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Analytical Methods

Characterisation of odour active volatile compounds of New Zealand sea urchin (*Evechinus chloroticus*) roe using gas chromatography–olfactometry–finger span cross modality (GC–O–FSCM) method

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ABSTRACT

Sea urchin roe is a high value product; however, previous attempts to market the roe from the New Zealand sea urchin (*Evechinus chloroticus*) have been unsuccessful due to its inconsistent sensory quality. The current study investigated the odour active volatile profiles of roe from male and female sea urchins, harvested from two locations in New Zealand, using solvent assisted flavour evaporation (SAFE) extracts and a gas chromatography–olfactometry–finger span cross modality (GC–O–FSCM) method. Panellists detected 81 odour active compounds, 18 of which were identified using GC–mass spectrometry (MS). Generalised procrustes analysis (GPA) of the data revealed that there were differences in the volatile profile that were location and gender specific. These differences may have contributed to the inconsistent sensory properties of sea urchin roe. The differences in volatile profiles between sea urchin populations and genders must be appreciated before developing effective strategies to produce consistent sea urchin roe quality.

1. Introduction

Sea urchin roe is a high value food product and roe value is dependent on quality as determined by its colour, appearance, taste, and flavour (McBride, Price, Tom, Lawrence, & Lawrence, 2004). Though New Zealand has dense populations of the sea urchin species *Evechinus chloroticus* (Andrew, 1988), previous attempts to export sea urchin roe has met with limited success due to their variable sensory properties (McShane, Stewart, Anderson, & Gerring, 1994).

Descriptive sensory analysis has been used to differentiate odour and flavour attributes of roe from *E. chloroticus* with respect to gender (Phillips et al., 2009) and harvest location (Phillips et al., 2010). Proton transfer reaction-mass spectrometry (PTR-MS) analysis revealed that the headspace volatile composition of sea urchin roe also varied with harvest location and gender (Phillips et al., 2010). While the PTR-MS data highlighted differences in volatile composition, it was not able to determine the relative importance of the mass ions giving rise to the differences observed. Volatile compounds in the headspace of roe from the sea urchin roe species *Paracentrotus lividus* have been reported to contain alcohols, aldehydes, aromatic hydrocarbons furans, hydrocarbons, ketones, nitrogen-containing compounds, sulphur-containing compounds, and terpenes (De Quirós, López-Hernandez, González-Castro, de la Cruz-García, & Simal-Lozano, 2001). However the contribution of these volatile compounds to the flavour of the sea urchin roe has not been determined. Potential sea urchin roe constituents that could contribute to volatile profiles have been identified (Liyana-Pathirana, Shahidi, & Whittick, 2002); however, there is scarce information on actual volatile compounds and their odour/aroma character.

Gas chromatography–olfactometry (GC–O) is a valuable tool for gaining an understanding of the important volatiles in a product and their relative contribution to odour and flavour attributes (Delahunty, Eyres, & Dufour, 2006). The cross modal odour intensity measurement methods are particularly attractive as the methodology typically uses a greater number of panellists, include replicates, provide measures of intensity, guides odour identification, intensity estimation is instinctive, and less training is required to achieve reproducible intensity ratings (Delahunty et al., 2006; Etiévant, Callement, Langlois, Issanchou, & Coquibus, 1999). By rating the intensity of compounds the data obtained can be correlated with sensory data and may possibly reveal potentially important contributors to the overall odour of the original food sample.

The objective of the current study was to use a GC–O–FSCM method in combination with GC–mass spectrometry (MS) analysis to investigate and identify the odour active volatile compounds

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responsible for the differences in the sensory properties and volatile profile of *E. chloroticus* roe due to gender and harvest location.

2. Materials and methods

2.1. Chemicals

Extraction of volatile compounds from the sea urchin roe was carried out using sodium dihydrogen orthophosphate (99%, Univar Analytical Reagent, Auburn, Australia), nanopure water, butylated hydroxyanisole (BHA) (99%, May & Baker Ltd., Dagenham, England), diethyl ether, anhydrous sodium sulphate, and ammonium sulphate (all three chemicals 99% from AnalR grade BDH, Poole, England). The salts ammonium sulphate (137.5 g), sodium dihydrogen orthophosphate (43.38 g), and Milli Q water (410 ml) were prepared as a saturated salt stock solution. The combination of salts and solvent served to disorder proteins to reduce enzyme activity (Iyer & Ananthanarayan, 2008). Eugenol (Fluka Analytical, Indonesia) was used as an internal standard.

Standards used for confirmation of volatile compound identifications were isovaleric acid, eucalyptol (both compounds 99%, Sigma–Aldrich, Steinheim, Germany), (*E*,*E*)-2,4-decadienal (85%, Fluka, Buchs, Switzerland), phenylacetaldehyde (50%), phenylethyl alcohol (99%) (both from Fluka AG, Buchs, Germany), butyric acid (98%, Merck, Hohenbrunn, Germany), hexanoic acid (99%, Nu-Chek-Prep Inc., Elysian, MN, USA), R + limonene (97%, Sigma Chemical Co., St. Louis, MO, USA), styrene (Ajax Finechem, Auckland, New Zealand), vanillin (99%, BDH, Poole, England), octanoic acid (99.5%), and indole (99%) (both from Acros Organics, Morris Plains, NJ, USA).

