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Physicochemical Properties and Fatty Acid Distribution Pattern in Lipids of *Eutropiichthys vacha* Hamilton-buchanan (Fam. Schilbeidae)

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Abstract: The present study describes the effect of season on the physicochemical properties and fatty acid distribution pattern of the lipids of *Eutropiichthys vacha*. Although no wide variability in fatty acids distribution profile was observed by the influence of seasonal changes but the fatty acids present were found to be mainly palmitic (C_{16:0}), oleic (C_{18:1}) and linoleic (C_{18:2}) acids besides small quantities of myristic (C_{14:0}), stearic (C_{18:0}), linolenic (C_{18:3}) and arachidic (C_{20:0}) acids. The physical and chemical characteristics viz. refractive index, specific gravity, acid value, saponification value, unsaponifiable matter, iodine value, reichert-meissel value of lipids have also been reported.

Key words: Physicochemical, lipid, fatty acid, *Eutropiichthys vacha* Hamilton-buchanan

Introduction

Eutropiichthys vacha, an excellent table-fish is found in lakes of Bangladesh, Pakistan, India, Burma and Thailand (Talwar and Jhingran, 1991). Effectiveness of fish oils to reduce cardiovascular problems has attracted scientists to extensively analyze the fish oils of both marine and fresh water fish (Oliver, 1981). The biochemical effects of fish-derivatives and plant lipids in cancer therapy have also been examined elsewhere (Burns and Spector, 1994; Horrobin, 2000). Fish and shellfish can be a generous source of supply of n-3 type polyunsaturated fatty acids such as icosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ackman, 1988).

Lipids are compound mixtures of fatty acids such as saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). The suitability of oils for a particular purpose, however, is determined by its fatty acid composition. The fatty acid composition of the endogenous fats plays an important role in determining shelf-life, nutrition and flavour of food products (Gaydou *et al.*, 1983). Oils from a single source is not suitable for all purposes since fatty acid composition of oil generally differs depending on sources of oils. This encourages to searching for new sources of novel oils. The patterns of fatty acids and total fat are calculated to comply with the regulation of food label declaration under US Nutrition Labeling and Education Act of 1991 (House *et al.*, 1994). PUFAs inhibit the biosynthesis of cholesterol in the liver, more the unsaturation, greater is the inhibition (David *et al.*, 1991). However, no attempts seem to have been made to decipher the fatty acid profiles, physicochemical characteristics and evaluate the biological value of fish lipid of this species. The present investigation was carried out to know the fatty acids distribution in different seasons in this subcontinent, under the influence of variegated climates, monsoons and humidity.

Materials and Methods

The fish were collected from the river Padma around Rajshahi region in three seasons (February-March, June-July and November-December) of the year. Total lipid content of fish was extracted following the method of Bligh and Dyer (1959). The physical and chemical contents of the lipid were determined by standard methods (Hildich, 1949; Williams, 1966; Lehninger, 1982).

The lipids were then converted into their respective fatty acid methyl esters (FAME) by *in situ* trans-esterification (ISTE) procedure (Park and Goins, 1994). It was carried out by heating lipid containing fish at 90°C for 10 min after adding 0.5N NaOH in methanol for methanolysis and continued heating for another 10 min for further methylation after adding 14% BF₃ in methanol. The FAME was then estimated on a Pye-unicam 4500U gas chromatograph (Williams, 1966). Fatty acids were separated on a

Quartz capillary column (i.d. 2 mm, length 1.5 m) equipped with a flame ionization detector (FID). Nitrogen was used as a carrier gas at a flow rate of 30 ml/min. Separations were performed on a column packed with 6% butanediol succinate polyester (BDS) on solid support Anakorm ABS (100/120 mesh). The temperature was programmed from 130-230 °C at a rate of 4° C rise per minute. The fatty acids were identified by comparing their retention times by plotting the log₁₀ of retention times against equivalent carbon length (ECL) with those of standards. The percentage of fatty acids was calculated by multiplying the peak height with the width at half height. Iodine values were calculated from fatty acid composition (Hashim *et al.*, 1993) using the following formula :

$$IV = (\% \text{ oleic} \times 0.8601) + (\% \text{ linoleic} \times 1.7321) + (\% \text{ eicosenoic} \times 0.7854)$$

Data on different contents of *Eutropiichthys vacha* were subjected to statistical analysis and mean values were compared using LSD (Gomez and Gomez, 1984) at 5% level of significance.

