Estimation of volatile organic compounds in farmed and wild rohu, *Labeo rohita*

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Abstract

Volatile organic compounds in farmed and wild rohu, *Labeo rohita*, were extracted by Likens-Nickerson concurrent distillation apparatus. The farmed raised rohu of two weight categories designated as FW_1 (1001-1200g) and FW_2 (501-700g) were procured from the Fish Seed Hatchery, Faisalabad. Concurrently, wild rohu of two weight categories designated as WW_1 (1001-1200g) and WW_2 (501-700g) were captured with gillnet from the Trimu Head. Quantitative and qualitative estimation was made by gas chromatography. In farmed and wild *L. rohita* of the weight group FW_1 and WW_1, 14 and 6 volatile compounds were extracted, respectively. In weight group FW_1, six compounds were identified as 3-methyl-1-butanol, 3-hexene-1-ol, heptanal, 3-octanol, decanal and 2-undecanone. In weight group WW_1, out of six compounds three were identified as heptanal, 1-octen-3-ol, 2-nonanone. In weight categories FW_2 and WW_2, 14 and 13 volatile compounds were detected, respectively. In weight category FW_2, out of 14 compounds, 3-hexene-1-ol, heptanal, 3-octanol, 2-undecanone and decanal were identified. In weight category WW_2, out of 13 compounds, 7 were identified as 3-methyl-1-butanol, 2-heptanone, heptanal, 3-octanol, 2-nonanone, decanal and 2-undecanone. The analysis of variance for retention time and concentration of volatile compounds showed no significant difference ($P>0.05$). The correlation coefficient between retention time and concentration was significant ($P< 0.05$).

Keywords: Farmed rohu, Wild rohu, Volatile organic compound, Retention time, *Labeo rohita*

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Introduction

Fish is an important source of protein and other nutrients in many parts of the world and some countries rely heavily on fish export for their income. Research and reports on fishing practices, fish storage, and fish quality are abundant, most performed with the fish processing factories and distributors in mind. Also, the health sector has performed extensive research on the healthiness of fish and fish oils and encourages consumers to eat more fish, either fresh or frozen. However, little research seems to be done on how to entice the consumers to buy and prepare fish and on their perception of fish as food.

The aromas are formed by the volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), which are present in trace amount. Aroma is considered more important than taste Volatile compounds contributing to the characteristic odor of fish can be measured and their quantity reflects the freshness of fish. Potential health hazards and environmental degradation resulting from the widespread use of volatile organic compounds have promoted increasing concern among scientists, industries and public (Elvevoll & James, 2003). They are light enough to breathe into the nose but heavy enough to be recognized by receptor. Almost all aromas are a result of various substances such as odorant, usually several hundred. Even though a few substances in an aroma may be the key contributors, it is still comprised all the odorants (Bell, 1996; Postel *et al*., 1996).

Freshness is one of the most important aspects of fish because of consumer's preference. There is a strong tendency to select fresh fish, as alteration of meat flavour negatively influences the consumer's acceptance (Venkateshwarlu *et al.*, 2000). 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. Immediately after death, muscles are totally relaxed 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 rigor. 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 (Kim *et al*., 2001). Freshness is a complex concept but can be estimated as a combination of several sensory attributes such as appearance, smell, taste and texture. Postel *et al.* (1996) concluded that volatile compounds are associated with freshness of fish.

Geosmin isolated from rainbow trout and identified as the principal compound responsible for the muddy flavor in farmed fish; saltwater fish contain bromophenol, but it is rarely present in freshwater fish species (Lindsay, 1990). Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar)* were found to be due to lipid oxidation products such as aldehydes and ketones. Several compounds (trimethylamine, dimethyl disulfide, 1-penten-3-ol, 3-methly-1-butanol and dimethyl disulfide) have been observed to increase continuously throughout storage and are of potential use as an indicator of flesh quality (Kim *et al*., 2001). The present study was designed to estimate the volatile constituents in the flesh of wild and farmed rohu under two different weight categories and to suggest their preference for consumption by these organic compounds. Rohu is preferred by the consumer from its aroma, so it was planned for qualitative and quantitative analysis of the volatile organic compounds of its flesh.

