



First Investigation on the Shelf life of Mediterranean Mussels (*Mytilus galloprovincialis*) on the Basis of Their Volatiles Profiles

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Abstract

Volatiles are critical for real and perceived quality of mussels. In the present study, we determined, for the very first time, the characteristic volatiles of fresh Mediterranean mussels (*Mytilus galloprovincialis*) and their variation during 4 days of storage at 6.0 ± 0.5 °C. During this time, volatile organic compounds (VOCs) were monitored using SPME-GC-MS. Twenty-seven VOCs were identified in mussel meat: eight esters, seven alcohols, three acids, three aldehydes, three ketons, one phenol, one sulfide, and one polycyclic aromatic hydrocarbon. While the molecular fingerprint of the fresh mussel was very simple, during storage, there was the onset of reliable shelf life markers. Two of them, namely 1-octen-3-ol and 2-nonanone, appeared after 1.5 days and increased during chilled storage up to the fourth day. Other seven compounds (three free acids, four esters, and one phenol derivative) were found only after 4 days. Shelf life markers monitoring enables correct transport and storing conditions and prevention of the distribution of stale mussels. This issue is obviously crucial for the food industry and catering. The technique is a rapid, green, and nondestructive monitoring tool during ongoing development of industrial food processing. The absence of sample manipulation assures the fact that its flavor is not influenced by pre-analytical steps. It could aid in the development of technologies that monitor and improve the processing product quality and consistency.

Keywords Mussels · Shelf life markers · Volatile organic compounds · HS-SPME-GC-MS · Flavor

Introduction

Mediterranean mussels are very popular shellfishes harvested in the Adriatic Sea. These bivalves are popular bivalves with an exceptional nutritional value since they are rich in minerals, vitamins, and polyunsaturated fatty acids, mainly omega 3, interestingly associated with a decreased risk of cardiovascular disease (Karakoltsidis et al. 1995).

They are highly appreciated from a gastronomical point of view. They are eaten as high-class food in restaurants; hence, their flavor is crucial.

The global mussel market is estimated to be slightly below 600,000 tons in equivalent live animal weight. The three main countries responsible for two thirds of all European mussel

production, which mostly comes from aquaculture, are Spain, France, and Italy. *Mytilus edulis* and *Mytilus galloprovincialis* are the principal species harvested all over Europe.

Microbial and biochemical degradation pathways result in a very short shelf life that is influenced also by the storage conditions. There are sensory and microbiological methods available to assess the quality of seafood. The former lack objectivity, while the latter are time-consuming and may not be able to promptly shed light on the presence of unpleasant off-flavors; hence, they are not practical for routine use. Fast, easy, green, and reliable methods are therefore necessary for assuring freshness specification of the mussels.

HS-SPME followed by GC-MS is a reliable technique increasingly used to sample and analyze volatile organic compounds (VOCs) in fresh seafood samples (Fratini et al. 2012). The same technique was used to assess the volatile profile of New Zealand Greenshell™ mussels during chilled storage (Tuckey et al. 2013); in that case, mussel samples were homogenized. In this time-consuming procedure, the contact between oxygen and mussel meat may result in lipid peroxidation, enzymatic and nonenzymatic changes, and new volatile compounds; hence, conclusions concerning the shelf life

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markers may not be adequate. There is another study about the comparison of volatiles between fresh and rotten mussels by GC-MS: the procedure comprised a homogenization step; hence, the criticism is the same (Yasuhara 1987). To the best of our knowledge, no other studies concerning the shelf life of mussels, even of different species, are available in the literature. Given the commercial importance of *M. galloprovincialis* and a dearth of shelf life data in the literature, the aim of the present paper is to assess for the first time a simple, a green, economical, easy, and nondestructive method to highlight molecular markers of shelf life, for routine use.

Materials and Methods

Mussel Samples and Their Handling

Fifty kilograms of Adriatic mussels was obtained live from a local culture operation in January 2017 (FAO Zone 37.2.1 sub zone 19.2A). On arrival at the facility, they were stacked inside a 50-L plastic bin and spaced so that filtered seawater (10 ± 1 °C) could flow (10 L min^{-1}) freely around each mussel. After recovering for 24 h in this system, 30 mussels whose shells closed firmly were randomly chosen to enter immediately the analytical procedure. This procedure guaranteed that all mussels were vital after standardized harvesting, transport, and storage.

