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# ESTIMATION OF VOLATILE CONSTITUENTS IN THE FISH FLESH FROM WILD AND FARMED CIRRHINA MRIGALA AND CYPRINUS CARPIO

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Abstract: Analysis of fish meat using gas chromatography is described. Flavor is the sensation arising from the interplay of the signals of sensing smell, taste and irritating stimuli from food stuff. For human, flavor and nutrition are inseparable. In fish, trace amount of volatile organic compounds (VOCs) are the major compounds to affect consumer's preference, which are produced during storage and spoilage. In the present study, volatile compounds were extracted by Likens-Nickerson concurrent distillation apparatus from wild and farmed Cirrhina mrigala and Cyprinus carpio. The quantitative and qualitative estimation of volatiles was made by gas chromatography. Wild and farmed fish of different fish sizes were compared for these compounds (appearing in the form of peaks), which were identified from their retention time by comparing with the standards. Fifteen major VOCs were found in these species which included hexadecane, 3-octanol, hexanal, decane, 3-hexene-1-ol, 2-undecanone, 2-heptanone, butanal, 2-nonanone, 1-heptanal, furaldehyde, 3-methyl-1-butanal, trans-3-hexene-1-ol, octanal and decanal. These compounds varied qualitatively and quantitatively in both wild and farmed fish of different fish sizes.

Key words: Volatile organic compounds; Retention time; Fish meat; Cirrhina mrigala; Cyprinus carpio
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Nowadays, fish consumption is increasing for the freshness and palatable flavour of fish meat. Flavour is the sensation arising from the interplay of signals of sensing smell, taste and irritating stimuli from foodstuffs. For human, flavour and nutrition are inseparable. It is primarily aroma that distinguishes the flavour of one food from the others. Aroma depends upon the vapour pressure of food constituents and on the interaction between volatile compounds and non-volatile compounds[1]. Aroma is formed in trace amount from volatile organic compounds (VOCs) and semi-volatile compounds, which is considered more important than taste volatile compounds because it can be measured and the quantity reflects the freshness of fish. Freshness of fish is important to consumer's preference and there is a strong tendency to select fresh fish as the alternative meat source for human[2]. First sensory change of fish during storage is concerned with the appearance and texture. The characteristic taste of species is normally developed in the first couple of days during storage in ice. The most dramatic change is the onset of rigor mortis. After death muscles are totally relaxed immediately and limp elastic texture usually persists for some hours, thereafter muscles will contract. When it becomes hard and stiffs the whole body becomes inflexible and the fish is in rigor mortis. This condition usually lasts for a day or more and then rigor resolves. The resolution of rigor mortis makes the muscles relax again and it becomes limp but no longer as elastic as before. The rate of onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical conditions of fish[3].

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One of the volatile compounds trimethylamine (TMA) may be produced which has very characteristic "fishy" smell. Flesh of healthy live or newly caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria get access to proliferate freely. They colonize on the skin surface and invade the flesh during storage by moving between the muscle fibers. Microorganisms are found on all outer surfaces (skin and gills) and in the intestine of live and newly caught fish. These organisms promote spoilage of fish. In the Pakistan fish spoilage mainly takes place during its transport. The present project will indicate the rate of spoilage. Fish from cold water has very lower number of these parasites than from warm waters. Fish spoil at very different rates and differences in surface properties have been proposed to explain this spoilage. Skins of fish have very different textures. Thus whiting (Merlangius merlangus) and cod (Gadus morhua), having very fragile integument spoil rapidly as compared to several flat fish such as Plaice that has very robust dermis and epidermis. Furthermore, latter has very thick slime layer containing several antibacterial components. Smaller and younger fish have less time to accumulate contaminants than older and larger one and are safer for consumption[4].

Volatile sulfur compounds are typical components of spoiled fish and most of the specific spoilage bacteria produce one or several volatile sulfides. The volatile sulfur compounds are very foul-smelling, their minimal quantity has a considerable effect on quality and they can be detected at ppb levels. Oxidation and hydrolysis of fish lipids are two distinct reactions for quality deterioration. They result in production of wide range of substances, among which some have unpleasant taste and smell, some may also contribute to texture changes by binding covalently to fish muscle proteins. Fatty fish are of course particularly susceptible to lipid degradation and can create a severe quality problem even on storage at subzero temperatures[5]. The present study was designed to estimate the volatile constituents in fish flesh from wild and farmed Cirrhina mrigala and Cyprinus carpio of three different fish sizes, to see the effect of body weight on the accumulation of volatiles and to suggest their preference for consumption of these organic compounds.

