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Study of the volatile compounds useful for the characterisation of fresh and frozen-thawed cultured gilthead sea bream fish by solid-phase microextraction gas chromatography–mass spectrometry

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ABSTRACT

A simple method, based on solid-phase microextraction gas chromatography–mass spectrometry, was developed for the study of the aromatic profile of cultured gilthead sea bream fish during frozen storage. The method was applied to the study of the volatile profile of fresh and frozen-thawed Italian and Spanish cultured gilthead sea bream fish over 266 days of frozen storage. Variations in the chromatographic responses of a number of volatile compounds during storage were detected and the observed differences evaluated on a biochemical basis. Correlations with oxidation parameters, e.g. peroxide value and thiobarbituric acid-reactive substance indices, indicated 1-octen-3-ol, 1-penten-3-ol, and Z-4-heptenal as markers for the differentiation between fresh and frozen-thawed fish.

1. Introduction

Fish and fish products fulfil an important role in human nutrition as a source of biologically-valuable proteins, fats and fat-soluble vitamins. Freezing is an efficient way of storing fish but, nowadays, consumer interest in fresh products is increasing. The trading of frozen-thawed as fresh products is a fraudulent practice, thus requiring the development of reliable analytical methods for freshness assessment. When freezing, storage and thawing are properly executed, the sensory properties of fish are very similar to those of fresh samples, thus making it very difficult to determine whether a fish has been previously frozen. Enzymatic, physical and physiological methods can be used for freshness assessment (Duflos, Le Fur, Mulak, Becel, & Malle, 2002); however, these methods are tedious and time-consuming. Recently, infrared and fluorescent spectroscopies, to differentiate frozen-thawed from fresh fish, have been also evaluated (Karoui, Thomas, & Dufour, 2006). To our knowledge, the analysis of the volatile compounds of frozen-thawed fish, for its differentiation from the fresh product has not yet been fully exploited. Up till now, only the volatile profiles produced over time for both fresh fish and fish stored on ice for a reduced time have been analysed. Dimethylamine, trimethylamine, volatile acids and aldehydes have been recognised to be fish degradation products (Bene, Hayman, Reynard, Luisier, & Villettaz, 2001; Iglesias & Medina, 2008; Ruiz-Capillas, Gillyon, & Horner, 2001; Ruiz-Capillas & Moral, 2002).

Many methods have also been developed for the analysis of non-volatile compounds. Biogenic amine accumulation has been studied as indicator of freshness and/or spoilage in Mediterranean hake during iced storage (Baixas-Nogueras, Bover-Cid, Veciana-Nogues, Marine-Font, & Vidal-Carou, 2005), whereas the use of amperometric biosensors has been proposed for freshness evaluation by Niculescu et al. (2000) and Hu and Liu (1997).

For comparisons between fresh fish samples and fish samples stored on ice, some studies have also been performed by monitoring the production of formaldehyde, demonstrating a strong increase in the production of this compound over the time (Bianchi, Careri, Musci, & Mangia, 2007).

Volatile compounds in fish can be generated by enzymatic reactions, lipid autoxidation or microbial action, but they can also be developed during fish processing or as a consequence of environmental contaminations (Ólafsdóttir & Fleurence, 1997). Taking into account that the action of microorganisms is negligible at frozen temperatures, volatiles formed as consequence of this activity should not be found in fresh/thawed fish. However, the progress of lipid oxidation leads to the formation of several volatiles resulting from degradation of polyunsaturated fatty acids (PUFA). Consequently, lipid oxidation has long been recognised as a leading cause of quality deterioration in fish muscle foods and is considered to be a critical parameter in determining their shelf-life (Frankel, 1993). Peroxide value (PV) and the 2-thiobarbituric acid-reactive

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substances (TBARS) are two of the commonly used chemical methods for the evaluation of oxidation. Recently, the evaluation of volatile compounds has become an additional indicator of lipid oxidation (Ross & Smith, 2006). Several volatiles, e.g. E-2-hexenal, Z-4-heptenal, (E,E)-2,4-heptadienal, have been associated with the characteristic odours and flavours of oxidised fish, described as rancid, painty, fishy and cod-liver like.