Materials and methods

Wild and farmed rohu carp, *L. rohita,* of two weight categories were analyzed for the estimation of volatile compounds. The farm raised specimens designated as FW_1 (1001-1200g) and FW_2 (500-700g) were procured from Fish Seed Hatchery, at Satiana Road, Faisalabad, Pakistan. Concurrently, wild specimens designated as WW_1 (1001-1200g) and WW_2 (501-700g) were captured with the help of gill net from Trimu Head, which is about 95km from Faisalabad. Fish were transported live from the catchments area to Fisheries Research Laboratory, Department of Zoology, GC University, Faisalabad.

The farmed *L. rohita* had been raised on a commercial diet (35% crude protein), whilst the wild ones had evidently fed on natural organisms consisting of crustaceans, insects, phytoplanktons and some other organisms.

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 in Flavor Research Laboratory, Nuclear Institute for Agriculture and Biology, Faisalabad, for detection of volatile components. In this project, concurrent distillation extraction method was used as described by Likens and Nickerson (1964).

The peaks for the compounds present in the volatile mixture were recorded and within half an hour chromatogram were completed. The retention times and concentrations of VOCs were noted by the GC, Perkin Elmer Model 3920 equipped with FID and Shimadzu C-R4A chromatopac integrator. Identification of the unknown compounds was made by comparing with the standards under identical working conditions as followings:

Column used 2m×2mm packed with 10% SE-30 on chromos orb. WAW 80-100 mesh; column temperature programmed at 80ºC for one minute and then rose at the rate of 8ºC /min up to 150ºC; injector temperature 150ºC; detector temperature 200ºC; carrier gas nitrogen; flow rate of carrier gas 25ml/min; hydrogen pressure 20psi; air pressure 50psi; volume injected 10µl.

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

Following standards were used:

Hexadecane, 3-octanol, hexanal, pentadecane, 3-hexene-1-ol, 2-undecanone, 2-heptanone, butanal, 2-nonanone, 1-heptanal, furaldehyde, 3-methyl-1-butanol, rans-3-hexene-1-ol, octanal and decanal (Merck, Germany).

The data thus obtained were subjected for statistical analysis by using MINITAB program through computer for analysis of variance and comparison of means.

Results

The volatile constituents of the wild and farmed rohu carp, *L. rohita*, flesh under two weight categories was estimated to suggest their preference for consumption.

The essence collected from fish samples, using Likens-Nickerson concurrent distillation extraction apparatus was analyzed by GC are presented in Fig. 1.

The chromatogram of the farmed *L. rohita* of the weight category FW_1 revealed 14 peaks, indicating the presence of 14 compounds, six of which identified as 3-methyl-1-butanol for peak 1, 3-hexene-1-ol, heptanal, 3-octanol, decanal, 2-undecanone; the other seven compounds could not be identified due to multiple reasons (Fig. 1a). The chromatogram of the wild *L. rohita* of the weight category WW1 indicated the presence of only six compounds, three of which were identified as heptanal, 1-octen-3-ol, 2-nonanone (Fig. 1b). The chromatogram of the farmed *L. rohita* of the weight group FW_2 showed that 14 volatile compounds were present (Fig. 1c), out of which 3-hexene-1-ol, heptanal, 3-octanol, 2-undecanone and decanal were identified. Whereas, the chromatogram of the wild *L. rohita* of the weight group WW_2 showed that 13 volatile compounds were present (Fig. 1d), out of which 7 were identified as 3-methyl-1-butanol, 2-heptanone, heptanal, 3-octanol, 2-nonanone, decanal and 2-undecanone

Statistical analysis showed that various interactions for retention time of volatiles determined in flesh of farmed and wild *L. rohita* remained non-significant ($P>0.05$). However, the interaction between farmed and wild *L. rohita* for the concentrations of volatile compounds were statistically significant ($P<0.05$) (Table 1). The correlation between retention time and volatile compounds remained significant ($P<0.05$) in *L. rohita* (Table 2).