## 1 Material and Methods

**1.1 Preparation of Fish Sample for Analysis** Farmed Cirrhina mrigala and Cyprinus carpio of three fish sizes designated as $F_1$ (500—800 g), $F_2$ (801—1100 g) and $F_3$ (1101—1400 g) were procured from Fish Hatchery, Satiana Road, Faisalabad, Pakistan. Whilst wild Cirrhina mrigala and Cyprinus carpio of three fish sizes designated as $W_1$ (500—800 g), $W_2$ (801—1000 g) and $W_3$ (1101—1400 g) were procured from Head Trimmu, Jhang. Fish were transported lively from the catchment to Fisheries Research Laboratory, Department of Zoology, GC University, Faisalabad, Pakistan.

Each fish sample was washed with tap water and then given longitudinal cut from the ventral side. Visceral organs were removed in order to avoid contamination by microbes from the viscera. Flesh was removed from the fish sample, weighed on electrical balance and cut into pieces before storage in freezer for further analysis. These samples were analyzed for detection of volatile components in Flavour Research Laboratory, Nuclear Institute for Agriculture and Biology, Faisalabad. Seven replicates were used for critical analysis. In this project, Liken-Nickerson concurrent distillation extraction method was used for extraction of volatile compounds as described previously[6].

**1.2 Identification of Components** The peaks for the compounds present in the volatile mixture were recorded in the given time of half an hour of chromatogram completion. The retention time and concentrations of VOCs were noted by gas chromatograph directly. Identification of these unknown compounds was made by comparing with the standards under identical working conditions.

Column used 2 m × 2 mm packed with 10% SE-30 on chromosorb WAW 80-100 mesh. Column temperature was programmed at 80 ℃ for one minute and then rose at the rate of 8 ℃ / min up to 150 ℃. Injector temperature 150 ℃, Detector temperature 200 ℃, Carrier Gas Nitrogen, Flow rate of Carrier Gas 25 mL/min, Hydrogen pressure 20 psi, Air pressure 50 psi.

Volume Injected 10μL. Gas Chromatograph Perkin Elmer Model 3920 Equipped with FID and Shimadzu C-R4A chromatopac Integrator.

Qualitative and quantitative estimation for each individual compound was made by gas chromatograph itself. The compounds represent in the form of peaks on the recorder and the concentration was given by the GC directly for each compound. The identification for each individual compound was done by comparing with that of standards.

Following standards were used: Hexadecane, 3-octanol, hexanal, pentadecane, 3-hexene-1-ol, 2-undecanone, 2-heptanone, butanal, 2-nonanone, 1-heptanal, furaldehyde, 3methyl-1-butanol, trans-3-hexene-1-ol, octanal and decanal (Merck, Germany). Two-way analysis of variance was performed by following (11).

## 2 Results

Chromatogram of wild Cirrhina mrigala of group $W_1$ showed that 24 volatile organic compounds were presented in the essence. However, only 10 compounds were identified, there were hexanal, trans-2-hexen-1-ol, 1-heptanal, octanal, 3-octanal, 2-nonanone, decanal, 2-undecanone, pentadecane and hexadecane (Fig. 1A). Decanal was the major volatile compound followed by 2-undecanone. Chromatogram obtained from the flesh of wild Cirrhina mrigala of $W_2$ revealed that 30 volatile compounds were presented in this group. While only 8 compounds were identified as 3-hexen-1-ol for peak 2, octanal for peak 9, 3-octanol for peak 10, 2-nonanone for peak 11, decanal for peak 13, 2-undecanone for peak 15, pentadecane for peak 18 and hexadecane for peak 20. In group $W_2$, 2-undecanone was the major compound closely followed by decanal and pentadecane (Fig. 1B).

The chromatogram of volatile compounds in the flesh of Cirrhina mrigala of group $W_3$ revealed that 23 compounds were present in this fish flesh. Among which, 7 compounds were identified as furaldehyde, trans-2-hexen-1-ol, 2-heptanal, 3-octanol, decanal, 2-undecanone and hexadecane. It has been observed that decanal and 2-undecanone was found in equal proportion and was closely followed by hexadecane while 2-heptanone was found in the least amount (Fig. 1C).

