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Microbiological spoilage and investigation of volatile profile during storage of sea bream fillets under various conditions

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ABSTRACT

Volatile organic compound (VOC) profile was determined during storage of sea bream (*Sparus aurata*) fillets under air and Modified Atmosphere Packaging (MAP — $CO_2/O_2/N_2$: 60/10/30) at 0, 5 and 15 °C. Microbiological, TVB-N (Total Volatile Base Nitrogen) and sensory changes were also monitored. Shelf-life of sea bream fillets stored under air was 14, 5 and 2 days (d) at 0, 5 and 15 °C respectively, while under MAP was 18, 8, and 2 d at 0, 5 and 15 °C respectively. At the end of shelf life, the total microbial population ranged from 7.5 to 8.5 log cfu/g. *Pseudomonas* spp. were among the dominant spoilage microorganisms in all cases, however growth of *Brochothrix thermosphacta* and Lactic Acid Bacteria (LAB) were favoured under MAP compared to air. TVB-N production was favoured at higher temperatures and under air compared to lower temperatures and MAP. TVB-N increased substantially from the middle of storage and its value never reached concentrations higher than 30–35 mg N/100 g, which is the legislation limit, making it a poor chemical spoilage index (CSI). A lot of alcohols, aldehydes, ketones and ethyl esters that were detected in the present study have been reported as bacterial metabolites, others as products of chemical oxidation while others as aroma constituents. VOCs such as 3-methylbutanal, acetic acid, ethanol, ethyl esters of isovaleric and 2-methylbutyric acids, 1-penten-3-ol, 1-octen-3-ol and cis-4-heptenal appeared from the early or middle stages and increased until the end of storage. From those only 3-methylbutanal, acetic acid, ethanol and the ethyl esters have been reported as microbial origin, making them potential CSI candidates of sea bream fillets.

1. Introduction

Fish is highly perishable product and spoil due to microbiological activity, chemical oxidation of lipids and autolysis (Gram and Huss, 1996). However, microbial spoilage is the main mechanism affecting fresh fish quality. Under particular storage conditions (e.g., atmosphere, temperature), a consortium of bacteria known as specific spoilage organisms (SSOs) produces metabolites (chemical spoilage indices—CSIs) responsible for off-flavours and causes the organoleptic rejection of the product (Dalgaard, 2003; Gram and Huss, 1996). Off flavours/off odour production is the major indicator used by consumers to evaluate fish freshness (Oehlenschläger, 2014). Compounds with characteristic smell such as trimethylamine (TMA), various nitrogenous (TVB-N) and sulphuric compounds, aldehydes, ketones, and esters are produced by various microorganisms during fish spoilage (Dalgaard, 2003; Gram and Huss, 1996; Olafsdottir et al., 1997). Many groups of microorganisms contain species and strains that contribute to fish spoilage under particular storage conditions. For example, under aerobic storage conditions, *Shewanella putrefaciens* has been recognised as potential spoilage organism of chilled fish from northern seas due to the ability to reduce Trimethylamine Oxide (TMAO) to TMA (Gram et al., 1987). In fish originated from Mediterranean Sea waters, *Pseudomonas* spp. are mainly involved in the spoilage of fish at low temperatures (Parlapani et al., 2013; Tryfinopoulou et al., 2002). However, under MAP, the dominant microbiota of sea bream fillets is influenced by the different storage conditions (Parlapani, 2013). It is known that, the succession of spoilage microorganisms as well as their metabolic activity is greatly influenced by temperature and type of packaging (Dalgaard, 2003; Gram and Huss, 1996). Indeed, the different gaseous atmospheres of MAP not only prolongs the shelf-life of fishery products but also affects the synthesis of spoilage microbiota and the profile of the metabolites produced leading to a different type of spoilage (Gram and Huss, 1996).

