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Volatile compounds of some popular Mediterranean seafood species

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Abstract

The volatile compounds of highly commercialised fresh Mediterranean seafood species, including seven fish (sand-smelt *Atherina boyeri*, picarel *Spicara smaris*, hake *Merluccius merluccius*, pilchard *Sardina pilchardus*, bogue *Boobps boops*, anchovy *Engraulis encrasicolus* and striped-mullet *Mullus barbatus*), squid (*Loligo vulgaris*), shrimp (*Parapenaeus longirostris*) and mussel (*Mytilus galloprovincialis*), were evaluated by simultaneous steam distillation-extraction and subsequent GC-MS analysis. A total of 298 volatile compounds were detected. The mussels contained the highest total concentration of volatile compounds, while pilchard among fish species contained the highest number and concentrations of volatile compounds. Individual patterns of volatile compounds have been distinguished. The fish species when compared to the shellfish species studied, contained 6 to 30 times more 1-penten-3-ol, higher quantities of 2-ethylfuran, and 2,3-pentanedione, which was absent from the shellfish species. Pilchard is characterized by a high concentration of alcohols, shrimps by the high presence of amines and S-compounds, while mussels by high amounts of aldehydes, furans, and N-containing compounds (pyridine, pyrazines and pyrroles). The fatty acid-originating carbonyl compounds in fish seem to be related to the species' fat content.

Keywords: Volatile compounds, flavour, Mediterranean fish, shrimp, squid, mussel.

Introduction

Volatile compounds largely define the flavour and subsequently the general organoleptic quality of seafood (Kawai, 1996; Ólafsdóttir & Jónsdóttir, 2010). They are those characterizing either species-related delicate fresh fish odours or spoilage related odours (Josephson *et al.*, 1984; Ólafsdóttir & Jónsdóttir, 2010).

The volatile compounds of various fresh fish (indicatively, Kawai, 1996; Prell & Sawyer, 1988; Morita *et al.*, 2003) and other raw seafood (Chung, 1999; Piveteau *et al.*, 2000; Pennarun *et al.*, 2002; Zhang *et al.*, 2010) have been studied. Sporadic data occur in the literature regarding the volatiles of Mediterranean fish species, referring mainly to sardine (Triqui & Bouchriti, 2003; Prost *et al.*, 2004), gilthead sea bream (Grigorakis *et al.*, 2003; Alasalvar *et al.*, 2005) and European sea bass (Leduc *et al.*, 2012). With respect to Mediterranean shellfish, some literature is available for mussel volatiles (Rasmussen *et al.*, 1993; Le Guen *et al.*, 2000a, b; Fuentes *et al.*, 2009).

Fish and other seafood share many similar volatile compounds, contributing to similarly general aroma notes, but they also possess species-specific aromas (Morita *et al.*, 2003). The aim of this study was to compare the volatile compounds of some very common, highly marketed Mediterranean seafood species. This

would definitely add to the knowledge regarding the flavour of seafood produced and consumed within this region. However, the importance of such knowledge is more general since most of these species are consumed world-wide. The choice of the studied species was based on their market popularity, i.e. the selected species are all widely consumed in the Mediterranean area, and on the fact that each one of them is traditionally cooked using several diverse methods. Therefore, this study aimed to provide data that would serve as a basis for further study on the effects of various cooking methods on seafood volatile compounds. Besides, excluding sardine and mussel, no data are available for the volatile compounds of the rest of the seafood species studied herein

Material and Methods

Specimens

The fish and shellfish species used for this study were purchased in January – February 2011 from the local fish market in Athens (Greece), while mussels were obtained from an aquaculture unit. The quantity of each sample was 2-4 kg, comprising individuals of commercial sizes, freshly harvested and stored on ice according to the hygiene regulations and requirements.

Mussels were kept alive. All seafood was of the same

freshness stage, characterized as Extra according to the EU freshness grading (Howgate *et al.*, 1992), thus implying that they were all received within a few hours post-harvest. Somatometric characteristics and origin of the studied species appear in Table 1. Adequate quantities were obtained, and upon arrival at the laboratory, samples were homogenized, wrapped in aluminium foil, which was subsequently sealed in plastic bags, in order to avoid transfers of volatile compounds from the air or from contact with materials, and immediately stored until analysis, at -80°C, in order to prevent further post-mortem formation of volatile compounds.

Extraction of volatile compounds

Volatile compounds were extracted from seafood tissues by simultaneous steam distillation - extraction (SDE). For this purpose, tissue samples of approx. 30 g, were homogenized with 60 mL 30% w/v NaCl solution prepared with double-distilled water, containing 100 ppm BHT as antioxidant. The homogenate was transferred to a Likens - Nickerson apparatus and was distilled-extracted with 25 mL CH₂Cl₂ (HPLC grade, Waters, Milford, MA). The solvent temperature was kept at 50-55°C by a water-bath. Gradual and homogenous temperature increase and gentle boiling was applied by the addition of a few boiling chips, constant magnetic stirring and heating by a mantel. Cooling of the extraction system was achieved with ethylene glycol kept at -5 °C. The extraction lasted 3 hours; thereafter, heating was interrupted and the system was left for 30 min to equilibrate. The organic phase was collected, dried by anhydrous Na₂SO₄ and subsequently concentrated first to 1-3 mL by a Kuderna-Danish apparatus and further to a volume of 0.5 mL by gentle nitrogen flow. The extracts were sealed in GC vials and stored at -20 °C until analysis. Two extractions per sample were conducted.

Volatile compounds analysis

An Agilent 7890 GC, equipped with an Agilent 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was employed for the analysis of volatile compounds, which were separated on an Agilent HP-5MS capillary column (30 m × 0.25 mm, coated with a 0.25 µm film thickness of 5% phenyl-95% methylsiloxane). For the analysis, 2 µL of each extract was injected into the GC/MS at splitless mode. Helium was used as a carrier gas at a flow rate of 1.8 mL/min. The injector and transfer line temperatures were 200 and 300 °C, respectively. The oven temperature program was: initially 35 °C, increased to 95 °C at 2 °C/min, then to 195 °C at 8 °C/min and finally to 240 °C at 12 °C/min, where it was held for 4 min.

