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A Study of Ultra-Violet Irradiation on Epithelial Tissue of Fresh Water Fish, "*Puntius Sophore*"

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ABSTRACT

The small indigenous fishes (SIFs) are known to be micronutrient rich. The gene Puntius comprises of about 134 beautiful species, out of which *Puntius Sophore* is an important species. Comprehensive nutrient profile of *Puntius Sophore* showed that it is rich in protein and minerals. In the present study we are investigating the effect of UV irradiation on epithelial tissue of fish, *Puntius Sophore*. The quantities of various mineral constituents, fat and amino acids were analyzed at different time intervals. This study was undertaken to evaluate the mineral and organic chemical constituents present in scale of fish, Puntius Sophore. The minerals (Na, Ca, Mg, P, and F), CO_2 and organic constituents (fat, protein and nitrogen) were determined before and after the time intervals of 1 hour, 10 hours and 20 hours of U.V. irradiation. All mineral constituents remain unchanged after U.V. irradiation. Total nitrogen, crude fat and crude protein showed continuous decrease with increase in the time of exposure. Percentage decrease in total nitrogen, crude fat and crude protein after 20 hour exposure was 2.784, 7.79 & 2.88 respectively. Likewise Amino acids content in fish scale decreases, maximum being in case of valine after 20 hours exposure. The reason for this decrease is the C-C bond cleavage with the formation of free radicals and evolution of ammonia. Exposure also reduced the weight of the scale powder.

Keywords: U.V. Irradiation, epithelial tissue, amino acid, crude fat, crude protein, fish *Puntius Sophore*.

INTRODUCTION

Puntius Sophore is one of the nutritionally superior SIFs[11, 16 - 18]. Nutritional composition of fish varies with the variables as zoogeography, size, season etc. The present study was undertaken to investigate the effect of U.V. radiation on essential mineral constituents, fat and amino acids present in scales of *Puntius Sophore*.

Ultraviolet Radiations may be understood as radiation beyond the violet region. Wavelength range of this region is 4000 to 20A^0. Radiations near ultra-violet region corresponding to 2000 Å (frequency 1.51X10^{15} cycles / seconds) have energy equal to 1.43X10^{15} calories and U.V. frequency 1.5X10^{15} cycles/ seconds have energy 1.43X10^7 calories (Bajpai & Mishra, 1990)[5], (Gurdeepraj, 1991)[12] William Kemp (1986)[15]. Ultraviolet light has

three wave length designations UV-A UV-B and UV-C. It has been proved that U.V. light is healthiest when it has trace amount of ultraviolet radiation. UV-B plays a vital role in the production of vitamin D_3 in our skin and is essential for the absorption of calcium into bones[4].

Tuberculosis and skin lesions, too, are cured by U.V. radiation[21]. In fact before the invention of penicillin in 1938, varieties of infectious disease were cured through exposure to the sun. The ultraviolet light was found very effective in stimulating the patient's immune system[20].

The amino acids are very important to life. In fact some of them are absolutely essential. The results of nutritional experiments on laboratory animals show that certain amino acids must be present in the diet, to obtain the normal growth of young and to maintain the natural state of health in adults. Some amino acids are synthesized in the animal body. The omission of any of essential

Table 1: Mineral Composition in Epithelial Tissue (scales) of fish *Puntius Sophore* before irradiation (gm/100gm of the dry matter)

Average of 10 Fish			Moisture	Ash	Mineral Values		Mineral Oxide / Fluoride Values		Ash (unac counted)
Length (cm)	Girth (cm)	Weight (gm)							
					Ca	10.49	CaO	14.6	
					Mg	0.33	MgO	0.55	
					Na	0.0026	Na_2O	0.0035	
8.66	8.52	89.9	10.74	27.6	P	4.68	P_2O_5	12.25	0.2065
								Total= 27.4035	
					F	0.0016	CaF_2	0.003	
							CO_2	1.003	

