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Food Chemistry 97 (2006) 465–471

Food Chemistry

www.elsevier.com/locate/foodchem

Odour active aroma compounds of sea fig (*Microcosmus sulcatus*)

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Received 6 December 2004; received in revised form 10 May 2005; accepted 10 May 2005

Abstract

Sea fig is an original shellfish appreciated for its powerful "marine, iodized" flavour. Different methods of analysis by gas chromatography, coupled with olfactometry (GC–O), were studied and compared. Subsequently, odour-active aroma compounds of sea fig extract were analyzed by OSME and CHARM analysis. Twenty-nine olfactive areas were observed by OSME, and 18 by CHARM analysis. Volatile compounds of the extract were analyzed by mass spectrometry and specific detectors associated with the GC. Twenty molecules, responsible for these odours were elucidated. Among them, 12 were directly identified by GC–MS, and the remaining 8 only by GC–O and standard sample injection. Moreover, 10 volatile sulfur-containing compounds were revealed as the major olfactive contributors. The two principal character-impact compounds possessed "marine, fresh" and "fishy, crustaceous" odours. The first could not be identified; however, trimethylamine was attributed to the other, and defined as a key compound of sea fig aroma.

Keywords: *Microcosmus sulcatus*; Aroma; Seafood; Odor active compounds; GC–O; CHARM; OSME

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1. Introduction

As the most important criterion of marine product acceptability is aroma, industrialists are very concerned with the identification of the flavour compounds in seafood. Consequently, they are widely studied (Shahidi, 1998; Spurvey, Pan, & Shahidi, 1998) and can be described as sweet, distinctly plant-like, often accompanied by metallic and fishy attributes. Aldehydes, ketones, nitrogen and sulfur-containing compounds are considered as the most important contributors to the odour of shellfishes. Aldehydes contribute to the plant-like aroma, and ketones to the sweet floral and fruity flavour of raw crustaceans. Alkyl-pyrazines with roasted notes, and sulfur-containing compounds with cabbage-like or meaty notes, contribute to the overall cooked odour of shellfish (Spurvey et al., 1998).

However, few reports have been published concerning raw shellfishes (De Quiros, Lopez-Hernandez, Gonzalez-Castro, De la Cruz-Garcia, & Simal-Lozano, 2001; Pennarum, Prost, & Demaimay, 2002a; Yasuhara, 1987). Moreover, only the character-impact compounds of fresh oyster have been investigated (Kirn et al., 2000; Pennarum, Prost, & Demaimay, 2002b; Pennarum, Prost, Haure, & Demaimay, 2003; Piveteau et al., 2000). These components: hex-3(*E*)-en-l-ol, decanal, undecan-2-one and nona-3,6(*E*,*Z*)-dien-1-ol, have been described as representative of freshness and sensory qualities of shells, with fresh and marine odours. Gas chromatography-olfactometry (GC–O), combining GC separating capabilities and human nose sensibility, is a complementary method for the detection of potent

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0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.05.026

fragrant molecules present in complex mixtures. Methodologies of GC–O can be classified into detection frequency methods: nasal impact frequency (NIF) (Pollien et al., 1997), intensity methods, odour specific magnitude estimation (OSME) (McDaniel, Miranda-Lopez, Watson, Micheals, & Libbey, 1990) and dilution methods, (Acree, 1993). Among dilution methods, combined hedonics of aromatic response measurement (CHARM) (Acree, Barnard, & Cunningham, 1984) and aroma extract dilution Analysis (AEDA) (Ullrich & Grosch, 1987) are generally used.

The aim of the present work was to apply olfactometric methods to sea fig extract to determine its most potent odourants, and to evaluate their importance among volatile and semi-volatile compounds from this shellfish. Sea fig (*Microcosmus sulcatus*) is a marine organism, an ascidia of the tunicates branch. This shellfish, commonly called "violet" in France, is consumed by Mediterranean people and is much appreciated for its powerful "marine" and "iodized" flavour. To our knowledge, sea fig's volatile compounds have never been studied. The purpose is to determine key components responsible for its specific organoleptic characteristics. These molecules were tentatively detected and identified by specific detectors: atomic emission detector (AED), pulsed flame photometric detector (PFPD), thermoionic specific detector (TSD) and mass spectrometry, associated with the GC. Retention indices on polar and apolar columns allowed us to identify a few of them only perceived by GC–O. Each structure was confirmed by injection of commercial or synthesized standard samples.

