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# Investigation of Sensory and Volatile Characteristics of Farmed and Wild Barramundi (*Lates calcarifer*) using Gas Chromatography−Olfactometry Mass Spectrometry and Descriptive Sensory Analysis

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Australian aquacultured and wild-caught barramundi (*Lates calcarifer*) were obtained for sensory evaluation and analysis by gas chromatography−olfactometry (GC-O) with simultaneous mass spectrometry. Aquacultured barramundi were sourced from commercial farms representing some typical Australian production methods: above-ground recirculation tank, in-ground lined pond, and in-ground earth pond cultivation. Wild barramundi were sourced from three river-mouth sites in Northern Australia: the Gulf of Carpentaria, the Arafura Sea in the Northern Territory, and the Coral Sea, Northern Queensland. Fish were filleted, minced into a homogeneous sample, and blast frozen for subsequent cooking and sensory and volatile analysis. Barramundi mince portions were grilled using a standardized method for sensory descriptive profiling and gas chromatography−mass spectrometry−olfactometry analysis. Volatiles from grilled fish were collected using dynamic headspace, and the extracts were subjected to direct-intensity olfactometry analysis by trained assessors. More than 30 odor-active compounds were present in the barramundi extracts, mostly with the same odor-active compounds detected in both wild and aquacultured samples. On average, the perceived GC-O odor intensities of most aroma volatiles were higher in aquacultured samples. This was also reflected by instrumental data, where most volatiles were present at higher concentrations in the aquacultured samples. Additional "muddy", "earthy", and "musty" flavor notes perceived in the lined and earth pond aquacultured samples were related to the presence of 2-methyl isoborneol and geosmin in these samples. Multivariate modeling was used to relate the sensory, olfactometry, and instrumental data; overall, there was good agreement between the data sets.

**KEYWORDS: Wild; aquaculture; *Lates calcarifer*; barramundi; olfactometry; descriptive analysis**

## INTRODUCTION

Barramundi (*Lates calcarifer*) is a premium quality eating fish that is in high demand in both Australia and overseas. This large predatory tropical fish is indigenous to the waters of South East Asia and Northern Australia and can tolerate a wide range of salinities. Barramundi can be found in diverse natural habitats such as open sea, brackish, estuarine, and freshwater environments (*1*). Barramundi can be successfully cultivated in a range of aquatic environments: freshwater ponds, recirculating water tank systems, and fresh or seawater cages. As a carnivorous species, barramundi in the wild eat a high-protein diet typically consisting of prawns, smaller fish, and insects. In Australia, aquacultured barramundi are fed standard commercial feeds composed primarily of marine (fish meal and fish oil) and terrestrial ingredients (soy/other vegetable protein/meat meal) and a cereal component of 20−25%. Normally, the feed is in the form of a semifloating pellet to allow observation of feed uptake by the fish (*2*−*4*).

One of the major objectives of aquaculture industries is to create an economically and environmentally sustainable product that retains excellent eating quality as compared to wild counterparts. There have been limited published studies where the sensory characteristics of wild and aquacultured product have been compared (*5*−*11*). In a study on sea- and freshwater-caught wild Atlantic salmon (*5*,*6*), no consistent sensory differences were measured between wild and aquacultured variants of the same. The sensory quality of wild and aquacultured rock lobster (*7*) did not differ significantly. In a more recent investigation (*8*) comparing the flavor of freshwater-farmed (purged and unpurged), marine-farmed, and freshwater wild (estuarine) barramundi, no significant sensory differences were found between wild and aquacultured fish except for high muddy flavor in the unpurged freshwater aquacultured samples. Differences in the fat content and fatty acid composition of wild and farmed Yellow Perch were

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**Table 1.** Description and Source of Wild and Aquacultured Barramundi Used in the Study

| code | description | no. of fish filleted/fillets used | details |
|---|---|---|---|
| LP-A | aquacultured in-ground lined pond | 25 × ~2 kg of fish | filleted and blast frozen in laboratory |
| RT-A | aquacultured above-ground recirculation tank | 40 × ~800−1000 g of fish | filleted and blast frozen in laboratory |
| EP-A | aquacultured in-ground earth pond | 20 × ~2 kg of fish | filleted and blast frozen in laboratory |
| NT-W | wild caught from the Arafura Sea, Northern Territory | 20 × individual ~1 kg fillets | filleted and frozen on-board from fish (15−30 kg) |
| GC-W | wild caught from the Gulf of Carpentaria, Northern Territory | 70 × 400 g portions | filleted and vacuum pack frozen on-board from fish (15−30 kg) |
| NQ-W | wild caught from the Coral Sea, North Queensland | 80 fillets | filleted from 40 fish (4−7 kg); fresh fish air freighted; filleted and blast frozen in laboratory |

reported; however, no flavor differences were found (*9*). Organoleptic and volatile differences were measured in a comparison of wild and farmed Sea Bream, with firmer texture and "more pleasant taste" reported for the wild fish (*10*, *11*). In contrast, there have been many investigations showing the effect of different compositions of aquaculture diets and dietary history on compositional aspects of the final product, including fat content, fatty acid profiles, amino acid profiles, and textural and sensory attributes (*12*−*16*). Most of these dietary studies demonstrated a direct influence of lipid type and source on the volatile and sensory profiles of farmed fish.

Although the Australian barramundi aquaculture industry has undergone rapid growth over the last 20 years, there have been few reported sensory investigations (*2*, *8*) and no studies using a gas chromatography−olfactometry (GC-O) approach. The purpose of this current investigation was to apply direct-intensity GC-O analysis and descriptive sensory analysis to compare barramundi flavor across a representative set of wild and aquacultured fish and also to attempt to identify the main odor-active volatiles responsible for barramundi aroma and for any sensory differences between the samples.

## MATERIALS AND METHODS

**Sourcing of Barramundi for Experiments.** Barramundi samples were obtained in March−April, 2006. A summary of the sources of barramundi samples used in this study is provided in **Table 1**. The aquacultured barramundi grown under the farming conditions described in this study were between 800 g and 2 kg. This is typical for aquacultured barramundi in Australia. In contrast, the wild-caught barramundi spanned a range of masses, between 4 and 30 kg; this range is not unusual for wild-caught barramundi. In this study, we did not attempt to match aquacultured and wild fish for size and weight, as this would not have been representative of what consumers receive.

**Aquacultured Barramundi.** Barramundi were sourced from commercial farms currently using the main methods of production: in-ground lined pond (LP-A), in-ground earth pond (EP-A), and above-ground recirculation tank (RT-A). Samples from sea cages (Bathurst Island, Queensland), another important cultivation method, were sought for this study, but severe adverse weather conditions during harvest meant that samples could not be obtained. Fish were obtained whole and transported on ice to the laboratory on the same day.

**Wild Barramundi.** Wild barramundi were obtained from individual commercial companies who regularly supply to the Australian domestic market; samples were sourced from three main areas for barramundi fishing (**Table 1**). In commercial fishing operations, barramundi are typically cleared from gill nets set in the creek mouth, filleted immediately on board, and then shatter-packed prior to being blast frozen. Fish samples were either received at the laboratory in frozen fillet form, or fresh fish were filleted and frozen in the laboratory.

**Sample Preparation.** Frozen fillets from individual fish were cut into ~3 $cm^3$ cubes and combined with cubed fillets from other fish. The combined fish flesh was then size reduced with a mince attachment (mesh size ~4 mm) on a Kenwood (KM201) mixer at medium speed. Mincing was done to provide homogeneous samples for descriptive sensory analysis, GC-O, and consumer testing (consumer data not reported here). Preparing the samples in this manner minimized textural differences between different-sized fish that might have influenced the sensory response; it has been established that small barramundi (~1 kg) have a softer texture than large wild-caught fish (*17*). Subsamples of minced flesh were packaged into 300 g lots and blast frozen (−40 °C) before storage at −20 °C. Samples remained frozen (3 weeks) until they were thawed for sensory and GC-O analysis. Corresponding sensory and GC-O analyses were carried out on the samples within the same 2 week period.

**Grilling of Fish Samples.** A grilling method was developed to enable freshly defrosted minced barramundi samples to be cooked to an extent that they were safe for consumption while still maintaining the underlying sensory character of the sample itself. Barramundi mince was prepared and packaged as above. After they were thawed overnight at 4 °C, 25 g samples of barramundi mince were made into a circular patty and placed into aluminum cooking cups (six samples simultaneously), covered with a sheet of aluminum foil, and placed onto the surface of a Silex grill (Meerbusch, Germany). The grill surface was heated to 195 °C and monitored using a hand-held Infrared thermometer (Fluke, Everett, WA). The patties were cooked for 200 s to attain an internal temperature of greater than 70 °C. These samples were then directly served in the aluminum cooking cups in the sensory booths for the descriptive sensory assessment with the trained panel. Panelists were provided with water and water crackers as palate cleansers.