The mass spectrometer was set at 70 eV in EI mode, and the detector operated with a mass range of 30-300 amu, with the repetition rate set at 2.73 scans/sec. Peaks identification was made by employing NIST 05 Mass

Spectral Library (Rev. D.05.01) (Agilent Technologies, USA) together with the peak deconvolution software AMDIS (Version 2.64), as well as by referring to the mass spectra of standards, that were used for recoveries estimation. Recoveries were calculated by applying the whole procedure in a mixture of standards comprised of (R)-(+)-limonene 97% (Sigma), nonanal 95% (Aldrich), 1-penten-3-ol 99% (Aldrich), 2,4 heptadienal (technical grade) 90% (Aldrich), pentadecane ≥99% (Aldrich), 1-heptanol (puriss. p.a., standard for GC, ≥99% (GC) (Fluka), 4-heptanone purum, ≥96% (GC) (Fluka), benzene anhydrous, 99.8% (Sigma- Aldrich), and toluene anhydrous, 99.8% (Sigma- Aldrich). Reference curves were constructed for each of the aforementioned standards (Table 2). Recoveries were calculated after standard addition of five increasing concentrations (0.1, 0.2, 0.5, 1 and 2 µg /Kg) of the reference compounds in 1 g samples of white fish muscle (gilthead sea bream, *Sparus aurata*), by following the whole SDE procedure and by subtracting, from the areas found, the respective areas obtained from the single fish-muscle sample; the recoveries obtained are shown in Table 2. Each volatile compound was subsequently quantified by comparing its peak area to the standard curve of a chemically similar reference compound (Table 2), and concentrations were corrected accordingly, taking into account the recoveries of the respective quantifying reference compounds. A 200 µg/L solution of isooctane, >99.0% (Agilent), was added to every sample just before SDE extraction to serve as internal standard for the quantification of all those volatile compounds not mentioned in the quantification list of Table 2. In this case, quantification took place by comparing the compounds peak area to that of the internal standard, essentially as described by Alasalvar *et al.* (2005).

Blanks were always run between samples in order to ensure that no volatile compounds occurred besides those characterizing the studied seafood.

Statistics

Cluster analysis was conducted to evaluate the proximity of the studied species as regards the profiles of their volatile compounds, using the Squared Euclidian Distance. The significance of the individual volatile compounds for grouping the samples was evaluated by factor analysis, employing the principal components extraction method. Because of the high number of identified compounds, only those present in at least half of the studied species have been evaluated. All the statistical analyses were conducted by means of the statistical program SPSS 13.0.

Results and Discussion

Among a total of 298 volatile compounds, which were detected in the seafood samples, the 75 most charac-

Table 1. Common and scientific names, somatometric characteristics and origin of the studied species.

| Common name | Scientific name | Weight (g) ^a | Total length (cm) ^a | Origin |
|--------------------------------|----------------------------------|-------------------------|--------------------------------|------------------------------|
| <i>Fish</i> | | | | |
| Big scale sand smelt | <i>Atherina boyeri</i> | 4.2 ± 1.3 | 7.5 ± 0.9 | Leros island (SE Aegean) |
| Picarel | <i>Spicara smaris</i> | 14.1 ± 2.3 | 9.5 ± 0.5 | Evoikos Gulf (W Aegean) |
| European hake | <i>Merluccius merluccius</i> | 43.6 ± 11.4 | 16.6 ± 1.5 | Lesvos island (NE Aegean) |
| European pilchard (or sardine) | <i>Sardina pilchardus</i> | 18.3 ± 5.1 | 10.6 ± 0.8 | Kavala (N Aegean) |
| Bogue | <i>Boops boops</i> | 128.6 ± 8.0 | 17.5 ± 0.5 | Chios island (NE Aegean) |
| European anchovy | <i>Engraulis encrasicolus</i> | 12.5 ± 1.8 | 10.4 ± 0.6 | Evoikos Gulf (W Aegean) |
| Striped mullet | <i>Mullus barbatus</i> | 17.9 ± 5.1 | 9.2 ± 0.7 | Kavala (N Aegean) |
| <i>Other seafood</i> | | | | |
| Squid | <i>Loligo vulgaris</i> | 68.1 ± 15.1 | 18.4 ± 1.9 | Chios island (NE Aegean) |
| Shrimp | <i>Parapenaeus longirostris</i> | 7.9 ± 2.4 | 12.1 ± 1.2 ^b | Saronikos Gulf |
| Mussel | <i>Mytilus galloprovincialis</i> | 22.2 ± 6.2 ^c | 6.2 ± 0.5 ^c | Saronikos Gulf (aquaculture) |

^a Average ± standard deviation, obtained from 20-40 individuals; ^b Includes cephalothorax; ^c Includes shell.

teristic and most abundant (found at concentrations >0.1 µg/kg) are shown in Table 3. Based on the fact that all species were extra fresh it is presumed that all volatiles found in the studied seafood are those characterizing the species, or some of them can alternatively be of environmental origin, especially in the case of filter feeders such as mussels (Fuentes *et al.*, 2009). In the case of seawater

fish, it has been shown that feeding history has some effects on volatile compound profiles when wild and cultured fish of the same species were compared (Grigorakis *et al.*, 2003; Alasalvar *et al.*, 2005). On the other hand, contradicting findings occur in studies comparing diets in farmed fish, showing minor or no effects in some cases (Grigorakis *et al.*, 2009; Silva *et al.*, 2012) and some ef-

Table 2. Standard curves (slope *a* and *R*²) and recoveries (%) for the authentic standards used for quantification of the volatile compounds.

