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clarez@ula.ve
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Lubes, Vito; Lubes, Giuseppe

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Artículo científico

Exploratory analysis of the volatile profile of Atlantic salmon (*Salmo salar*) and the rainbow trout (*Oncorhynchus mykiss*) by HS-SPME-GC/MS

Vito Lubes, Giuseppe Lubes*

Laboratorio de Equilibrios en Solución. Universidad Simón Bolívar (USB),
Apartado 89000, Caracas 1080 A, Venezuela

(*) glubes@chemist.com

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Resumen

Análisis exploratorio del perfil de volátiles del salmón del Atlántico (*Salmo salar*) y la trucha arcoíris (*Oncorhynchus mykiss*) por HS-SPME-GC/MS. El perfil de compuestos volátiles del salmón Atlántico (*Salmo salar*), proveniente de dos orígenes diferentes, y de la trucha arcoíris (*Oncorhynchus mykiss*) ha sido analizado por medio de micro extracción en fase sólida (SPME, por sus siglas en inglés) y GC–MS. Sesenta y seis compuestos químicos fueron aislados, de los cuales cincuenta de ellos fueron identificados basados en sus espectros de masas y sus índices de retención de Kovats. Hidrocarburos, alcoholes y cetonas fueron los compuestos más abundantes encontrados en *S. salar*, mientras que los aldehídos eran de mayor importancia en *O. mykiss*.

Keywords: *Salmo salar*, *Oncorhynchus mykiss*, GC-MS, data mining, SPME.

Abstract

The volatile profile of the Atlantic salmon (*Salmo salar*), from two different origin, and rainbow trout (*Oncorhynchus mykiss*) has been analyzed by solid phase micro-extraction (SPME) and GC–MS. Sixty-six substances, from different chemical classes, were isolated but only fifty were identified based on their mass spectral and Kovats indexes. Many of these compounds were found in both samples but showing quantitative differences. Hydrocarbons, alcohols and ketones were the most abundant compounds in *S. salar* samples while aldehydes were predominant in *O. mykiss*.

Keywords: S*almo salar, Oncorhynchus mykiss*, GC-MS, chemometrics, SPME.

Introduction

Two of the most studied species of fishes and with major acceptance by consumers, are represented by the genera *Salmo* and *Oncorhynchus*[1,2]. The Atlantic salmon (*S. salar*) and the rainbow trout (*O. mykiss*) are commercially farmed from aquaculture around the world, being Norway one of the biggest producers followed by Chile[2]. Only in Chile, the salmonid aquaculture arose a value of US$3.8 billion[3], and due to the beneficial health effects of omega-3 polyunsaturated fatty acids (PUFA), the fish market of fatty fish, such as salmon and trout, shows an increasing trend[4].

Aroma, on the other hand, is one of the key attributes that brings important organoleptic characteristics to fish products and enhance or reduce the consumer's acceptance[5]. Although in freshly harvested and/or processed fish have been identified several hundred of volatile organic compounds (VOCs), from different chemical classes, only a few numbers are essentially characteristic of the fish aroma[6]. The volatile profile of the raw trout, for example, contains alcohols, aldehydes, hydrocarbons, esters and phenol derivatives[7]. Aldehydes and alcohols such as *n*-hexanal, *n*-heptanal, *n*-nonanal, (*Z*)-4-heptenal, *n*-octanal, (*E*)-2-nonenal, *n*-decanal, benzeneacetaldehyde, (*E,E*)-2,4-decadienal, 1-octen-3-ol and (*E*)-1,5-octadien-3-ol are potent fish aroma compounds on the basis of their odour activity values (OAVs)[8]. Other compounds can be produced by enzymatic reaction, lipid autoxidation, microbial action, etc., and they can be important contributors to the fish aroma profile[9,10].

Solid phase micro-extraction (SPME) in combination with gas chromatography-mass spectrometry (GC-MS) are the preferred techniques for the analysis of VOCs in the determination of freshness in fish and the evaluation of different processes that can affect their aromatic composition[7,11,12]. In the present study, we employed these techniques to analyze the aromatic profile of two commercially important fish species, *Salmo salar* and *Oncorhynchus mykiss*.