**Dynamic Headspace Volatile Extraction.** Further lots of 25 g patties were grilled in an identical manner to those cooked for sensory evaluation. Immediately after grilling, six barramundi patties were transferred into water (22 °C) at a ratio of 2:1 water:fish (300 g of water:150 g of fish) and briefly homogenized with a hand-held food processor. A 60 g mass of the slurry was transferred into headspace sampling flask with a magnetic stir bar. An aliquot of 4-methyl-1-pentanol was added as an internal standard (IS). Preconditioned traps packed with Tenax-TA (60/80 mesh, Supleco) were attached to Dressel tubes fitted to the neck of the flask with a Teflon O-ring. Ultrahigh purity nitrogen gas (BOC Gases, Australia) was introduced through the Dressel tube to achieve a flow of 60 mL/min through the Tenax trap. Trapping was continued for 15 min at 37 °C with stirring. Traps were "dry purged" with nitrogen (60 mL/min) for 30 s and subsequently sealed with Teflon-lined caps until desorption onto the gas chromatograph and sniffing experiments (~less than 2 h). Samples were desorbed at 270 °C with a TD-4 Thermal desorption unit (Scientific Instruments Services, NJ) directly into the hot injector (250 °C) of the gas chromatograph with simultaneous cryofocusing with liquid carbon dioxide.

**Gas Chromatography/Mass Spectrometry−Olfactometry (GC/MS-O).** A Varian 4000 gas chromatograph ion trap mass spectrometer (GC/MS) (Varian Inc., Mulgrave, Australia) was connected to an ODO-II sniffing port (SGE, Ringwood, Australia), such that approximately 25% of the column gas effluent was directed to the mass spectrometer and 75% to the olfactory port. A BP-20-Wax-Forte capillary column was used (0.32 id, 30 m, 0.5 $\mu$m, SGE, Ringwood, Australia); during sample desorption, the injector was held at 250 °C in split mode (1:10) with a pressure pulse of 20 psi. The GC column oven was programmed as follows: 50 °C (hold 5 min) and then 5 °C/min to 240 °C (hold 5 min). Mass spectrometer conditions were applied as follows: 0−15 min, 40−200 $m/z$ (0.43 s/scan, 3 $\mu$scans) and 15−45 min, 50−280 $m/z$ (0.58 s/scan, 3 $\mu$scans). Selected samples were also run in methanol chemical ionization (CI) mode, where the presence (or absence) of a distinct $M + H^+$ ion was used to assist the interpretation of EI mass spectra. A prominent $M + H^+$ ion is

generally formed for organic volatiles except for *n*-alkanes and primary alcohols. Most of the compounds could be tentatively identified based on EI mass spectra, linear retention index (RI) values, odor quality (OQ), and in many cases by the presence of an unambiguous $M + H^+$ molecular mass peak by methanol CI. In addition, the identity of many compounds was confirmed by authentic standard reference compounds (RC).

The variation in peak area for the IS was always within 5%, indicating that the sampling and desorption processes were highly reproducible. All chemical data were normalized according to IS area, and semiquantitative estimates of the concentrations of compounds were calculated in $\mu$g/kg. Replicate peak areas for chemical compounds for each of the barramundi samples were subjected to analysis of variance (ANOVA) using GenStat 10th Edition (VSN International Ltd., Hemel Hempstead, United Kingdom). RCs were obtained to confirm identification of the volatile compounds.

**Chemicals.** Ultrapure water (Milli-Q water, Millipore Australia, North Ryde) was used for all dilutions. GC-flavor reference standards were dissolved in dichloromethane (Hypersolve-), and a 1 $\mu$L aliquot was dissolved onto blank Tenax-TA tubes and desorbed with the TD4 thermal desorber. Retention indices were determined using a standard solution of C7−30 saturated alkanes (Supelco, Bellefonte, PA, 1000 $\mu$g/mL in hexane). All flavor chemicals were purchased from Sigma Aldrich (Australia) unless stated otherwise: 2,3-butanedione (diacetyl, 97%), 2,3-pentanedione (98%), hexanal (98%), *R*-(+)-limonene (97%), 1-penten-3-ol (99%), styrene (98%), 1-octanal (99%), 1-pentanol (95%), 1-octen-3-one (98%), (*E*)-2-penten-1ol (95%), dimethyl trisulfide (Fluka, 98.5%), 1-nonanal (95%), (*E*)-2-octenal (94%), 1-octen-3-ol (99%), 3-(methylthio)-propionaldehyde (methional, Fluka, 99%), 2-ethyl-1-hexanol (Fluka, 95%), (*E*,*E*)-2,4-heptadienal (90%), benzaldehyde (95%), 1-octanol (95%), (*E*,*E*)-2,6-nonadienal (93%), 2-methyl isoborneol (MIB)/(±)-geosmin (GSM) (100 $\mu$g/mL in methanol), and 4-methyl-2-pentanol (Fluka, 95%).

**GC-O Assessor Selection and Training.** Six trained assessors, each with a minimum of 20 h of previous GC-O sniffing experience (on nonseafood products), were selected to take part in the experiments. Assessors had been prescreened with "in-house" and commercial olfactory tests (Sniffin' Sticks, Berghardt, Wedel, Germany) to demonstrate "normal" olfactory acuity (*18*). All GC-O assessors had also previously taken part in the barramundi sensory descriptive analysis training and had actively contributed to the development of the descriptive sensory vocabulary.

**GC-O Direct Intensity (DI) Measurement and Data Processing.** DI measurement was recorded using the time−intensity application in Compusense Five (Compusense Inc., Guelph, Ontario, Canada). The DI GC-O procedure has been described in detail elsewhere (*19*, *20*). GC-O assessors were encouraged to describe the OQ, by clearly describing the percieved odor and talking into a microphone. MPEG-1 Audio Layer 3 sound files were recorded for each sniffing run, and odor descriptors were annotated next to DI output (*20*). For practical reasons, two Tenax traps per batch of cooked barramundi sample (2 × 60 g of slurry) were produced at once, enough for two sniffing experiments by two GC-O assessors on the same day. The order of GC-O experiments was according to a random design, such that no more than two sniffs of the same fish sample occurred on the same day. Six assessors performed one DI GC-O sniff for each of the six barramundi samples (total of $n = 36$ GC-O samples). For statistical purposes, each of the six traps was considered as a unique experimental replicate from a homogeneous bulk minced fish sample. Statistical analyses were performed on the replicate samples for each barramundi source; mean intensity ratings were used for multivariate data modeling.

**Sensory Analysis.** Fish samples for sensory assessment were cooked as described above and immediately presented to the panel of assessors (~70 °C). The 10-member sensory panel developed a consensus vocabulary to describe the important sensory properties of the samples over 10 days of panel training. The descriptive vocabulary intentionally excluded texture attributes; size reduction was used to minimize the influence of textural differences on aroma perception. Throughout training, the panel were presented all of the barramundi samples a total of 10 times. A trial profile on a subset of barramundi samples was used to refine the developing terms, and thereafter, the vocabulary was defined by consensus, and where necessary, reference standards were introduced to clarify confusing terms. The final descriptive vocabulary separated the perceptual differences into odor (O), flavor (F), and aftertaste (AT) modalities across five attributes. The five sensory attributes were "impact" (the initial overall intensity), "fishy" (intensity of fishy odor—reference tuna in salt water), "prawn" (intensity of the sweetness of cooked prawn), "muddy" (intensity of the muddiness, dirtiness, earthiness—reference mud from a pond), and "seawater" (saltiness, greenness, and seaweed from a seawater spray—reference seawater with sea weed). Quantitative ratings were collected in triplicate (subsets of samples) for each of the barramundi sources, using the sensory vocabulary. The experimental design used was produced using the design generation package CycDesigN Version 2 (*21*). All samples were blind-coded with random three-digit codes, and the order of the sample presentation was balanced across the sessions to account for first-order and carryover effects. Attributes were rated on 100 mm unstructured line scales anchored at 5 (low) and 95 (high), respectively, with extremes for each descriptive term. Data were recorded and stored using Compusense. All sensory assessments were conducted in purpose-built sensory booths, which conforms to international standards or sensory analysis (ISO 1988: 8587; General guidance for the design of a test room).

**Sensory Data Analysis.** Descriptive analysis data were analyzed using the general liner model (GLM, SPSS v 12.0, SPSS Inc., Chicago, IL) with product ($n = 6$) and assessor ($n = 10$) as main treatment factors. Estimated means for significant ($p < 0.05$) main effects were produced along with standard errors of difference (SED). Double the SED (SED × 2) was taken as equivalent to a least significant difference (LSD) posthoc measurement and was used as a cautious indication of the minimum value necessary for differences between means.

**Multivariate Data Processing.** Sensory, GC-O, and volatile data were analyzed by principal components analysis (PCA) and partial least-squares (PLS) analysis using the Unscrambler Software (Version 9.1, CAMO Australia, St. Peters). For PLS modeling, the barramundi sample names were coded as dummy variables using the "passive" option function within Unscrambler. Either the mean GC-O odor peak maxima values or the mean integrated volatile peak area data were considered as *X* variables, and the mean sensory attribute data were considered *Y* variables in PLS models. For both PCA and PLS models, both *X* and *Y* data were normalized using the 1/SDEV transform to remove assessor scale effects and, in the case of the chemical data, to treat all peaks as having equal potential influence. The validity of PLS models was assessed based on the criteria outlined by others (*22*, *23*); $R^2$ for calibration curves (Cal. $R^2$) > 0.66 and $R^2$ for internal cross-validation curves (Val. $R^2$) > 0.33. For the purposes of understanding the relationship between *X* variables and all sensory attributes (multiple *Y* data), PLS-2 was employed. Subsequently, further refined modeling with PLS-1 was explored using *X* data and one *Y* variable at a time.