| Authentic standard | <i>a</i> | <i>R</i> ² | Recovery % | Volatile compounds quantified by means of each authentic standard |
|--------------------|----------|-----------------------|------------|---|
| 2-Butanone | 23074 | 0.9938 | 98.27 | Ketones with retention times from 2.9 to 7.3 min |
| Benzene | 31939 | 0.9799 | 82.15 | Benzene, alkyl-benzenes, p-xylene, o-xylene, ethers, amines, furans |
| 1-Penten-3-ol | 25391 | 0.9895 | 97.66 | Non-aliphatic alcohols |
| Toluene | 30421 | 0.9806 | 77.07 | Toluene, alkyl-toluenes, pyrazines, pyridines, pyrroles, pyrrolidines |
| 4-Heptanone | 28916 | 0.9850 | 99.84 | Ketones with retention times from 7.31 to 56.3 min, esters |
| 1-Heptanol | 10643 | 0.9838 | 99.90 | Aliphatic alcohols |
| D-Limonene | 18866 | 0.9834 | 99.90 | Terpenes, alicyclic hydrocarbons |
| Nonanal | 9167.7 | 0.9635 | 99.90 | Aldehydes |
| Octadecane | 5970.3 | 0.9344 | 60.27 | Alkanes, alkenes, alkadienes |

Table 3. Main volatile compounds* ($\mu\text{g}/\text{kg}$ fresh weight) characterizing the studied Mediterranean seafood species. The retention times (Rt) and characteristic ions for each compound are also provided (nd stands for not detected; tr for traces).

