Comparison of aroma characteristics of 16 fish species by sensory evaluation and gas chromatographic analysis

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Abstract: The aroma properties of fish broths prepared from 16 fish species (10 saltwater, three freshwater, two anadromous and one brackish water species) were described quantitatively by reference to 10 sensory attributes. Principal component analysis (PCA) and cluster analysis of sensory attributes classified the fish into four groups. Group 1, characterised by a strong ‘green’ odour, comprised all three freshwater species (loach, pond smelt and carp), two saltwater whitefish species (snapper and conger) and eel. Group 2 included migratory coastal species (sardine, banded blue-sprat and mackerel) and was distinguished by strong ‘fish oil’ and ‘grilled fish’ notes. Group 3 consisted of swordfish, sablefish and salmon, which exhibited a strong ‘fried chicken’ note. Group 4 included flounder, cod, tuna and goby, which were scored high for ‘cooked fish’, ‘roasted soy sauce’, ‘canned tuna’ and ‘sweet’ aromas. Partial least squares regression (PLSR) models derived from selected influential peaks in the gas chromatograms of the volatile components in the broths for each attribute were highly predictive ($R^2 > 0.936$). The selected peaks corresponded well to each sensory attribute.

Keywords: fish; sensory attribute; chemometrics; quantitative descriptive analysis

INTRODUCTION

The number of identified fish species currently exceeds 22 000.1 Regardless of whether raw or cooked, various fish species commonly possess similar aroma notes, but they also have specific aromas inherent to the species. Raw fish generally has a weak smell, but a strong, desirable aroma is produced thermally during cooking. Cooked fish aroma varies depending on the fish species and cooking procedure.

Fresh fish are characterised by sweet, mild, green, plant-like, metallic and fishy aromas, and volatile compounds contributing to these aromas are generated mainly by oxidative enzymatic reactions and autoxidation of lipids.2,3 For example, 5-, 6-, 8-, 9- and 11-carbon alcohols and carbonyls are derived enzymatically from polyunsaturated fatty acids (PUFAs), while autoxidative degradation of PUFAs produces 6-, 7-, 8- and 10-carbon carbonyls.2,3 Trimethylamine is generated by micro-organisms from trimethylamine oxide (TMAO).2,3 Aroma extract dilution analysis (AEDA) has been applied to identify the aroma-active compounds in boiled trout, cod, salmon and carp, and the influence of storage conditions and diet on fish aroma has been investigated.4–7 Josephson et al8 reported that volatile carotenoid-related oxidation products played important roles in cooked salmon flavour, and found a single compound exhibiting a cooked salmon loaf-like aroma. Horiuchi et al9 analysed the volatile compounds obtained from fish oil heated with cysteine and/or TMAO and concluded that TMAO promoted the oxidative degradation of fish oil and that cysteine generated compounds contributing to cooked fish flavour.

Using descriptive sensory analysis, Sawyer et al10 and Prell and Sawyer11 compared the characteristics of 18 species of snapper and rockfish and 17 species of North Atlantic fish respectively. Josephson et al12 studied the occurrence of 14 volatile compounds, including 6-, 8- and 9-carbon alcohols and carbonyls, in 16 fresh and saltwater fish species. They detected four compounds common to both freshwater and saltwater species, but 10 compounds were found only in freshwater species.

In flavour research, sensory evaluation is essential because of its high sensitivity, and the ability to describe sensory properties and correlate them with instrumental data helps in understanding the sense of flavour. Chemometric methodologies are effective in extracting useful information from complicated multivariate data.13 Khayat14 applied multiple linear regression analysis to the gas chromatographic (GC) peaks of canned tuna aroma to predict off-odour.

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(Received 27 March 2002; revised version received 6 June 2002; accepted 16 July 2002)
scores. Girard and Nakai\textsuperscript{15} successfully classified different grades of canned pink salmon by discriminant analysis using three selected peaks. Bak et al\textsuperscript{16} correlated sensory attributes of frozen cold-water shrimp with their GC profiles by multivariate analysis.