Materials and methods

Sample preparation

Fresh fish fillets (250 g each approx.) from *Salmo salar* (from two different origins) and *Oncorhynchus mykiss*, were purchased from local markets and transported within one hour with isothermal bags to the laboratory for GC analysis. Each sample was grinded with the help of a mortar. 1 g of sample was weighed and placed in 20 mL round-bottomed headspace vial; afterwards, 4.95 mL of a NaCl aqueous solution (20 %

w/v) were poured into the vial. A solution of cinnamaldehyde (50 µL, 100 mg.L^{-1}) was added as Internal Standard (IS). Subsequently, vial was tightly capped with a polytetrafluorethylene (PTFE) septum.

SPME extraction

For extraction of VOCs from the headspace of fish samples the 50/30 µm divinylbenzene/carboxen on PDMS (DVB/CAR/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was employed. Each vial was equilibrated at 65 °C for 10 min, followed by the VOCs extraction during 30 min. The VOCs were thermally desorbed for 3 min into injector port (splitless mode) heated to 220 °C equipped with a SPME liner (0.75 mm i.d., Supelco, Bellefonte, PA, USA).

GC-MS analysis and data acquisition

The VOCs were separated on an Agilent instrument 6890A gas chromatograph–5973N mass spectrometer (GC–MSD, Agilent Technologies, Palo Alto, CA) equipped with a fused silica capillary column DB-WAX (J&W; 60 m×0.25 mm i.d.) with a 0.25 µm film thickness. Agilent ChemStation software controlled the GC–MSD system for data acquisition. The GC oven temperature program applied was: initial temperature: 40 °C for 3 min followed by a linear thermal gradient of 6 °C/min to 230 °C and held for 15 min resulting in a run time of 49.67 min. The GC–MS interface temperature was set at 230 °C. Helium was used as a carrier gas with column flow rate of 1.5 ml/min (total flow of 104.3 ml/min, pressure: 157.8 kPa). The separated VOCs peaks were analyzed with quadrupole mass spectrometer working in electron ionization (EI) mode at 70 eV at 150 °C. The mass spectra were scanned in the range of 35–350 *m/z*. The preliminary identification of individual VOCs was based on the comparison of their mass spectra with NIST02 database using a similarity index (SI, > 70 %) and comparison of retention times of available analytical standards. The data were analyzed using OpenChrom software, version 1.1.0 (Diels)[13]. Retention indices were calculated by using a *n*-alkane mixture (C7-C40 from Sigma Aldrich, Germany) and through the equation: $Ix = 100n + 100(tx-tn)/(tn+1 - tn)$; where n, n+1 refer to the hydrocarbons eluting immediately before and after the compound of interest and x to the compound of interest[14].

Pre-processing and data analysis

Five step algorithms already installed in OpenChrom were employed for pre-processing the chromatographic data. *Step 1:* a defined set of selected ions are removed preliminarily to reduce the noise and optimize the mass spectrometric data. It consists of removing water (*m/z* 18); nitrogen (*m/z* 28), SPME bleed (*m/z* 73), argon (*m/z* 44) and column bleed (*m/z* 207). *Step 2*: The Savitzky-Golay filter was applied using a smoothing degree of two and a width of seven. *Step 3*: baseline detection using the predefined parameters from the software. *Step 4*: peak detection using a first derivative algorithm and a min S/N ratio of 10. *Step 5*: peaks were integrated with Peak Integrator Trapezoid algorithm selecting a min S/N ratio of 10. All the pre-processed chromatograms were exported into .txt format for peak alignment. Alignment of peak was done at peak-table level with GCAligner 1.0 software[15] and using a weight parameter alpha (α) of 0.125 and the internal standard, found in all the chromatograms, as peak reference. From the output, CSV file, signals from a blank chromatogram were used to subtract artifacts and false peaks coming from packaging in all the chromatograms. The output data was subsequently analyzed as recommended in literature[16,17], for that, the final data matrix containing the peak areas of 66 VOCs found in nine samples was subjected to statistical multivariate analysis in Clustvis web tool[18]. Principal Component Analysis was performed using auto-scaled data and under NIPALS algorithm. The heatmap dendrogram was generated for illustrate overall similarity between samples based on VOCs profiles. The normalized similarity distances were based on the Euclidean distance calculated using Ward linkage clustering analysis.