Results and Discussion

The physical and chemical contents of the lipid of *Eutropiichthys vacha* obtained in the investigation and other fats and oils like linseed, soybean, cottonseed, butter is presented (Table 1) for comparison (Agarwal, 1996-97). The fatty acids distribution of *Eutropiichthys vacha* fish lipid in different seasons (Table 2) along with some of said vegetables fats and oils (Agarwal, 1996-97). Higher refractive index in fats and oils (lipid) is consistent with the value of highly unsaturated lipid (Swern, 1964) i.e. soybean (1.4723-1.4756) but inconsistent with less unsaturated oil. Specific gravity was normal in comparison with other fats and oils. The Reichert-Meissel value indicates the lower fatty acid content of the lipid, which is also in agreement with the high saponification value. A bit high saponification value indicates that the lipid contains high percent of lower fatty acids which is similar to butter fat (Table 2). The results clearly indicate that the *Eutropiichthys vacha* fish lipid contained fatty acids of lower molecular weight i.e. C_{16:0} and below. The iodine value indicates that the lipid is unsaturated but not highly unsaturated. The per cent of unsaponifiable matter indicates the presence of small amount. With few exceptions, vegetable oils contain an average of 0.2-1.5% unsaponifiable compounds. The sterols are components of unsaponifiable lipids and are important to identify the blends of fats and oils (Belitz and Grosch, 1987; Mariani *et al.*, 1994).

The results showed that there was no significant influence on the ratio of saturated to monounsaturated to polyunsaturated fatty acids with changes in season (Table 3). The major fatty acids present are palmitic (C_{16:0}), oleic (C_{18:1}) and linoleic acids (C_{18:2}) besides small amount of myristic (C_{14:0}), stearic (C_{18:0}), linolenic

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Table 1: Physical and chemical contents of *Eutropiichthys vacha* fish lipid.

Name of sample	Seasons	Refractive index, 30°	Specific gravity, 30°	Acid value	Saponification value	Unsaponifiable matter	Iodine value	Reichert-Meissel value
<i>Eutropiichthys vacha</i> fish lipid	Feb.-Mar.	1.47	0.923	1.993	216.37	0.640	96.20	0.920
	Jun.-Jul.	1.45	0.926	1.992	216.26	0.637	96.21	0.920
	Nov.-Dec.	1.45	0.926	1.991	216.41	0.640	96.18	0.913
LSD value at 5%		0.00689	0.00155	NS	NS	NS	NS	NS
Fats and oils	Linseed	1.479-1.480	0.931-0.938	4.0	189-195	1.0-1.5	175-200	0.95
	Soybean	1.4723-1.4756	0.922-0.928	1.27-1.54	190-195	0.7-1.6	129-137	0.5-2.5
	Cotton seed	1.4743	0.921-0.945	1.0-5.0	192-198	0.8-1.8	102-114	0.95
	Butter	1.460	0.936-0.944	0.45-35.5	210-230		26-28	24-33

NS = Not significant

Table 2: Fatty acids distribution of *Eutropiichthys vacha* fish lipid and those of some fats and oils (Weight %). (Figure in mean sum square)