Chromatogram in flesh of farmed Cirrhina mrigala of group $F_1$ showed that 23 volatile compounds were detected. Among which, 7 compounds were identified as 3-methyl-1-butanol, 3-hexen-1-ol, 1-heptanal, 3-octanal, 2-undecanone, butanal and hexadecane. 2-Undecanone was in abundance and was followed by 3-hexen-1-ol and butanal (Fig. 1D).

Chromatogram of farmed fish of group $F_2$ showed that there were 7 compounds presented in this fish size. Among which, 8 compounds were identified as furaldehyde, trans-2-hexen-1-ol, 2-undecanone, 3-octanol, 2-nonanone, 2-heptanopne, butanal, and hexadecane. Maximum concentration was recorded for trans-2-hexen-1-ol (Fig. 1E).

Chromatogram of farmed Cirrhina mrigala of $F_3$ showed that 18 compounds were presented in fish meat of this fish. 7 compounds were identified as trans-2-hexen-1-ol, octanal, 3-octanal, 2-nonanone, decanal, 2-undecanone and hexadecane. Trans-2-hexen-1-ol and 2-nonanone showed almost in same concentration closely followed by 2-undecanone. 3-Octanal occurred in the least amount (Fig. 1F).

Chromatogram obtained from the analysis of flesh sample from wild Cyprinus carpio of $W_1$ showed that 24 compounds were presented in the fish but 15 were observed by the recorder phase of GC. From these 15 compounds, 6 compounds were identified. They were furaldehyde, 1-heptanone, 3-octanol, 2-nonanone, 2-undecanone, hexadecane. From these compounds, 1-heptanone was found in considerable amount closely followed by 2-nonanone (Fig. 1G).

Chromatogram obtained from the analysis of flesh sample from wild Cyprinus carpio of $W_2$ indicated that 14 compounds were presented in the sample. Out of those, 9 compounds were identified. They were trans-2-hexen-1-ol, 1-heptanal, octanal, 3-octanol, 2-nonanone, decanal, 2-undecanone, butanal and hexadecane. 2-nonanone was found in the maximum concentration (Fig. 1H).

Chromatogram obtained from the analysis of fish meat from wild Cyprinus carpio of $W_3$ represented that 26 volatile compounds were presented in the fish. From these total 26 volatile compounds, 8 compounds have

been identified as furaldehyde for peak 1, trans-2-hexen-1-ol for peak 3, 3-octanal for peak 5, 2-nonanone for peak 8, decanal for peak 12, 2-undecanone for peak 15, pentadecane for peak 19 and hexadecane for peak 20. In this group, decanal was the major compound followed by pentadecane (Fig. 1 I).

Chromatogram obtained from the analysis of flesh from farmed Cyprinus carpio of $F_1$ showed 24 volatile compounds. Out of those total 24 volatile compounds, 9 compounds were identified. They were 3-methyl-1-butanol, hexanal, 1-heptanal, octanal, 2-nonanone, decanal, 2-undecanone, butanal and hexadecane. Volatile compound 2-nonanone was dominating (Fig. 1 J).

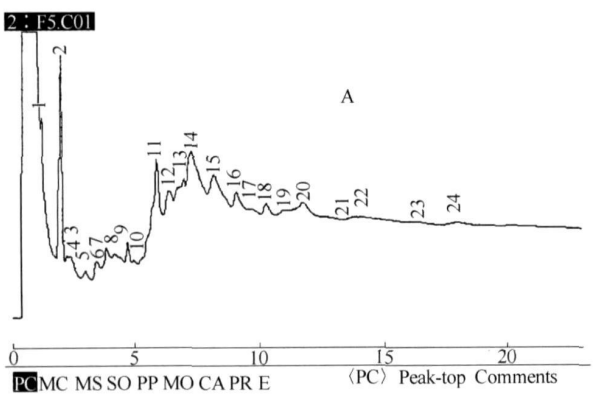
Chromatogram obtained from the analysis of essence from farmed Cyprinus carpio of $F_2$ showed that 22 volatile compounds were presented in fish flesh. Out of those 22 volatile compounds, 6 volatile compounds were identified. They were hexanal, octanal, 3-octanol, 2-nonanone, 2-undecanone and hexadecane (Fig. 1 K).