Results and discussion

The volatile composition of *S. salar* and *O. mykiss* is listed in Table 1. Fifty VOCs of sixty-six isolated compounds were identified considering a match library higher than 70 % and comparing their Linear Retention Index with reported ones in http://webbook.nist.gov/ for this type of column. Those compounds that did not satisfied the criteria for their identification were classified as unknown (uk). From this table, it can be seen different classes of compounds where hydrocarbons, ketones and aldehydes characterized both fish samples. Compounds like 2,2,4,6,6-pentamethyl-heptane (ranging from 795.10 to 413.88 µg.kg^{-1}), *n*-pentadecane (from 273.88 to 120.66 µg.kg^{-1}), styrene (ranged from 233.92 to 143.04 µg.kg^{-1}), ethylbenzene (from 188.78 to 161.55 µg.kg^{-1}), 2,4-octadiene (from 69.27 to 25.04 µg.kg^{-1}) are the main hydrocarbons presents in both samples.

1-penten-3-ol, ranged from 42.95 to 16.61 µg.kg^{-1}, was the most abundant alcohol in both fish species, followed by 4-methylpentanol (from 42.98 to 0 µg.kg^{-1}) and 1-pentanol (from 17.21 to 8.85 µg.kg^{-1}). In particular, 1-penten-3-ol has been reported as a marker of lipid-oxidation in chilled Atlantic horse mackerel muscle, but its origin is still not clarified[19]. Among ketones, the most abundant ones are 3,5-octadien-2-one (from 165.02 to 8.80 µg.kg^{-1}), 3-undecen-2-one (from 17.03 to 4.32 µg.kg^{-1}) and 2,3-octanedione (from 50.40 to 19.97 µg.kg^{-1}), being the first and the last one reported as well as important indicators of freshness in *Merlangius merlangus*[20].

On the other hand, eleven aldehydes were identified in both samples. In this case, compounds like *n*-hexanal (most abundant in *O. mykiss* with a concentration of 187.64 µg.kg^{-1}), benzaldehyde (ranging from 102.07 to 45.65 µg.kg^{-1}), 2-hexenal (from 76.39 to 32.32 µg.kg^{-1}), (*E,E*)-2,4-heptadienal (from 63.35 to 43.87 µg.kg^{-1}) were the most abundant ones.

Table 1: Volatile compounds quantified in *S. salar* and *O. mykiss* samples expressed as IS equivalent (in µg.kg^{-1}).