| Compound | Rt (min) | Characteristic ions (m/z) | <i>A. boyeri</i> | <i>S. smaris</i> | <i>M. merluccius</i> | <i>S. pilchardus</i> | <i>B. boops</i> | <i>E. encrasiolus</i> | <i>M. barbatus</i> | <i>L. vulgaris</i> | <i>P. longirostris</i> | <i>M. galloprovincialis</i> |
|---------------------------------------|----------|---------------------------|------------------|------------------|----------------------|----------------------|-----------------|-----------------------|--------------------|--------------------|------------------------|-----------------------------|
| Tetrahydrofuran | 3.042 | 42,72,71 | 0.06 | 0.05 | 0.04 | 0.17 | 0.04 | nd | 0.23 | 0.13 | 0.06 | 0.82 |
| <i>trans</i> -2-Butenal | 3.279 | 70,39,69 | nd | nd | Nd | 0.22 | nd | nd | 0.02 | nd | nd | 0.29 |
| 3-Methylbutanal | 3.298 | 44,41,43 | 3.01 | 0.39 | 0.22 | 1.43 | 0.27 | 1.30 | 0.96 | 1.14 | 0.45 | 3.94 |
| 2-Methylbutanal | 3.395 | 41,57,58 | 1.38 | nd | 0.18 | 0.72 | nd | 0.56 | 0.56 | nd | nd | 2.49 |
| N,N,N',N'-Tetramethyl-methanedi-amine | 3.432 | 58,42,44 | nd | nd | nd | nd | nd | nd | nd | nd | 0.35 | nd |
| 1-Penten-3ol | 3.590 | 57,41,39 | 1.33 | 0.88 | 0.60 | 2.63 | 0.60 | 0.77 | 0.95 | 0.06 | 0.13 | 0.12 |
| 1-Methoxy-2-propanol | 3.612 | 45,47,43 | nd | 0.49 | nd | 4.48 | nd | nd | nd | 0.82 | nd | nd |
| 3-Penten-2-ol | 3.633 | 71,43,41 | 0.03 | 0.17 | 0.02 | nd | nd | nd | nd | 0.01 | nd | nd |
| 2-Pentanone | 3.694 | 43,45,48 | nd | nd | nd | 0.14 | 0.03 | 0.03 | nd | nd | 0.02 | nd |
| 2,3-Pentanedione | 3.767 | 43,57,100 | 0.21 | 0.27 | 0.13 | 1.03 | 0.21 | 0.46 | 0.23 | nd | nd | nd |
| Heptane | 3.767 | 43,71,56 | nd | nd | nd | nd | nd | nd | nd | 0.23 | nd | 1.91 |
| Pentanal | 3.785 | 44,58,45 | 0.45 | 0.30 | 0.28 | 0.84 | 0.26 | 0.32 | 0.29 | 0.06 | nd | 3.78 |
| 2-Ethyl-furan | 3.822 | 81,96,53 | 0.72 | 0.41 | 0.28 | 2.31 | 0.42 | 0.57 | 0.44 | 0.02 | 0.02 | 0.15 |
| 3-Hydroxy-2-butanone | 3.987 | 45,43,88 | 1.19 | 2.18 | 1.42 | 1.17 | 0.50 | 3.29 | 1.63 | 1.92 | 0.81 | 0.88 |
| 3-Methyl-1-butanol | 4.432 | 57,70,42 | 0.11 | 0.02 | nd | nd | nd | nd | 0.08 | 0.01 | nd | nd |
| Pyridine | 4.624 | 79,52,51 | nd | 0.03 | 0.01 | 0.01 | 0.03 | nd | 0.02 | 0.04 | 0.07 | 1.54 |
| 2-Methoxy-furan | 4.694 | 98,83,69 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.20 |
| <i>cis</i> -2-Pentenal | 4.846 | 55,83,84 | 0.29 | 0.26 | 0.20 | 0.91 | 0.22 | 0.23 | 0.28 | nd | nd | nd |
| Pyrrrole | 4.919 | 67,41,39 | nd | nd | nd | nd | nd | nd | 0.04 | nd | 0.03 | 0.13 |
| Toluene | 5.035 | 91,92,65 | 0.33 | 0.15 | 0.23 | 11.12 | 0.01 | 2.06 | 6.90 | 3.49 | 0.29 | 22.24 |
| 1-Pentanol | 5.125 | 42,55,41 | 0.03 | 0.01 | nd | nd | 0.01 | nd | 0.01 | nd | nd | 0.21 |
| <i>trans</i> -2-Penten-1-ol | 5.188 | 57,41,44 | 0.07 | 0.07 | nd | 0.24 | 0.04 | tr | 0.02 | nd | nd | nd |
| 3-Methyl-2-butenal | 5.596 | 84,55,41 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.40 |
| Octane | 5.901 | 43,85,57 | 0.14 | 0.22 | 0.23 | 0.21 | 0.12 | 0.09 | 0.15 | 0.08 | 0.04 | 2.14 |
| Hexanal | 5.962 | 44,56,41 | 0.25 | 0.38 | 0.19 | 1.03 | 0.40 | 0.12 | 0.33 | 0.02 | nd | 0.49 |
| 3-Pentanol | 6.029 | 59,107,45 | nd | 0.03 | 0.02 | 0.10 | 0.02 | 0.04 | 0.05 | 0.02 | nd | 0.07 |
| 2-Hydroxy-3-pentanone | 6.248 | 45,57,59 | 0.11 | 0.01 | 0.03 | 0.05 | 0.01 | 0.05 | 0.03 | nd | nd | 0.04 |
| Unknown | 6.474 | 58,42,61 | nd | nd | nd | nd | nd | nd | nd | 0.56 | 1.70 | nd |
| 2-4-Dimethyl-heptane | 6.596 | 43,85,57 | 0.15 | 0.25 | 0.12 | 0.50 | 0.21 | 0.25 | nd | nd | nd | 0.82 |
| Furfural | 7.059 | 96,95,39 | 0.03 | 0.01 | 0.01 | 0.02 | 0.02 | 0.05 | 0.01 | 0.04 | tr | 0.36 |
| 3-Methoxy-1-butanol | 7.096 | 59,43,89 | nd | nd | nd | 0.42 | nd | nd | nd | nd | nd | nd |
| 2-4-Dimethyl-1-heptene | 7.285 | 43,70,55 | 0.05 | 0.08 | 0.04 | 0.09 | 0.05 | 0.10 | 0.02 | 0.05 | 0.02 | 0.27 |
| 2-Methyl-1H-pyrrole | 7.352 | 80,81,53 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.18 |
| 2-Hexenal | 7.803 | 41,69,55 | 0.12 | 0.11 | 0.05 | 0.50 | 0.08 | 0.11 | 0.08 | nd | nd | 0.09 |
| 2-Furanmethanol (furfuryl alcohol) | 7.980 | 98,97,41 | 0.14 | nd | nd | nd | 0.01 | nd | nd | nd | nd | 1.40 |
| 4-Methyl-octane | 8.163 | 43,85,71 | 0.15 | 0.19 | 0.11 | 0.35 | 0.17 | 0.23 | 0.02 | 0.10 | 0.04 | 0.62 |
| <i>p</i> -Xylene | 8.345 | 91,106,105 | 0.04 | nd | nd | 0.02 | 0.02 | 0.06 | nd | 0.02 | nd | 0.26 |
| 1-Methoxy-2-propyl-acetate | 8.716 | 43,45,72 | nd | nd | nd | 0.13 | nd | nd | nd | 0.06 | nd | nd |
| Styrene | 9.345 | 104,103,78 | nd | 0.01 | 0.06 | 0.08 | nd | 0.03 | 0.27 | 0.16 | 0.01 | 0.06 |
| 4-Heptanone | 9.449 | 43,58,71 | 0.02 | 0.02 | 0.02 | 0.06 | 0.01 | 0.01 | 0.03 | nd | 0.02 | 0.14 |
| <i>trans</i> -4-Heptenal | 9.796 | 41,55,68 | 0.17 | 0.12 | 0.08 | 0.45 | 0.05 | 0.08 | 0.05 | 0.02 | nd | 0.25 |
| 1-(2-Furanyl)-ethanone | 10.382 | 95,100 | tr | tr | tr | nd | nd | 0.01 | nd | nd | nd | 0.15 |
| 2-3-Dimethyl-pyrazine | 10.746 | 108,67,40 | nd | nd | nd | nd | nd | nd | nd | nd | 0.03 | 0.02 |
| Unknown | 10.894 | 57,42,86 | nd | nd | nd | nd | nd | nd | nd | nd | 0.212 | nd |
| Unknown | 11.827 | 95,126,96 | 0.09 | nd | nd | 0.03 | 0.01 | nd | nd | nd | nd | 0.13 |
| 5-Methyl-2-furancarboxaldehyde | 12.296 | 110,109,53 | 0.26 | 0.01 | nd | 0.03 | 0.07 | nd | nd | nd | nd | 0.48 |
| Benzaldehyde | 12.790 | 106,105,77 | 0.21 | 0.11 | 0.15 | 0.26 | 0.14 | 0.15 | 0.12 | 0.15 | 0.12 | 0.96 |

(continued)

(continued)