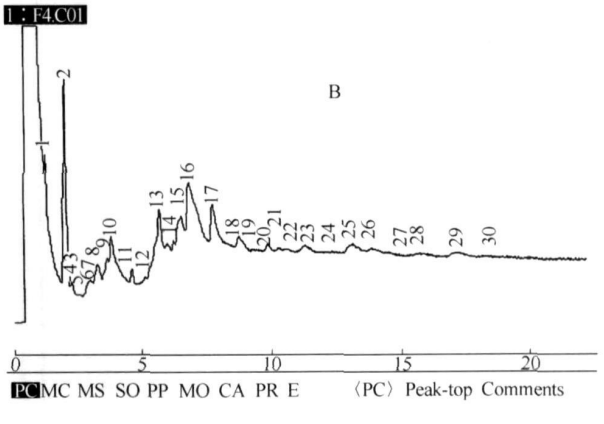
Chromatogram obtained from the analysis of essence from farmed Cyprinus carpio of $F_3$ showed that 28 volatile compounds were presented in this fish sample. Out of those 28 volatile compounds, 7 were identified. They were 3-methyl-1-butanal, hexanal, 2-nonanone, decanal, 2-undecanone, butanal and hexadecane. Hexanal was found in maximum concentration (Fig. 1 L).

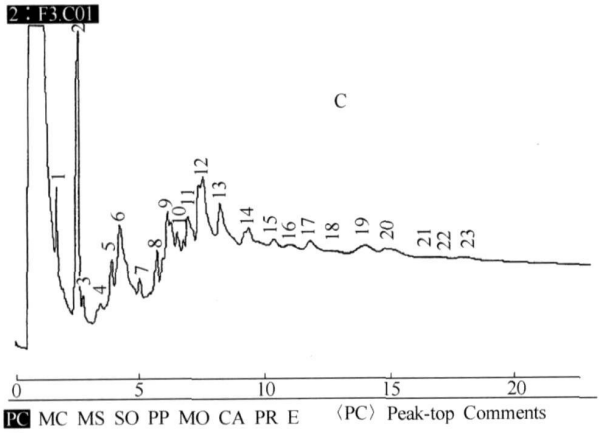
Figures (M-P) indicate comparative studies between wild and farmed and in between different species.

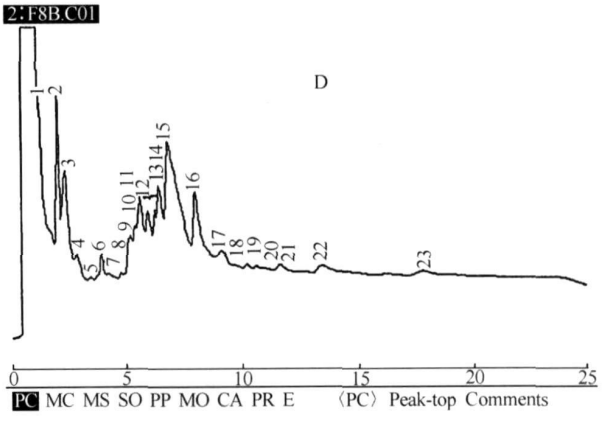
## 3 Discussion

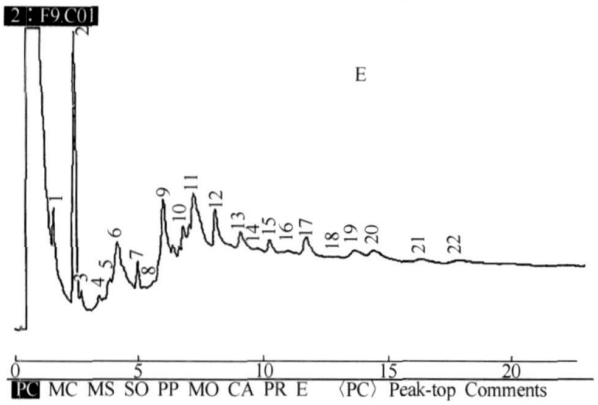
Taste is usually thought to be perceived in the mouth and is due to non-volatile constituents, while aroma is usually thought to be perceived in nose and mainly due to volatile constituents of the food[7]. However some volatile compounds not only affect taste (flavour by mouth) but also aroma (flavour by nose). Although odour or taste perception by human is not normally necessary for survival, we are still quite sensitive to volatile substances. Certain foods are liked or

