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Analysis of volatile compounds of wild gilthead sea bream (*Sparus aurata*) by simultaneous distillation–extraction (SDE) and GC–MS

Serkan Selli *, Gonca Gul Cayhan

Department of Food Engineering, Faculty of Agriculture, Cukurova University, 01330-Adana, Turkey

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The volatile compounds of wild gilthead sea bream (*Sparus aurata*) were analyzed by sensory and instrumental analyses. Simultaneous distillation and extraction (SDE) with dichloromethane were used for extraction of volatile components. According to sensory analysis, the aromatic extract obtained by SDE was representative of sea bream odour. A total of 46 compounds were identified and quantified in sea bream. Aldehydes and alcohols were the most dominant volatiles in sea bream, as they accounted for the largest proportion. Hexanal, heptanal, nonanal, (*Z*)-4-heptenal, octanal, (*E*)-2-nonenal, decanal, benzenacetaldehyde, (*E,E*)-2,4-decadienal, 1-octen-3-ol and (*E*)-1,5-octadien-3-ol were potent aroma compounds on the basis of odour activity values (OAVs). Within these, decanal (OAV: 1110) and (*E*)-2-nonenal (OAV: 447.5) were the most powerful contributors to the aroma of the sea bream.

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1. Introduction

The gilthead sea bream (*Sparus aurata*) is a common fish in the Mediterranean Sea, present along the Eastern Atlantic coasts from Great Britain to Senegal, and rare in the Black Sea [1]. The principal fisheries for sea bream are in Spain, Turkey, Italy and Egypt. The total capture production of sea bream in Turkey was 759 tons in 2007, with Mediterranean Sea the leading captured coast with 423 tons followed by Aegean Sea with 326 tons [2]. Production of sea bream from capture fisheries remains relatively static.

The aroma is one of the important factors to determine the character and quality of fish species. Fish has a characteristic and delicate aroma which is influenced by the species and the conditions used for its post-harvest handling and storage. Important aroma compounds, characteristic of fresh fish species, are lipid derived volatile compounds generated mainly by oxidative enzymatic reactions and autooxidation of lipids. The action of enzymes (lipoxygenases) on polyunsaturated fatty acids, produces carbonyls, aldehydes and ketones which are responsible for some of the fresh like odours of fish [3,4]. Among the fish species, the popularity of wild gilthead sea bream in the world is mainly due to its distinctive aroma, desirable taste, and high nutritional quality [5]. In addition, consumers perceive the wild sea bream to be of better aroma and taste than farmed. Differences in volatile compounds are to be expected between wild and farmed fish due to dietary differences and environmental conditions [3].

Several extraction methods have been tried previously for the analysis of fish volatile components including simultaneous distillation and extraction (SDE) [4], vacuum steam distillation [6], dynamic headspace [7], supercritical fluid extraction [8], solid phase micro extraction [9], and microwave assisted distillation-solvent extraction [10]. The first step in the study of aroma is the selection of an extraction technique for the volatile components which gives an aromatic extract as close as possible to the original product [6,11]. In present study the aroma extraction method selected was simultaneous distillation and extraction using dichloromethane. This method has already shown its reliability for the extraction of volatile components of different fish species, such as mackerel, tuna, swordfish, eel, sablefish, flounder [4], fresh and smoked salmon [12], and brown trout (*Salmo trutta* L.) fillet [13].

No work has yet been published in the literature to determine the volatile composition of gilthead sea bream (*S. aurata*) captured from the Eastern Mediterranean Sea of Turkey. Therefore, the aim of the present study was, first, to assess the representativeness of sea bream aromatic extract obtained by simultaneous distillation and extraction using similarity and intensity test and second to determine the volatile composition of this fish species.

2. Material and methods

2.1. Samples and chemicals

Wild gilthead sea bream (average mass 300 g) were bought from the Karatas province, Eastern Mediterranean Sea of Turkey. Fish were caught and manually slaughtered on the same day by immersing in ice-cold water and transported under ice in insulated polystyrene boxes to a biotechnology laboratory at the Department of Food Engineering, University of Cukurova (Adana province)—Turkey. For

* Corresponding author. Fax: +90 322 338 66 14.
E-mail address: sselli@cu.edu.tr (S. Selli).

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the volatile analysis, samples were manually eviscerated and filleted and mechanically peeled at the same day.

All water was purified by using a Millipore-Q system (Millipore Corp., Saint-Quentin, France). Dichloromethane, sodium chloride, and sodium sulphate were obtained from Fluka (Buchs, Switzerland). All standard compounds were purchased from Aldrich (Steinheim, Germany) except octanal and 1-octanol, which came from Merck (Darmstad, Germany).

2.2. Extraction of volatile compounds

The volatile compounds were extracted by SDE with dichloromethane in a Likens-Nickerson apparatus. 30 g of cubes of gilthead sea bream was placed in a 500 mL round-bottom flask with 60 mL of purified water containing 40 mg of 4-nonanol as internal standard, and the flask attached into the appropriate arm of the SDE apparatus. A 30 mL round-bottom flask containing 25 mL of dichloromethane was linked to the other arm of the SDE apparatus. The steams were cooled thanks to the circulation of polyethylene glycol at −5 °C. The contents in the sample and solvent flasks were heated to a boil. The temperature of dichloromethane flask was maintained by a water bath at 50 °C. The distillation–extraction was continued for 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent thanks to a Vigreux apparatus and to 1 mL under a gentle cold stream of nitrogen. Sample was extracted in triplicate and the concentration of volatiles, as 4-nonanol equivalents, was obtained as a mean of three repetitions. The extracts were then stored at −20 °C in a glass vial equipped with a Teflon-lined cap before the analysis.