| Compound | Rt (min) | Characteristic ions (m/z) | <i>A. boyeri</i> | <i>S. smaris</i> | <i>M. merluccius</i> | <i>S. pilchardus</i> | <i>B. boops</i> | <i>E. encrasicolus</i> | <i>M. barbatus</i> | <i>L. vulgaris</i> | <i>P. longirostris</i> | <i>M. galloprovincialis</i> |
|--|----------|---------------------------|------------------|------------------|----------------------|----------------------|-----------------|------------------------|--------------------|--------------------|------------------------|-----------------------------|
| 2-thiophenecarboxaldehyde | 14.564 | 111,112,83 | 0.15 | 0.04 | 0.03 | 0.07 | 0.02 | 0.04 | 0.10 | 0.04 | nd | 0.09 |
| 2-Pentyl furan | 14.771 | 81,82,138 | 0.03 | 0.02 | 0.03 | 0.09 | 0.04 | 0.02 | 0.04 | nd | nd | 0.03 |
| 2-Octanone | 14.850 | 43,58,59 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.04 |
| 2-Ethyl-6-methyl-pyrazine, | 15.210 | 121,122,39 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.01 |
| <i>trans</i> -2-(2-Pentenyl) furan | 15.368 | 107,136,68 | 0.03 | 0.02 | 0.02 | 0.07 | 0.03 | 0.02 | 0.02 | nd | nd | nd |
| Trimethyl pyrazine | 15.411 | 122,42,81 | nd | nd | nd | nd | nd | nd | nd | nd | 0.07 | nd |
| Octanal | 15.521 | 43,41,57 | 0.07 | 0.08 | 0.05 | 0.16 | 0.04 | 0.04 | 0.05 | 0.01 | nd | 0.35 |
| <i>trans,trans</i> -2,4-Heptadienal | 15.954 | 81,110,53 | 0.16 | 0.21 | 0.12 | 0.71 | 0.21 | 0.17 | 0.32 | 0.01 | nd | 0.08 |
| N,N-dimethylmethanethioamide | 16.624 | 89,44,42 | nd | nd | nd | 0.02 | nd | nd | nd | 0.08 | 0.10 | nd |
| Unknown amine | 16.686 | 58,44,42 | nd | nd | nd | nd | nd | nd | nd | nd | 0.60 | nd |
| Phenylacetaldehyde | 17.911 | 91,92,120 | 2.14 | 0.27 | 0.09 | 0.24 | 0.16 | 0.08 | 0.43 | 0.12 | nd | 2.71 |
| 3,4-Dimethyl-2,5-hexanedione | 18.222 | 43,131,88 | nd | nd | nd | nd | nd | nd | nd | 0.14 | nd | nd |
| Unknown | 18.362 | 58,42,72 | nd | nd | nd | nd | nd | nd | nd | nd | 0.11 | nd |
| 4,7-Dimethyl-undecane | 18.959 | 57,43,71 | 0.04 | 0.06 | 0.04 | 0.15 | 0.07 | 0.05 | nd | 0.02 | nd | 0.13 |
| 4-Methoxy-2,5-dimethyl-3(2H)-furanone | 19.240 | 142,43,71 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.09 |
| <i>cis</i> -5-Nonenal | 20.368 | 79,41,55 | 0.11 | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1,2,4-Trithiolane | 20.587 | 124,78,45 | nd | nd | nd | nd | nd | nd | nd | nd | 0.23 | 0.07 |
| N,N,N'-Trimethyl-1,2-ethanediamine | 21.221 | 58,42,59 | nd | nd | nd | nd | nd | nd | nd | nd | 0.45 | nd |
| 2-Nonanone | 21.373 | 43,58,71 | 0.02 | 0.02 | 0.01 | 0.07 | 0.01 | 0.03 | nd | nd | nd | 0.10 |
| Unknown aromatic ketone | 21.666 | 107,79,135 | nd | nd | nd | 0.332 | nd | 0.13 | nd | nd | nd | nd |
| Nonanal | 22.105 | 57,41,56 | 0.14 | 0.41 | 0.46 | 0.42 | 0.14 | 0.10 | 0.12 | 0.12 | nd | 1.92 |
| Dimethylaminohexane | 24.123 | 58,45,42 | nd | nd | nd | nd | nd | nd | 1.03 | nd | nd | nd |
| 2,6,6-Trimethyl-2-cyclohexene-1,4-dione | 24.568 | 68,96,152 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.20 |
| <i>cis,trans</i> -2,6-Nonadienal | 25.324 | 41,70,69 | 0.05 | 0.06 | 0.04 | 0.22 | 0.04 | 0.05 | 0.06 | nd | nd | 0.04 |
| 2-Phenylpropanal | 25.360 | 103,104,132 | 0.36 | 0.06 | 0.02 | 0.34 | 0.02 | 0.55 | 0.12 | 0.22 | nd | nd |
| 4-Ethyl benzaldehyde | 25.702 | 104,133,105 | 0.07 | 0.07 | 0.03 | 0.24 | 0.06 | 0.07 | 0.07 | nd | nd | nd |
| N,N-dimethyl-1-Butanamine | 25.797 | 58,45,42 | nd | nd | nd | nd | nd | nd | nd | nd | 2.71 | nd |
| 5,6-Dihydro-5-methyl-4H-1,3,5-dithiazine | 27.744 | 135,44,57 | nd | nd | nd | nd | nd | nd | nd | nd | 0.49 | nd |
| Decanal | 28.902 | 57,43,41 | 0.03 | 0.04 | 0.04 | 0.04 | 0.03 | nd | 0.03 | nd | nd | 0.11 |
| Unknown amine | 29.726 | 58,42,44 | nd | nd | nd | nd | nd | nd | nd | nd | 0.22 | nd |
| Unkown hydrocarbon | 37.834 | 57,42,177 | 1.39 | 0.31 | 0.34 | nd | nd | nd | nd | nd | nd | nd |
| Pentadecane | 38.260 | 57,43,71 | nd | nd | nd | 0.43 | 0.22 | nd | nd | nd | nd | nd |
| Unknown (1,4 dimethyl piperidine) | 39.160 | 112,57,42 | nd | nd | nd | nd | nd | nd | nd | nd | 0.14 | nd |
| Unknown thiole | 39.636 | 42,162,120 | nd | nd | nd | nd | nd | nd | nd | nd | 5.09 | nd |
| Heptadecane | 41.498 | 57,71,43 | nd | 0.04 | nd | 1.68 | nd | 0.15 | nd | 0.03 | 0.02 | 0.09 |
| 2,6,10,14-Tetramethyl pentadecane | 41.598 | 57,71,43 | 0.06 | 0.04 | nd | 0.03 | 1.19 | nd | 0.15 | 0.03 | nd | 0.02 |
| 2-Pentadecanone | 41.857 | 58,43,71 | nd | nd | nd | nd | nd | nd | 0.10 | nd | nd | nd |
| <i>cis</i> -1-Octadecene | 42.800 | 83,97,55 | 0.05 | nd | 0.08 | 0.10 | 0.05 | 0.07 | 0.10 | 0.03 | 0.02 | 0.11 |
| Octadecane | 43.070 | 57,71,43 | 0.26 | 0.71 | nd | 0.94 | 1.22 | 0.34 | nd | 0.07 | 0.20 | nd |
| 1-Nonadecene | 45.049 | 55,83,97 | nd | nd | 0.07 | nd | nd | 0.06 | 0.09 | 0.07 | nd | 0.14 |
| Octadecanal | 45.409 | 57,43,82 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.96 |
| Octadecanoic acid ethyl ester | 46.872 | 88,101,43 | 0.02 | 0.02 | 0.01 | 0.10 | 0.11 | 0.01 | nd | 0.01 | 0.01 | 0.02 |