Volatile Profile

Mussels were removed from their shells and the seawater mussel liquor discarded. Tissue was weighted (3.0000 ± 0.0100 g) in 8 ml headspace vials and closed through a PTFE/silicone septum. Volatiles in the headspace were measured through HS-SPME and GC-MS. A Hewlett Packard GC-MS, G1800C-GCD Series-II (Palo Alto, CA) equipped with a HP-5MS column $30 \text{ m} \times 0.25 \text{ mm I.D.} \times 0.25 \text{ } \mu\text{m}$ film thick was used. Chromatographic conditions are described elsewhere (Cecchi 2014; Cecchi and Alfei 2013; Vincenzetti et al. 2017). The experimental design was run in quadruplicate (samples A/B/C/D) and measurements were run in triplicate. Volatiles of the fresh samples were adsorbed onto the SPME fibers for 12 h at 6.0 ± 0.5 °C. After extraction, injections provided the fiber thermal desorption. After the first injection, the fiber was exposed to the same sample headspace for additional 24 h and then the procedure was repeated. After the second injection, the fiber was exposed to the same sample headspace for additional 60 h and then the procedure was repeated again. This way, the same mussel sample was analyzed three times, that is after 12, 36, and 96 h of refrigerated storage (thereby obtaining samples A1, A2, A3....D1, D2, D3) from the moment they were still vital and they were removed from the shell. Absolute peak areas were recorded in area counts. Samples were spiked with $1.33 \text{ } \mu\text{g/ml}$ chlorobenzene (internal

standard in refined oil) that was used to normalize peak areas. In this context, it is crucial to underline that we are not interested in the amount of the shelf life marker but on its appearance with increasing storage time. Only compounds with a signal to noise ratio higher than 5 were considered. Blank runs were done periodically during the study to reveal possible carryover. The identification of the constituents was based on comparison of the retention times with those of authentic samples obtained from Sigma Aldrich (Milan, Italy), if available. The identification was also based on computer matching against NIST 1998 library. In the absence of the commercial standard, the identity of the spectra at 98% was needed for identification (Cecchi and Alfei 2013; Vincenzetti et al. 2017). Table 1 details the base peak of the mass spectra and two other main peaks in order of decreasing relative abundance that were used for the identification. Volatile compounds were also identified by comparison of their linear retention indices, relative to n-alkanes, calculated using straight-chain alkanes mixture C6-C19, with the averaged values reported in the bibliography for chromatographic columns similar to that used (<http://webbook.nist.gov/chemistry/>).

In order to ground the possible presence of odorants in laboratory ambient air, a control sample that comprises only the laboratory air in the headspace vial was analyzed (Cecchi 2014): in this case, limonene was detected, among other terpenes.

Results and Discussion

Identification of VOCs in Fresh Samples

The volatile profiles of fresh (A1-D1 samples) and chilled stored mussels at 36 h (A2-D2) and 90 h (A3-D3) are detailed in Table 2. Table 2 also details the odor type of each analyte. Twenty-seven compounds were identified: eight esters, seven alcohols, three acids, three aldehydes, three ketons, one phenol, one sulfide, one polycyclic aromatic hydrocarbon (PHA). Limonene was found in some mussel samples; since it is a widespread airborne contaminant, also found in our laboratory air (Cecchi 2014), it was not included in Table 2 because its link with mussels was not sure.