In this study, quantitative descriptive analysis and gas chromatography/mass spectrometry (GC/MS) analysis were applied simultaneously to compare the aroma properties of boiled fish of various species. In order to classify the species according to their sensory properties and to correlate sensory and instrumental data, chemometric techniques were applied to the two data sets.

**EXPERIMENTAL**

**Sample preparation**

Fourteen fish species were purchased from a local market in Tokyo and two (loach and eel) from a local market in Chiba (Table 1). All the species except loach were gutted, and tuna and swordfish were also skinned. Small fish species such as banded blue-sprat, sardine, loach, pond smelt and goby were boiled whole, while the others were cut into 3–4 cm lengths before being boiled, as in ordinary domestic cooking. Each sample (200 g) was placed in a three-necked separable 3 l round flask along with 200 ml of boiling water, then heated with refluxing for 30 min by a mantle heater. The fish broth (350 ml) obtained after filtering the boiled mixture through three layers of cotton gauze (Hakujuji Co, Tokyo, Japan) was divided into 35 ml aliquots in 50 ml glass vials. The headspace air in the vials was replaced by N\textsubscript{2} gas, and the broth was stored at −50° C to await sensory evaluation and aroma extraction and concentration. The broths were thawed by immersing the vials in water at 40° C and used in the following steps.

**Sensory evaluation**

Quantitative descriptive analysis was applied for evaluation of the samples, using a 15 cm line scale, by a well-trained panel consisting of 14 females aged 21–29 years. The panellists were chosen for their sensitivity in detecting 2-phenylethyl alcohol, skatol, isovaleric acid, \( \gamma \)-undecalactone and cyclotene (Daiichi-Yakuhin-Sangyo Co, Tokyo, Japan) at their threshold levels and for their ability to identify the aroma properties of these compounds, which are widely used for selecting sensory panellists as well as for diagnosis of olfactory disorders in Japan. Fish broth (35 ml) prepared from 20 g of fish was placed in a 260 ml disposable plastic cup, covered with a plastic petri dish and served to the panellists at 40° C. Prior to quantitative descriptive analysis, panellists discussed the aroma properties of the samples during three preliminary sessions, each lasting 90 min, until they had agreed on their use as sensory attributes. Quantitative descriptive analysis was performed for the 10 attributes listed in Table 2, using a 15 cm line scale. In order to tune the scales used by each panellist for each attribute, panellists evaluated five representative samples from the 16 samples and discussed their results in respect of each attribute. For any attribute, if the score awarded by one or a few panellists differed from that of the majority, the minority was expected to accept the majority assessment, though this was not mandatory. Quantitative descriptive analysis was then performed on all the samples, divided randomly into four sessions involving three or four samples each. Each sample was coded with a three-digit random number. In each session, samples were presented randomly to each panellist. All samples were evaluated once.

| Table 1. Species of fish investigated |
|--------------------------------------|
| **Scientific name** | **Common name** |
|---------------------|-----------------|
| Saltwater fish | | |
| Migratory coastal fish | | |
| Spratelloloides gracilis | Banded blue-sprat |
| Sardinops melanosticta | Sardine, pilchard |
| Scomber japonicus | Mackerel, chub mackerel |
| Coastal bottom fish | | |
| Microstomus achne | Slime flounder |
| Gadus macrocephalus | Cod, Pacific cod |
| Pagrus major | Snapper, red sea bream |
| Conger myriaster | Conger, common Japanese conger |
| Pelagic fish | | |
| Thunnus thynnus | Tuna, blue-fin tuna |
| Xiphias gladius | Swordfish |
| Deep-sea fish | | |
| Anoplolepis fimbria | Sablefish |
| Freshwater fish | | |
| Misgurnus anguillicaudatus | Loach, oriental weatherfish |
| Hypomesus nipponensis | Pond smelt |
| Cyprinus carpio | Carp |
| Anadromous fish | | |
| Oncorhyncus keta | Chum salmon |
| Anguilla japonica | Japanese eel |
| Brackish water fish | | |
| Acanthogobius flavimanus | Goby, yellowfin goby |