RT (Min)	*KI*	*Compounds*	*S. salar (East)*	*± s.d.*	*S. salar (West)*	*± s.d.*	*O. mykiss*	*± s.d.*
3.35	500	*n*-pentane	5.95	0.94	5.96	3.77	4.89	1.39
3.64	522	1-amino-2-propanol	9.85	0.50	3.16	1.21	1.53	0.23
4.41	582	trimethylamine	14.16	0.92	0.00	0.00	0.00	0.00
4.73	800	*n*-octane	15.72	4.39	4.61	3.48	11.77	1.93
5.52	835	uk 1	4.41	0.45	1.69	0.12	5.41	1.56
6.19	900	*n*-nonane	4.76	0.53	5.70	2.48	3.83	0.90
6.60	918	(?)-2,4-octadiene	69.27	6.84	27.24	2.27	25.04	4.73
6.83	918	(?)-3,5-octadiene	27.32	5.23	5.03	0.04	9.35	1.84
6.97	934	ethanol	7.11	1.17	52.86	4.96	0.00	0.00
7.30	958	2-ethylfuran	34.00	3.99	17.43	4.46	45.92	12.37
7.44	968	2,2,4,6,6-pentamethyl-heptane	795.10	81.20	413.88	18.10	753.60	30.65
7.87	999	uk 2	32.49	0.56	16.86	8.20	23.32	8.98
8.66	1048	uk 3	93.60	7.31	65.43	9.46	111.05	0.51
9.19	1075	2,6,7-trimethyl decane	78.22	1.72	58.52	0.54	102.15	32.74
9.40	1085	2-butenal	27.41	0.49	0.00	0.00	8.12	0.00
9.60	1094	uk 4	13.29	1.03	9.27	3.52	16.30	2.00
9.77	1101	uk 5	53.40	15.42	36.84	18.15	45.95	13.28
10.10	1116	uk 6	5.94	3.68	3.83	0.63	9.59	2.00
10.39	1129	*n*-hexanal	75.53	22.36	100.88	23.40	187.64	25.28
10.89	1149	3-p-Menthene	37.96	1.63	21.38	1.58	37.25	3.48
11.43	1167	ethylbenzene	188.78	33.69	161.55	11.76	187.39	68.74
11.68	1175	(*E*)-2-pentenal	24.46	6.30	25.78	4.57	52.01	23.81
11.80	1180	(?)-xylene	37.00	9.62	14.10	1.49	18.36	14.93
12.55	1182	1-penten-3-ol	42.95	0.29	16.61	7.40	28.12	7.36
12.65	1186	(?)-xylene	12.87	2.91	10.37	5.14	10.13	0.93
12.98	1197	*n*-heptanal	36.01	1.31	9.85	0.90	16.87	9.86
13.07	1200	*n*-dodecane	15.13	3.81	9.49	1.90	22.33	7.60
13.26	1206	limonene	49.32	5.99	25.54	3.10	11.87	3.05
13.60	1217	propylbenzene	31.55	5.20	16.92	0.96	12.18	0.00
13.96	1224	2-hexenal	32.32	5.99	34.24	2.82	76.39	32.49
14.02	1225	1-ethyl-3-methylbenzene	8.78	0.64	57.51	6.51	24.43	0.00
14.12	1227	uk 7	104.23	11.02	33.81	0.00	23.51	0.00
14.38	1232	bicyclo(3,2,1)oct-2-ene	57.16	4.44	30.64	2.07	14.63	1.50
14.48	1234	(?)1,2,4-trimethylbenzene	4.15	3.59	12.09	1.45	13.76	7.66
14.78	1240	*n*-pentanol	17.21	6.56	8.85	4.77	12.17	7.89
15.19	1248	styrene	233.92	39.83	143.04	31.61	148.94	62.13
15.50	1254	(?)1,3,5-trimethylbenzene	38.82	6.37	6.00	0.00	0.00	0.00
15.66	1258	Octanal	17.89	4.13	3.30	0.00	9.67	0.00
15.91	1263	trans-2-(2-pentenyl)furan	13.86	0.52	11.90	1.00	17.42	7.70
16.43	1273	2,3-octanedione	38.21	1.27	19.97	2.37	50.40	29.48
16.60	1276	(*E*)-2-heptenal	19.54	13.30	18.65	5.31	30.69	10.35
16.75	1279	uk 8	9.21	2.01	6.08	1.02	8.38	3.43
16.84	1281	1-tridecene	4.50	3.43	2.90	0.31	1.51	2.62
17.22	1289	4-methylpentanol	42.98	3.17	0.00	0.00	5.40	11.45
18.08	1297	2-nonanone	12.61	6.47	1.47	0.00	6.30	1.31
18.17	1299	*n*-octanal	43.57	10.08	20.58	8.06	50.68	15.37
18.26	1300	*n*-tridecane	4.93	2.32	6.40	2.20	2.07	1.81
19.84	1435	(*E,E*)-2,4-heptadienal	54.24	1.51	43.87	6.52	63.35	21.40
20.17	1493	benzaldehyde	66.83	42.75	45.65	0.92	102.07	47.68
20.48	1500	*n*-pentadecane	273.88	91.73	120.66	2.38	176.56	130.23
20.74	1506	uk 9	17.69	1.05	25.20	2.52	32.77	1.07
20.92	1510	uk 10	4.53	0.97	0.00	0.00	2.09	3.61
21.05	1516	(*?*) -3,5-octadien-2-one	165.02	45.18	27.01	3.43	93.02	23.61
21.40	1524	uk 11	8.46	7.43	2.97	2.53	11.69	5.95
21.58	1525	uk 12	17.97	0.53	14.58	1.21	19.67	3.49
22.16	1538	(*?*)-3,5-octadien-2-one	64.77	17.64	8.80	2.22	44.00	11.87
22.48	1546	2,6-nonadienal	27.13	10.28	9.34	0.27	51.47	11.71
24.32	1643	uk 13	101.02	1.77	58.85	10.22	151.34	47.06
24.56	1698	*n*-heptadecane	49.92	18.24	77.37	10.72	69.58	36.16
25.23	1716	3-undecen-2-one	15.09	1.17	4.32	0.00	17.03	14.50
28.06	1884	benzyl alcohol	11.23	3.42	1.50	0.00	54.33	90.34
28.73	2014	benzothiazole	45.35	23.18	6.17	1.55	34.68	2.33
30.79	2075	uk 14	4.59	1.70	0.00	0.00	8.36	0.23
32.40	2125	uk 15	14.52	9.09	0.00	0.00	1.11	1.92
32.75	2136	nonanoic acid	17.55	7.04	9.37	4.36	31.78	3.46
34.33	2386	uk 16	60.53	6.36	43.14	3.98	66.00	3.54

uk: unknown compounds
s,d: standard deviation
KI: calculated linear retention index on a DB-Wax column

The presence of *n*-hexanal can be related to the fatty acid composition of fish since its formation is due to the oxidation of linoleic acid[21]. While, (*E,E*)-2,4-heptadienal and *E*-4-heptenal follow different oxidation pathways. The first one is originated by the autoxidation of eicosapentaenoic acid (EPA) whereas (*Z*)-4-heptenal can be produced via 2,6-nonadienal by the action of 12-lipoxygenase on EPA[21]. The presence of aldehydes is important because they generally present a low odour-threshold; therefore, they produce according to their volatile-proportions, characteristics aroma to the fishes[8].