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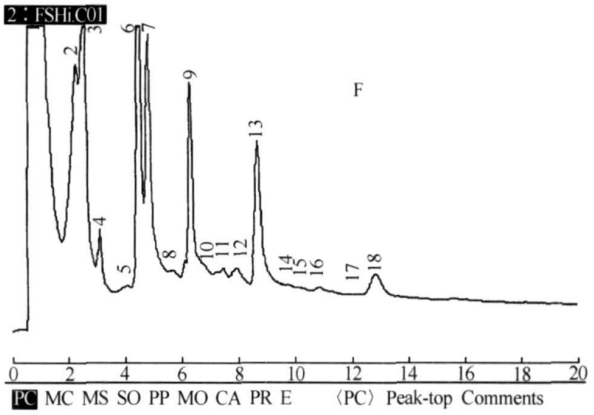
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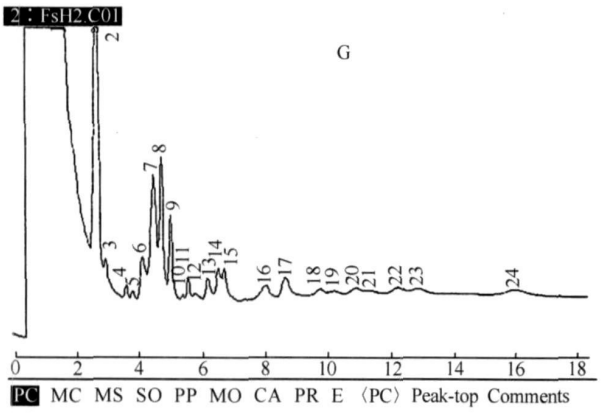
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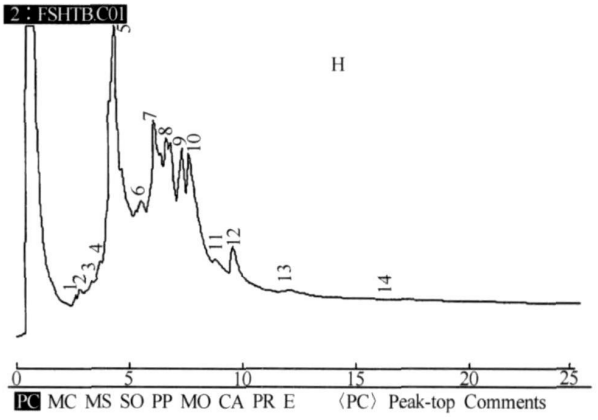
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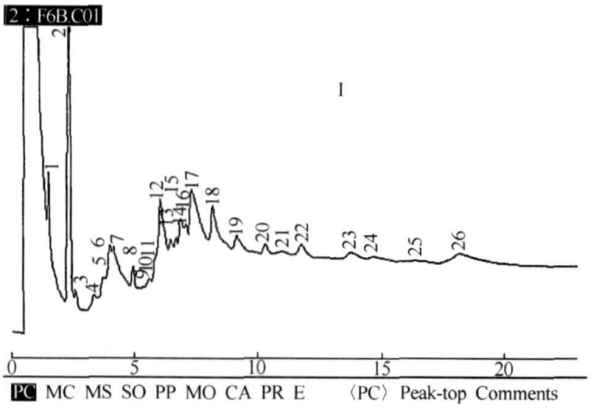
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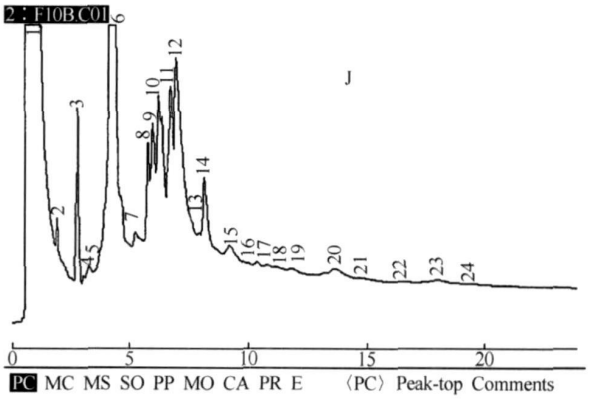
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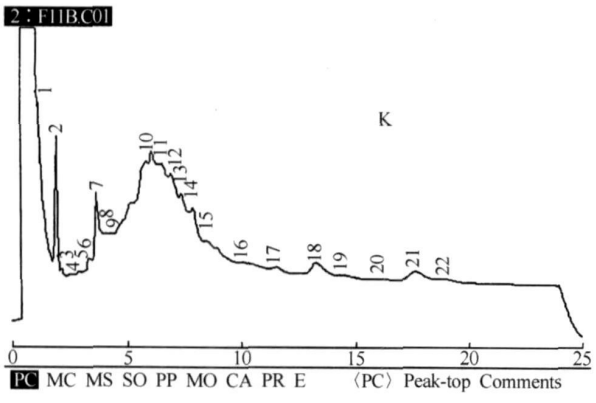
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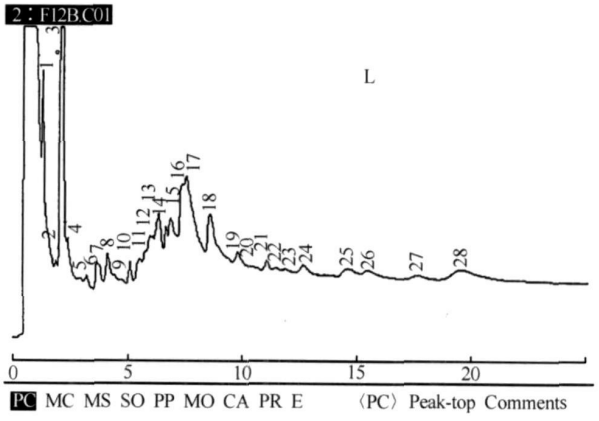
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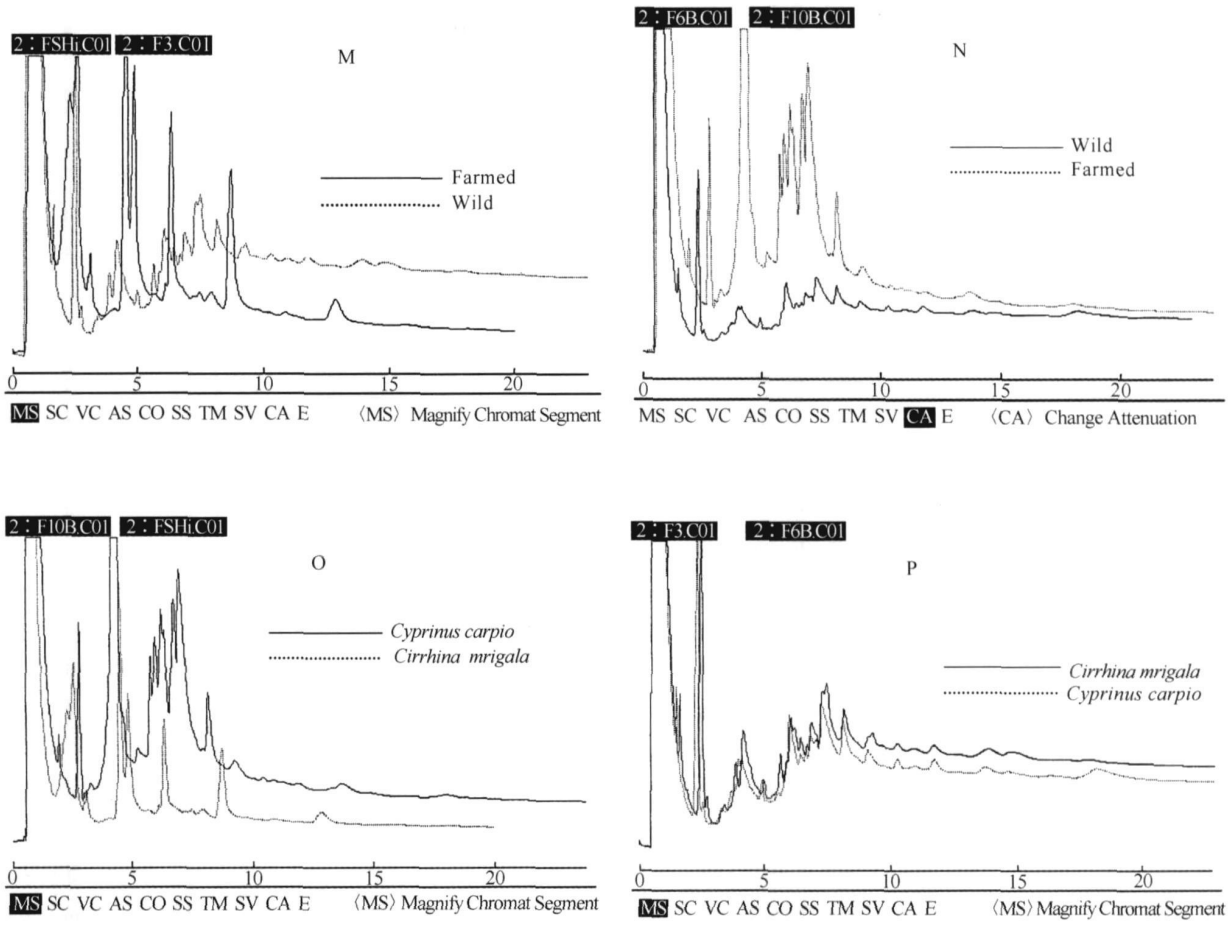