**a** All scientific and common names and classifications according to living areas are based on Ref 1.

**b** Italicised names are those used throughout the paper.
Preparation of aroma concentrates from fish broths

Fish broth (105ml) prepared from 60g of fish was subjected to simultaneous distillation and extraction (SDE) in a modified Likens–Nickerson apparatus. The extracting solvent was 80ml of redistilled dichloromethane, and extraction was carried out for 2h. The extract was transferred to a round-bottomed flask, to which was added 1ml of dichloromethane containing 2.1ppm methyl decanoate as internal standard (IS), and placed in a water bath at 45°C so that the dichloromethane was slowly evaporated off at atmospheric pressure. The extract was finally concentrated to 0.5ml under a gentle stream of N₂ gas. The concentrate was transferred to a 0.8ml test tube using a Pasteur pipette. A gentle stream of N₂ gas was introduced at the top of the test tube through the tip of a Pasteur pipette until the weight of liquid decreased to about 10mg (8µl of dichloromethane weighs about 10mg).

GC/MS analysis

An HP 5972 mass spectrometer connected to an HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) was used with a DB-WAX fused silica capillary column (60m × 0.25mm, film thickness 0.25µm; J & W Scientific Inc, Folsom, CA, USA). The oven temperature was held at 60°C for 4min and then raised to 180°C at 2°Cmin⁻¹. The injection temperature was 200°C, and He at a flow rate of 1.0mlmin⁻¹ was used as carrier gas. GC/MS was carried out at an ionisation voltage of 70eV and an ion source temperature of 150°C. All samples were analysed once. Peak components were identified by matching their mass spectra with those in the Wiley mass spectral library (Hewlett Packard) and on the basis of their retention indices. The ratio of each peak area to the IS peak area was calculated, and these ratios were used in the subsequent statistical analyses.

Statistical analysis

Analysis of variance (ANOVA), principal component analysis (PCA) and cluster analysis were performed by SPSS v 9.0 (SPSS Inc, Chicago, IL, USA). In the cluster analysis the distances between pairs of samples were compared on the basis of the squared Euclidean distance, and the resulting clusters were connected using Ward’s method, which merges those two clusters for which the total sum-of-squares error is smallest. Partial least squares regression (PLSR) analysis was performed by Unscrambler v 7.01 (CAMO ASA, Trondheim, Norway).

RESULTS AND DISCUSSION

Flavour profiles of fish species

Fig 1 compares the sensory profiles of the broths of the 16 fish species. ‘Fish oil’ and ‘grilled fish’ scores for migratory coastal fish species, ie banded blue-sprat, sardine and mackerel, were higher than those for other species. These three species, together with eel and loach, also showed higher ‘fishy’ scores. Scores of ‘cooked fish’ for flounder, cod and goby were high, as were those of ‘canned tuna’, ‘sweet’ and ‘roasted soy

\[ \text{Figure 1. Comparison of sensory profiles of broths of 16 fish species: (a)} \]

migratory coastal fish, pelagic fish and deep-sea fish; (b) coastal bottom fish; (c) freshwater fish, anadromous fish and brackish water fish (*** p < 0.001).
sauce’. ‘Fried chicken’ scores for swordfish, sablefish and salmon and ‘green’ scores for carp, eel, loach and snapper were higher than those for other species.

**Relationships between fish species and attributes**

PCA was performed to explore the relationship between fish species and sensory attributes. Two principal components (PCs) explained 79.7% (PC1, 54.5%; PC2, 25.2%) of the total variance. As shown in Fig 2, four groups were observed in a biplot. Snapper, conger, eel and freshwater species, ie loach, pond smelt and carp, were grouped around ‘green’. Migratory coastal fish species, ie sardine, banded blue-sprat and mackerel, were located close to ‘fish oil’, ‘grilled fish’, ‘sea breeze’ and ‘fishy’. Swordfish, sablefish and salmon were located close to ‘fried chicken’. Flounder, cod, tuna and goby were grouped around ‘cooked fish’, ‘sweet’, ‘canned tuna’ and ‘roasted soy sauce’.