Since the aroma is one of the most typical features of a food, the characterisation of the volatile profile is a useful tool for evaluating the organoleptic quality and it could be used to guarantee food authenticity. In fact, the volatile profile can be considered as a chemical fingerprint of the product, as the nature and the relative amount of the compounds present in the volatile fraction are distinctive features of the product (Berard et al., 2007; Bianchi et al., 2007).

Solvent-free techniques, e.g. dynamic headspace (DHS) and solid-phase microextraction (SPME), are widely used for the extraction of volatile compounds from different matrices, whereas volatile identification is usually achieved by using gas chromatography–mass spectrometry (GC–MS).

As for SPME, an excellent description of theory and practice has been given by Pawliszyn, who developed this technique in the early 90's (Pawliszyn, 1997). SPME offers several advantages: it is a fast, easily automated solvent-free technique, which can be used for *in situ* sampling.

The aim of this work was to characterise volatile compounds of fresh and frozen-thawed Italian and Spanish cultured gilthead sea bream fish, one of the main marine fish species aqua-cultured around the Mediterranean coast. The volatile profile was studied over 266 days of frozen storage and the changes in the volatile composition were finally related to peroxide value and thiobarbituric acid-reactive substances indexes.

2. Materials and methods

2.1. Chemicals and materials

Acetaldehyde, propanal, benzaldehyde, acetone, methylene chloride, ethyl acetate, 2-ethylfuran, 2-pentylfuran, tetrahydrofuran, 3-hexanone, 3-hydroxy-2-butanone, 3-octanone, acetophenone, α-pinene, limonene, 1,8-cineole, linalool, pentanal, E-2-pentenal, 1-penten-3-ol, 2,3-pentanedione, toluene, 1-pentanol, 1-hexanol, 1-heptanol, 2-ethyl-1-hexanol, hexanal, heptanal, E-2-hexenal, E-2-heptenal, Z-2-heptenal, ethylbenzene, o-, m-, p-xylene, styrene, allylbenzene, octanal, (E,E)-2,4-heptadienal, nonanal, hexane, heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, ferrous chloride, ferric chloride, ammonium thiocyanate, 1,1,3,3-tetraethoxypropane 2-thiobarbituric acid and trichloroacetic acid were from Sigma–Aldrich (Steinheim, Germany). All solvents were of analytical grade.

The fibres: carboxen/polydimethylsiloxane 75 μm (CAR–PDMS), polydimethylsiloxane/divinylbenzene 65 μm (PDMS–DVB), polydimethylsiloxane 100 μm (PDMS) and carboxen/polydimethylsiloxane/divinylbenzene 2 cm 50/30 μm (CAR–PDMS–DVB) were from Supelco (Bellefonte, PA, USA).

2.2. Samples

Fresh cultured gilthead sea bream were obtained from Italian and Spanish commercial markets and cultivated in net cages located in Tuscany, Italy, and Aguiño, Spain, respectively. An 8 kg amount of fresh gilthead sea bream *(Sparus aurata)* was deboned, eviscerated and the white muscle separated and minced. Fresh fish samples were immediately analysed, whereas frozen fish samples

Table 1
Volatile compounds identified in the gilthead sea bream fish samples.