The volatile profile of fresh *M. galloprovincialis* mussels from the Adriatic region detailed in Table 2 is very simple, much simpler than that of *Perna canaliculus* mussel (Tuckey et al. 2013) and that of *M. galloprovincialis* from Spain (Fratini et al. 2012). This is not surprising because source location is expected to influence physico-chemical properties of mussels (Fuentes et al. 2009) but also because the homogenization procedure is a flavor generating step. The aroma of fresh mussel (not manipulated at all) is mainly characterized by the presence of dimethyl sulfide and benzaldehyde. Dimethyl sulfide may possibly come from dimethylsulfoniopropionate, which

Table 1 MS base peak and two other main peaks in order of decreasing relative abundance, used for the identification procedure

Molecule	MS base peak and two other main peaks in order of decreasing relative abundance
Dimethyl sulfide	47, 45, 46
1-Penten-3-ol	57, 29, 27
Ethyl propanoate	29, 57, 27
Propanoic acid	74, 28, 45
Ethyl butanoate	71, 43, 29
1-Hexanol	56, 43, 41
Butanoic acid	60, 73, 41
Propyl butanoate	43, 71, 27
2-Methylbutanoic acid	74, 57, 29
Benzaldehyde	77, 106, 105
1-Heptanol	70, 56, 43
1-Octen-3-ol	57, 43, 72
3-Octanone	43, 57, 72
Butyl butanoate	71, 43, 56
Hexyl acetate	4, 56, 55
2-Ethyl-1-hexanol	57, 41, 43
Ethyl-2-hexenoate	55, 97, 99
1-Octanol	56, 55, 41
4-Methyl-phenol	108, 107, 77
2-Nonanone	43, 58, 41
Nonanal	57, 41, 43
2,3,4,5,6,7,8,9-Octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene	267, 211, 282
Ethyl octanoate	88, 101, 57
Decanal	43, 41, 57
Ethyl 2-octenoate	55, 29, 125
2-Undecanone	58, 43, 59
2-Undecanol	45, 43, 55

originates in algae on which the mussels feed (Dacey et al. 1994). Benzaldehyde comes from amino acid degradation (Piveteau et al. 2000) and its odor has been described as candy, sweet, and almond (Turchini et al. 2010).

Some volatiles, such as 1-penten-3-ol, 2-ethyl-1-hexanol, 1-octanol, decanal, 2-undecanone, 2-undecanol, were sporadically detected in different fresh samples. 1-penten-3-ol is a degradation product due to the action of lipoxygenases on ω -3 PUFA; this oxidation marker imparts a desirable heavy plant-like aroma to fish muscle (Iglesias and Medina 2008). The biochemical pathways through which this and other compounds are produced in various seafood have been reviewed previously (Kawai 1996), and therefore it will not be discussed in detail. Anyhow, the simple volatile profile of fresh mussel is not surprising: fresh saltwater fish were similarly found to be nearly odorless because they contain a small quantity of volatiles. Their monotonous volatile constitution was likely associated to an unknown antioxidation system restraining the fish from oxidizing (Kawai 1996). Generally,

most compounds detected here have been previously identified in seafood (Iglesias and Medina 2008).

Changes in VOCs During Chilled Storage

Table 2 also illustrates changes of the sample volatile profiles upon storage. It can be observed that dimethyl sulfide usually increases with storage thereby indicating that microbial metabolism was likely to be the dominant dimethyl sulfide production pathway (Tuckey et al. 2013). In two samples, it reaches a maximum and then it decreases. No clear trend can be observed for benzaldehyde.

It can be observed that some molecules are not present in the volatile profile of fresh mussels but they are invariably linked to storage in all samples; hence, they are highly eligible shelf life markers.

We can divide these markers in two groups. The first group includes two compounds, namely 1-octen-3-ol and 2-nonanone, whose onset is after 1.5 days of storage. Their

Table 2 Volatiles (and their odor type) of fresh (A1-D1 samples) and chilled stored mussels at 36 h (A2-D2) and 90 h (A3-D3). Measurements were done in triplicate and the average area counts were given