Fig. 1 Volatile compounds are shown in the form of peaks (each peak represent particular type of compound) in the chromatograms above (A) Volatile compounds in flesh from wild Cirrhina mrigala under weight category $W_1$ (500-800 gm); (B) volatile compounds in flesh from wild Cirrhina mrigala under weight category $W_2$ (801-1100 gm); (C) volatile compounds in flesh from wild Cirrhina mrigala under weight category $W_3$ (1101-1400 gm); (D) volatile compounds in flesh from farmed Cirrhina mrigala under weight category $F_1$ (500-800 gm); (E) volatile compounds in flesh from farmed Cirrhina mrigala under weight category $F_2$ (801-1100 gm); (F) volatile compounds in flesh from farmed Cirrhina mrigala under weight category $F_3$ (1101-1400 gm); (G) volatile compounds in flesh from wild Cyprinus carpio under weight category $W_1$ (500-800 gm); (H) volatile compounds in flesh from wild Cyprinus carpio under weight category $W_2$ (801-1100 gm); (I) volatile compounds in flesh from wild Cyprinus carpio under weight category $W_3$ (1101-1400 gm); (J) volatile compounds in flesh from farmed Cyprinus carpio under weight category $F_1$ (500—800 gm); (K) volatile compounds in flesh from farmed Cyprinus carpio under weight category $F_2$ (801—1100 gm); (L) volatile compounds in flesh from farmed Cyprinus carpio under weight category $F_3$ (1101-1400 gm); M-P show comparison of VOCs of two samples in a single chromatogram; (M) volatile compounds in flesh from farmed and wild Cirrhina mrigala; (N) volatile compounds in flesh from farmed and wild Cyprinus carpio; (O) Chromatogram for comparison of volatile compounds in flesh from farmed Cirrhina mrigala and Cyprinus carpio; (P) volatile compounds in flesh from wild Cirrhina mrigala and Cyprinus carpio