2. Materials and methods

2.1. Material

Fresh commercial sea figs (1 kg), *M. sulcatus,* were obtained from the Bay of Sete on the Mediterranean coast of France in November 2003. Extraction was performed immediately after purchasing.

2.2. Obtaining extract

Twenty individual sea figs (1 kg) were shelled and the flesh with internal remaining sea water (300 g) was extracted using dichloromethane (HPLC Grade, 400 g) under ultrasonic treatment (47 kHz, 25 °C, Branson 3510, Branson ultrasonics, Danbury, Connecticut) for 1.5 h. The organic layer was dried over anhydrous sodium sulfate and concentrated to 2 ml using a RapidVap evaporator (Labconco, Kansas City, MO). The obtained extract was filtered by HPLC syringe filter (25 mm, 0.45 μm PVDF, Alltech associate, USA). The extract was stored at low temperature (4 °C) prior to analysis.

2.3. Chemicals

Methanethiol, butane-2,3-dione, dimethyldisulfide, pent-2(*Z*)-enol, butyric acid, hex-3(*Z*)-enol, hept-4(*Z*)-enal, furfurylthiol, oct-l-en-3-one, eucalyptol, *p*-cresol, 2-acetyl-2-thiazoline, nona-2,6(*E*,*Z*)-dienal and nona-2,6(*E*,*Z*)-dienol were purchased from Sigma–Aldrich (Saint Quentin, France), trimethylamine from Merck Eurolab (Briare, France), 3-methylbut-2ene-l-thiol, and 2-methyl-3-furanthiol from Oxford Chemicals (Cleveland, United Kingdom). 2-Methylthiazolidine (Fernandez, Dunach, Fellous, Lizzani-Cuvelier, & Loiseau, 2002) and octa-l,5-dien-3-ol (Lin, Welti, Vera, Fay, & Blank, 1999) were synthesized using published procedures and characterized by GC–MS and ^{1}H, ^{13}C NMR.

2.4. GC–O

GC–O was conducted on a 6890 GC (Agilent Technologies, Massy, France), equipped with a Flame lonization Detector (FID) and a sniffing port ("Sniffer 9000", Brechbuhler Scientific Analytical Solutions, Grand-Lancy, Swiss) equipped with a HP 7683 Series Injector, using a HP-1 (polymethylsiloxane, J & W Scientific) column (50 m × 0.32 mm; film thickness, 0.52 μm) or an HP-INNOWAX (polyethylene glycol, J & W Scientific) column (60 m × 0.32 mm; film thickness, 0.5 μm). The oven was started from 40 to 130 °C at 2 °C/min, then from 130 to 250 °C at 4 °C/min and held at 250 °C for 25 min for the apolar column, and from 60 to 220 °C at 2 °C/min, then held at 220 °C for 10 min, for the polar column. Conditions were as follows: temperature conditions for injector and detector: 250 °C; gas vector: He; constant flow: 1.5 ml/min. Retention indices were determined by calculation within a range of alkanes used as standards, starting from C5 to C28.

OSME analyses was performed with 8 trained panellists. For CHARM analyses, 5 dilutions with a factor of 3 were applied. For each dilution analysis, two panellists were required, each one replacing the other every twenty minutes.

2.5. GC–MS analysis

GC–MS analysis was carried out using an Agilent 6890/5973A system (Agilent technologies, Massy, France), equipped with a multifunction automatic sampler (Combi-Pal, CTC Analytics, Zwingen, Swiss), using an HP-1 (polymethylsiloxane, J & W Scientific) column (50 m × 0.20 mm; film thickness, 0.5 μm) and an HP-INNOWAX (polyethylene glycol, J & W Scientific) column (60 m × 0.25 mm; film thickness, 0.5 μm). Helium was used as carrier gas at a constant flow rate of 1.5 ml/mm. GC conditions were the same as above for GC–O, respectively, with apolar and polar columns. Mass spectra analyses (electronic impact) were per-