Dendrograms obtained from cluster analysis applied both to samples and to sensory attributes, using the squared Euclidian distance and Ward’s method, are shown in Fig 3. Four and five groups were observed for fish species and for attributes respectively. The four groups of fish species (group 1: loach, pond smelt, carp, eel, snapper and conger; group 2: sardine, banded blue-sprat and mackerel; group 3: swordfish, sablefish and salmon; group 4: flounder, cod, tuna and goby) corresponded to their classification by PCA (Fig 2). Except for one group consisting of ‘fishy’ and ‘sea breeze’, four groups of attributes corresponded to the four groups of fish species, ie ‘green’ for group 1, ‘fish oil’ and ‘grilled fish’ for group 2, ‘fried chicken’ for group 3 and ‘canned tuna’, ‘cooked fish’, ‘roasted soy

**Figure 2.** Biplot of principal component (PC) scores and factor loadings from principal component analysis applied to aromas of broths of 16 fish species (+ roasted soy sauce).

**Figure 3.** Clustering of (a) 16 fish species and (b) 10 sensory attributes.
| Peak no | Compound                                      | Banded blue-spray | Mackerel | Tuna | Swordfish | Conger | Snapper | Flounder | Cod | Carp | Loach | Pond smelt | Eel | Salmon | Goby | Sablefish |
|--------|----------------------------------------------|-------------------|----------|-----|----------|-------|--------|----------|-----|------|-------|------------|-----|--------|------|-----------|
| 2      | 1-Methylpyrrole                              | 3                 | 0        | 4   | 0        | 0     | 43     | 0        | 0  | 0   | 0     | 0          | 0   | 0      | 0   | 0         |
| 3      | (E)-2-Penten-2-one                           | 5                 | 2        | 7   | 5        | 0     | 9      | 11       | 9  | 5   | 3     | 7          | 7   | 16     | 1   |           |
| 5      | 3-Penten-2-ol                                | 6                 | 3        | 12  | 20       | 16    | 0      | 9        | 7  | 5   | 9     | 0          | 17  | 0      | 4   |           |
| 7      | Pyridine                                     | 0                 | 0        | 0   | 0        | 6     | 0      | 8        | 2  | 0   | 0     | 0          | 0   | 0      | 0   |           |
| 9      | Unknown                                      | 0                 | 0        | 0   | 0        | 0     | 17     | 0        | 0  | 0   | 2     | 0          | 4   | 0      | 0   |           |
| 10     | 3-Methyl-1-butanol                           | 0                 | 0        | 0   | 0        | 34    | 11     | 13       | 0  | 0   | 6     | 10         | 2   | 0      | 0   |           |
| 13     | Unknown                                      | 4                 | 0        | 5   | 7        | 0     | 0      | 4        | 2  | 0   | 0     | 5          | 7   | 5      | 0   | 2         |
| 15     | N,N-Dimethylaminocetoni trile                | 0                 | 0        | 7   | 15       | 0     | 5      | 0        | 0  | 10  |
| 17     | 1-Methylthiopropane                          | 6                 | 0        | 2   | 0        | 0     | 0      | 0        | 0  | 3   | 2     | 0          | 0   | 0      | 0   |           |
| 21     | Unknown                                      | 7                 | 11       | 0   | 0        | 18    | 2       | 0        | 3  | 0   | 5     | 0          | 7   | 0      | 0   |           |
| 24     | 2,4,4-Trimethyl-2(3H)-furanone               | 0                 | 0        | 0   | 0        | 6     | 0      | 6        | 7  | 3   | 0     | 3          | 19  | 0      | 0   | 0         |
| 26     | 2-Methylpyrazine                             | 0                 | 0        | 0   | 0        | 0     | 3      | 0        | 0  | 0   | 3     | 0          | 4   | 0      | 0   |           |
| 37     | 1-Hydroxy-2-butanone                         | 29                | 7        | 49   | 34       | 0     | 74     | 166       | 67 | 42  | 30     | 37         | 33   | 84     | 22  | 18        |