Samples	Season	Myristic C _{14:0}	Palmitic C _{16:0}	Stearic C _{18:0}	Oleic C _{18:1}	Linoleic C _{18:2}	Linolenic C _{18:3}	Arachidic C _{20:0}	Unidentified
<i>Eutropiichthys vacha</i> fish lipid	Feb.-Mar.	2.90	49.20	1.10	24.27	17.80	1.30	0.53	3.080
	Jun.-Jul.	3.10	46.30	0.95	25.30	20.30	1.53	0.47	2.030
	Nov.-Dec.	3.20	44.47	0.81	26.10	22.10	1.60	0.28	1.100
LSD value at 5%		0.200	0.209	0.123	0.236	0.200	0.179	0.109	0.155
Standards Fats and Lipids	Palm	1.1	41.1	4.2	38.4	10.7	-	-	4.500
	Soybean	-	6.5	4.2	32.2	49.3	2.2	0.70	4.900
	Linseed	-	-	-	5.0	48.5	34.1	-	12.400
	Cotton seed	0.3	19.1	1.9	33.2	39.4	-	0.60	5.500
	Butter	22.6	26.6	11.4	27.4	-	-	-	5.800

Reference source for standard: Agarwal (1997)

Table 3: Saturated, monounsaturated, polyunsaturated fatty acids percent and ratio of *Eutropiichthys vacha* fish lipid.

Fatty acids	Name of Seasons			
	Feb.-Mar.	Jun.-Jul.	Nov.-Dec.	Average
TSFA C _{14:0} + C _{16:0} + C _{18:0} + C _{20:0}	53.73	50.82	48.76	51.10
TMUFA C _{18:1}	24.27	25.30	26.10	25.22
TPUFA C _{18:2} + C _{18:3}	19.1	21.83	23.7	21.54
TSFA/TMUFA ratio	2.21	2.00	1.86	2.02
TMUFA/TPUFA ratio	1.27	1.16	1.10	1.18

TSFA= Total saturated fatty acid; TMUFA= Total monounsaturated fatty acid; TPUFA= Total polyunsaturated fatty acid

(C_{18:3}) and arachidic acids (C_{20:0}) are shown in the Table 2. It is well known that dietary fats, rich in linoleic acid (C_{18:2}), prevent cardiovascular disorders such as coronary heart diseases, atherosclerosis, high blood pressure and also C_{18:2} fatty acid derivatives serve as precursors of some metabolic regulatory compounds (Vles and Gottenbos, 1989). Since the oil is non-toxic and rich in polyunsaturated acids like linoleic acid, it can be classified as an oil containing essential fatty acids which prevent atherosclerosis and high cholesterol accumulation or synthesis (Mead and Howton, 1958; Thomson, 1953). The high linoleic acid (C_{18:2}) content makes the lipid of *Eutropiichthys vacha* nutritionally valuable.

The results indicate that MUFA (C_{18:1}; C_{18:1}) and PUFA (C_{18:2}; C_{18:3}) in fish lipid increased gradually with the change in seasons (Table 2). All these saturated and unsaturated fatty acids are commonly observed in the lipids of other fish species (Viswanathan Nair and Gopakumar 1978; Anonymous, 1989). The lipids are characterized by their high content of palmitic acid (C_{16:0}). The fatty acid composition of the total fish lipids of *Eutropiichthys vacha* reveals that oleic acid and linoleic acid are the predominant unsaturated fatty acids. This particular finding is encouraging because it is a desirable feature in human food. The high content of linoleic acid, low amount of saturated acids, high value and minor percent of linolenic acid constitute an oil which forms drying, non-yellowish films that have a very good through dry and low wrinkling characteristics (Kneeland, 1966). Examination of the effects of W-3 PUFA supplementation and EFA deficiency revealed that both dietary manipulation led to reduced release of NO₂⁻/ NO₃⁻ at all the concentrations of lipopolysaccharide (LPS) tested (Boutard, 1994). The results obtained in the present study will help in suitability for edible

purpose with respect to SFA, MUFA and PUFA. The results showed minor percent of unidentified fatty acids, which could not be identified due to the nonavailability of suitable standards in the laboratory. No significant changes in physical and chemical characteristics and per cent of fatty acid profile were observed among three different seasons.

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