*: Only compounds present at concentrations >0.1 µg/kg are included.

fects on some volatiles in other cases (Sérot *et al.*, 2001).

In this study, mussels exhibited the highest total concentration of volatiles, while among fish pilchard was the species with the highest number of volatile compounds and with the highest total concentrations (Table 4).

Fatty fish species like pilchard, anchovy, and picarel contained higher amounts of carbonyl compounds (12.5, 8.0 and 5.6 µg/Kg, respectively), when compared to the lean species bogue and hake (3.4 and 3.9 µg/Kg, respectively). The existing literature confirms the high lipid contents in sardines (Kalogeropoulos *et al.*, 2004; Zlatanov & Laskaridis, 2007), and anchovy and picarel, from the same origin as in our study, by the end of winter (Zlatanov & Laskaridis, 2007). The increased contents of lipid-originating carbonyl compounds in fatty fish when compared to lean fish has been reported previously (Iglesias *et al.*, 2010).

Distinct patterns of volatiles were observed among the different seafood. All finfish species were found to contain 2,3-pentanedione, which was absent from the shellfish studied, and up to 30 times higher 1-penten-3-ol concentrations. The low concentrations or absence of 1-penten-3-ol have been confirmed in mussels (Le Guen *et al.*, 2000a) and pre-cooked shrimp (Soncin *et al.*, 2008). The non-fish seafood was also characterized by the absence of *trans*-2-pentenal, *trans*-2-(2-pentenyl) furan,

and 4-ethyl-benzaldehyde. Finfish also contained much higher quantities of 2-ethyl furan compared to shellfish, in agreement with the findings of Giogios *et al.* (2009) who reported higher 2-ethylfuran and furans concentrations in fish oils when compared to shrimp oil. Among shellfish, only mussels contain significant quantities of this compound, which were in any case lower than that of fish species. The volatiles of pilchard contained the highest amounts of 2-ethylfuran, being more than 5 times higher than in the other species. A potential mechanism for 2-ethylfuran formation in fish muscle has been proposed, including n-3 fatty acid β-oxidation to produce conjugated dienes radicals, which are further oxidized to produce vinylhydroperoxides; these in turn lose a hydroxyl radical and undergo cyclization to form 2-ethylfuran (Medina *et al.*, 1999).

Pilchard volatiles are characterized by the presence of significant amounts of alcohols when compared to the other fish and seafood species (Table 4), with 1-penten-3-ol, 1-methoxy-2-propanol, *trans*-2-penten-1-ol and 3-pentanol predominating (Table 3). These alcohols have been confirmed previously in sardines of various origins (Prost *et al.*, 2004; Ganeko *et al.*, 2008). With respect to the other pilchard volatiles, the profound presence of various carbonyls (2,3-pentanedione, pentanal, 2-butanone, *cis*-2-pentenal, hexanal, *trans,trans*-2,4-heptadienal and

Table 4. Concentrations of volatile compound classes (µg/kg fresh weight) characterizing the studied Mediterranean seafood species.

| | <i>A. boyeri</i> | <i>S. smaris</i> | <i>M. merluccius</i> | <i>S. pilchardus</i> | <i>B. boops</i> | <i>E. encrasicolus</i> | <i>M. barbatus</i> | <i>L. vulgaris</i> | <i>P. longirostris</i> | <i>M. galloprovincialis</i> |
|------------------|------------------|------------------|----------------------|----------------------|-----------------|------------------------|--------------------|--------------------|------------------------|-----------------------------|
| Alcohols | 1.75 | 1.71 | 0.69 | 8.05 | 0.77 | 0.82 | 1.15 | 0.94 | 0.12 | 0.64 |
| Aldehydes | 9.27 | 3.09 | 2.32 | 9.68 | 2.54 | 4.10 | 4.47 | 2.04 | 0.70 | 19.47 |
| Ketones | 1.58 | 2.54 | 1.62 | 2.78 | 0.86 | 3.93 | 2.17 | 2.17 | 0.85 | 2.19 |
| Furans | 1.29 | 0.53 | 0.40 | 2.78 | 0.64 | 0.68 | 0.74 | 0.19 | 0.09 | 3.55 |
| Amines | 0.01 | 0.01 | 0.01 | 0.02 | tr ^a | 0.02 | 0.00 | 0.45 | 0.92 | nd ^b |
| Pyrazines | nd | nd | tr | nd | 0.12 | 0.09 | 0.11 | 0.18 | 1.71 | 1.64 |
| Pyridines | nd | 0.27 | 0.01 | 0.01 | 0.05 | nd | 0.02 | 0.03 | 0.06 | 1.54 |
| Pyrroles | nd | nd | nd | nd | nd | nd | tr | 0.01 | 0.11 | 0.42 |
| Hydrocarbons | 2.06 | 2.17 | 1.26 | 17.09 | 3.97 | 4.17 | 8.15 | 5.08 | 0.83 | 30.14 |
| Sulfur compounds | 0.27 | 0.06 | 0.04 | 0.24 | 0.05 | 0.12 | 0.05 | 0.27 | 0.90 | 0.36 |
| Ethers | 0.26 | 0.21 | 0.21 | 0.23 | 0.05 | 0.07 | 0.12 | 0.12 | 0.07 | 0.24 |
| Esters | 0.12 | 0.10 | 0.07 | 0.22 | 0.31 | 0.14 | 0.10 | 0.01 | 0.13 | 0.10 |
| Acids | 0.01 | nd | tr | nd | nd | 0.01 | nd | nd | nd | nd |
| Others | 0.07 | 0.06 | 0.04 | 0.09 | 0.03 | 0.14 | 0.02 | 0.10 | 0.05 | 0.05 |
| Unknown | 2.00 | 0.51 | 0.47 | 0.85 | 0.49 | 0.84 | 1.34 | 2.53 | 11.5 | 1.50 |
| TOTAL | 18.7 | 11.22 | 7.17 | 42.04 | 9.79 | 15.06 | 18.38 | 17.07 | 16.54 | 60.35 |