disliked because of such compounds present in very small concentration. Typically fish do not contain level of contaminants, they are enough to cause a remarkable threat to the health even after a few meals. Risk from persistent organic contaminants increases for those persons regularly consuming larger fish and predatory fish from areas of contaminations. In the present study, wild fish was caught from the river and farmed fish was procured from hatchery ponds to generate the information for the consumers. It is evident from the results that wild fish contains more number of volatile organic volatile organic compounds as compared to farmed

fish[8]. Their findings are in line with the results of the present study. It has been recorded that the amount of VOCs slightly increases with increase in body weigh[9]. They were documented as products of oxidation of fatty acids in the fish flesh just after death and declared as one of the major cause of off-flavor in fish. Flavor is created by aromatic substances which are produced from mass grown in nature. Aromatic substances with important odour and taste affect the human palate with relish, zest and sense[7]. Chromatograms obtained from the analysis of wild and farmed Cirrhina mrigala indicated presence of a large number of VOCs in even fresh flesh. They may affect negatively on the consumers preference as has been found in the present study. Soil also contains large number of VOCs[10]. As this fish species is a bottom feeder and is exposed to almost all contaminants which rest upon bottom and are absorbed by plants there. These plants, crustaceans may be consumed by this fish species and may get accumulated in their muscles. These organic chemicals cause sensory changes in fish linked with appearance and texture. It is also evident from the findings that freshness of the fish can be detected by VOCs present in the fish. The similar attempts have been made in the present study as the information in the literature is non existent about fish flavour storing and quality improvements of indigenous Indian major carps. These findings may be considered as the initial information about these fish species for consumers and researchers.