Many methods have been used to assess fish and seafood quality based on physical, microbiological and chemical changes during storage (Olafsdottir et al., 1997). Sensory evaluation is the most common way of assessing the freshness of fish and fish products (Howgate, 1982). Various sensory attributes (appearance of the skin, eyes, mucus and gills, colour, odour and texture) have been used to estimate the overall quality of fish (Cakli et al., 2006; Kyrana et al., 1997; Özogul et al., 2007;

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Rodriguez et al., 2003). The reliability of sensory analyses is decreased in fish products, such as fillets, due to the diminished number of assessment attributes (Duflos et al., 2006). Additionally, sensory methods have to be carried out by trained assessors, hence it is expensive to perform and difficult to standardize, while microbiological methods used by now are retrospectively expensive and time consuming (Dainty, 1996; Dalgaard, 2003). Thus, there is a need to implement new and rapid methods for fish and fish product freshness/spoilage monitoring based on determination of microbial metabolites (Dainty, 1996; Dalgaard, 2003; Edirisinghe et al., 2007; Ellis and Goodacre, 2001).

Traditional chemical methods to monitor microbial activity in fish include the determination of TVB-N and TMA. However, TVB-N and TMA increase in fish occurs only at the late stages of storage, hence those two parameters cannot be used as freshness indicator (Oehlenschläger, 2014). Additionally, TMA development in Mediterranean fish is not significant, presumably due to the low level of precursor compound TMAO (Drosinos et al., 1997; Koutsoumanis and Nychas, 1999; Kyrana and Lougovois, 2002).

Solid Phase MicroExtraction coupled with gas chromatography/mass spectrometry (SPME–GC/MS) is an analytical method which has been used to study volatile organic compounds (VOCs) in seafood in order to evaluate the degree of seafood spoilage, by providing us with valuable information which can be used for the identification of potential CSIs for rapid quality assessment and estimation of the remaining shelf-life (Duflos et al., 2006; Edirisinghe et al., 2007; Joffraud et al., 2001; Jonsdottir et al., 2008; Jorgensen et al., 2001; Leduc et al., 2012; Noseda et al., 2012; Soncin et al., 2008; Wierda et al., 2006). According to Jay (1986) a metabolite that can be used for spoilage assessment has to (i) be absent or at least present at only low levels in food, (ii) increase during storage, and (iii) be produced by the dominant flora and show good correlation with sensory score. Obviously, it is essential to find out which metabolites fulfil those requirements, hence a preliminary investigation has to focus on the VOC profile throughout storage and record which of them appear at the early stages of storage and have the tendency to increase until the end of shelf-life. Afterwards in a future study, documentation of which VOCs are produced by spoilage microbiota has to be done before setting up trials with various product batches and determine accurately the concentrations of the VOCs of interest throughout storage stages and then correlate them with bacterial counts and sensory changes.

Gilthead sea bream (*Sparus aurata*) is one of the main fish species farmed in Greece and other Mediterranean countries. Greece is the leading producer in the world with approximately 45% of the total production (FAO, 2012). To our knowledge, there is no study regarding microbiological spoilage analysis and investigation of VOCs production of sea bream fillets stored at various temperatures and atmospheric conditions. The aims of this work were to (i) determine the microbiological changes and shelf-life of sea bream fillets stored under air and MAP with a commercial gaseous mixture used by Hellenic Aquaculture Industry, at 0, 5 and 15 °C, and (ii) carry out a preliminary investigation of VOC profile using SPME–GC/MS, in order to reveal any potential CSIs of sea bream fillet spoilage/freshness. This study will give valuable information regarding spoilage of sea bream fillets which is an important added-value product of seafood market.

2. Materials and methods

2.1. Sea bream fillet provision and storage

Packages from two different batches containing two sea bream fillets of approximately 120 g each were taken from the Fish Processing Plant of Dias Aquaculture SA (Magoula, Attica, Greece). Sea bream was farmed in the geographical area designated as FAO 37, 3.1 (Aegean Sea) and captured on March of 2011. Fillets were packaged in polystyrene boxes (Sirap Gema S.p.A., Italy) under air or MAP. The MAP gas concentrations were CO_2: 60%, O_2: 10%, and N_2: 30%, which is one of the commercial gas composition used by the Hellenic seafood industry for sea bream fillets, while the MAP film was the BDF 8050F (Cryovac-Sealed Air Ltd, Athens, Greece). The samples were transferred to the laboratory within 4 h after packaging using insulated boxes with melted ice. The samples were stored in incubators operating at 0, 5 and 15 °C.