tr stands for "traces"; nd stands for "not detected".

trans-4-heptenal) and 2-ethylfuran in sardines have been reported previously and have been considered to contribute to a characteristic sardine-fishy odour (Triqui & Bouchriti, 2003; Prost *et al.*, 2004; Ganeko *et al.*, 2008).

To the best of our knowledge, there are no published data for the volatile compounds of the other fish species of this study, with the exception of carbonyls in the European anchovy (Iglesias *et al.*, 2010).

Squid was herein characterized by high concentrations of 1-methoxy-2-propanol, 3-hydroxy-butanone, an unknown compound (retention time $R_t=12.043\text{min}$), *N,N*-dimethyl methanethioamide, 2,4-dihydroxy-3,4-dimethyl-2,5-hexanedione, and also by the very low furan content and the almost complete absence of esters. No previous data exist regarding the volatile compounds of fresh squid. The only available data refer to processed (Giri *et al.*, 2011) or cooked (Kubota *et al.*, 1996) squid, or to offensive volatile compounds from decaying squid (Kim *et al.*, 2009).

The shrimp is characterized by the presence of amines and S-compounds, low concentrations of hydrocarbons, alcohols and carbonyls and by the almost complete absence of furans. Mussels, on the contrary, are characterized by high contents of hydrocarbons, aldehydes, furans and furan-containing compounds. What shrimp and mussels have in common is the presence of S-compounds and N-containing heterocyclic compounds (mainly pyrazines).

Among the S-compounds found in shrimp, the most abundant is an unknown thiol (Table 3). The S-containing compounds, are considered to be enzymatically produced in seafood from methionine and cysteine (Varlet & Fernandez, 2010). Thioamides, herein present in shrimp, have also been reported for crab (Chung, 1999) and dried squid (Kawai *et al.*, 1991). Thiazoles, have been found in cuttlefish oil (Shen *et al.*, 2007) and cooked squid (Kubota *et al.*, 1996) and have been reported as heating products in seafood (Varlet & Fernandez, 2010).

The N-containing heterocyclic compounds have been confirmed for various crab species (Chung 1999; Chen & Zhang, 2006), and they have been positively related to prawn-shrimp aroma notes (Morita *et al.*, 2001).

Previous studies on mussels have also indicated the existence of pyrazines and pyridine-containing compounds (Le Guen *et al.*, 2000a), and shown the presence of significant amounts of carbonyls, in particular hexanal, benzaldehyde, and 2-heptanone (Le Guen *et al.*, 2000a; Fuentes *et al.*, 2009).

On the other hand, some differentiations were found in relation to literature data. *Trans*-4-heptenal, which has been found in great quantities (Fuentes *et al.*, 2009) and recognized as one of the most potent odorants in mussel (Le Guen *et al.*, 2000b), is present in relatively small quantities in our mussels. Also 2,3-diones, indicated by Le Guen *et al.* (2000a, 2000b) as major mussel odorants, are almost absent in our mussels but present in the fish species. The same also applies for 2,4-octadienal, that

was absent from mussels in our study, but has been reported as being a major mussel odorant contributor (Le Guen *et al.*, 2000b). On the other hand, the presence of significant quantities of *cis*-2-butenal, 2-methyl- and 3-methyl- butanal, pentanal, 1-(2-furanyl)-ethanone and 5-methyl-2-furancarboxaldehyde has not been reported previously in the respective literature.

Although no profound justification occurs for these differences, possible explanations can be found. Given that the volatile compounds' isolation methodology followed in the literature (Le Guen *et al.*, 2000b; Fuentes *et al.*, 2009), was the same as ours, these differences can only be justified by the origin of the samples. Mussels, being filter feeders, may be prone to origin-specific environmental impacts on their volatile compounds (Fuentes *et al.*, 2009). Another possible explanation for the observed differences between literature data and our results can be the different sampling season, since seasonality in seafood volatile compounds is known to occur (Ólafsdóttir & Jónsdóttir, 2010). In the works of Le Guen *et al.* (2000a) and Fuentes *et al.* (2009), sampling was conducted during a different period of the year than this study, while in other cases the sampling season is not specified (Le Guen *et al.*, 2000b).

Cluster analysis showed that the volatiles pattern of pilchard primarily, and mussel to a lesser degree, followed by shrimp, differentiate them from the rest of the studied seafood (Fig. 1). These three species also exhibited the highest squared Euclidian distances (6216.6 and 6016.4 between pilchard, and mussel and shrimp respectively). Four fish species, namely hake, mullet, picarel and bogue, are grouped together (low squared Euclidian distances, ranging from 74.9 between mullet and hake to 345.4 between picarel and bogue). The squid and sand smelt differentiate from this group, while anchovy exhibits even higher differentiation.