A principal component analysis (PCA) was performed to reduce the dimensionality of the VOCs dataset and forming groups between samples based on their similarities and differences without losing significant information, X and Y-axis, from the figure 1, show the principal component 1 and the principal component 2 that explain 29.3 % and 20.8 % of the total variance, respectively. In this graph it can be seen that samples from *S, salar* can be discriminated based on their origin, e.g., samples from West Europe cluster together (green pyramids) while those from East Europe belongs to the blue cluster, and both are separated from samples of *O, mykiss* (the red dotted cluster) which are from a different species.

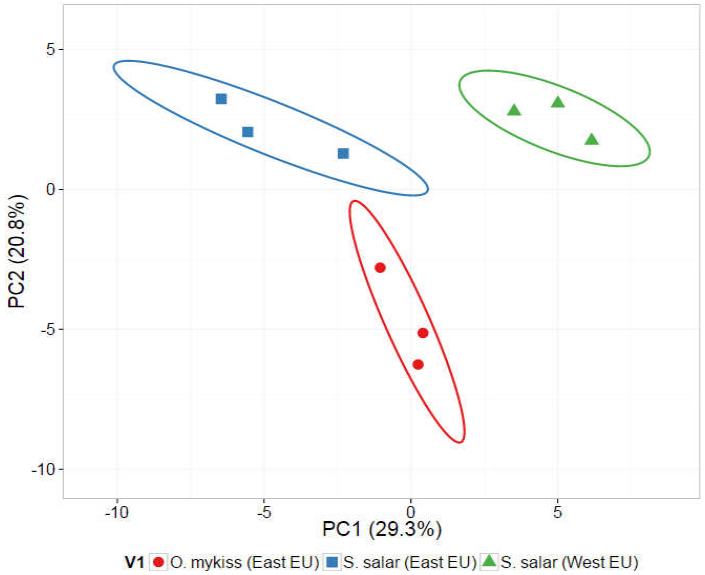

Fig. 1: Principal component score-plot clusters based on the volatile composition of the Salmonidae samples. X and Y axis show principal component 1 and principal component 2 that explain 29.3 % and 20.8 % of the total variance, respectively. Prediction ellipses are such that with probability 0.95, a new observation from the same group will fall inside the ellipse.