| 38     | 3-Methyl-2-pentanol                          | 0                 | 0        | 4   | 8        | 6     | 7      | 3        | 0  | 2   | 4     | 4          | 0    |
| 46     | 2,6-Dimethylpyrazine                         | 0                 | 0        | 2   | 7        | 5     | 0      | 4        | 2  | 0   | 4     | 5          | 7   | 5      | 0   | 2         |
| 50     | 2-Acetylthiazole                             | 0                 | 0        | 0   | 0        | 0     | 0      | 0        | 0  | 0   | 0     | 0          | 0   | 0      | 0   |           |
| 58     | Benzaldehyde                                 | 0                 | 0        | 12  | 9        | 12    | 48     | 22        | 4  | 14  | 9     | 19         | 13   | 14     | 3   | 4         |
| 69     | 2-(2-Butoxyethoxy)ethanol                    | 23                | 33       | 12   | 0       | 0     | 6      | 0        | 3  | 0   | 8     | 4          | 0    | 0      | 3   | 0         |
| 74     | 1-Methylthiopropene                          | 0                 | 0        | 0   | 0        | 0     | 0      | 3        | 4  | 0   | 4     | 0          | 0    | 0      | 0   | 1         |
| 99     | 2,4,4-Trimethyl-3-hydroxy-2-phenylethanol    | 10                | 0        | 4    | 0        | 0     | 4      | 0        | 0  | 0   | 0     | 0          | 0    | 0      | 0   | 0         |
| 100    | 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate| 0                 | 0        | 0   | 0        | 0     | 0      | 0        | 0  | 0   | 0     | 0          | 0    | 0      | 0   | 0         |
| 101    | 2,4,4-Trimethyl-5-hydroxy-2-phenylethanol    | 10                | 0        | 5    | 4        | 4     | 2      | 21        | 4  | 3   | 5     | 1          | 0    | 4     | 8   | 0         |
| 105    | 2,4-Undecadienal                             | 0                 | 0        | 10  | 3        | 0     | 0      | 10       | 0  | 5   | 0     | 6          | 3    | 0      | 0   |           |
| 109    | 2-(2-Ethoxyethoxy)ethanol                    | 12                | 21       | 22   | 17       | 24    | 20     | 35       | 45 | 28  | 0     | 36         | 34   | 25     | 16  | 29        |
| 114    | 4-Ethylbenzaldehyde                          | 0                 | 0        | 0   | 0        | 0     | 12     | 0        | 0  | 27  | 26     | 7          | 0    | 0      | 0   | 0         |
| 117    | 3-Methylthiopropene                          | 0                 | 0        | 0   | 0        | 0     | 12     | 0        | 0  | 27  | 26     | 7          | 0    | 0      | 0   | 0         |
| 120    | Octanoic acid                                | 4                 | 5        | 3    | 1        | 2     | 0      | 2        | 0  | 2   | 0     | 8          | 2    | 0      | 5   | 3         |
These classifications support the observations of Prell and Sawyer,11 who classified 17 North Atlantic fish species into four groups, using sensory data, and found that cod and flounder belonged to the same group, having a ‘sweet’ aroma, while mackerel belonged to another group characterised by a ‘fish oil’ note, and swordfish was independent of the others. On the other hand, Josephson3 postulated that thermally processed fish flavours can be classified into at least five groups, namely saltwater whitefish, freshwater whitefish, tuna and mackerel, trout and char, and salmon. However,
in our study, saltwater whitefish species were further separated into ‘green’ and ‘sweet’ aroma groups, and the aromas of tuna and mackerel were described differently. Although the saltwater and freshwater species investigated in this study were not differentiated clearly, all the freshwater species belonging to group 1 were characterised by a strong ‘green’ odour. Furthermore, group 1 was divided into two subgroups (group 1a: loach and eel; group 1b: snapper, carp, conger and pond smelt). Fish species belonging to group 1a had high scores only for ‘green’ and ‘fishy’, whereas those in group 1b showed high or moderate scores for other attributes as well.