## RESULTS

**GC/MS Data.** More than 30 volatile compounds were detected by GC/MS in the Tenax headspace concentrates over the six barramundi samples (**Table 2**). In most cases, the identity of compounds was confirmed through the use of reference standards. The remaining volatiles were tentatively identified through a combination of criteria previously described and are denoted by an asterisk in **Table 2**. All of the volatile compounds listed have been previously reported in other freshwater and marine creatures, except for 1,4,9-decatriene, tentatively identified in the aquacultured samples. Quantitatively, the volatile profiles were dominated by hexanal (~6−24 mg/kg) followed by 1-octen-3-ol (~34−108 $\mu$g/kg), 2,3-pentanedione (20−132 $\mu$g/kg), 1-penten-3-ol (12−96 $\mu$g/kg), heptanal (~15−27 $\mu$g/kg), and styrene (~2.7−30 $\mu$g/kg). There were no significant differences in the concentration of 2,3-pentanedione, hexanal, heptanal, and 1-octen-3-ol between wild and aquacultured samples. 1-Penten-3-ol and styrene were present at higher concentrations in the aquacultured barramundi samples. Furthermore, lower concentration volatile compounds were measured; they are listed together with their likely identity and estimated concentration in **Table 2**. Significant differences ($p < 0.05$) were found in the concentration of more than 20 volatiles between barramundi

**Table 2.** Estimated Mean Concentrations and Odor Attributes of the Major Volatile Compounds in Wild and Aquacultured Barramundi Samples[a]

| | | | | | | estimated concentration (μg/kg) | | | | | | statistical significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RI | most likely compd | method of identification | odor description | literature ref[b] | threshold (μg/kg) | EP-A | LP-A | RT-A | GC-W | NQ-W | NT-W | DI[c] | all[d] | WvA[e] |
| <900 | (*E*,E)-2,4-octadiene[f] | MS, CI, RI | | 4, 8 | 15[g] | ND | 33.3 | 4.3 | 9 | ND | ND | | *** | *** |
| 982 | 2,3-butanedione | MS, CI, RI, RC, OQ | sweet, caramel | 1,2 | 6.5[h] | 0.64 | 1.3 | 0.46 | 53 | 5.2 | 25.2 | ** | NS | * |
| 1061 | 2,3-pentanedione | MS, CI, RI, RC, OQ | sweet, caramel, malty | 1, 2, 4−6 | 30 | 45.7 | 68.3 | 132.0 | 20.5 | 22.4 | 16.6 | * | *** | NS |
| 1083 | hexanal | MS, CI, RI, RC, OQ | fresh, green, grass | 1−6, 8 | 4.5−5[h] | 7829 | 15898 | 8900 | 24457 | 17312 | 6884 | NS | NS | NS |
| 1103 | (*E*,*E*)-1,3,5-octatriene[f] | MS, CI, RI | | 4, 7 | NA | 3.23 | 14.90 | 5.18 | ND | ND | ND | *** | *** | NS |
| 1194 | limonene | MS, CI, RI, RC | | 4, 5 | 10[h] | 16.7 | ND | 0.9 | 4.5 | 1.8 | 6.3 | NS | NS | NS |
| 1192 | heptanal | MS, CI, RI, RC | green, floral | 1, 3, 4, 8 | 3[h] | 15.3 | 26.8 | 17.27 | 27.4 | 25 | 23.8 | NS | NS | NS |
| 1208 | 1-penten-3-ol | MS, RI, RC, OQ | plastic, green | 4, 5 | NA | 63 | 96 | 95 | 12.78 | 32 | 12.78 | NS | *** | *** |
| 1262 | 1,4,9-decatriene[f] | MS | | | NA | 1.7 | 34.6 | 2.6 | ND | ND | ND | NS | *** | *** |
| 1268 | styrene | MS, CI, RI, RC | | 4, 5 | 730[h] | 30.9 | 38.0 | 29.6 | 10.7 | 11.1 | 2.7 | NS | *** | *** |
| 1280 | (*Z*)-4-heptenal[f] | MS, CI, RI, OQ | fish oil, stale dried fish, raw fish | 1−4, 6−9 | 0.8[h] | 0.52 | 1.70 | 1.71 | ND | 0.18 | ND | *** | ** | *** |
| 1305 | *n*-octanal | MS, CI, RI, RC, OQ | sweet, orange, floral | 1, 3−6 | 0.7[h] | 3.63 | 10.20 | 8.11 | 8.41 | 10.95 | 6.97 | NS | NS | NS |
| 1313 | 1-pentanol/ 1-octen-3-one [i] | MS, CI, RI/ RI, RC, OQ | mushroom, earthy | 1, 3−5, 8 | 4000/0.005[h] | 3.97 | 11.96 | 9.26 | ND | ND | 1.84 | NS | *** | *** |
| 1353 | 2,3-octanedione[f] | MS, CI, RI | savory, cooked | 8 | NA | 15.1 | 43.3 | 27.9 | 36.5 | 43.1 | 26.4 | NS | NS | NS |
| 1384 | (*E*)-2-penten-1-ol | MS, RI, RC, OQ | raw fish, metallic, green, marine | 2−5, 7, 8 | NA | 7.08 | 21.17 | 13.86 | ND | 3.84 | 1.59 | *** | *** | *** |
| 1408 | dimethyl trisulfide | MS, CI, RI, RC, OQ | garlic, savory, rotten, metallic | 4−6 | 0.005−0.01[h] | 12.1 | 19.7 | 13.7 | 18.6 | 16.7 | 13.8 | NS | NS | NS |
| 1422 | nonanal | MS, CI, RI, RC, OQ | geranium, raw fish, plastic, marine | 1, 3−5, 8, 9 | 1[h] | 7.3 | 19.5 | 10.9 | 8.8 | 14.0 | 9.8 | NS | * | NS |
| 1468 | (*E*)-2-octenal | MS, CI, RI, RC | savory, fatty | 1−4, 6, 8 | 10.7[j] | 1.20 | 3.13 | 0.15 | 3.04 | 2.50 | 0.78 | NS | *** | NS |
| 1501 | 1-octen-3-ol | MS, RI, RC | mushroom, fermented | 3−5, 8, 9 | 1.2[g] | 34.1 | 82.6 | 56.9 | 108.7 | 91.9 | 57.4 | NS | *** | NS |
| 1508 | 1-heptanol | MS, RI, RC | savory, fermented | 2, 4, 5 | 3[h] | 1.45 | 5.86 | 4.57 | 8.76 | 6.37 | 3.8 | NS | NS | NS |
| 1510 | methional | RI, RC, OQ | cooked potato | 1, 2, 4, 6, 8 | 0.2[h] | ND | ND | ND | ND | ND | ND | NS | NS | NS |
| 1513 | (*E*,*Z*)-2,4-heptadienal | MS, CI, RI | savory, fatty | 3, 8 | 19[j] | 25.1 | 37.6 | 15.4 | 11.9 | 7.0 | 0.1 | NS | *** | NS |
| 1545 | 2-ethyl-1-hexanol | MS, RI, RC | | 4−6, 9 | 270000[h] | ND | ND | ND | 11.66 | 8.67 | 6.11 | NS | *** | ** |
| 1547 | (*E*,*E*)-2,4-heptadienal | MS, CI, RI, RC, OQ | oxidized fat, cooking | 3, 5, 6, 8, 9 | 19[j] | 10.6 | 47.5 | 13.7 | ND | 3.5 | ND | * | *** | *** |
| 1566 | (*E*,*Z*)-3,5-octadien-2-one[f] | MS, CI, RI | green, floral, cucumber | 3, 5, 8 | 0.04[k] | 3.56 | 7.19 | 8.01 | 1.65 | 1.58 | ND | NS | *** | *** |
| 1570 | benzaldehyde | MS, CI, RI, RC | | 4, 5 | 350−3500[h] | 6.03 | 12.2 | 9.33 | 9.22 | 7.8 | 4.7 | NS | * | ** |
| 1602 | (*E*,*E*)-2,4-octadienal[f] | MS, CI, RI | sweet, rosewater, cucumber | 2, 6, 8, 9 | NA | 1.3 | 6.9 | 0.62 | ND | 0.30 | 0.00 | * | *** | *** |
| 1603 | 1-octanol | MS, RI | | 4 | 110−130[h] | 1.07 | 2.57 | 2.07 | 3.99 | 3.37 | 2.59 | NS | NS | NS |
| 1614 | (*E*,*E*)-3,5-octadien-2-one[f] | MS, CI, RI | sweet, rosewater, nail polish | 3, 5 | NA | 1.98 | 4.86 | 6.18 | ND | 0.68 | 0.10 | NS | *** | *** |
| 1623 | (*E*,*Z*)-2,6-nonadienal | MS, CI, RI, RC, OQ | cucumber, floral | 1, 2, 8 | 0.2[j] | 0.36 | 1.23 | 0.12 | ND | 0.10 | 0.00 | NS | ** | * |
| 1641 | 2-MIB | MS, RI, RC, OQ | muddy, musty | 4, 9 | 0.1[k] | 1.37 | 0.21 | ND | ND | ND | ND | *** | *** | ** |
| 1657 | (*E*)-2-octen-1-ol[f] | MS, RI | | 2, 3 | NA | 2.05 | 3.22 | 1.46 | 5.07 | 2.14 | 1.35 | NS | NS | NS |
| 1720 | (*E*,*Z*)-3,6-nonadien-1-ol[f] | MS, RI | melted plastic, oily, | 7 | NA | 2.07 | 10.19 | 8.30 | 0.66 | 1.29 | 0.18 | *** | *** | *** |
| 1783 | (*E*,*Z*)-2,4-decadienal[f] | MS, CI, RI | roasted, plastic, popcorn | 2, 3, 8, 9 | 0.2[j] | 2.92 | 7.93 | 3.29 | 1.52 | 2.16 | 0.39 | NS | *** | * |
| 1819 | (*E*,*E*)-2,4-decadienal[f] | MS, CI, RI | plastic, fatty, oily | 1, 2, 3, 8 | 0.2[j] | 0.98 | 2.32 | 0.84 | 0.91 | 0.42 | ND | ** | * | NS |
| 1820 | GSM | MS, RI, RC, OQ | musty, muddy, earthy | 4, 9 | 0.25[k] | 0.24 | 0.45 | ND | ND | ND | ND | *** | *** | NS |