Compounds	Identification
Alcohols	
1-Penten-3-ol[I,S]	MS; RT
1-Pentanol[I,S]	MS; RT
2-Penten-1-ol[I,S]	MS
1-Hexanol[I,S]	MS; RT
1-Butoxy-2-propanol[I]	MS
1-Heptanol[I]	MS; RT
1-Octen-3-ol[I,S]	MS
1-Octanol[S]	MS
2-Octen-1-ol[S]	MS
2-Nonen-1-ol[S]	MS
2-Ethyl-1-hexanol[S]	MS; RT
1,5-Octadien-3-ol[I]	MS
3,5-Octadien-3-ol[I]	MS
Aldehydes	
Acetaldehyde[S]	MS; RT
Propanal[S]	MS; RT
Pentanal[S]	MS; RT
E-2-pentenal[I,S]	MS; RT
Hexanal[I,S]	MS; RT
Heptanal[I,S]	MS; RT
E-2-heptenal[I,S]	MS; RT
Z-4-heptenal[I,S]	MS; RT
Benzaldehyde[I,S]	MS; RT
Octanal[I,S]	MS; RT
(E,Z)-2,4-heptadienal[I,S]	MS
(E,E)-2,4-heptadienal[I,S]	MS; RT
E-2-octenal[I]	MS
Nonanal[I,S]	MS; RT
2,6-Nonadienal[I]	MS
3-Phenyl-2-propenal[I]	MS
E-2-hexenal[I]	MS; RT
Aromatic hydrocarbons	
Toluene[I,S]	MS; RT
Ethylbenzene[I,S]	MS; RT
m-+p-xylene[I,S]	MS; RT
o-xylene[I,S]	MS; RT
Styrene[I,S]	MS; RT
Propylbenzene[I,S]	MS
Not identified *m*/*z* 105, 120[I,S]	MS
Not identified *m*/*z* 105, 120[I,S]	MS
Not identified *m*/*z* 105, 120	MS
Not identified *m*/*z* 105, 120	MS
Not identified *m*/*z* 105, 120	MS
Not identified *m*/*z* 105, 120	MS
Not identified *m*/*z* 119, 134	MS
Allylbenzene	MS; RT
(?)-dimethylbenzene	MS
(?)-methyl-(?)-propylbenzene[I,S]	MS
(?)-dimethyl-(?)-ethylbenzene	MS
Not identified *m*/*z* 119, 134	MS
Not identified *m*/*z* 119, 134	MS
Not identified *m*/*z* 119, 134	MS
Not identified *m*/*z* 119, 134	MS
Esters	
Ethylacetate[I,S]	MS; RT
Furans	
Tetrahydrofuran[I,S]	MS; RT
2-Ethylfuran[I,S]	MS; RT
2-Pentylfuran[I,S]	MS; RT
Hydrocarbons	
3-Methylpentane[I]	MS
Hexane[I,S]	MS; RT
Not identified[I]	MS
Heptane[I,S]	MS; RT
Octane[I,S]	MS; RT
Not identified[I]	MS
Nonane[I]	MS; RT
2,2,4,6,6-Pentamethylheptane[I,S]	MS
n-Decane[I]	MS; RT
Not identified[I]	MS
Undecane[I]	MS; RT
Dodecane[I]	MS; RT

Table 1 (*continued*)

Compounds	Identification
Tridecane[I,S]	MS; RT
Not identified[I]	MS
Tetradecane[I]	MS; RT
Pentadecane[I,S]	MS; RT
Hexadecane[I,S]	MS; RT
Heptadecane[I,S]	MS; RT
Ketones	
Acetone[I,S]	MS; RT
3-Hexanone[I]	MS; RT
3-Hydroxy-2-butanone[I,S]	MS; RT
2,3-Pentandione[I,S]	MS; RT
3-Octanone[I]	MS; RT
2,3-Octanedione[I,S]	MS
2,5-Hexandione[I]	MS
Acetophenone[I,S]	MS; RT
3-Octen-2-one[I]	MS
Miscellaneous	
Carbondisulphide[I,S]	MS
Dichlorometane[I,S]	MS; RT
Terpenes	
α-Pinene[I]	MS; RT
Not identified[I]	MS
Limonene[I,S]	MS; RT
1,8-Cineole[I]	MS; RT
Linalool[I]	MS; RT

I: compound detected in the Italian fish samples.
S: compound detected in the Spanish fish samples.
MS: identification by comparison with NIST mass spectrum.
RT: identification by injection of pure standards.

were maintained at the temperature of −20 °C over a 266 day storage period.

2.3. SPME analysis

All the SPME experiments were performed by using a manual injection device. The CAR–PDMS–DVB fibre was exposed to the headspace of a 100 ml vial containing 20 g of minced fish muscle for 30 min at 40 °C. Prior to extraction, samples were equilibrated for 15 min in a water bath at 40 °C. Desorption was carried out at the temperature of 260 °C for 2 min. Prior to use, the fibre was conditioned in the injection port of the gas chromatograph at 270 °C under helium flow for 1 h. A fibre blank was always run between samples to avoid carry-over effects.

Analyses were performed on days 0, 18, 28, 39, 60, 120 and 266. For each day, 3 replicated measurements were performed.