Molecule	Odor type	A1	A2	A3	B1	B2	B3	Chemical class
Dimethyl sulfide	Sulfurous	7.46E+07	9.47E+07	1.45E+08	6.37E+07	1.06E+08	1.22E+08	Sulfide
1-Penten-3-ol	Green							Alcohol
Ethyl propanoate	Fruity		6.69E+06	1.06E+07			1.52E+07	Ester
Propanoic acid	Acidic			1.85E+07			1.41E+07	Acid
Ethyl butanoate	Fruity			4.34E+07				Ester
1-Hexanol	Herbal		4.34E+06					Alcohol
Butanoic acid	Cheesy			1.39E+08			8.10E+07	Acid
Propyl butanoate	Fruity			2.81E+07			8.14E+06	Ester
2-Methyl-butanoic acid	Acidic			2.81E+07			2.66E+05	Acid
Benzaldehyde	Fruity	1.37E+06	3.10E+06	6.06E+06	5.72E+05	4.67E+05	1.96E+06	Aldehyde
1-Heptanol	Green			7.14E+06				Alcohol
1-Octen-3-ol	Earthy		1.93E+06	5.24E+06		2.05E+05	2.61E+05	Alcohol
3-Octanone	Herbal		1.47E+06	3.62E+06				Ketone
Buryl butanoate	Fruity			6.22E+06			1.16E+01	Ester
Hexyl acetate	Fruity	5.85E+05	5.10E+05	2.30E+05				Ester
2-Ethyl-1-hexanol	Citrus			4.01E+06			1.46E+06	Alcohol
Ethyl-2-hexenoate	Fruity		5.55E+05	1.96E+06		2.01E+05	3.14E+05	Ester
1-Octanol	Waxy			8.99E+05			2.41E+05	Alcohol
4-Methyl-phenol	Phenolic		5.67E+05	8.94E+06		2.91E+05	1.51E+06	Phenol
2-Nonanone	Fruity							Ketone
Nonanal	Aldehydic			2.43E+06			4.36E+05	Aldehyde
2,3,4,5,6,7,8,9-Octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene	—							PHA
Ethyl octanoate	Waxy			2.86E+06			1.22E+06	Ester
Decanal	Aldehydic		1.81E+05	7.26E+05		1.57E+05	2.62E+05	Aldehyde
Ethyl 2-octenoate	Fruity			8.94E+06			9.30E+06	Ester
2-Undecanone	Fruity		1.08E+06	5.33E+06		9.82E+05	2.70E+06	Ketone
2-Undecanol	Fruity		6.84E+05	1.53E+06		5.34E+05	9.62E+05	Alcohol
Molecule	Odor type	C1	C2	C3	D1	D2	D3	Chemical class
Dimethyl sulfide	Sulfurous	6.10E+07	1.27E+08	1.23E+08	5.81E+07	7.82E+07	5.70E+07	Sulfide
1-Penten-3-ol	Green	3.58E+06						Alcohol
Ethyl propanoate	Fruity							Ester
Propanoic acid	Acidic			1.57E+04			2.71E+07	Acid
Ethyl butanoate	Fruity			2.69E+07			1.10E+07	Ester
1-Hexanol	Herbal		2.82E+06			5.39E+05		Alcohol
Butanoic acid	Cheesy			1.08E+08			2.87E+07	Acid

Table 2 (continued)

Propyl butanoate	Fruity	1.23E+07				9.10E+05	Ester
2-Methyl-butanoic acid	Acidic	2.05E+06				4.55E+05	Acid
Benzaldehyde	Fruity	6.83E+06	1.91E+06	3.38E+05		2.07E+06	Aldehyde
1-Heptanol	Green	6.96E+06				2.58E+06	Alcohol
1-Octen-3-ol	Earthy	4.86E+06	1.60E+06			2.72E+05	Alcohol
3-Octanone	Herbal	2.27E+06	1.28E+06			2.88E+05	Keton
Butyl butanoate	Fruity	1.90E+06					Ester
Hexyl acetate	Fruity						Ester
2-Ethyl-1-hexanol	Citrus						Alcohol
Ethyl-2-hexenoate	Fruity	9.90E+06					Ester
1-Octanol	Waxy						Alcohol
4-Methyl-phenol	Phenolic	5.69E+05				2.89E+05	Phenol
2-Nonanone	Fruity	5.13E+06	6.71E+05			1.58E+06	Keton
Nonanal	Aldehydic	1.70E+06	8.67E+05			6.60E+05	Aldehyde
2,3,4,5,6,7,8,9-Octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene	—	1.49E+06				4.03E+05	PHA
Ethyl octanoate	Waxy	1.60E+06				4.79E+05	Ester
Decanal	Aldehydic	4.45E+05	3.41E+05	5.71E+04		3.37E+05	Aldehyde
Ethyl 2-octenoate	Fruity	8.14E+06				1.80E+06	Ester
2-Undecanone	Fruity	2.70E+06	1.04E+06	1.50E+05		1.50E+06	Keton
2-Undecanol	Fruity	1.13E+06	7.61E+05	1.25E+05		9.27E+05	Alcohol

