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Lipid variations in Different Tissues (Muscle and Brain) of fresh water cat fish. Heteropneustes fossils (Bloch)

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ABSTRACT
Qualitative studies on characterization and identification of lipids by thin layer chromatography in different tissues of H. fossilis showed a considerable variation. The number of lipid spots was highest in brain compared to other tissues. The R_f values of spots indicate the regions of similarity between tissues. Comparison of R_f values with those of standards indicated that the presence of phospholipids, choline lipids, gyolopids, ninhydrine, amino group containing lipids were present. muscle showed showed more amount of glycolipids and low amount of ninhydrine containing lipids, whereas brain showed glycolipids, ninhydrine containing lipids, Iodine containing lipids and choline lipids too. The present study revealed that the brain contain highest amount of different classes of lipids. The muscle tissue has shown less number of lipids. Glyco lipids and general class of lipids are present in both tissues with minor variations, choline lipids present in brain but not in muscle tissue, while Phospho lipids are present in brain only

Keywords H.fossilis, Fatty acid, Lipid class,Glycolipids.

I. INTRODUCTION

Thin-layer chromatography (TLC) is a technique that has been routinely used for the separation and identification of lipids. Here we describe an optimized protocol for the steady state labeling, separation, and quantification of yeast phospholipids using TLC. There is a considerable variation between the tissues of H. fossilis was observed during our investigation, the fast moving spots are probably the neutral lipids or cholesterol esters. The staining of gill tissue of H. fossilis was deeply stained and moderately stained with sulphuric acid and ninhydrine reagents respectively. The biochemical and pharmacological properties and the lipid composition of epidermal secretions from various catfish of the Arabian Gulf have been investigated [1]. However, there has been a very little mention about the fatty acid (FA) composition of muscle of catfish lipid, with the report by [2], being the only publication on FA composition in Arius catfish n-3 polyunsaturated FA (n-3 PUFA) in the diet has been recognized to have important beneficial properties for the prevention of human coronary artery disease [3].

Sea and freshwater fish, which constitute the majority of water products, make up an important part of animal food sources for human. Fish are quite different from the other animal food sources, because they provide low energy and have high-level proteins, which contain all essential amino acids. So they are beneficial nutrition sources [4]) Fish are not only beneficial protein source but also contain considerable amount of unsaturated fatty acids, and thus the studies on lipid biochemistry have been considered so important recently [5,,6,7,8,9,10],The lipids are the most important biochemical compounds of fish [11]. Fish store the lipids in various organs; particularly in muscles and liver. On the contrary, the mammals store in adipose tissue. A great amount of these lipids are transferred to the different parts of the body to be used for various physiological actions [12]. The freshwater fish constitute a great food potential for human. A large part of these fish species are cultivated forms. Since some other new food sources which have the same content are found there is a growing need for information about the chemical composition of the various species and their food contents. For the cultivation of these fish, some important characteristics, such as nutrition properties, biochemical structure and growth conditions need to be known. It is of great importance to know the seasonal variations of the lipids and the lipid amount of the fish, which is economically important and willingly consumed.

Overall, adequate accuracy was obtained in this study. The proposed method provides a fast and efficient means of identifying fish and feed for quality control purposes.

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II. METHODOLOGY

The tissues were collected from the fish i.e gill, liver , intestine, muscle and brain of Heteropneustus fossilis were collected by the method of Folch et al., (1957) as follows.

The different tissues were collected from various parts of the body and tissues were weighed to nearest milligram and homogenized with chloroform methanol mixture (v/v) (2:1) to a final dilution of 10 fold the weight of tissue i.e., the homogenate 1 gm of tissue was diluted to a volume of 10 ml of choloform methanol mixture solution. The mixture was homogenized and centrifuged at 2000 rpm for 5 mins. The organic layer was collected and evaporated in vaccum at 35^0C and the residue was dissolved in 0.5ml of solvent (Chloform : Methanol. 2:1) .

The TLC was run on a readymade silica gel plates (E- merck) in a solvent system of Chloroform, methanol, water (65:25: 10) system. The plates were dried at room temperature. The plates were observed under UV lamp and the spots were marked with pencil. For routine analysis of plates were sprayed with 1) Iodine 2) H_2SO_4 3) Ninhydrine 4) Dittmer- Lester and 5) Dragendorff's reagent. R_f values of all the spots were determined immediately.

Identification of lipids

Protocol

Tissues collected

↓

Tissues was weighed to the nearest milligram

↓

Homogenized the tissues with (2:1) Chloroform : Methanol mixture
(1gm of tissue was diluted to a volume of 10ml of Chloroform Methanol mixture solution)

↓

The mixture was homogenized and centrifuged at 2000 rpm for 5 minutes

↓

Collect the organic layer (Upper Layer)

↓

And kept it and evaporated and vacuumed at 35^0C

↓

Sediment (residue) was dissolved in 0.5 ml of solvent (Chloroform: Methanol, 2:1)

↓

The TLC plates were run on readymade silica gel plates in a solvent system
Choloroform, methanol, water (65:25:10)

↓

The plates were dried at room temperature

↓

The plates were dried under UV lamp and the spots were marked with pencil.