2.4. GC–MS analysis

Analyses were carried out on a Thermo Finnigan ThermoQuest (San Jose, CA) gas chromatograph equipped with a split/splitless injector and coupled with a Trace quadrupole mass detector (Thermo Finnigan ThermoQuest). Compounds were separated on a 30 m × 0.32 mm × 1 μm film thickness, fused silica DB-1701 (Folsom CA) capillary column. The GC oven temperature programme was: 35 °C for 3 min, followed by an increase of 3 °C/min to 70 °C; then an increase of 10 °C/min to 200 °C and finally an increase of 20 °C/min to a final temperature of 260 °C, held for 5 min. Helium, with a constant flow of 1.5 ml/min, was used as the carrier gas. Transfer line temperature was maintained at 265 °C. The quadrupole mass spectrometer was operated in the electron impact mode and the source temperature was set at 200 °C. Acquisition was performed in the 35–200 amu mass range with a scan rate 0.220 s/scan. All the analyses were performed setting ionisation energy at 70 eV, filament emission current at 150 μA and the electron multiplier voltage at 500 V.

The identification of the volatile compounds was achieved by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. Pure standards were also injected to confirm MS identifications.

In order to evaluate relative quantitative differences in the aromatic profiles of the samples investigated, GC peak areas of the compounds identified were calculated as total ion current (TIC).

2.5. Peroxide value

Peroxide value (PV) of fish muscle was determined using the ferric thiocyanate method (Chapman & McKay, 1949) and expressed as mmol oxygen/kg lipid. All the analyses were performed on days 0, 18, 28, 39, 60, 120 and 266.

2.6. TBARS index

The thiobarbituric acid-reactive substances (TBARS) index (mg malonaldehyde/kg muscle) was determined according to Vyncke

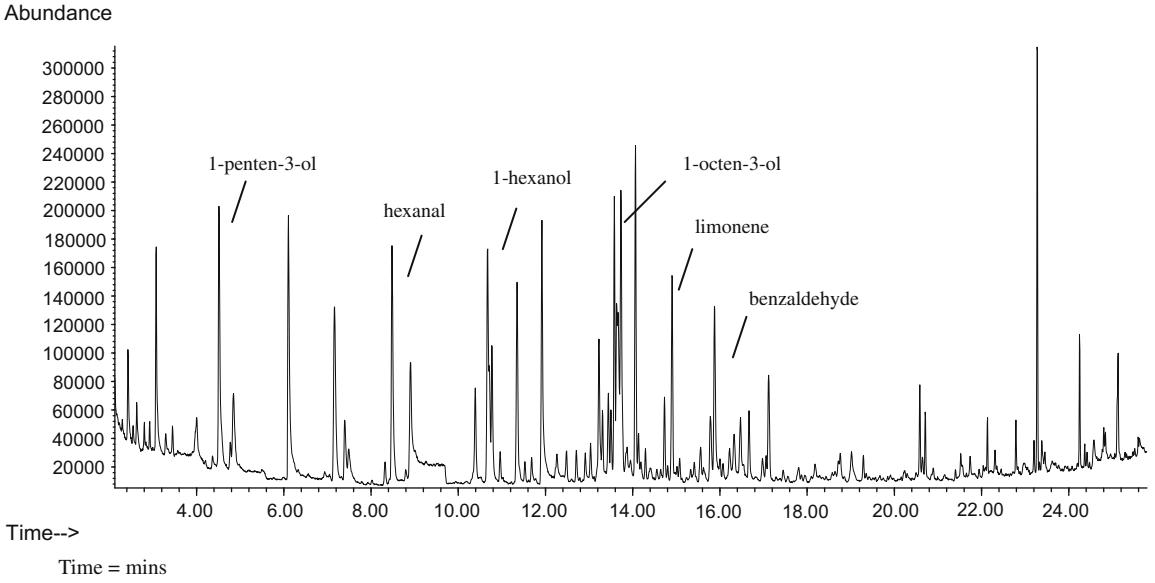

Fig. 1. GC–MS (full scan) chromatogram of a fresh gilthead sea bream fish sample.

Table 2
Occurrence of volatile compounds correlated with PV and TBARS whose concentration increased during frozen storage.

