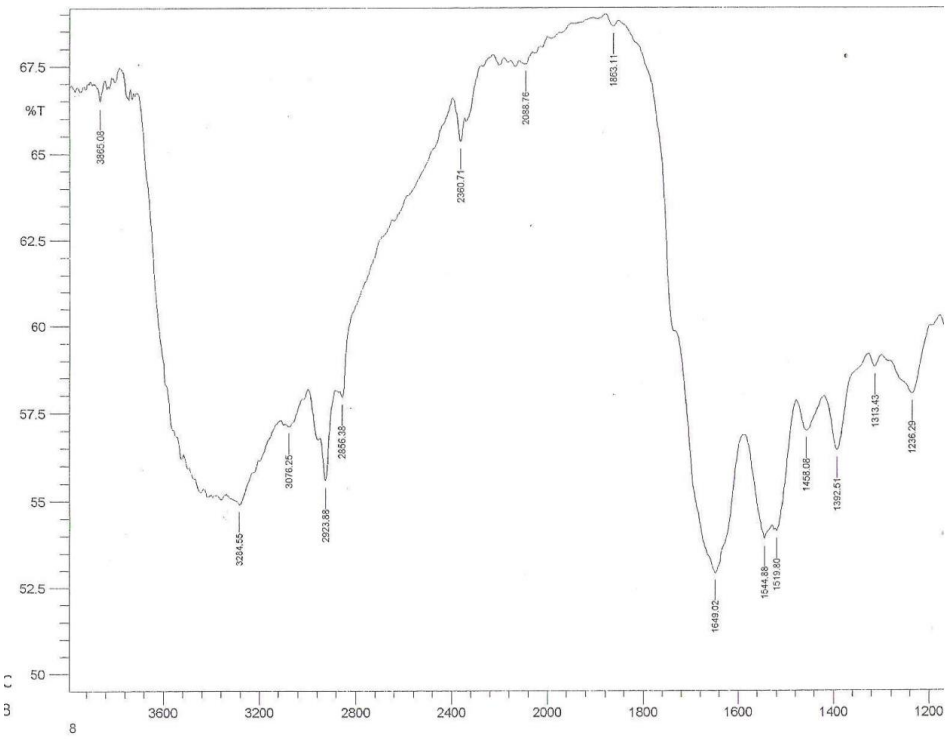

Figure3. **FTIR** spectrum represents secondary protein of *Bagrusbagrus*

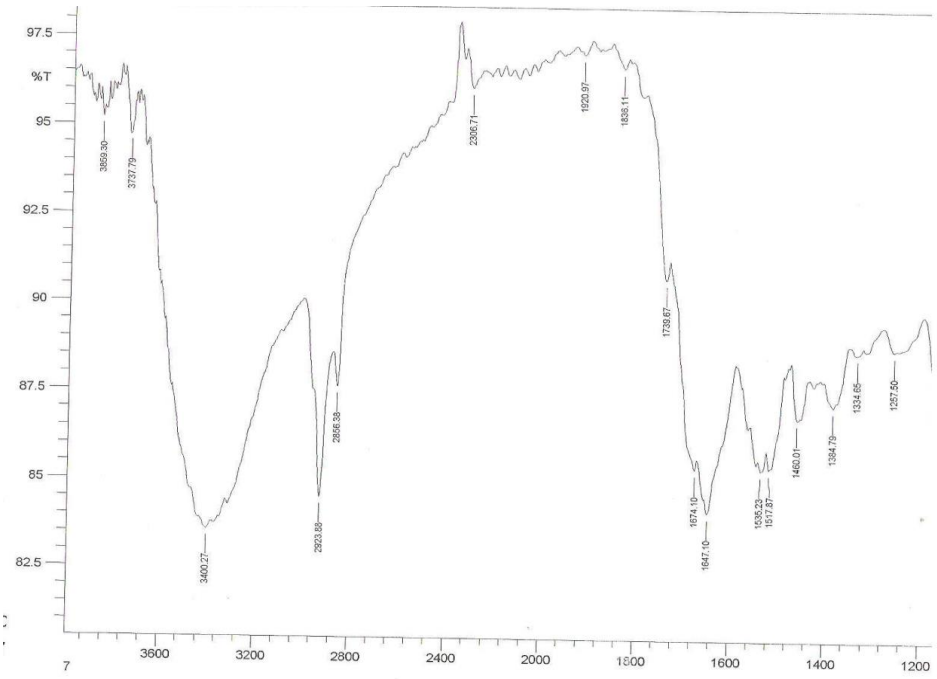

Figure4. **FTIR** spectrum represents secondary protein of *Clarias*

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