## 4 Conclusions

We extracted fifteen volatile compounds by Likens-Nickerson concurrent distillation apparatus from wild and farmed Cirrhina mrigala and Cyprinus carpio. For this purpose, fish were cooked and volatiles were collected in the organic solvents. Quantitative and qualitative estimation was made by gas chromatography. Wild and farmed fish of different fish sizes were compared by these compounds (appearing in the form of peaks), which were identified from their retention time by comparing with the standards. The compounds were hexadecane, 3-octanol, hexanal, pentadecane, 3-hexene-1-ol, 2-undecanone, 2-heptanone, butanal, 2-nonanone, 1-heptanal, furaldehyde, 3-methyl-1-butanal, trans-3-hexene-1-ol, octanal and decanal, which were the major volatile organic compounds in these species. These compounds varied qualitatively and quantitatively in both wild and farmed fish of different weight groups. The statistical analysis indicated that there were significant differences among the species. There were significant variations among different compound concentrations when we dealt at compound level. The results are shown in the tables 1-3. From our findings we can conclude that these compounds are responsible for the off-flavor of wild and farmed Cirrhina mrigala. Further, detailed study can explore the mechanism of these volatile compounds in the spoilage of Cirrhina mrigala and Cyprinus carpio.

Tab. 1 Analysis of variance for concentration (% age) of volatile compounds from wild and farmed Cirrhina mrigala and Cyprinus carpio of three different fish sizes

| Source of Variation | DF | SS | MS | F | Probability |
|---|---|---|---|---|---|
| Species | 1 | 45.31 | 45.31 | 1.45 | 0.23$^{NS}$ |
| Wild, Farmed | 1 | 0.02 | 0.02 | 0.00 | 0.98$^{NS}$ |
| Fish sizes | 2 | 26.11 | 13.06 | 0.42 | 0.66$^{NS}$ |
| Species× Wild, Farmed | 1 | 59.45 | 59.45 | 1.90 | 0.17$^{NS}$ |
| Species× Fish size | 2 | 110.21 | 55.11 | 1.76 | 0.17$^{NS}$ |
| Wild, Farmed× Fish size | 2 | 51.32 | 25.66 | 1.82 | 0.44$^{NS}$ |
| Species× Wild, Farmed× Fish size | 2 | 6.70 | 3.35 | 0.11 | 0.99$^{NS}$ |
| Error | 238 | 7447.73 | 31.29 | | |
| Total | 249 | | | | |

Tab. 2 Comparison of means of concentration (% age) of volatile compounds from wild and farmed Cirrhina mrigala and Cyprinus carpio of three different fish sizes

| Fish Species | Fish size | Wild Conc. ± SE | Farmed Conc. ± SE | t value | Probability |
|---|---|---|---|---|---|
| Cirrhina mrigala | $W_1$, $F_1$ | 4.35±0.86 | 4.76±1.46 | −0.24 | $0.81^{NS}$ |
| Cirrhina mrigala | $W_2$, $F_2$ | 3.78±0.58 | 4.54±0.75 | −0.81 | $0.43^{NS}$ |
| Cirrhina mrigala | $W_3$, $F_3$ | 4.08±0.67 | 5.95±1.38 | −1.21 | $0.24^{NS}$ |
| Cyprinus carpio | $W_1$, $F_1$ | 6.67±2.26 | 4.16±1.23 | 0.97 | $0.34^{NS}$ |
| Cyprinus carpio | $W_2$, $F_2$ | 7.14±1.97 | 6.25±2.30 | 0.30 | $0.77^{NS}$ |
| Cyprinus carpio | $W_3$, $F_3$ | 3.99±0.82 | 4.46±1.10 | −0.34 | $0.74^{NS}$ |

Tab. 3 Overall comparison of means of concentration (% age) for volatile compounds from wild and farmed and three different fish sizes

| Variables | Mean Conc. ± SE | Mean Conc. ± SE | t value | Probability |
|---|---|---|---|---|
| Wild Farmed | 4.70±0.45 | 4.91±0.54 | −0.30 | $0.76^{NS}$ |
| $W_1$, $F_1$ | 7.63±0.72 | 7.22±0.71 | 0.41 | $0.68^{NS}$ |
| $W_2$, $F_2$ | 8.28±0.75 | 8.21±0.76 | 0.06 | $0.95^{NS}$ |
| $W_3$, $F_3$ | 7.89±0.66 | 7.25±0.58 | 0.72 | $0.47^{NS}$ |

SE= Standard Error; $W_1$, $W_2$, $W_3$ = 1st, 2nd and 3rd Weight Categories respectively for wild fish;
$F_1$, $F_2$, $F_3$ = 1st, 2nd and 3rd Weight Categories respectively for farmed fish

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