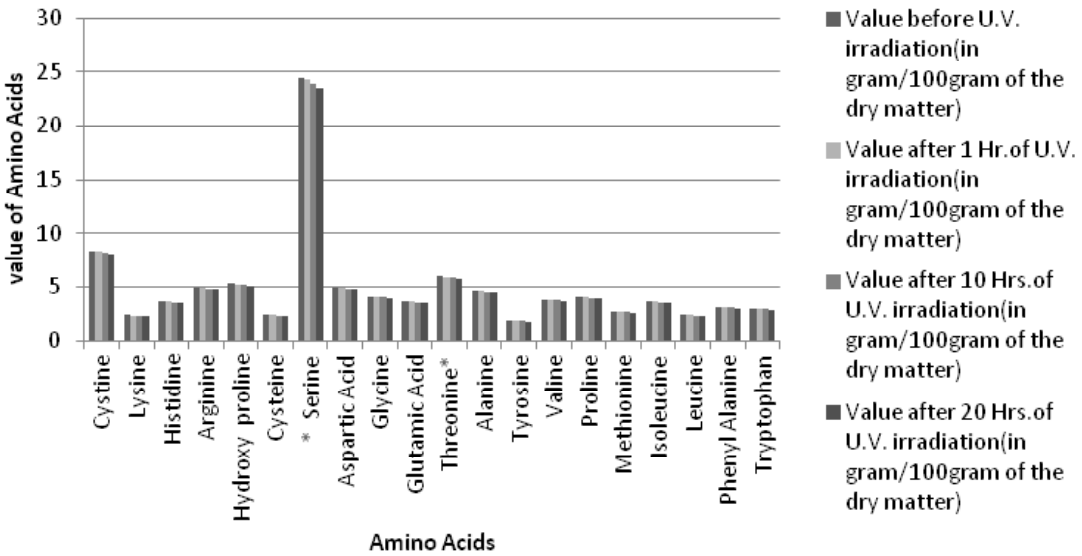

Fig.1: Comparative study of amino acid content before and after U.V. irradiationof different time exposure

amino acids from the diet leads to one or more manifestations of malnutrition. Argine, for example, is synthesized in the organism, but the rate of its formation is insufficient to permit normal growth. Some quantity of it must be provided in the food supply. [Ray Q. Brewster & William E. Mcewen (1968)][23].

MATERIAL AND METHODS

A good quality Puntius Sophore fish were provided by Al-Shah enterprises Delhi. Scales from the body of fish, *Puntius Sophore* were pulled out with the help of forceps. To obviate the effect of habitat and environment on the result, samples for the study were obtained from the same place during the same period of the year i.e. the first fortnight of January. Scales of fish were washed thoroughly several times with distilled water and air dried at room temp for 36 hours. Whole scales of fish were ground separately in an electrical grinder.

Moisture and Ash

Moisture was determined by keeping 1 gram of the air dried material in an aluminum cup in an electric oven at 110^{0}C for 24 hours and then weighing the oven dried material. Ash content was determined by keeping 1gram of the material in a muffle furnace at 900^{o}C till the weight of the ash become constant.

Minerals

About 100 mg of ash was dissolved in the minimum quantity of HCl, calcium was precipitated

Table 2: Mineral composition in Epithelial Tissue (Scales) of Fish *Puntius Sophore* after irradiation (gm/100 gm of the dry matter)

Time of irradiation	Moisture	Ash	Minerals			Mineral oxides	Ash (unaccounted)
1 HOUR	10.01	27.61	Ca	10.49	CaO	14.6	
			Mg	0.33	MgO	0.55	
			Na	0.0026	Na_2O	0.0035	0.2065
			P	4.68	P_2O_5	12.25	
						TOTAL =27.4035	
			F	0.0016	CaF_2	0.0033	
					CO_2	1.003	
10 HOURS	9.30	27.61	Ca	10.49	CaO	14.6	
			Mg	0.33	MgO	0.55	
			Na	0.0026	Na_2O	0.0035	
			P	4.68	P_2O_5	12.25	0.2065
						TOTAL=27.4035	
			F	0.0016	CaF_2	0.0033	
					CO_2	1.0003	
20 HOURS	9.01	27.61	Ca	10.49	CaO	14.6	
			Mg	0.33	MgO	0.55	
			Na	0.0026	Na_2O	0.0035	
			P	4.68	P_2O_5	12.25	0.2065
						TOTAL=27.4035	
			F	0.0016	CaF_2	0.0033	
					CO_2	1.003	

as calcium oxalate. Calcium was determined volumetrically using standard $KMno_4$ solution after liberating free oxalic acid by dissolving the precipitate in dilute H_2SO_4. Fluorine was determined by the method reported by Snell & Snell, 1967[26]. Mg was determined calorimetrically after removing calcium as calcium sulphate precipitate by taking about 500 mg of ash (Snell and Snell, 1967). Na was determined as sodium zinc uranyl acetate precipitate. The colour of precipitate was intensified with a little H_2O_2. Sodium was estimated in Eel's colorimeter using the green filter (Snell and Snell, 1967) Phosphorus was determined by dissolving 20 mg of ash in minimum quantity of HNO_3. Phosphorus in canary yellow ammonium phosphomolybdate was determined by alkali metric method (Cumming and Kay, 1956) [7]. CO_2 was determined with the help of Schrotter's apparatus (Cumming and Kay, 1956 revised).