Volatile compounds	Day (chromatographic response: mean ± std. dev. – arbitrary units)						
	0	18	28	39	60	120	266
1-Octen-3-ol	7.13 ± 1.24	17.6 ± 3.35	24.0 ± 3.17	40.7 ± 16.8	22.7 ± 3.04	61.6 ± 5.19	105 ± 20.5
1-Penten-3-ol	27.5 ± 6.48	76.8 ± 15.7	89.5 ± 4.87	119 ± 28.7	84.0 ± 6.10	148 ± 4.80	255 ± 14.7
Z-4-heptanal	n.d.	3.27 ± 0.50	8.09 ± 7.17	8.20 ± 3.56	4.61 ± 1.31	12.2 ± 1.88	22.1 ± 3.42
2-Penten-1-ol	2.65 ± 1.86	5.66 ± 2.20	5.70 ± 2.02	6.97 ± 2.51	4.32 ± 1.22	7.77 ± 0.37	12.0 ± 2.78
Propanal	1.72 ± 0.15	32.3 ± 5.65	35.6 ± 8.28	40.0 ± 13.7	35.3 ± 2.03	50.3 ± 3.19	54.4 ± 13.1
Hexanal	12.6 ± 4.32	276 ± 69.9	411 ± 73.2	489 ± 205	406 ± 29.3	416 ± 44.8	606 ± 213
Limonene	2.93 ± 1.24	3.90 ± 1.72	6.57 ± 1.68	8.81 ± 1.11	3.03 ± 0.74	7.00 ± 0.66	12.8 ± 1.85
Heptanal	0.43 ± 0.11	7.36 ± 1.69	14.7 ± 1.90	25.6 ± 16.5	11.4 ± 0.74	15.7 ± 0.82	26.4 ± 6.11
Pentanal	0.32 ± 0.17	5.63 ± 0.99	8.09 ± 2.70	11.9 ± 6.40	8.02 ± 0.73	9.29 ± 0.75	11.3 ± 6.01
(E,E)-2,4-heptadienal	n.d.	0.69 ± 1.20	n.d.	3.07 ± 0.68	1.17 ± 0.39	1.79 ± 0.17	2.61 ± 1.16
2-Pentylfuran	0.03 ± 0.06	2.57 ± 0.53	1.57 ± 0.51	9.94 ± 0.80	5.93 ± 4.10	7.24 ± 2.83	3.20 ± 0.73
Nonanal	6.30 ± 2.57	11.4 ± 4.82	15.5 ± 1.99	28.7 ± 5.43	11.6 ± 3.73	19.8 ± 1.42	16.8 ± 5.82
2,3-Pentanedione	n.d.	5.99 ± 1.24	12.3 ± 0.81	9.20 ± 1.46	6.23 ± 1.29	6.92 ± 0.39	8.67 ± 1.09
2-Ethylfuran	4.46 ± 1.31	13.8 ± 3.19	20.5 ± 1.07	19.6 ± 6.50	11.5 ± 1.42	12.3 ± 1.75	18.1 ± 3.73
2,3-Octanedione	0.20 ± 0.35	3.78 ± 0.78	6.35 ± 3.38	8.58 ± 4.88	4.30 ± 0.65	5.80 ± 1.48	4.45 ± 1.54
Octanal	n.d.	4.61 ± 1.17	10.2 ± 3.71	12.6 ± 5.20	4.78 ± 0.71	7.27 ± 1.18	6.58 ± 1.87
E-2-Pentenal	0.50 ± 0.25	1.45 ± 0.94	5.62 ± 4.12	2.85 ± 0.77	0.95 ± 0.76	1.46 ± 1.13	2.41 ± 0.31

n.d. not detected.

(1970). The analyses were performed on days 0, 18, 28, 39, 60, 120 and 266.

2.7. Statistical analysis

One-way-analysis of variance (ANOVA) was performed using the SPSS statistical package (Version 15.0, SPSS Inc., Chicago, Illinois, USA).

Correlations were performed with the statistical package, Statgraphics Plus for Windows V. 5.1.