[a] MS, electron impact mass spectrum match with NIST library reference; CI, correct $M + H^+$ ion by methanol chemical ionisation; RI, retention index agreement with literature values for the same on a WAX-Forte column; RC, confirmed by reference compound; OQ, odor quality matches literature; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; ND, not detected; NS, not significant; and NA, no reliable data available. [b] Referred to in the following published studies: 1, ref *36*; 2, ref *40*; 3, ref *39*; 4, ref *28*; 5, ref *10*; 6, ref *34*; 7, ref *38*; 8, ref *33*; and 9, ref *31*. [c] Significant difference in replicate DI scores between wild and aquacultured samples by ANOVA. [d] Significant differences between samples by integrated chemical peak areas by ANOVA. [e] Significant differences between wild and aquacultured samples by integrated chemical peak areas by ANOVA. [f] The OQ was not confirmed by a RC; the indentity remains tentative. [g] Values were obtained from ref *30*. [h] Values were obtained from ref *29*. [i] Coeluting compounds. [j] Values were obtained from ref *32*. [k] Values were obtained from ref *44*.

**Table 3.** Estimated Mean Scores for Significant ($p \leq 0.05$) Barramundi O, F, and AT Attributes[a]

| | O attributes | | | | | F attributes | | | | | AT attributes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample | impact | fishy | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water |
| NT-W | 70.8 | 55.9 | 36.2 | 17.4 | 33.5 | 53.8 | 49.3 | 38.2 | 6.6 | 37.5 | 43.7 | 34.4 | 31.5 | 5.2 | 27.8 |
| NQ-W | 69.1 | 56.4 | 43.6 | 13.8 | 42.7 | 61.4 | 56.0 | 59.8 | 4.5 | 50.3 | 53.7 | 43.3 | 49.5 | 2.2 | 40.2 |
| GC-W | 63.6 | 59.1 | 37.8 | 11.8 | 52.9 | 57.9 | 48.8 | 45.0 | 5.7 | 57.5 | 44.2 | 37.1 | 40.8 | 2.7 | 42.6 |
| LP-A | 67.1 | 67.6 | 25.0 | 33.5 | 46.2 | 72.9 | 67.8 | 33.7 | 33.9 | 55.6 | 66.0 | 56.5 | 28.2 | 33.0 | 45.2 |
| EP-A | 69.6 | 65.0 | 22.9 | 35.1 | 41.1 | 74.6 | 67.5 | 35.1 | 38.6 | 51.0 | 66.6 | 59.6 | 22.9 | 32.6 | 40.5 |
| RT-A | 67.6 | 77.5 | 17.6 | 8.2 | 45.9 | 63.2 | 64.6 | 15.1 | 6.7 | 49.2 | 55.0 | 54.0 | 16.6 | 4.0 | 36.91 |
| Sig | 0.33 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.01 | 0.001 |
| LSD | 2.35 | 2.46 | 4.67 | 4.35 | 3.34 | 2.30 | 2.62 | 4.16 | 4.07 | 3.18 | 2.24 | 2.88 | 3.99 | 3.50 | 3.30 |

[a] Attributes were assessed on a 100 mm line scale.

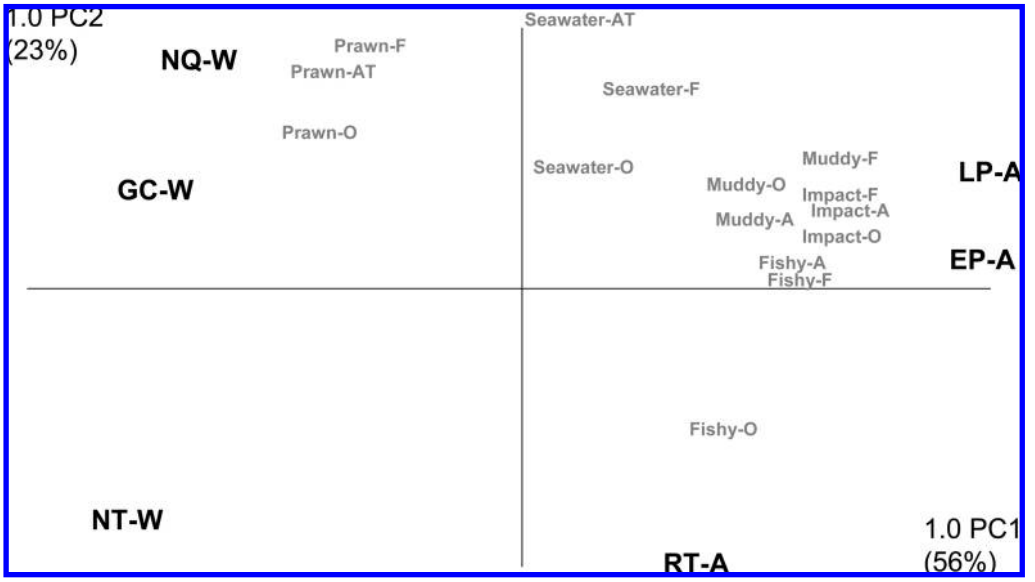


**Figure 1.** Principal components biplot of PC1 (56%) and PC2 (23%) showing the relationship between the barramundi samples and the sensory O, F, and AT attributes: prawn, muddy, fishy, seawater, and impact. The aquacultured samples were clearly separated from the wild samples on PC1.

samples, and at least 18 volatiles differed in comparisons of wild and aquacultured samples.

**Sensory Profiling.** Estimated means for O, F, and AT sensory attributes are shown in **Table 3**. Significant differences were measured for all attributes except odor impact. All of the farmed samples were perceived to have significantly higher fishy O, F, and AT. Within the farmed samples, the two pond-cultivated samples (LP-A and EP-A) were found to be high in muddy/earthy odor when compared to the other farmed sample (RT-A) and the three wild samples. Similarly, the RT-A farmed sample was significantly lower in prawn odor than all of the other farmed and wild samples. Significantly higher prawn O, F, and AT were reported in the wild samples as compared to farmed samples. The seawater attribute (O, F, and AT) was lowest in the NT-W and highest in the GC-W sample. No clear differences between the seawater attribute in wild and aquacultured barramundi were evident. The NT-W sample had a different odor profile to the other wild samples and was primarily associated with a lower impact, fishy, prawn, and seawater attributes. Overall, the NT-W sample was rated as lowest in perceived odor impact and seawater odor. The aquacultured samples were associated with higher fishy odor and odor impact. The relationship of the barramundi samples to these significant sensory attributes is summarized graphically in the biplot of principal component 1 (PC1) and principal component 2 (PC2) (**Figure 1**). The model described 56 and 23% on PC1 and PC2, respectively. Clear separation of the wild (left-hand side) from the aquacultured (right-hand side) barramundi samples on PC1 was apparent.

**Multivariate Modeling of Chemical Data and Sensory Attributes.** The relationship between the volatile concentration data and the significant sensory attributes was initially modeled by PLS-2. **Figure 2** shows the correlation loadings plot PC1 vs PC2, for mean chemical concentrations, and O, F, and AT sensory attributes. The PLS-2 model explained 53 and 28% of the data variance with the first two PCs. In **Figure 2**, the outer ellipse describes 100% explained variance, and the inner ellipse indicates 50% of explained variance. Most variables were contained within these limits. The aquacultured samples were separated from wild counterparts on PC-1, mainly on the basis of greater impact and fishy sensory attributes in the aquacultured samples. The weighted regression coefficients from PLS-1 calibrations for prediction of individual sensory attributes from volatile concentration data are also shown in **Table 4**. Impact, fishy, and seawater attributes (O, F, and AT) were positively correlated with the majority of volatile peaks. In the case of impact and fishy attributes, acceptable PLS-2 models (**Figure 2**) were obtained. The PLS models could be improved in some cases by using a subset of highly correlated volatiles (highlighted in bold in **Table 4**) and performing PLS-1 models on one sensory attribute at a time. The improved PLS-1 model correlation and cross-validation prediction $R^2$ values are shown at the bottom of **Table 4**—denoted as Opt. Cal $R^2$ and Opt Val. $R^2$. In contrast to the other sensory attributes, a negative correlation existed