Fat, Protein and Amino acids

Crude fat was extracted in a soxhlet extractor using petroleum ether (40-60^{0}C), time of extraction being 6-8 hrs. Total nitrogen was determined by Kjeldhal method[2]. Crude protein was determined by multiplying the total N content by 6.25 and amino acids were determined using high performance liquid chromatography[1, 10].

RESULTS AND DISCUSSION

Minerals are required for the normal life processes, and all animals including fish, need these inorganic elements. Fish may derive these minerals from the diet and also from ambient water. Although the minerals are required in very trace amounts but, they are vital for maintaining proper homeostasis inside the body. *Puntius Sophore* has significantly higher level of calcium concentration[16] which if quite high in comparison to Indian major carps (*L. rohita, C. catla, C mrigala*)[24]. SIFs are the rich source of calcium and other micronutrients as they are eaten whole with bone head and eye[11]. The bioavailability of calcium in *Puntius Sophore* is as high as that from milk[13]. Deficiency of calcium may be associated with rickets and osteomalacia.

Table 3 :Crude Fat, Nitrogen and Crude Protein Contents of Fish *Puntius Sophore* before irradiation (gm/100gm of dry matter)

Average of 10 fish			Crude	Total	Crude
Length (cm)	Girth (cm)	Weight (gm)	Fat	Nitrogen	Protein
8.66	3.91	89.9	0.308	11.53	72.08

Total of Ash + Crude Protein + Crude Fat= 99.998

Table 4: Crude fat, Total Nitrogen and Crude protein content in scales of Fish *Puntius Sophore* after U.V. irradiation (gm/100 gm of the dry matter)

Time of irradiation	Crude Fat	% decrease of crude fat	Total Nitrogen	Decrease of nitrogen After irradiation	Crude protein (N×6.25)	Crude protein (after irradiation)	% decrease of crude protein
1 hour	0.301	2.27	11.474	0.520	72.08	71.68	0.554
10 hours	0.291	5.51	11.294	2.047	72.8	70.56	2.108
20 hours	0.284	7.79	11.209	2.784	72.8	70.00	2.88

Total (Ash+ Crude fat + Crude protein) = 99.991 (After 1 hour of exposure)
Total (Ash+ Crude fat + Crude protein) = 99.98 (After 10 hour of exposure)
Total (Ash+ Crude fat + Crude protein) = 99.97 (After 20 hour of exposure)

Na and F are present in traces, Mg content is too low and ash accounted for 0.2065 (Table. 1&2), calcium and phosphorus are the major constituents of the fish scale. CO_2 content is also low as compared to calcium and phosphorus oxides (Table. 1). After 1 hour U.V. irradiation moisture content of air dried scale decreases extensively while the minerals (Na, Ca, P, Mg, and F) and CO_2 remain unchanged. After 20 hours of U.V. exposure appreciable decrease in moisture content is observed in air dried scales but minerals (Na, Ca, P, Mg and F) and CO_2 still remain unchanged (Table. 2).

The fish is a rich source of fat soluble vitamins like A, D, E and K. *Puntius Sophore* contains saturated fatty acids (20.02%), monounsaturated fatty acids (37.12) and high amount of polyunsaturated fatty acids (PUFAs)[16]. PUFAs have potential in reducing coronary heart disease, atherosclerosis, cancer, arthritis, hypertension and Alzheimer[3, 8, 14, 22, 25, 29, 30]. The percentage decrease in fat content after 1, 10 and 20 hours of exposure are 2.72, 5.51 and 7.79 respectively. This decrease is observed due to degradation of fat through free radical formation (Table. 4).

Table 5: Percentage of Amino Acids in scales of fish *Puntius Sophore* before irradiation (gm/100 gm of the dry matter).