TLC plates

Readymade marketed (E. Merck AG, Darmstadt, Germany) silica gel-G coated on aluminium plates with thickness of the layer 0.2mm were used, the solution of total lipids prepared in (Chloroform : Methanol, 2:1) was

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applied on the plates at a distance of 1 cm from lower edge of the plate. The loaded plates were developed in the solvent system mentioned above.

Reagents preparation

Following reagent systems were employed in identifying the lipid fractions in accordance with the procedures given by Kates (1986).

Iodine vapours

Iodine vapours were used as a general purpose detector for all lipids, since both saturated and unsaturated lipids absorb iodine by simple dissolution. Iodine crystals were packed between the blotting papers and wetted with Chloroform. The TLC plates dried at room temperature and covered with Petri dish. Lipid spots were turned into yellow on exposure to Iodine vapours. The spots were scanned immediately and their R_f values were noted on after exposure to Iodine vapours.

Sulphuric acid reagent

Sulphuric acid – di chromate reagent was prepared by dissolving 0.6gm of $K_2Cr_2O_7$ in 55% H_2SO_4. The reagent was sprayed on to the developed plate and then the plates were heated on hot plate from low heat and gradually increasing to high heat for 5-10 minutes. All lipids were charred and appeared as black spots. Glycolipids and sterols appeared as red spots upon low heating.

Nin hydrin Reagent

This was used to detect Phosphatides or lipids containing a free amino group. Phosphatidyl ethanolamine (PE), Phosphatidyl serine (PS) and Phosphatidyl inositol (PI) were detected by this reagent.

Reagent preaparation

0.25 gm of reagent grade Ninhydrin was dissolved in 100ml of Acetone. After spraying the reagent, reagent plates were heated at 60^0C for 3-5 minutes the spots appeared purple in colour.

Dittmer – Lester Reagent (Modified by vaskovasky and kostetsky)

This reagent was used to detect phospholipids for which it is specific, and can detect as little as 10µg of lipid. The phospholipids appeared as blue spots on a white background with few minutes without heating.

Reagent preparation

Solution A

16gm of Ammonium molybdate in 120 ml distilled water.

Solution B

40 ml of Con, HCL, 10ml of mercury and 80 ml of sol. A were added, shaken for 30 minutes and filtered. To reminder of the solution (A) 200 ml of Con. H_2SO_4 and all the solution (B) were added, cooled and stored. This was bright green coloured solution and was stabled at room temperature. Just before use this solution was diluted with distilled water (1:3 v/v). the final spray reagent was Amber colored.

Dragendorff's reagent

The stock solution (5ml) was mixed with 15 ml of distilled water immediately before use.

Solution- I

2.7 gm of Bismath nitrate in 100 ml of Acetic acid.

Solution- II

10 gm in 25 ml of Potassium Iodide.

Before use 20 ml of solution _I and 5 ml of solution-II were mixed in and diluted with 70 ml of distilled water.

III. RESULTS

The pattern of lipids observed through TLC in the two tissues under examination is presented in **Figure.1 and 2** R_f values of individual spots and the staining patterns with different reagents Viz., Dittmer reagent, Dragendorff's, Sulfuric acid, Iodine and Ninhydrin were presented in **Table. 1 and 2**

Muscle

Lane-1 of muscle tissue showed spots with R_f values were 80±0.5 and 90±0.5 and were not clearly visible in chromatogram, when treated with Dittmer-Litster reagent (Lane-1). Lane-2 showed treated with Dragendorff's reagent lipid spot, as R_f value 90±0.5 was not clearly visible, lipid spots with R_f values 30±0.5, 60±0.5, 70±0.5 and 80±0.5 were faintly stained when treated with Sulfuric acid reagent (lane-3). Lane-4 of muscle tissue showing seven purple color lipid spots when treated with iodine reagent the spots with R_f value 60±0.5, 70±0.5 and 90±0.5 were not clearly visible Lane five of muscle showed seven purple color spots with R_f values 30±0.5, 40±0.5, 50±0.5, 60±0.5 and 70±0.5 which were moderately stained and spots

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with R_f value 80±0.5 and 90±0.5 were deeply stained (**Table. I** and **Figure. I**).

Brain

Lane-1 of brain tissue showed lipid spots with R_f value 40±0.05, 50±0.05, 60±0.05, 70±0.05, 80±0.05, 90±0.05 treated with Dittmer reagent exhibited blue color spots; in Lane-2 the lipid spots with R_f value 10±0.05, 20±0.05, 30±0.05, 50±0.05 were not visible clearly. The lipid spots with R_f value10±0.05 & 90±0.05 were faintly stained with Dragendorff's reagent and showed nine orange color spots of Choline lipids, in Lane-3 six lipid spots were exhibited and deeply stained, when treated with sulphuric acid, their R_f values are 20±0.05, 30±0.05, 50±0.05, 60±0.05, 80±0.05, 90±0.05 (Glycolipid spots). Lane-4 when treated with Iodine vapors eight yellow color lipid spots were observed, all these spots were moderately stained. Lane-5 staining with Ninhydrine reagent showed seven purple color spots for amino group with R_f a vlues10±0.05, 20±0.05, 30±0.05, 40±0.05, 70±0.05, 80±0.05, 90±0.05. All these spots were deeply stained (**Table.2** and **Figure. 2**).