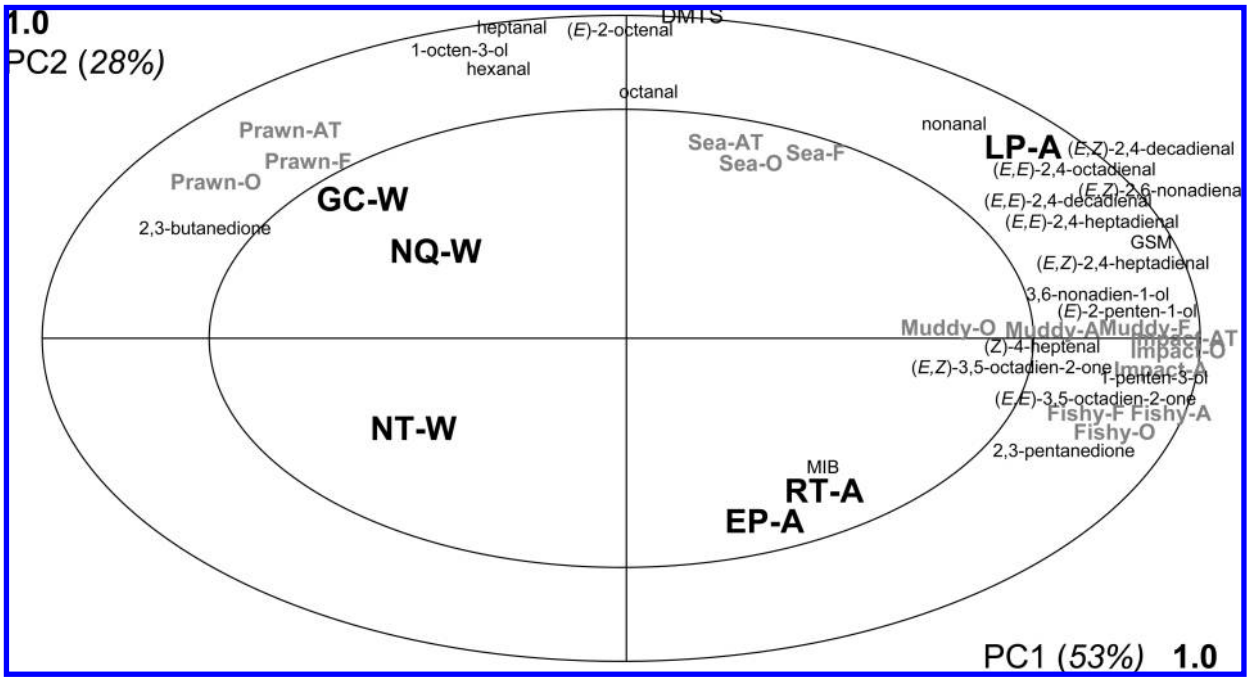


**Figure 2.** PLS-2 correlation loadings plot (PC1 vs PC2) for the mean GC/MS integrated area data for volatiles that had odor activity identified by GC-O and their relationship with significant O, F, and AT sensory attributes: prawn, muddy, fishy, seawater, and impact. The three wild samples (left-hand side) were separated from the aquacultured samples (right-hand side) on the plot. The wild samples were more strongly associated with prawn odor and less with the other odor attributes (shown in gray). The aquacultured samples were more strongly associated with fishy and impact attributes. Similarly, the relationship between barramundi samples and odor-active volatiles can also be seen.

**Table 4.** List of Weighted Regression Coefficients from PLS-2 Calibrations for Significant Sensory Attributes and Estimated Volatile Concentration Data[a]

| | O attributes | | | | F attributes | | | | | AT attributes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| volatile compound | fishy | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water |
| 2,3-butanedione | −0.04 | 0.04 | −0.04 | 0.04 | −0.05 | −0.06 | **0.03** | −0.04 | 0.01 | −0.06 | −0.06 | 0.04 | −0.04 | −0.03 |
| 2,3-pentanedione | **0.08** | −0.10 | −0.01 | 0.03 | 0.03 | 0.05 | −0.08 | −0.09 | 0.01 | 0.03 | 0.05 | −0.08 | −0.09 | −0.01 |
| hexanal | −0.03 | 0.07 | −0.02 | **0.10** | −0.01 | −0.03 | 0.05 | −0.03 | **0.07** | −0.02 | −0.03 | 0.07 | −0.04 | 0.08 |
| (*E*,*E*)-1,3,5-octatriene | 0.05 | −0.04 | 0.06 | 0.03 | 0.05 | 0.06 | −0.04 | 0.06 | 0.04 | 0.05 | 0.05 | −0.04 | 0.07 | 0.02 |
| heptanal | −0.04 | 0.11 | −0.02 | 0.04 | −0.03 | −0.04 | **0.06** | −0.06 | 0.02 | −0.03 | −0.05 | 0.07 | −0.04 | 0.04 |
| 1-penten-3-ol | **0.07** | −0.08 | 0.04 | 0.02 | **0.06** | 0.07 | −0.07 | 0.02 | 0.03 | **0.06** | 0.07 | −0.07 | 0.01 | 0.02 |
| (*Z*)-4-heptenal | **0.07** | −0.08 | 0.02 | 0.03 | 0.04 | 0.06 | −0.07 | −0.03 | 0.03 | 0.05 | 0.06 | −0.07 | −0.03 | 0.01 |
| octanal | −0.01 | 0.09 | −0.04 | 0.04 | −0.02 | −0.01 | **0.03** | −0.16 | 0.03 | −0.01 | −0.02 | 0.05 | −0.14 | 0.07 |
| (*E*)-2-penten-1-ol | **0.06** | −0.05 | 0.04 | 0.02 | **0.05** | 0.06 | −0.05 | 0.02 | 0.03 | 0.06 | 0.06 | −0.06 | 0.02 | 0.02 |
| dimethyl trisulfide | −0.01 | 0.07 | 0.00 | **0.08** | 0.00 | −0.01 | **0.04** | −0.03 | **0.06** | 0.00 | −0.01 | 0.05 | −0.03 | 0.07 |
| nonanal | 0.01 | 0.05 | 0.02 | 0.02 | 0.02 | 0.03 | 0.01 | −0.03 | 0.03 | 0.03 | 0.02 | 0.01 | −0.02 | 0.06 |
| (*E*)-2-octenal | −0.03 | 0.09 | 0.02 | **0.07** | 0.01 | −0.01 | **0.06** | 0.07 | **0.07** | 0.01 | −0.01 | 0.07 | 0.06 | 0.09 |
| 1-octen-3-ol | −0.03 | 0.09 | −0.04 | **0.08** | −0.03 | −0.04 | 0.05 | −0.10 | **0.05** | −0.03 | −0.04 | 0.07 | −0.10 | 0.07 |
| (*E*,*Z*)-2,4-heptadienal | 0.04 | −0.05 | 0.07 | 0.05 | **0.07** | 0.06 | −0.03 | 0.15 | **0.06** | **0.07** | 0.06 | −0.04 | 0.14 | 0.04 |
| (*E*,*E*)-2,4-heptadienal | 0.04 | −0.02 | 0.06 | 0.03 | **0.05** | 0.05 | −0.03 | 0.07 | 0.04 | **0.05** | 0.05 | −0.04 | 0.08 | 0.03 |
| (*E*,*Z*)-3,5-octadien-2-one | **0.08** | −0.09 | 0.02 | 0.05 | **0.05** | 0.06 | −0.07 | −0.03 | 0.04 | **0.05** | 0.06 | −0.07 | −0.03 | 0.01 |
| (*E*,*E*)-2,4-octadienal | 0.03 | −0.01 | 0.07 | 0.02 | **0.05** | 0.05 | −0.02 | 0.10 | 0.04 | **0.05** | 0.04 | −0.02 | 0.11 | 0.03 |
| (*E*,*E*)-3,5-octadien-2-one | **0.08** | −0.09 | 0.01 | 0.03 | 0.04 | 0.06 | −0.08 | −0.05 | 0.02 | 0.04 | 0.06 | −0.07 | −0.05 | 0.00 |
| (*E*,*Z*)-2,6-nonadienal | 0.03 | −0.01 | 0.07 | 0.02 | **0.06** | 0.05 | −0.02 | 0.12 | 0.04 | **0.06** | 0.05 | −0.03 | 0.12 | 0.03 |
| 2-MIB | 0.01 | −0.06 | **0.08** | −0.02 | 0.06 | 0.04 | −0.01 | **0.24** | 0.01 | 0.05 | 0.05 | −0.04 | **0.22** | 0.00 |
| (*E*,*Z*)-3,6-nonadien-1-ol | **0.07** | −0.06 | 0.02 | 0.04 | 0.04 | 0.06 | −0.06 | −0.03 | 0.03 | 0.05 | 0.05 | −0.06 | −0.03 | 0.01 |
| (*E*,*Z*)-2,4-decadienal | 0.04 | −0.02 | 0.06 | **0.04** | 0.06 | 0.06 | −0.03 | 0.07 | **0.05** | **0.06** | 0.05 | −0.03 | 0.07 | 0.05 |
| (*E*,*E*)-2,4-decadienal | 0.04 | −0.03 | 0.06 | **0.07** | 0.06 | 0.05 | −0.03 | 0.10 | **0.07** | **0.05** | 0.05 | −0.03 | 0.10 | 0.05 |
| GSM | 0.02 | −0.02 | **0.09** | 0.01 | 0.06 | 0.05 | −0.02 | **0.18** | 0.04 | 0.06 | 0.05 | −0.03 | **0.18** | 0.03 |
| PLS-1 Cal. $R^2$ | **0.86** | **0.96** | **0.75** | **0.67** | **0.83** | **0.91** | **0.79** | **0.99** | **0.68** | **0.85** | **0.88** | **0.86** | **0.99** | **0.61** |
| PLS-1 Val. $R^2$ | **0.48** | **0.74** | −0.47 | −0.27 | **0.44** | **0.77** | 0.31 | 0.27 | 0.07 | **0.49** | **0.66** | **0.57** | 0.23 | −0.41 |
| PLS-1 Opt Cal. $R^2$ | **0.98** | NI | **0.96** | **0.73** | **0.76** | NI | **0.81** | **0.98** | **0.83** | **0.81** | NI | NI | **0.99** | NI |
| PLS-1 Opt Val. $R^2$ | **0.92** | NI | **0.92** | 0.27 | **0.57** | NI | **0.48** | **0.92** | **0.41** | **0.65** | NI | NI | **0.97** | NI |