3. Results and discussion

3.1. Method development

Preliminary experiments were carried out in order to evaluate the appropriate conditions for SPME measurements. As expected the CAR–PDMS–DVB fibre showed the highest extraction capability, whereas PDMS–DVB and PDMS fibres proved less effective in the extraction of volatiles of the samples investigated (Guillén, Errecalde, Salmerón, & Casas, 2006; Iglesias & Medina, 2008). For the desorption time, 2 min were sufficient to avoid carry-over effects. The influence of sample amount on extraction efficiency was determined by varying the amount of minced fish muscle (10 and 20 g) in a 100 ml vial. GC responses increased according to the increase of sample amount and 20 g amounts were selected for subsequent experiments. A heating temperature of 40 °C and an exposure time of 30 min were selected as extraction conditions in order to avoid oxidation of the sample. Fibre position in the injector was tested after the injection of the same samples with depths of 3, 4 and 5 cm, but no significant differences were observed among the three chromatograms. Finally, a depth of 4 cm was chosen. Under these conditions, many volatiles belonging to different chemical classes could be extracted, giving good GC responses.

3.2. Identification of volatiles in gilthead sea bream

The developed method was then applied to the determination of the volatile compounds of fresh and frozen-thawed cultured gilthead sea bream fish samples. The volatiles identified in the analysed samples are listed in Table 1. Fig. 1 shows a GC–MS chromatogram obtained from the SPME analysis of a fresh gilthead sea bream fish sample.

As a general comment, it can be stated that the Italian fish samples were characterised by the highest number of volatile compounds, mainly as hydrocarbons, terpenes and aromatics. 1-Penten-3-ol was the most abundant compound in both the fresh fish species, followed by hexanal and 1-octen-3-ol in the Italian samples and hexanal and 2-ethyl-1-hexanol in the Spanish fish. Taking into account that the aim of this study was the identification of markers for differentiating frozen-thawed from fresh fish, the changes in the volatile profile during frozen storage were followed for all the investigated compounds.

3.3. Changes of volatiles during frozen storage

Aldehydes were found to be the compounds showing the highest peak areas increase during the frozen storage (Table 2). Among them, propanal can be obtained from the 16-hydroperoxide formed by autoxidation of methyl linolenate and from the 15-hydroperoxide formed by photosensitized oxidation of methyl linolenate (Frankel, 1998). Hexanal was the aldehyde showing the highest increase of the GC area. The high levels obtained for this compound can be related to the fatty acid composition of fish since it is formed

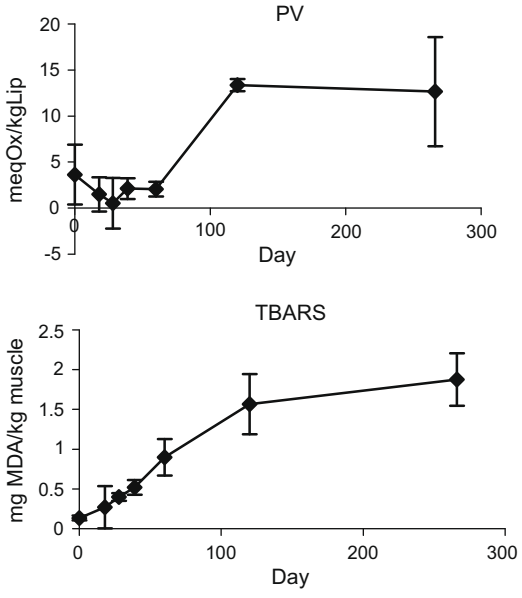

Fig. 2. Rate of lipid oxidation indices during frozen storage of the gilthead sea bream fish samples.

Table 3
Pearson coefficients calculated between the lipid oxidation indices and analyses of the volatile compounds.