Table 1.TLC of lipids in Muscle tissue stained with different reagents

	Muscle tissue treated with different reagents for lipid identification R_f values								
Reagent	**10±0.5**	**20 ± 0.5**	**30 ±0.5**	**40 ±0.5**	**50± 0.5**	**60± 0.5**	**70± 0.5**	**80± 0.5**	**90± 0.5**
Dittmer-Lister	-	-	-	-	-	-	-	±	±
Dragon-Draft	±	±	±	±	±	±	±	-	±
Sulphuric Acid	-	-	+	-		+	+	+	
Iodine	-	-	-	-		±	±	±	±
Ninhydrine	-	-	++	++	++	++	++	+++	+++

Table 2.TLC of lipids in Brain tissue stained with different reagents

	Brain tissue treated with different reagents for lipid identification R_f values								
Reagent	**10±0.5**	**20 ± 0.5**	**30 ±0.5**	**40 ±0.5**	**50± 0.5**	**60± 0.5**	**70± 0.5**	**80± 0.5**	**90± 0.5**
Dittmer-Lister	-	-	-	+	+	-	+	+	+
Dragon-Draft	+	+	+	+	+	+	+	+	+
Sulphuric Acid		+++	+++		+++	+++	-	+++	+++
Iodine	++	++		++	++	++	++	++	++
Ninhydrine	+++	+++	+++	+++	-	-	+++	+++	+++

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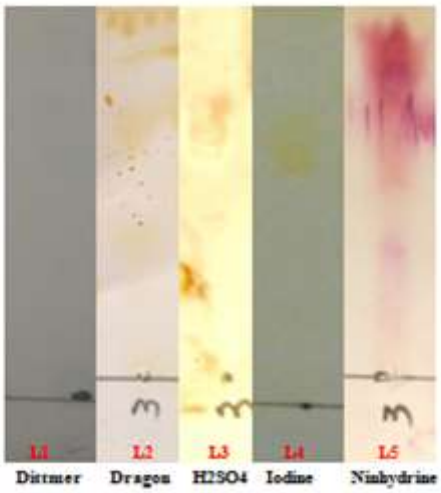

Figure.1.. TLC of lipids in Muscle tissue stained with different reagents

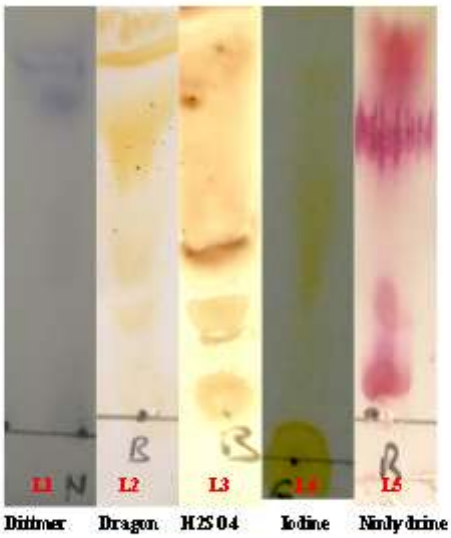

Figure.2.TLC of lipids in Brain tissue stained with different reagents

IV. DISCUSSION

There is a considerable variation between the tissues of H. fossilis was observed during our investigation, the fast moving spots are probably the neutral lipids or cholesterol esters. The staining of gill tissue of H. fossilis was deeply stained and moderately stained with sulphuric acid and ninhydrine reagents respectively. The biochemical and pharmacological properties and the lipid composition of epidermal secretions from various catfish of the Arabian Gulf have been investigated (Ali et al., 1989; Al-Hassan at al., 1986). However, there has been a very little mention about the fatty acid (FA) composition of muscle of catfish lipid, with the report by Sinclair et al., 1983, being the only publication on FA composition in Arius catfish n-3 polyunsaturated FA (n-3 PUFA) in the diet has been recognized to have important beneficial properties for the prevention of human coronary artery disease [13]

A similar study carried out by [14]) in muscle and liver of rainbow trout (Oncorhynchus mykiss) determined that the variations of the levels of lipids in liver and other organs are the results of irregular seasonal variations which affect the fish diet. However, it may be noted that along with seasonal variation, habitat conditions also influence fat synthesis in the fish. In lotic habitat, more amount of lipid content was observed in liver during winter season but significantly lower during

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the monsoon but in lentic habitat, apparently low lipid content was observed during winter season [15]

The fishes selected in the present study are of high consumer demand having good taste. Present study will provide a detailed understanding of the necessity of the healthy life for the fish consuming people. This information also may be helpful to evaluate the nutritional significance of the fishes. We concluded that fish lipid should not be avoided in human nutrition; its consumption should be minimized by individuals and populations at risk of cardiovascular diseases and its associated squeal.

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