[a] Bold signifies coefficients used in refined PLS-1 models. NI, no improvement using PLS-1. Cal. $R^2$, $R^2$ value for calibration model; Val. $R^2$, $R^2$ value for the cross validation model; Opt cal. $R^2$, $R^2$ for PLS-1 optimized model; and Opt. val. $R^2$, $R^2$ for PLS-1 optimized model.

between the prawn odor and the majority of volatile compounds. Overall, the prawn attributes (O, F, and AT) were negatively correlated with all volatiles except for 2,3-butanedione, hexanal, heptanal, dimethyl trisulfide, nonanal, (*E*)-2-octenal, and

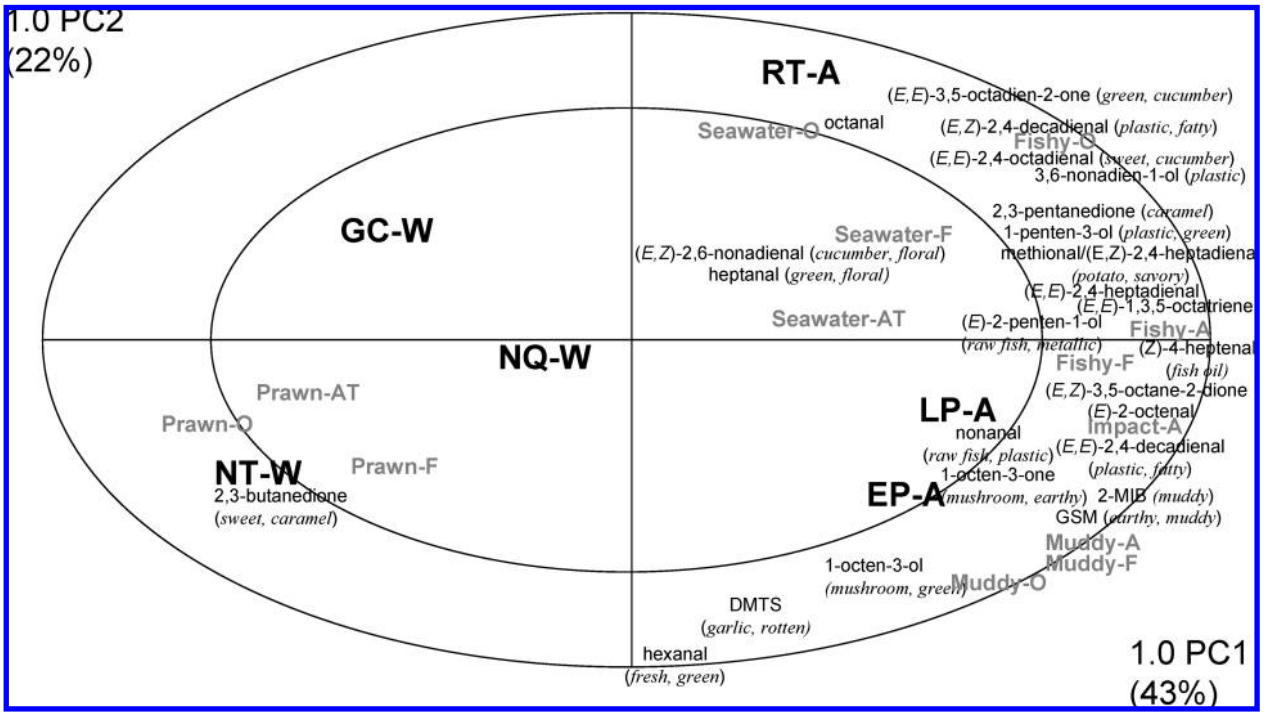


**Figure 3.** PLS-2 correlation loadings plot (PC1 vs PC2) for the mean DI GC-O aromagram peaks and their relationship with significant O, F, and AT sensory attributes: prawn, muddy, fishy, seawater, and impact. The three wild samples (left-hand side) were separated from the aquacultured samples (right-hand side) on the plot. The wild samples were more strongly associated with prawn odor and less with the other odor attributes (shown in gray). The aquacultured samples were more strongly associated with fishy and impact attributes. Similarly, the relationship between barramundi samples and odor-active volatiles together with indicative OQ can be seen. The complete list of frequent odor descriptors associated with volatiles is listed in **Table 2**.

1-octen-3-ol. With the exception of 2,3-butanedione, these compounds did not differ significantly between wild and aquacultured samples. The seawater sensory attribute (O, F, and AT), while positively correlated to nearly all volatiles, could not be successfully modeled by a PLS approach. Although all of the fish samples were rated as having baseline muddy odor (**Table 2**), MIB and GSM were only detected by GC/MS in those samples (LP-A and EP-A) with the highest muddy attribute scores. PLS-2 revealed positive correlations between muddy attributes and a number of volatiles: the isomeric 2,4-heptadienals, 2,4-octadienal, (*E*,*Z*)-2,6-nonadienal, and the two isomers of 2,4-decadienal; however, the strongest relationship was found with MIB and GSM in all cases. The association of these compounds with muddy flavors is well-known and discussed later.

**GC-O Aromagram Data.** More than 30 odor-active peaks were reported in the aromagrams of the barramundi headspace extracts (aromagrams not shown). In most cases, compounds responsible for a given odor were confirmed using RCs and the criteria discussed previously. For odor-active volatiles for which standards were not available, a tentative identification was made (**Table 2**). As expected, a set of generic odors were present in all samples, and the main aromagram differences were in the relative odor intensity and detection frequency in different barramundi extracts. In most cases, odors were sufficiently separated for assessors to be able to easily discriminate, but some odors occurred close together and may have been due to two or more coeluting compounds, for example, methional and (*E*,*Z*)-2,4-heptadienal. The aromagram profiles were complex with a range of odor intensities; there was an overall higher perceived intensity of most odors in the aquacultured samples as well as the presence of additional odors such as the muddy odor peaks corresponding with GSM and MIB in some samples. The most frequently used odor descriptors from the GC-O assessors are listed in **Table 2**. In most cases, the descriptors used agreed with those found in literature. In some cases, odor qualities were not in agreement with literature or standards, indicating the possibility of unidentified coeluting odor-active compounds at the same retention time. The main odor impact compounds across all of the barramundi samples, in order of decreasing average odor intensity and together with frequently used odor descriptors, were as follows: "raw fish, metallic, green, marine" [(*E*)-2-penten-1-ol]; "garlic, savory, rotten, metallic" (dimethyl trisulfide); "fresh raw fish, herbal, geranium, plastic, marine" (nonanal); "cooked potato, fatty, savory" (methional/2,4-heptadienal); "sweet, caramel" (2,3-pentanedione); "orange, sweet" (octanal); "mushroom" (1-octen-3-one); and "mushroom, fermented" (1-octen-3-ol). Furthermore, moderate and lower intensity odor compounds also contributed to the aromagram profiles of barramundi. The complete list of odor-active peaks and their corresponding odor descriptors are listed in **Table 2**. In many cases, the average perceived intensity for a given peak was higher in the aquacultured samples. Significantly higher ($p < 0.05$) average DI responses were measured for a number of odor peaks in acquacultured barramundi including: 1-penten-3-ol, (*E*)-2-penten-1-ol, (*Z*)-4-heptenal, (*E*,*E*)-2,4-heptadienal, and others. In addition to differences in the perceived intensity of common odor-active compounds, some unique odors with descriptors such as musty, muddy, and earthy were shown to be associated with both GSM and MIB. Not only were these compounds detected by GC-O, but they were also positively identified by analytical (GC/MS) measurement in the LP-A and EP-A samples.