2.3. Representativeness of the extract

2.3.1. Sample preparation and presentation

The panel was composed of seven judges from our laboratory (two females and five males between 23 and 42 years old) trained in sensorial characterization of food products. Different methods can be used to evaluate the representativeness of the odour of aromatic extracts depending on the type of investigation. We used a cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lyas, France) for the checking representativeness of the extract obtained simultaneous distillation and extraction with dichloromethane. Smelling strip has already given good results for the representativeness test of cooked mussel [14] and oyster *Crassostrea gigas* extract [15]. 1 g of small cubes of cooked sea bream flesh was placed in a 15 mL brown coded flask as a reference for representativeness tests. An aliquot of the fish aromatic extract was adsorbed onto a cardboard smelling strip. After 1 min (the time necessary for solvent evaporation) the extremities of the strips were cut off, then placed in dark coded flasks (15 mL) and presented to the panel after 15 min. Dichloromethane is a very volatile solvent. After evaporation, no panellists detected the odour of the solvent. All of the samples were assessed at room temperature (20 °C) in neutral conditions.

2.3.2. Similarity test

A similarity test was performed to evaluate the closeness between the odour of extract and the cooked sea bream (reference sample). The panellists were instructed to sniff and memorize the aroma of the reference sample and for extract, to sniff the smelling strip odour and to determine the similarity of their odours. A 100 mm unstructured scale was used anchored with “very different from the reference” on the left and “identical to the reference” on the right. The position of the sample on the unstructured scale was read as the distance in millimeters from the left anchor.

2.3.3. Odour intensity evaluation

The panellists were asked to assess the odour intensity of the extract. A 100 mm unstructured scale was used anchored with “no odour” on the left and “very strong odour” on the right. The position of the sample on the unstructured scale was read as the distance in millimetres from the left anchor.

2.4. GC-FID and GC–MS and analysis of volatile compounds

The gas chromatography (GC) system consisted of an Agilent 6890 chromatograph equipped with a flame ionisation detector (FID) (Wilmington, DE, USA), an Agilent 5973-Network-mass-selective detector (MSD) (Wilmington, DE, USA). GC effluent was split 1:1 among the FID and MSD. Volatile compounds were separated on DB-Wax (30 m length × 0.25 mm i.d. × 0.5 µm thickness, J&W Scientific Folsom, CA, USA) capillary column. Each extract (2 µL) was injected in splitless mode into capillary column. Injector and FID detectors were set at 270 °C and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min^{-1}. The oven temperature of the DB-Wax column was first increased from 50° to 200 °C at a rate of 5 °C min^{-1} and then to 260 °C at 8 °C min^{-1} with a final hold at 260 °C for 5 min.

The same oven temperature programs were used for the mass-selective detector. The MS (electronic impact ionisation) conditions were as follows: ionisation energy of 70 eV, mass range *m*/*z* of 30–300 a.m.u., scan rate of 2.0 scan s^{-1}, interface temperature of 250 °C, and source temperature of 180 °C.

The volatile compounds were identified by comparing their retention index, mass spectra with those of a commercial spectra database (Wiley 6 and NIST 98) and of an internal library of the laboratory. Some of the identifications were confirmed by the injection of the chemical standards into the GC–MS system. Retention indices of the compounds were calculated by using an n-alkane series [16].

3. Results and discussion

3.1. Similarity and intensity evaluation of aromatic extract

The aim of the similarity and intensity evaluation tests was to check the representativeness of the odour of aromatic extract with that of the initial products. The similarity score of the aromatic extract obtained by SDE was found to be 53.5 mm on a 100 mm unstructured scale. The similarity score of the SDE extract was found to be in an acceptable level in comparison to the literature. In our previous study, the similarity score of rainbow trout extract obtained by vacuum steam distillation was 51.1 mm [6]. When we compared it to other studies, similarity score of the edible red algae (*Palmaria palmata*) was found to be between 25.9 and 42.3 mm by Le Pape et al. [7]; and for cooked silurus (*Silurus glanis*) flesh between 22 and 55 mm by Hallier et al. [17]. With regard to intensity evaluation, the intensity score of aromatic extract was found to be 50.5 mm on a 100 mm unstructured scale. The intensity score of the extract was not high, but acceptable. According to similarity and intensity results of the aromatic extract, SDE was found to be a reliable extraction method in order to determine the sea bream volatile compounds.

3.2. Volatile composition of wild gilthead sea bream

The volatile compounds identified in gilthead sea bream (*S. aurata*) and linear retention index values on the DB-Wax column for these compounds are listed in Table 1. Mean values (µg L^{-1}) of the GC analyses of triplicate extractions and standard deviations are reported. A total of 46 compounds were identified and quantified in sea bream. The fish had 5723.8 µg L^{-1} volatile compounds, which included aldehydes (16), alcohols (15), ketones (5), aromatics (4), alkanes (3), terpene (1), ester (1), and pyrazine (1). Aldehydes were the most dominant volatiles in sea bream, as they accounted for the largest proportion (34.8%) of the total volatile compounds. Alcohols were the second largest (32.6%) volatile group in the sea bream. Table 2 presents the potent volatile

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