Volatile compound	PV (*p*-value)	TBARS (*p*-value)
1-Octen-3-ol	0.833 (0.020)	0.916 (0.004)
1-Penten-3-ol	0.757 (0.049)	0.892 (0.007)
Z-4-heptenal	0.755 (0.050)	0.882 (0.009)
2-Penten-1-ol	0.714 (0.072)	0.823 (0.023)
Propanal	0.558 (0.193)	0.791 (0.034)
Hexanal	0.385 (0.393)	0.699 (0.080)
Limonene	0.593 (0.161)	0.685 (0.090)
Heptanal	0.396 (0.379)	0.618 (0.139)
Pentanal	0.335 (0.459)	0.617 (0.138)
(E,E)-2,4-heptadienal	0.480 (0.285)	0.609 (0.152)
2-Pentylfuran	0.167 (0.723)	0.319 (0.485)
Nonanal	0.195 (0.675)	0.306 (0.504)
2,3-Pentanedione	−0.020 (0.966)	0.268 (0.561)
2-Ethylfuran	0.172 (0.926)	0.225 (0.635)
2,3-Octanedione	−0.009 (0.993)	0.217 (0.633)
Octanal	−0.050 (0.922)	0.158 (0.728)
E-2-pentenal	−0.232 (0.610)	−0.071 (0.877)

by oxidation of linoleic acid (Kawai, 1996), the most abundant fatty acid in the cultured fish muscle. The presence of this compound can also be related to a degradation process of pre-formed volatiles, such as 2-octenal (Josephson & Lindsay, 1987; Koelsch, Downes, & Labuza, 1991). The presence of 2,4-heptadienal can be ascribed to the autoxidation of eicosapentaenoic acid (20:5ω-3) (EPA) (Kawai, 1996), whereas *Z*-4-heptenal can be produced *via* 2,6-nonadienal by the action of 12-lipoxygenase on EPA (Aro, Tahvonen, Koskinen, & Kallio, 2003; Duflos, Coin, Cornu, Antinelli, & Malle, 2006).

Among alcohols, the GC response of 1-penten-3-ol and 1-octen-3-ol increased during frozen storage, whereas a decrease was observed for 2-ethyl-1-hexanol. Similar behaviour has already been observed in previous studies (Alasalvar, Taylor, & Shahidi, 2005; Aro et al., 2003). The increases of 1-penten-3-ol and 1-octen-3-ol can be explained by taking into account both the action of 15-lipoxygenase on EPA (20:5n-3) (Alasalvar et al., 2005; Kawai, 1996) and of 12-lipoxygenase on arachidonic acid (Hsieh & Kinsella, 1989). In particular, 1-octen-3-ol is an important contributor to off-flavours due to its low odour threshold, whereas 1-penten-3-ol has recently been used as a marker of lipid oxidation in chilled Atlantic horse mackerel muscle (Iglesias & Medina, 2008).

Among furans, tetrahydrofuran, 2-ethylfuran and 2-pentylfuran were detected and identified. The formation of 2-ethylfuran can be explained by taking into account that the 12-hydroperoxide of linolenate (18:3n-3), the 14-hydroperoxide of eicosapentaenoate (20:5n-3) and the 16-hydroperoxide of docosahexaenoate (22:6n-3) can undergo β-cleavage to produce a conjugated diene radical which can react with oxygen to produce a vinyl hydroperoxide. Cleavage of the vinyl hydroperoxide, by loss of a hydroxyl radical, forms an alkoxyl radical, that undergoes cyclisation, thus producing 2-ethylfuran (Medina, Satue-Gracia, & Frankel, 1999).

For aromatic hydrocarbons, a variability of the GC responses over the storage period was observed. However, the origin of these compounds has not been completely clarified, although their presence has been reported in different fish (Rodriguez-Bernaldo De Quirós, Lopez-Hernandez, Gonzalez-Castro, de la Cruz-Garcia, & Simal-Lozano, 2001; Aro et al., 2003).

Among ketones, an increase of the GC responses was observed for 2,3-octanedione and 2,3-pentanedione, the latter being recently reported as an indicator of lipid oxidation in chilled fish muscle (Iglesias & Medina, 2008).

The absence of sulphur-containing compounds, e.g. methanethiol, dimethyl disulphide and dimethyl trisulfide, in all the analysed frozen fish samples is remarkable. These compounds, mainly related to bacterial contamination, have been previously identified in fish stored in ice, showing a rapid increase over time, with a negative effect on fish aroma as a consequence of their undesirable odour (Alasalvar, Aishima, & Quantick, 1995; Alasalvar et al., 2005).