**PLS Modeling of GC-O Peak Intensities to Sensory Attributes.** The relationship between the average GC-O peak intensity and the sensory attributes was initially modeled by PLS-2. **Figure 3** shows the correlation loadings plot of PC1 vs PC2 for GC-O odor intensities (designated by chemical name and odor attribute) and the significant sensory attributes. The PLS model explained 43 and 22% of the data variance with the first two PCs. In most

**Table 5.** List of Weighted Regression Coefficients from PLS-2 Calibrations for Sensory Attributes Using DI Data[a]

| | O attributes | | | | F attributes | | | | | AT attributes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | fish | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water | impact | fishy | prawn | muddy | sea water |
| 2,3-butanedione | −0.08 | **0.08** | 0.03 | −0.03 | −0.04 | −0.06 | **0.09** | −0.03 | −0.02 | −0.04 | −0.08 | **0.09** | 0.00 | 0.00 |
| 2,3-pentanedione | **0.07** | −0.06 | 0.05 | 0.11 | **0.06** | 0.05 | −0.06 | 0.06 | **0.10** | 0.05 | 0.05 | −0.05 | 0.08 | 0.05 |
| hexanal | −0.05 | 0.01 | 0.14 | 0.10 | 0.03 | 0.02 | 0.05 | 0.06 | −0.03 | 0.07 | 0.03 | 0.01 | 0.11 | −0.01 |
| (*E*,*E*)-1,3,5-octatriene | **0.07** | −0.07 | 0.02 | 0.01 | **0.08** | **0.09** | −0.05 | 0.06 | 0.04 | **0.08** | **0.09** | −0.06 | 0.03 | 0.06 |
| heptanal | −0.01 | **0.04** | −0.05 | **0.09** | 0.01 | −0.02 | **0.07** | −0.01 | **0.08** | 0.02 | −0.02 | **0.08** | −0.06 | **0.14** |
| 1-penten-3-ol | **0.07** | −0.06 | 0.05 | **0.10** | **0.07** | 0.06 | −0.05 | 0.06 | 0.09 | **0.07** | 0.07 | −0.05 | 0.07 | 0.07 |
| (*Z*)-4-heptenal | **0.08** | **-0.09** | 0.09 | 0.04 | **0.08** | **0.09** | **-0.08** | 0.09 | 0.05 | **0.08** | 0.09 | **-0.09** | 0.11 | 0.02 |
| octanal | 0.04 | 0.00 | −0.07 | **0.13** | 0.02 | 0.00 | 0.00 | −0.02 | **0.09** | 0.00 | −0.01 | 0.02 | −0.06 | 0.11 |
| 1-octene-3-one | 0.03 | −0.05 | 0.10 | −0.05 | **0.06** | 0.07 | −0.04 | 0.07 | −0.01 | **0.09** | 0.09 | −0.06 | 0.10 | −0.02 |
| (*E*)-2-penten-1-ol | **0.06** | −0.06 | 0.00 | −0.05 | **0.06** | **0.08** | −0.05 | 0.05 | −0.01 | 0.06 | 0.06 | −0.06 | 0.00 | 0.03 |
| dimethyl trisulfide | −0.05 | 0.03 | 0.13 | −0.02 | 0.03 | 0.01 | 0.07 | 0.06 | 0.02 | 0.06 | 0.00 | **0.05** | 0.10 | 0.05 |
| nonanal | 0.01 | −0.01 | 0.03 | −0.02 | 0.06 | 0.06 | 0.03 | 0.05 | 0.03 | 0.09 | 0.05 | 0.01 | 0.01 | **0.11** |
| (*E*)-2-octenal | 0.04 | −0.05 | 0.06 | −0.01 | 0.06 | 0.07 | −0.04 | 0.07 | 0.03 | 0.05 | 0.05 | −0.04 | 0.06 | 0.04 |
| 1-octen-3-ol | 0.00 | −0.04 | 0.11 | −0.11 | 0.05 | 0.07 | −0.02 | 0.07 | −0.05 | 0.07 | 0.07 | −0.06 | 0.09 | −0.05 |
| (*E*,*Z*)-2,4-heptadienal | 0.06 | −0.05 | −0.06 | −0.04 | 0.04 | 0.07 | −0.05 | 0.02 | −0.01 | 0.04 | 0.05 | −0.05 | −0.05 | 0.04 |
| (*E*,*E*)-2,4-heptadienal | 0.03 | −0.02 | −0.02 | 0.05 | 0.05 | 0.04 | 0.02 | 0.03 | 0.06 | 0.07 | 0.05 | 0.01 | −0.03 | **0.12** |
| (*E*,*Z*)-3,5-octadien-2-one | **0.09** | **-0.08** | −0.03 | **0.10** | 0.03 | 0.04 | **-0.11** | 0.01 | 0.06 | 0.00 | 0.05 | −0.08 | 0.01 | −0.01 |
| (*E*,*E*)-2,4-octadienal | **0.09** | **-0.08** | −0.04 | 0.04 | 0.04 | **0.07** | **-0.10** | 0.02 | 0.02 | 0.03 | **0.07** | −0.09 | −0.01 | 0.00 |
| (*E*,*E*)-3,5-octadien-2-one | 0.05 | −0.05 | 0.11 | 0.07 | **0.07** | 0.05 | −0.05 | 0.08 | 0.07 | 0.06 | 0.05 | −0.04 | 0.12 | 0.03 |
| (*E*,*Z*)-2,6-nonadienal | 0.02 | −0.02 | −0.04 | 0.00 | 0.01 | 0.02 | −0.01 | −0.01 | −0.01 | 0.03 | 0.04 | −0.02 | −0.04 | 0.01 |
| 2-MIB | 0.02 | −0.05 | **0.15** | 0.00 | 0.08 | 0.07 | −0.02 | **0.10** | 0.05 | **0.10** | 0.07 | −0.04 | **0.15** | 0.04 |
| (*E*,*Z*)-3,6-nonadien-1-ol | **0.09** | −0.08 | −0.03 | 0.05 | 0.05 | **0.07** | −0.10 | 0.03 | 0.04 | 0.02 | 0.06 | −0.08 | 0.00 | 0.02 |
| (*E*,*Z*)-2,4-decadienal | 0.04 | −0.05 | 0.09 | 0.00 | **0.09** | **0.08** | −0.02 | 0.09 | 0.04 | **0.10** | **0.08** | −0.04 | 0.09 | 0.06 |
| (*E*,*E*)-2,4-decadienal | **0.07** | −0.05 | −0.01 | **0.14** | 0.05 | 0.03 | −0.05 | 0.02 | **0.10** | 0.04 | 0.05 | −0.04 | 0.02 | 0.07 |
| GSM | 0.02 | −0.05 | **0.16** | 0.00 | 0.08 | 0.08 | −0.02 | **0.11** | 0.04 | 0.11 | 0.08 | −0.05 | **0.16** | 0.02 |
| PLS-1 Cal. $R^2$ | **0.91** | **0.88** | **0.99** | 0.86 | 0.96 | 0.99 | 0.80 | 0.92 | 0.78 | 0.99 | 0.99 | 0.81 | 0.99 | 0.78 |
| PLS-1 Val. $R^2$ | **0.46** | **0.41** | **0.47** | −0.63 | 0.72 | 0.97 | −0.16 | 0.45 | −0.62 | 0.89 | 0.95 | 0.01 | 0.56 | −0.61 |
| PLS-1 Opt Cal. $R^2$ | **0.91** | **0.93** | **0.96** | **0.80** | **0.94** | **0.99** | **0.84** | **0.98** | **0.82** | **0.98** | **0.98** | **0.88** | **0.99** | **0.93** |
| PLS-1 Opt Val. $R^2$ | **0.67** | **0.82** | **0.92** | **0.59** | **0.85** | **0.98** | **0.43** | **0.96** | 0.31 | **0.96** | **0.96** | **0.63** | **0.99** | **0.85** |

[a] Bold signifies coefficients used in refined PLS-1 models. NI, no improvement using PLS-1; Cal. $R^2$, $R^2$ value for calibration model; Val. $R^2$, $R^2$ value for the cross-validation model; Opt cal. $R^2$, $R^2$ for PLS-1 optimized model; and Opt val. $R^2$, $R^2$ for PLS-1 optimized model.

cases, the PLS-2 models were improved by using a subset of highly correlating odor peaks to build a PLS-1 model for individual attributes (**Table 5**). Similar to PLS model based on volatile concentration data, the aquacultured samples were clearly differentiated from the wild samples on PC1 on the basis of average GC-O odor peak scores. The aquacultured samples were more strongly associated with impact, fishy, seawater, and muddy sensory attributes and less associated with prawn sensory attributes. Across the wild samples, NT-W and GC-W were found to have lower association with fishy and impact attributes as compared to the NQ-W sample and a high association with prawn attributes. Nearly all of the odor peaks were positively correlated with fishy (**Table 5**), with a strong correlation with 1-penten-3-ol, 2,3-pentanedione, (*E*,*E*)-3,5-octadien-2-one, and (*E*,*Z*)-3,6-nonadien-1-ol. Most odor peaks were also negatively correlated with the prawn attributes with the exception of 2,3-butanedione, hexanal, heptanal, and dimethyl trisulfide and a number of other compounds. Importantly, the same four volatile compounds were also correlated positively with the prawn attributes (**Table 4**). Two of the aquacultured samples (LP-A and the EP-A) were significantly higher in muddy attributes as compared to the remaining samples (**Table 3**). Muddy odors corresponding to both GSM and MIB were clearly detected by GC-O.

## DISCUSSION

Most of the identified volatiles, especially the subset of odor-active compounds, are derived from the oxidative breakdown of polyunsaturated fatty acids (PUFAs) and highly unsaturated fatty acids (HUFAs). PUFAs are present mainly in the form of linoleic (18:2 n-6) and linolenic (18:3 n-3) acids, derived mainly from vegetable sources in barramundi diets. Fish lipids are rich in 5- and 6-bond $\omega$-polyenic HUFAs, docosahexaenoic acid (DHA; 22:6 n-3), and eicosapentaenoic acid (EPA; 20:5n-3); these may come from a variety of sources in the wild diet but mainly from fish meal and oil in aquaculture diets. The contribution of these lipid substrates to fish aroma volatiles is well-known (*24*−*28*).