What is interesting in this clustering as regards the fish species is that the two littoral species inhabiting very shallow waters, i.e. the pilchard and anchovy differ as regards their volatile compounds from the rest of the fish species that are pelagic, inhabiting deeper waters, demersal or benthic (www.fishbase.org). The fact that all studied fish are carnivorous (www.fishbase.org) and that they originate from the same geographic region, indicates that the ecology of the fish species plays an important role in their volatile compounds profile.

Principal components analysis (PCA) for individual volatile compounds extracted nine components explaining almost 100 % of total variance in seafood species (Fig. 2). The contribution of the volatiles to each component is detailed in Appendix Table 1. In the first component, explaining 34.4% of total variability, numerous carbonyls are present, including aliphatic, non aliphatic and heterocyclic ones, three carbonyl-furans and one S-containing aldehyde. Also, two alcohols (1-pentanol, 3-pentanol), one furan (tetrahydrofuran), numerous

* * * H I E R A R C H I C A L C L U S T E R A N A L Y S I S * * *

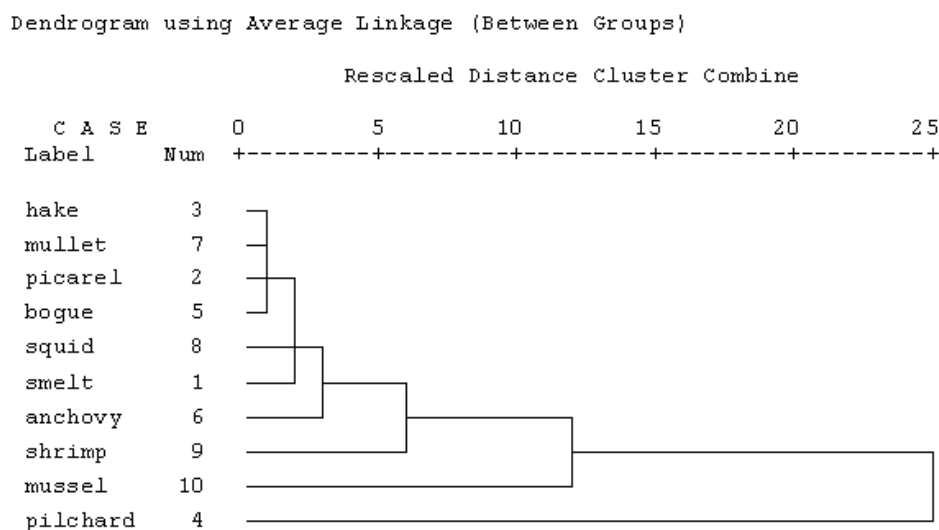


Fig. 1: Cluster analysis: dendrogram using average linkage between seafood species based on their volatile compound profiles.

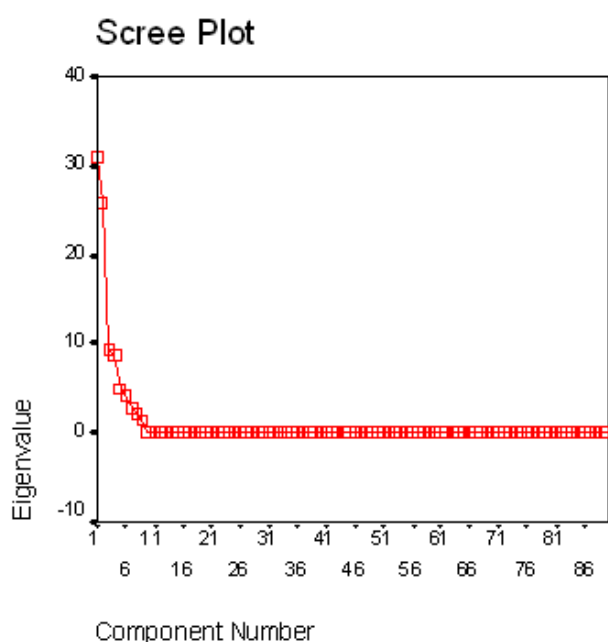


Fig. 2: Scree plot obtained from PCA of volatile compounds of the studied species.

saturated, unsaturated, aromatic hydrocarbons, and one S-containing (1-methylthiopropene), a terpene (d-limonene), and one N-containing compound (pyridine) are present in the 1st component. The second component, explaining, cumulatively with the first one, 63.2% of total variability includes non aliphatic and cyclic carbonyls, four furans, two alcohols (1-penten-3-ol, *trans*-2-penten-1-ol), six non aliphatic and aromatic hydrocarbons, one ether (2-methoxy-2-methyl-butane), one ester (hexadecanoic acid-ethylester), and one S-compound (dimethyl-trisulfide). And the third component that, cumulatively with

the other two, explains 73.4% of total variability contains three esters, two cyclic carbonyls (acetophenone and thiophenecarboxaldehyde), one alcohol (1-octadecanol) and eight hydrocarbons. The contribution of carbonyls of six, eight and nine carbon atoms, in the three first components underlines the importance of those substances. They are mostly lipoxygenase-generated (Ólafsdóttir & Jónsdóttir, 2010), considered typical of fresh seafood and have been associated with aroma notes characterized as light, green, delicate, marine plant and iodised (Josephson *et al.*, 1984; Lindsay, 1990; Ólafsdóttir & Jónsdóttir, 2010)

Conclusions

Besides the common seafood-characterizing carbonyls, some of the studied fish-species have distinct volatile compound patterns. Thus, the pilchard is characterized by a higher number and content of volatiles and in particular alcohols. The non-fish species also differ, with mussel showing the most distinct pattern of volatiles, characterized by high contents of aldehydes, furans and furan-containing compounds, and N-containing heterocyclic compounds (pyridines, pyrazines and pyrroles), and shrimp being rich in amines and S-compounds. An interesting observation is that the clustering of fish species according to their volatile compound profiles may be related to their ecological inhabitants.

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