S.No	Name of amino acid	Value
1	Cystine	8.35
2	Lysine	2.41
3	Histidine	3.71
4	Arginine	4.95
5	Hydroxy proline	5.28
6	Cysteine	2.46
7	* Serine	24.44
8	Aspartic Acid	4.91
9	Glycine	4.14
10	Glutamic Acid	3.71
11	Threonine*	6.01
12	Alanine	4.64
13	Tyrosine	1.92
14	Valine	3.85
15	Proline	4.13
16	Methionine	2.78
17	Isoleucine	3.66
18	Leucine	2.46
19	Phenyl Alanine	3.17
20	Tryptophan	3.02
	Total	100.00

Proteins are essential to all life. In animals they help from supporting and protective structures such as cartilage, skin, nail, hairs. They are major constituents of enzymes, antibodies, hormones and body fluids. Essential amino acids meet the demand of growth during childhood. Fish protein contains essential amino acids that are required for the human nutrition and thus improve the overall protein quality of a diet[19]. The predominant amino acids present in *Puntius Sophore* are histidine followed by glutamic acid, aspartic acid, serine, threonine and lucine[16]. Several reports are available to check the effect of U.V radiation on the fish collagen; the U.V radiation causes the progressive degradation of the collagen molecules into smaller molecular fragments[6, 27]. Collagen peptide is beneficial as a dietary supplement to suppress UV-B-induced skin damage and photo aging[28].

Continuous decrease in protein content is observed after 1 (0.554), 10 (2.108) and 20 (2.88) hours of radiation (Table. 3, 4). Amount of different amino acids in fish scales have also been determined after 1, 10 and 20 hours of U.V. radiation, after one hour of exposure maximum decrease is observed for glutamic acid (1.704%) while cystine shows minimum decrease (0.369%), after 10 hours of exposure the maximum percentage of phenylalanine (2.821) decreases maximum and percentage of lysine (1.754) decreases minimum, valine decreases maximum (4.736%) while cystine decreases minimum (3.367%) after the exposure of 20 hours of radiation (Table. 6). Table 2 clearly indicates that there is no change in mineral constituents after 1, 10 and 20 hours of radiation; the change is only in moisture content. Amino acids are decreased continuously with the time of exposure (Figure. 1). U.V. radiation was found useful to improve the gel strength of fish gelatin[9].

CONCLUSION

The entire study leads to the conclusion that moisture content shows a decrease as result of U.V. irradiation. Crude protein, crude fat and total

Table 6: Percentage of Amino Acids of scales of fish *Puntius Sophore* after iradiation (gm/100 gm of the dry matter)

Amino Acid	Values after irradiation at different time intervals					
	1 hour		10 hours		20 hours	
	value	% decrease	Value	% decrease	Value	% decrease
Cystine	8.319	0.369	8.147	2.431	8.069	3.367
Lysine	2.393	0.697	2.368	1.754	2.327	3.441
Histidine	3.672	1.021	3.615	2.554	3.541	4.550
Arginine	4.913	0.747	4.837	2.277	4.755	3.933
Hydroxy Proline	5.217	1.195	5.143	2.598	5.059	4.188
Cysteine	2.431	1.171	2.393	2.716	2.352	4.375
*Serine	24.312	0.524	23.881	2.288	23.524	3.749
Aspartic Acid	4.888	0.454	4.786	2.518	4.730	3.665
Glycine	4.103	0.903	4.048	2.221	3.971	4.077
Glutamic acid	3.647	1.704	3.615	2.554	3.541	4.550
Threonine*	5.926	1.397	5.856	2.568	5.767	4.042
Alanine	4.609	0.665	4.532	2.332	4.452	4.056
Tyrosine	1.899	1.075	1.871	2.538	1.846	3.829
Valine	3.824	0.674	3.768	2.130	3.668	4.736
Proline	4.075	1.337	4.023	2.601	3.971	3.845
Methionine	2.748	1.160	2.704	2.741	2.666	4.100
Isoleucine	3.621	1.053	3.577	2.266	3.529	3.591
Leucine	2.431	1.171	2.393	2.716	2.352	4.375
Phenyl Alanine	3.140	0.937	3.081	2.821	3.035	4.249
Tryptophan	2.988	1.049	2.953	2.209	2.884	4.518
TOTAL	99.157		97.591		96.040	

N- Terminal residue not determined
-$CONH_2$ not determined
*Corrected for the loss during hydrolysis

nitrogen decrease with the time of exposure. Minerals remain unchanged as a result of U.V. irradiation. The individual amino acids also decrease with the time of exposure.

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