Italian fish showed an important increase in the volatile composition after just 6 days of frozen storage. By the sixth day, high increments in peak areas were obtained for toluene (784% of increment), hexanal (311% of increment) and 2,3-pentanedione (106% of increment). The first significant increment of volatiles in Spanish samples was detected after 62 days of frozen storage. Hexanal (4706%), 1-octen-3-ol (1367%) and 1-penten-3-ol (826%) were

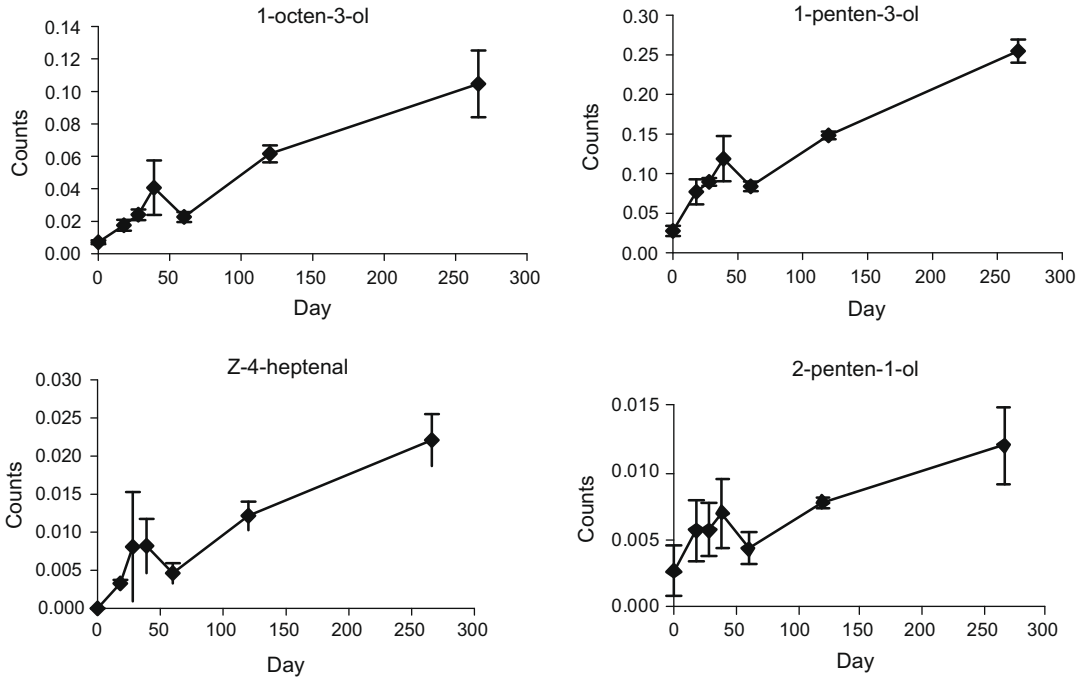

Fig. 3. Time course of volatile compounds formation in gilthead sea bream fish samples analysed by the developed SPME–GC–MS method.

the volatiles which showed the highest increments during frozen storage. Again, high levels were obtained for hexanal, according to the high levels of linoleic acid (15.5% of the total fatty acids).

PV and TBARS were also calculated to evaluate possible correlations between these indices and the development of some volatiles.

As shown in Fig. 2, the development of oxidation by-products, such as aldehydes, during frozen storage was strongly related to these parameters. Table 3 show the Pearson coefficients calculated between these oxidation indices and the concentrations of different volatiles. The highest correlations were obtained between the volatiles and TBARS index, since their measurements are related to secondary lipid oxidation products. Four compounds, namely 1-octen-3-ol, 1-penten-3-ol, Z-4-heptenal and 2-octen-1-ol, showed Pearson coefficients higher than 0.8 with the TBARS index. Time courses for these compounds is shown in Fig. 3. Accordingly, these volatiles can be proposed as markers for lipid oxidation occurring during the frozen storage, thus allowing differentiation between fresh and frozen-thawed fish.

4. Conclusions

SPME–GC–MS proved to be a useful tool for the evaluation of the effect of frozen storage on the volatile compounds of cultured gilthead sea bream fish samples. Comparison of the changes in the volatile profiles over a long frozen storage period, as well as their correlation with chemical indices for evaluation of lipid oxidation, allowed the identification of marker compounds that could possibly be used as quality indicators for differentiating frozen-thawed from fresh fish samples.

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