2.2. Sensory analysis

Sensory evaluation was carried out by five trained panellists according to ISO 8586-1 (1993). The sensory attributes that were evaluated were appearance of the skin and flesh (translucent, glossy, natural colour, opaque, dull, discoloured) and odour of flesh (marine, fresh, neutral, sour, stale, spoiled, putrid). The rating of each sensory attribute was scored using a 1 to 9 descriptive hedonic scale (9 being the highest quality score and 1 the lowest). A score of 5 was taken as the average score for minimum acceptability (Tsironi and Taoukis, 2011). The aim of the sensory evaluation was the determination of shelf-life of fish fillets.

2.3. Microbiological analysis

All microbiological media were supplied by LAB M (Lancashire, UK), apart from streptomycin sulphate, thallus acetate, cycloheximide (actidione) agar (STAA) which was supplied by Biolife Italiana srl (Milano, Italy). Iron agar (IA) was prepared according to Gram et al. (1987) by mixing the following ingredients: peptone 20 g/L, meat extract 3.0 g/L, yeast extract 3.0 g/L, ferric citrate 3.0 g/L, sodium thiosulphate 0.3 g/L, NaCl 5 g/L, L-cysteine 0.6 g/L, agar 14 g/L, before pH was adjusted to 7.4.

At every sampling point 4 packages (2 from each batch) were opened and one fillet from each package was taken for microbiological analyses. Twenty-five (25) gram sample from each fillet was transferred aseptically to stomacher bags with 225 mL MRD (Maximum Recovery Diluent, 0.1% w/v peptone, 0.85% w/v NaCl) and homogenized for 2 min using a Stomacher (Bug Mixer, Interscience, London, UK). Samples of 0.1 mL of serial dilutions in MRD were spread on the surface of dried media in Petri dishes for enumeration of (a) total microbial population as aerobic plate counts (APC) on TSA (Tryptone Soy Agar), incubated for 48–72 h at 25 °C, (b) *Pseudomonas* spp., on cetrimide–fucidin–cephaloridine agar (CFC), incubated for 48 h at 25 °C and (c) *Brochothrix thermosphacta*, on STAA, incubated for 48–72 h at 25 °C. Samples of 1 mL of serial dilution in MRD were used for the pour plate technique for enumeration of (a) H_2S producing bacteria (presumptive *S. putrefaciens*) on IA by counting only black colonies, after incubation at 25 °C for 72 h., (b) Enterobacteriaceae on Violet Red Bile Glucose agar (VRBGA), incubated at 37 °C for 24 h and (c) Lactic Acid Bacteria (LAB) on Mann, Rogosa, Sharpe agar (MRS) after incubation at 25 °C for 72 h. The results were expressed as mean log cfu g^{-1} ± standard deviation of 4 replicates.

2.4. Determination of TVB-N

At every sampling point, portions of 10 g of flesh were taken from 4 different packages (2 from each batch) and homogenized in trichloroacetic acid (TCA) 6% w/v and filtered using Whatman No. 1 filter paper in a 100 mL volumetric flask. Fifty milliliters in duplicates was taken for TVB-N analysis using the steam-distillation procedure according to Vyncke et al. (1987). The results were expressed as mean TVB-N mg N/100 g ± standard deviation of 4 replicates (2 replicates from each batch of fish).

2.5. VOC determination by headspace SPME–GC/MS analysis

A slight modification of the method described by Iglesias et al. (2009) was used. At every sampling point, a total amount of 50 g of fillet flesh was removed from 4 different packages (2 from each batch) and