2.2. Sea urchin samples

Live sea urchins were harvested from either northern or southern locations in New Zealand. Sea urchins from the northern region were harvested from Matheson's Bay (36°18′S, 174°48′E) of the North Island. The sea urchins were air couriered to the University of Otago, Dunedin, in polystyrene boxes filled with wet newspapers within 24 h of harvest and were held at 4 °C on arrival prior to dissection. Sea urchins from the southern region were harvested from Doubtful Sound (45°15′S, 166°51′E) on the south east coast of the South Island. These samples were transported in seawater to the Portobello Marine Laboratory, Dunedin, where they were held in sea cages and fed the seaweed *Macrocystis pyrifera* until required. Roe was recovered as previously described by Phillips et al. (2009) and stored in sterile Whirl-pak® bags at −80 °C until further processing. Sea urchin gender was determined using the procedure of Walker and Lesser (1998).

2.3. Solvent assisted flavour evaporation (SAFE) of sea urchin roe volatiles

Roe samples from northern males (19.87 g), northern females (20.42 g), southern males (19.92 g), and southern females (16.11 g) were individually homogenised. The individual roe samples were placed in aluminium foil covered Schott bottles (500 ml) that contained salt solution (25 ml), BHA (1 ml of 2 mg/ml in diethyl ether), and diethyl ether (200 ml). The samples were shaken for 2 min and held at 2 °C for 48 h. The resulting suspensions were centrifuged (2800*g* at 3 °C for 25 min), the supernatant recovered, and distilled using the solvent assisted flavour evaporation (SAFE) apparatus at a pressure of 10^{-6} mbar for 90 min. The frozen distilled extract in the receiver flask was defrosted prior to dehydration with anhydrous sodium sulphate (1 g) and filtration through Celite. A volume of internal standard, eugenol (2 mg/ml) was added prior to concentration to achieve a final concentration of 50 ppm in the concentrated extract. The extracts were concentrated until a 50-fold concentration was achieved. Northern female, northern male, and southern female extracts were concentrated to 400 μl, and the southern male extract was concentrated to 300 μl under a gentle stream of oxygen-free nitrogen (120 ml/min). The concentrated extracts were stored at −20 °C until further analysis.

2.4. GC–O analysis and evaluation

GC–O analyses were carried out using the GC–olfactometry–flame-ionisation-detector (O–FID). The GC–O–FID (Hewlett Packard 5896 Series II plus, Wilmington, DE, USA) was installed with a BPX5 column (25 m × 0.32 mm × 0.5 μm) (SGE, Melbourne, Australia Ptg Ltd.) and an olfactometer (DATU Inc., Geneva, NY, USA). All GC–O–FID splitless injections (1 μl) were carried out manually with a 5 μl syringe (SGE, Melbourne, Australia). The oven temperature programme used an initial temperature of 60 °C that was increased to 250 °C at a rate of 6 °C/min, with a final holding time of 20 min. The injector port was at 200 °C and the detector at 300 °C. Retention indices (RI) of odour active compounds were calculated using an alkane series (C8–C20 and C21–C40, 99%, Fluka, Buchs, Switzerland) run under the same conditions used for analyses of sea urchin roe extracts, except that a 10:1 split injection was used.

A modified finger span board for the GC–O–FSCM method was developed by SCL Ltd. (Dunedin, New Zealand) based on the prototype developed by Etiévant et al. (1999). In the modified board a rotating bar was used in place of a sliding peg, with intensity being related to the extent of axis rotation of the bar as the finger span increased, rather than to the extent of finger movement along a rheostat slider used in the original board.

The University of Otago Human Ethics Committee granted ethical approval for GC–O work carried out in this study. Six trained panellists, comprising of one male and five females ranging in age from 25 to 60 years, assessed odour intensities of sea urchin roe extracts in triplicate using the GC–O–FSCM method over 12 sessions. Panellists were instructed to verbally describe odours based on the odour and flavour lexicon compiled by Phillips et al. (2009). In each session, GC–O runs for each sample were divided into two sessions (0–30 min and 31–51.67 min) to prevent panellist fatigue. The data was later combined to provide a complete aromagram using SpecAlign (Cartwright group, University of Oxford) and odour descriptions from the audio files were recovered using Garageband'08 version 4.1.2. (Apple Inc.). Based on retention time, the odour intensity ratings extracted were matched with the odour descriptions.

2.5. Gas chromatography–mass spectrometry (GC–MS) analysis

Volatile compounds of each sample were separated using an Agilent 6890N GC (Agilent Technologies, Palo Alto, CA, USA) equipped with an MS (Agilent Technologies Inc., 5975B VL MSD, Wilmington, DE, USA), an auto-sampler (Agilent Technologies Inc., 7683 series injector, Wilmington, DE, USA) and an HP5 column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies Inc., Wilmington, DE, USA).

The injection port was 250 °C and the temperature program used an initial temperature of 60 °C that was increased at a rate of 6 °C/min to 250 °C with a 20 min hold time. The gas flow was set at 1 ml/min of helium and used a 10:1 split injection. The MS quadrupoles and source were set at 150 °C and 230 °C, respectively, and the data acquisition parameters used were within an ion range of 20–300 amu scan mode, detector voltage of 1564.7 V, and a solvent delay of 2 min.