Hexanal, quantitatively the most abundant volatile in headspace profiles, is readily formed through the oxidation of both linoleic and linolenic acids (*24*), and both hexanal and heptanal are formed during the oxidation of conjugated linoleic acids (*25*). The flavor active alcohols, 1-octen-3-ol and 1-penten-3-ol, have been found previously in fish and fish oil and have been shown to be typical products of oxidation of DHA and EPA (*25*, *26*). Styrene, found at a higher concentration in the aquacultured samples, is likely to be an exogenous packaging contaminant that has been reported in aquacultured and fresh fish previously (*10*, *27*). Further typical HUFA oxidation volatile compounds were identified in the barramundi samples at lower concentrations (**Table 2**). In nearly every instance, the relative concentration of lipid-derived volatile compounds was significantly higher ($p < 0.05$) in the aquacultured samples as compared to the wild samples, for example: (*E*,*E*)-1,3,5-octatriene, (*E*)-2-penten-1-ol, 1-penten-3-ol, the two isomers of 3,5-octadiene-2-one, 2,4-heptadienal, and 2,4-decadienal. Many of these compounds have relatively low odor thresholds. On the basis of estimations of their concentration and published odor threshold data (shown in **Table 2**, where available), most of these volatiles were expected to be odor-active in the fish samples (*29*−*32*). For example, in the LP-A and RT-A samples, (*Z*)-4-heptenal was calculated to be present at or above the olfactory threshold. In the wild samples, this volatile was estimated to be absent or below threshold concentration. Two odor-active compounds of

nonlipid origin, 2,3-butanedione (diacetyl) and 2,3-pentanedione, were measured in the samples. 2,3-Butanedione was present at a higher ($p < 0.05$) concentration in the wild samples and estimated to be present at a concentration close to or above its reported odor threshold. In the aquacultured samples, the same compound was estimated to be present at a subthreshold concentration. The relative odor activity of 2,3-butanedione was expected to be greater than 2,3-pentanedione in the barramundi aroma based on its threshold data, although the latter was present at a higher concentration (**Table 2**).

Overall, the discrimination of data based on average GC-O odor peaks was similar to that obtained using volatile concentration data, and both data sets complemented the sensory findings. (*E*)-2-Penten-1-ol has been cited as a major odor impact compound in a number of fish, including trout (*14*, *33*), turbot (*15*), catfish (*28*), and cooked mussels (*34*). The current study confirmed its importance in barramundi where it was asssociated with odor qualities described as "raw fish, metallic, green, marine". This unsaturated alcohol is formed in the oxidation of n-3 HUFAs (*35*). The odor peak identified as nonanal was associated with a strong plastic, geranium, raw fish, and marine odor based on qualitative reports from the trained GC-O panelists. The closely eluting compounds methional and (*E*,*Z*)-2,4-heptadienal were associated with savory, cooked potato, fried fish, and fatty aromas. Methional has previously been shown to be an important impact compound in mussels (*34*) and carp (*36*). In the present study, methional was identified as an impact compound in all of the barramundi samples. Dimethyl trisulfide was also detected as a high impact compound in all of the samples; the corresponding odor was described as garlic, savory, rotten, and metallic. This compound was also identified in mussel aroma (*34*). Both methional and dimethyl trisulfide are generated through the breakdown of the amino acid methionine (*36*, *37*).

Many of the compounds identified in barramundi, including (*Z*)-4-heptenal, (*E*,*Z*)-2,4-heptadienal, (*E*,*E*)-2,4-octadienal, and (*E*,*Z*)-2,6-nonadienal and the two isomers of 3,5-octadien-2-one have been associated with fishy aroma (*27*). When present at low concentrations, most of these lipid oxidation products have been positively associated with fresh fish flavor. However, when present at elevated concentrations, compounds such as (*Z*)-4-heptenal and various isomeric dienals have been associated with undesirable flavors and increased fishy flavor attributes. Of particular interest was (*Z*)-4-heptenal, which has been shown to be a major odor impact compound in a number of fresh marine creatures (*38*, *39*), including catfish (*28*) and mussels (*34*). This compound has also been shown to increase during storage, contributing to increased fishy flavors (*27*, *40*). Oxidation of vegetable derived n-6 PUFAs produces characteristic odor-active volatiles including hexanal, (*E*)-2-octenal, 2,4-decadienal, and pentanal (*26*). These compounds were found in both wild and aquacultured barramundi, and although (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal were elevated in the aquacultured fish, there was little evidence of differences in the concentration of other n-6 PUFA breakdown compounds between wild and aquacultured samples.

The stronger fishy flavor apparent in the aquacultured barramundi samples may be explained by differences in diet. In addition to fish oil and marine protein meal, most commercial feeds include a percentage of cereal and plant-based fat. There was little evidence that differences in the odor-active volatile profiles were due to breakdown products plant-based lipids but rather marine n-3 HUFAs. It has been shown that aquacultured fish often have higher n-3 HUFA contents than wild counterparts due to the higher fish meal/oil content of the diet (*10*, *12*). There are active research programs in Australia and worldwide attempting to reduce the proportion of marine-derived n-3 HUFAs in commercial feeds (*13*, *41*). Replacement of fish meal and fish oil in aquaculture feeds may have sensory consequences for the final product.

2,3-Butanedione has been previously reported as a moderate impact compound in a variety of seafoods, mussels (*34*), and fresh sardines (*40*). 2,3-Butanedione was an important impact aroma volatile present at a significantly higher concentration in wild turbot as compared to cultured (*15*), in agreement with findings in this study. In addition, 2,3-butanedione was positively correlated with sweet prawn-like flavor attributes. Interestingly, the same compound was a top impact odorant in crustacean aroma (spiny lobster tail meat) (*42*). Both 2,3-butanedione and 2,3-pentanedione have similar caramel, sweet odors. 2,3-Butanedione originates from the decarboxylation of intermediates in the citric acid cycle. Both compounds are also known to be generated in the reaction of glucose with amino acids in the Maillard reaction (*43*).

MIB and GSM are lipid-soluble potent odorants that have been associated with muddy odors and flavors in freshwater fish. MIB and GSM are metabolites produced by algae and cyanobacteria found in fresh water (*8*, *44*, *45*). The sensory thresholds of MIB and GSM are reported to be between 0.1−0.2 and 0.25−0.5 $\mu$g/kg, respectively, in catfish flesh (*44*); however, different values have been reported in other fish species (*44*−*46*). According to these thresholds and the estimated concentrations of MIB and GSM in the barramundi, both compounds were estimated to be above threshold values in both the EP-A and the LP-A samples—both "in-ground" cultivation methods. In contrast, the farmed barramundi from the recirculation tank (RT-A) were rated with the lowest muddy odor (**Table 3**). The dynamic headspace extraction and the GC-O method were not specifically designed for quantitative measurement of GSM and MIB; it is well-known that the extraction and accurate instrumental quantification of these compounds from lipid rich matrices are challenging and require sensitive analytical approaches (*8*, *44*, *45*). The data in **Table 2** are only semiquantitative; the actual concentration of these volatiles may have been somewhat different than those reported. It has been demonstrated that larger fish have a higher concentration of fat as a proprtion of total body mass and that fat is distributed differently within the flesh (*4*, *8*). It has been hypothesized that a higher lipid content of larger fish may also increase susceptibility to taint by GSM and 2-MIB (*8*). In this study, the wild samples were clearly larger (**Table 1**) and presumably higher in fat than the aquacultured samples; yet, muddy taint was not detected to any extent in the wild samples. On the other hand, it has been shown that the overall fat content of aquacultured fish is often higher than that in wild counterparts when controlled for size and weight (*10*, *11*). As stated previoulsy, the primary purpose of these set of experiments was to understand any potential flavor differences in barramundi typically available to consumers; commercially aquacultured barramundi are rarely grown beyond 2 kg, and wild barramundi are rarely filleted under ~5 kg. In any case, in the current study reflecting the commercial reality, the significant muddy, dirt, and musty flavor notes reported by the sensory panel in the EP-A and LP-A samples were also refelected by the GC-O data and the analytical data. As with many fresh water cultivated species, both MIB and GSM can be removed from fish with sufficient purging with fresh untainted water for a period before harvesting (*8*, *46*). This, however, imposes an additional cost to aquaculture production. The purging effect may have been partly responsible for the low muddiness of the RT-A samples. The recirculated water system employed sand filters, which is known to facilitate control and biodegradation of GSM and MIB (*47*).

This paper describes an integrated application of a trained sensory panel for both descriptive profiling and GC-O characterization of aroma. This information together with

chemical data could be used in a systematic way to help guide the development of new aquaculture diets and feed formulations for optimal sensory quality. In summary, this study provides an extensive investigation of the sensory properties of wild and farmed barramundi and additionally goes further by diagnosing the underpinning chemical and volatile changes that lead to differences in the perception between the farmed and the aquacultured product. Although many of the volatiles identified in this investigation have been described previously in other seafood and finfish species, this is the first time odor-active volatiles have been systematically reported in barramundi together with an indication of their relative odor intensity. The commercial samples obtained for this initial investigation were intentionally obtained "as-is" with no explanation as to the intended use or application of the samples, other than for general research. Although the findings of this study are based on a limited number of samples, which may not reflect the full range of variation within "typical" wild and aquacultured barramundi, the applied techniques proved successful for differentiating wild from aquacultured barramundi. Future investigations will require sourcing a larger range of samples, preferably at different seasons.

Importantly, this study demonstrates the good complimentarity of DI GC-O, sensory descriptive profiling, and volatile data. In many cases, specific odor-active volatiles could be associated with specific sensory attributes. The purpose of the multivariate data modeling was not to develop a predictive model but rather to assist in interpretation of the relationship between GC-O and analytical data to sensory data. In future studies, the effects of new diet formulations on the sensory characteristics of aquacultured produce could be systematically characterized in a similar manner.

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