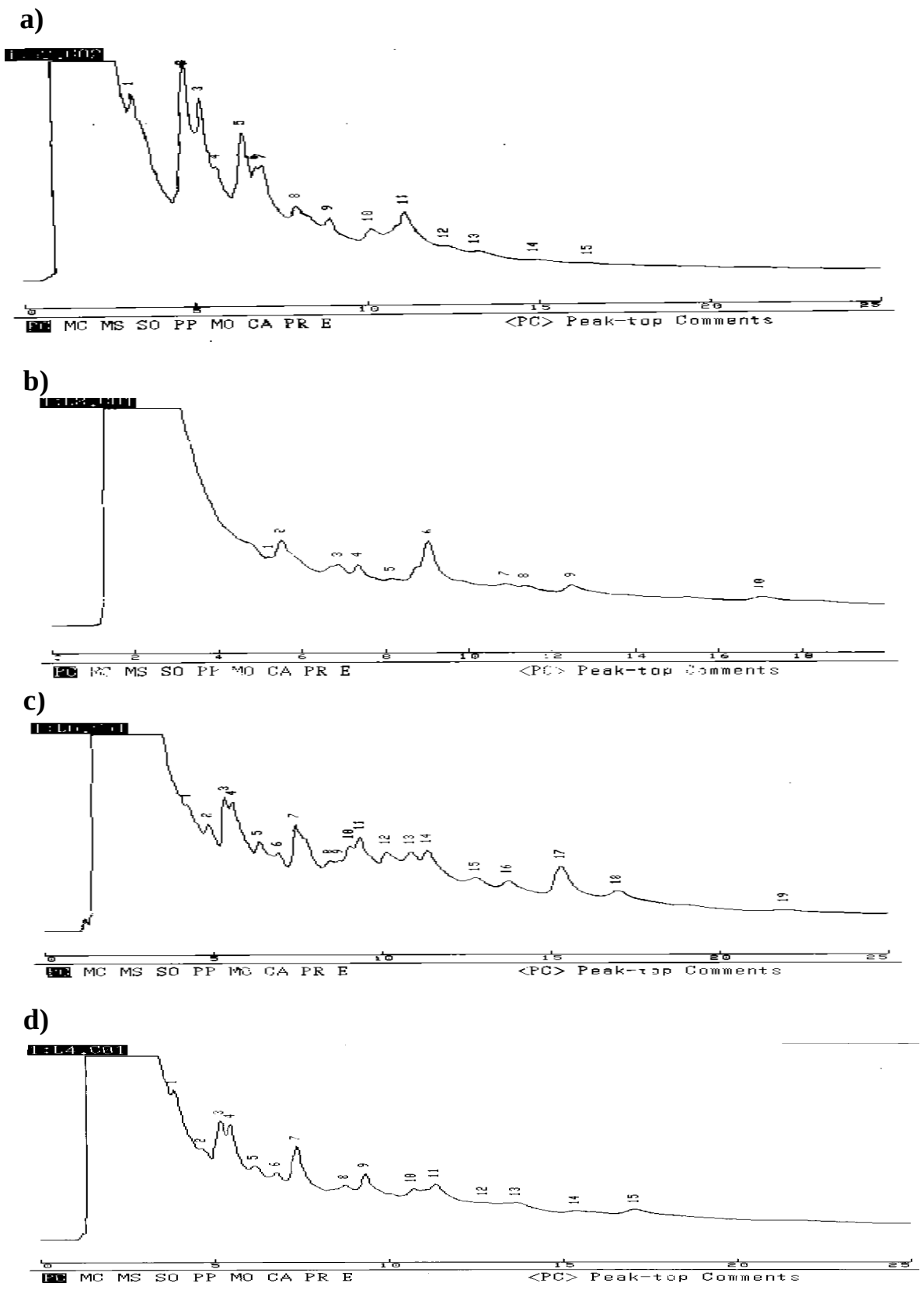

Figure 1: Chromatograms of volatile compounds in the flesh of farmed and wild *L. rohita* under weight categories W_1 (10001-1200g) and W2 (501-700g)

Table 1: Comparison of means for retention time (minutes) of volatiles in *L. rohita*

Weight Category	Farmed Minutes ±SE	Wild Minutes ±SE	T value	Prob.
W_1	9.79 ± 1.50	9.73 ± 1.80	0.83	0.98
W_2	7.80 ± 0.61	9.34 ± 1.10	- 1.18	0.25

W_1=Weight category (1001-1200g).

W_2 =Weight category (501-700g).