**Relationships between attributes and volatile components**

The 120 major peaks common to more than two fish species were used as variables in the multivariate statistical analysis. Prior to statistical analysis, sardine was eliminated, because its hydrocarbon peaks were so large that other peaks could not be found in the GC profile. The peak ratios of the 120 peaks in the GC profiles of the remaining 15 fish species were used as predictors in PLSR analysis. By comparing the PLS loading weights on the peaks, 20 peaks were selected to construct PLSR models for each attribute. The PLSR models derived from the 20 influential peaks for each attribute were highly predictive ($R^2 = 0.970$ for ‘fish oil’, $R^2 = 0.973$ for ‘roasted soy sauce’, $R^2 = 0.964$ for ‘sweet’, $R^2 = 0.964$ for ‘grilled fish’, $R^2 = 0.945$ for ‘green’, $R^2 = 0.978$ for ‘fishy’, $R^2 = 0.964$ for ‘canned tuna’, $R^2 = 0.973$ for ‘sea breeze’, $R^2 = 0.993$ for ‘fried chicken’ and $R^2 = 0.936$ for ‘cooked fish’). In Table 3, selected peaks contributing highly to each sensory attribute and the peak area ratios of compounds in the 15 fish species are listed. Compounds relating closely to each attribute are shown in Table 4. For ‘fish oil’ and ‘grilled fish’, which were located close to each other on the biplot (Fig 2), 12 common peaks were selected. For ‘roasted soy sauce’, ‘sweet’, ‘canned tuna’ and ‘cooked fish’, which were clustered on the biplot, seven common peaks were selected. Regarding four attributes corresponding to groups 1, 2, 3 and 4, ie ‘green’, ‘grilled fish’, ‘fried chicken’ and ‘cooked fish’ respectively, plots of the peaks with heavy loadings on PLS components 1 and 2 are shown in

![Graphs showing relationships between observed and estimated scores for green, grilled fish, fried chicken, and cooked fish aromas.](image-url)
Fig 4. Fig 5 shows the relationships between observed and estimated scores based on PLSR models for the four attributes.

‘Green’ odour was characteristic of freshwater species (Fig 5(a)) and was located separately from other attributes on the biplot in Fig 2. Fig 4 and Table 4 show that three ketones and 2-acetylpyrrole (cookie- or mushroom-like) were negatively correlated with ‘green’ odour, while 5-methylfurfural (sweet-spicy, burnt) and aldehydes such as \((E,E)-2,4\)-nonadienal, \((E,Z)-2,4\)-decadienal and \((E,E)-2,4\)-decadienal were positively correlated. \((E,E)-2,4\)-Nonadienal (green, tallowy, melon) and \((E,E)-2,4\)-decadienal (fatty, fried) have been determined by AEDA as potent odorants in boiled trout aroma,\(^4\) boiled carp aroma\(^7\) and crayfish waste.\(^{20}\)

‘Grilled fish’ aroma was strongest in group 2 species, followed by group 4 species (Fig 5(b)). As Fig 4 and Table 4 indicate, for ‘grilled fish’ aroma, many sulphur- and/or nitrogen-containing compounds widely known as cooked flavour compounds generated in the Maillard reaction\(^{21,22}\) were found to relate positively. 3-Ethyl-2,5-dimethylpyrazine and 3-methylthiopropanol have a roasted/nutty/baked potato and sweet/soup-like and a meat-like aroma respectively.\(^{19}\) Phenol (medicinal) and benzaldehyde, with an almond/fruity/creamy/nutty aroma, have been found to be flavour-active in crayfish waste\(^{20}\) and retort salmon,\(^{23}\) and here the former contributed positively but the latter negatively to ‘grilled fish’ aroma.