amount increases during chilled storage till the fourth day. 1-octen-3-ol is produced from the action of 12-lipoxygenase on arachidonic acid (Hsieh et al. 1988); it is a marker of lipid oxidation in fish, rising in conjunction with peroxide value during chilled and frozen storage (Iglesias et al. 2009); it contributes a heavy, plant-like, and mushroom-like aroma to Atlantic and Pacific oysters (Josephson et al. 1985).

The second group includes volatiles detectable only after 4 days of storage; it is clear that their production needs a longer lag phase. Propanoic, butanoic, and 2-methylbutanoic acids, esters such as ethyl butanoate, propyl butanoate, ethyl octanoate, ethyl 2-octenoate, and other semivolatiles such as 4-methyl-phenol and 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene, belong to this group.

The eligibility of free fatty acids and esters as shelf life markers is not surprising since, during storage, lipases cleave triacylglycerols and phospholipids, forming free fatty acids that can be further esterified.

Carboxylic acids were already found to increase upon rotting of *Mytilus edulis* and they are actually highly malodorous (Yasuhara 1987); 4-methylphenol, another malodorous compound, was already detected only in rotten mussel (Yasuhara 1987).

2,3,4,5,6,7,8,9-Octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene is a PHA derivative. It has been related to pollution (Chamorro et al. 2013). Mussels can be used to compare PAHs pollution between different sites (León et al. 2013). The appearance of 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene only after 4 days of storage can be explained by matrix effects: this analyte is probably released by the tissue and partitioned into the headspace only after its staling. Since it is related to pollution, it is not eligible as a storage marker.

There are other compounds that appear in the volatile profile during storage but they are not ubiquitous to all samples; for this reason, they will not be considered shelf life markers even if they are probably linked to staling. This is the case of other esters such as ethyl propanoate, ethyl-2-hexenoate, butyl butanoate, hexyl acetate, and ethyl-2-hexenoate; alcohols such as 1-hexanol, 1-heptanol, and 1-octanol; and carbonyl compounds such as nonanal and 3-octanone. The primary production pathway for alcohols and aldehydes is lipid oxidation mediated by lipoxygenase and lyase enzymes (Kawai 1996).

Contribution of VOCs to Odor and Flavor

It has to be emphasized that no sensory panels were used in this study because the properties of many of the detected compounds have been described previously. The impact of each VOC on odor and flavor depends on both concentration and odor threshold. Therefore, small amounts of low odor

threshold VOCs may have a stronger sensory impact than huge amounts of high odor threshold compounds.

Therefore, since alcohols usually have high odor thresholds, their contribution might be limited (Alasalvar et al. 2005). Anyhow, this is not true for 1-octen-3-ol, which has a low odor threshold, a mushroom-like aroma, and contributes to off-flavors (Iglesias et al. 2009; Kawai 1996).

The aldehydes and ketones commonly have low odor and flavor thresholds (Alasalvar et al. 2005; Kawai 1996) and consequently are likely to have a much stronger influence on mussel positive sensory properties, contributing with fruit and vegetables notes with descriptions such as green/fruity/planty and earthy; nonanal in particular has a fruity-like and plant-like aroma (Alasalvar et al. 2005; Kawai 1996; Tuckey et al. 2013).

Cooking, storage conditions and time, and oxygen levels are all likely to influence mussel volatile compounds. The interactions of these parameters are amenable to be studied by HS-SPME GC-MS.

Compliance with Ethical Standards

Conflict of Interest Teresa Cecchi declares that she has no conflict of interest. Luca Sacchini declares that he has no conflict of interest. Alberto Felici declares that he has no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed Consent Not applicable.

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