Table 2: Correlation between retention time and concentration of various volatiles in wild and farmed *L. rohita*

Weight Categories	Variables	Correlation
Farmed W_1	Rt. Time – Conc.	$- 0.449^{NS}$
Wild W_1	Rt. Time – Conc.	0.022*
Farmed W_2	Rt. Time – Conc.	$- 0.524^{NS}$
Wild W_2	Rt. Time – Conc.	$- 0.452^{NS}$

W1=Weight category (1001-1200g); W2=Weight category (501-700g)
* = Significant; NS=Non-significant; Rt=Retention; Conc=Concentration

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 (Fischer & Widder, 1997). However, some volatile compounds not only affect taste (flavor by mouth) but also aroma (flavor by nose). Although odor or taste perception by human is not normally necessary for survival, we are still quite sensitive to volatile substances. Although flavor perception has been researched widely throughout the years, it is not yet fully understood. Aromatic substances create flavor which has been developed from things grown in nature. Microbial spoilage odor, oxidized odor, environmentally derived odor and processing odor are also related to fresh fish odor. The latter is not interesting when dealing with fresh fish. The fresh fish odor is prevalent during the first few days after catching, after which oxidation products and microbial metabolism dominate aroma of fish.

The presence of compounds mostly 6, 8, and 9 carbon compounds might be derived from the unsaturated fatty acids characteristic of fish by lipoxygenase activities (Grigorakis *et al*., 2003). The higher number of volatile compounds in the farmed fish might be due to the artificial fish feed which was comprising of fish meal, plant material, blood meal and rice bran (Whitefield *et al*., 1997). During the storage, the compound responsible for the very fresh fish flavors might be deteriorated through autolytic or microbial reactions. Flavor is created by aromatic substances which are produced from mass grown in

nature. Aromatic substances with important odor and taste affect the human palate with relish, zest and sense (Fischer & Widder, 1997). Chromatograms obtained from the analysis of wild and farmed *L. rohita* indicated presence of a large number of VOCs in fresh flesh. They may affect negatively the consumers' preference as has been found in the present study. It is also evident from the findings of Raatikainen *et al*. (2001) 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 flavor, storing and quality improvements of indigenous Indian major carps.

It is concluded that the number of volatiles compounds are greater in farmed *L. rohita o*f weight categories FW_1 and FW_2, which could be the reason of production of off- flavor, low taste and less acceptability by the consumer and preference of consumer for wild fish in our local market, which have been confirmed in this study from the volatile composition of the fish flesh.

References

Bell, G.A., 1996. Molecular mechanism of olfactory perception: Their potential for future technologies. Trends in Food Science and Technology. 7**(12):** 425-431.

Fischer, N. and Widder, S., 1997. How proteins influence food flavor? Food Technology. **51:**68-70.

Elvevoll, E. and James, D., 2003. Potential benefit of fish for material, fatal and neonatal nutrition. A review of literature. Food Nutrition and Agriculture. 8**(27):**28-37.

Grigorakis, K., Taylor, K.D.A. and Alexis, M.N., 2003. Organoleptic and volatile aroma compounds of wild and cultured gilthead sea bream (*Sparus aurata*): Sensory differences and possible chemical basis. Aquaculture. **225:**109-119.

Kim,Y.H., Nam, K.C., Ismail, H.A., Hur, S.J., Du, M. and Ahn, D.U., 2001. Volatile profiles, lipid oxidation and sensory characteristics of irradiated meat species. Meat Science. **61:**257-265.

Likens, S.T. and Nikerson, G.B., 1964. Study detection of certain hop oil constituents in brewing products. American Society of Brewing Chemists Proceeding. 5-13.

Lindsay, R.C., 1990. Fish Flavor. Food Reviews International. 6**(4)**:437-455.

Postel, R.T., Ladoucceur, M., Hobert, D. and Gallagher, M.L., 1996. Texture and flavour of hybrid striped bass fed soybean meal diets. Journal of Aquatics Food Production Technology. pp.83-91.

Raatikainen, O., Pursiainen, J., Hyvonen, P., Von Wright, A., Reinkainen, S.P. and Muje, P., 2001. Fish quality assessment with ion mobility based gas detector. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biology Wet. 66**(3b):**475-80.

Venkateshwarlu, G., Meyer, A.S., Let, M.B. and Jacobsen, C., 2000. GC olfactrometric characterization of odor impact volatiles in fish oil enriched milk 16 drinks. Journal of Agriculture and Food Chemistry. **52:**311-317.

Whitifield, F.B., Helidoniotis, F., Shaw, K.J., Svoronos, D., 1997. Distribution of bromophenols in Australian wild-harvested and cultivated prawns (shrimp). Journal of Agriculture and Food Chemistry. **45:**4398-4405.