‘Fried chicken’ scores were highest for group 3 species (Fig 5(c)). According to Fig 4 and Table 4, many sulphur- and/or nitrogen-containing compounds contributed positively to ‘fried chicken’ aroma. Among them, 2,5-dimethylpyrazine (popcorn-like/nutty), 2,3-dimethylpyrazine (nutty/green), 2,3,5-trimethylpyrazine (musty/nutty) and 3-methylthiopropanol (boiled potato) have been determined by AEDA as potent odorants in trout,\(^4\) crayfish waste,\(^{20}\) squid,\(^{24}\) clam\(^{25}\) and crustaceans.\(^{26}\) 3-Methyl-1-butanol (dark chocolate)\(^{26}\) was found to contribute negatively to ‘fried chicken’ aroma by PLSR analysis. Girard and Nakai\(^{15}\) classified different grades of canned pink salmon by discriminant analysis using three peaks, including 3-methyl-1-butanol, and found that lower-grade samples contained higher concentrations of this alcohol.

As shown in Fig 5(d), ‘cooked fish’ aroma was characteristic of goby, flounder and cod. ‘Sweet’ aroma in pond smelt was as strong as in goby (Fig 1), but the ‘cooked fish’ score was low. As already shown in Fig 2, ‘roasted soy sauce’, ‘canned tuna’, ‘cooked fish’ and ‘sweet’ were close to each other, and some common compounds were selected by PLSR. Among them, seven compounds, including four ketones and a furanone, contributed positively and three aldehydes negatively to both ‘sweet’ and ‘cooked fish’ odours. Most of these compounds influenced ‘green’ odour in the opposite sense. Ketones contribute to the sweet aroma of many crustaceans.\(^{27}\)

Figure 6. Dendrograms of 15 fish samples based on (a) 120 gas chromatographic peak ratios and (b) 69 peaks selected by partial least squares regression.
Pyridine (burnt\textsuperscript{19}) and 2,6-dimethylpyrazine (green nutty\textsuperscript{20}/nutty\textsuperscript{25}) positively influenced ‘cooked fish’ aroma.

**Clustering of fish species by GC profiles**

First, cluster analysis was carried out on the basis of 120 peak ratios. However, no distinct freshwater fish cluster was observed, as indicated in Fig 6(a). Josephson \textit{et al}\textsuperscript{12} compared 14 volatile compounds, including 6, 8 and 9-carbon alcohols and carbonyls, in 12 freshwater and four saltwater fish species. They detected four common compounds in both groups, but 10 compounds were found only in freshwater species. In the dendrogram the distance between mackerel and banded blue-sprat was small. Cod, flounder and goby made one cluster, while swordfish and sablefish made another. These classifications were partly similar to those based on the sensory data. Next, clustering of the 15 fish species on the basis of 69 peaks selected by PLSR (listed in Table 3) is shown in Fig 6(b). Group 1 (banded blue-sprat and mackerel) and group 3 (swordfish, sablefish and salmon) each made one cluster, but clusters corresponding to groups 2 and 4 were not clearly observed. However, clustering based on the selected peaks looks more like the dendrogram formed by sensory data (Fig 3(a)) than that based on all the peaks. This similarity can be easily understood, because the 69 peaks were selected according to their high contributions to the sensory attributes by PLSR analysis.

**CONCLUSIONS**

Quantitative descriptive sensory analysis succeeded in clearly depicting the aroma characteristics of the broths of 16 fish species using 10 sensory attributes. Both PCA and cluster analysis were useful in classifying the sensory data, resulting in four distinct groups. Although freshwater and saltwater species were not clearly differentiated, all the freshwater species belonged to a cluster characterised as ‘green’. Our classification agreed generally with those of previous studies. PLSR models calculated using influential GC/MS peaks for each attribute were highly predictive. Considering the aroma characteristics of individual compounds, PLSR selection of influential compounds for each attribute seems theoretically meaningful.

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