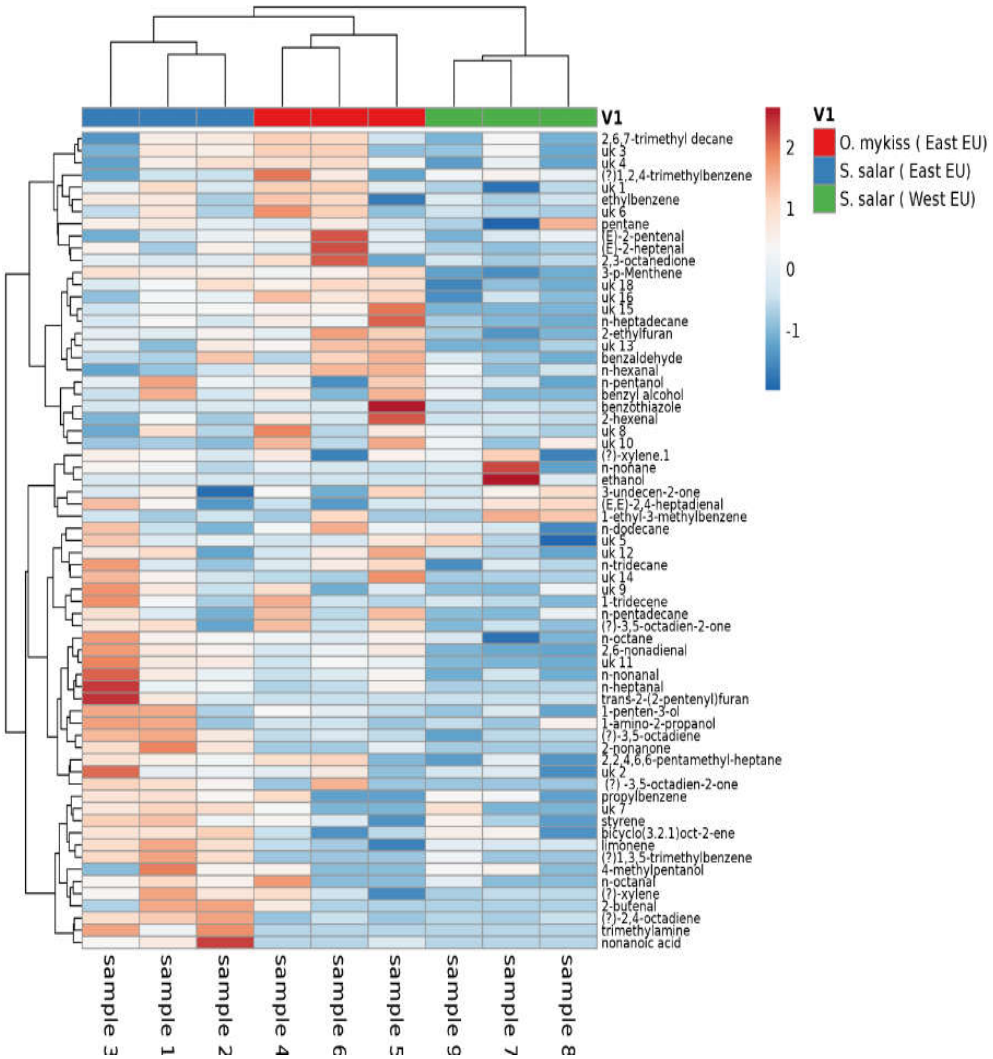

Fig. 2: Heatmap plot showing the formation of clusters based on the volatile composition of the Salmonidae samples. Red one represents cluster formed with the trout *O. mykiss* while cluster in blue represents samples of *S. salar* (East Europe) and the green one *S. salar* (West Europe).

Hierarchical Cluster Analysis (HCA) is another unsupervised technique often used to determine visual relationship among samples of the dataset. All samples are located into columns according to their similarity and forming, therefore, clusters between the nearest object. While, VOCs are located into rows forming cluster as well, according to the same criteria mentioned before. The figure 2, represent the heatmap obtained using the information of the VOCs composition identified in S. salar and O. mykiss samples, using Ward´s linkage as clustering method and Euclidian distances to the establishment of the clusters[22]. Blue and red colors indicate the extreme distance values of 0 and 2, respectively. In the figure 2, it can be seen that columns are grouped according to the specie and provenience. The first cluster contains aromatics hydrocarbons, like styrene, alkyl-benzenes and ketones (3,5-octadien-2-one and 2-nonanone), which are the most abundant compounds found in the specie S. salar (East EU). While on the other hand, in the second cluster compounds like benzothiazole, benzylalcohol and benzalhdehyde and 2-alkenals compounds (e.g., 2-pentenal, 2-hexenal and 2-heptenal), are predominantly characteristics and present in the specie *O. mykiss*. In this figure, it can be also appreciated that samples from *S. salar* from West EU origin presented a more balanced profile that its counterpart from East EU.

Conclusion

The implementation of GC-MS techniques in combination with exploratory data mining, allows researchers to access in detail to the volatile profile of, for example in this case, two commercially important fish species. Finding characteristics markers of each specie or origin can be applied in food control for the authentication of products.

Other authors have analyzed the VOCs composition of these two species but with other objectives in mind. Thus, GC-MS has been used for the determination of the odor-active and off-odor components in fish[5], as well as for the comparison of volatiles emitted by raw fish meat and smoked one[7]; similarly, in the area of food control to find VOCs associated to freshness or spoilage[11,12], or even to identify fresh fish from frozen one[23]. In this work, the volatile profile of the fish species *Salmo salar* and *Oncorhynchus mykiss* was determined. Fifty volatile compounds were identified in the headspace of fish samples through SPME and GC-MS. Hydrocarbons, aldehydes, ketones and alcohols were found as the majority classes of compounds in both species. According to the heatmap, the specie *S. salar* is rich in hydrocarbons, alcohols and ketones, while alkenals were the class of compounds predominantly present in *O. mykiss*. Further investigation in their VOCs profile could lead the determination of markers for the differentiation of these two salmonids species.

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