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Identification and sensory evaluation of flavour enhancers in Japanese traditional dried herring (*Clupea pallasii*) fillet

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ABSTRACT

Flavour-enhancing components of dried herring fillet (migaki-nishin in Japanese) were isolated and evaluated for their effects on sensory perception. Sensory evaluation revealed that addition of dried herring fillet water-soluble extracts to Japanese noodle soup significantly ($P < 0.05$) enhanced soup flavour characters, such as thickness, mouthfulness and continuity. The extracts were fractionated by dialysis and chromatography. Fractions containing flavour enhancers were isolated by sensory perception. Results from instrumental analyses showed that the *kokumi* flavour enhancers in dried herring fillet were creatine and creatinine.

1. Introduction

Dried herring (*Clupea pallasii*) fillet (DHF, Japanese, migaki-nishin) is a traditionally popular food item in Japan due to its remarkable flavour-enhancing properties. In particular, addition of DHF to noodle soup enhances flavour characters, such as thickness, mouthfulness and continuity. These flavour characters are often called *kokumi* in Japanese. Compounds added to enhance existing flavour characters, such as thickness, mouthfulness and continuity, of a food, without imparting a typical taste and aroma of their own, have been described as *kokumi* flavour enhancers (Ueda, Sakaguchi, Hirayama, Miyajima, & Kimizuka, 1990; Ueda, Yonemitsu, Tsubuku, Sakaguchi, & Miyajima, 1997).

The flavour of fish and shellfish principally originates from extractive components (Konosu & Yamaguchi, 1982). In general, most flavour components of foods are water-soluble. These include nucleotides, amino acids, peptides, organic acids and bases, and inorganic ions. Other than amino acids, peptides and nucleotides, creatine, creatinine and lactate were found to be taste-active components in dried skipjack (Fuke & Konosu, 1991). Lactate, succinate, creatine, creatinine and hypoxanthine were also recognised as taste-active in stewed beef juice (Schlichtherle-Cerny & Grosch, 1998).

It is well known that amino acids and peptides contribute to the taste of a wide variety of foods. The taste quality produced by amino acids and peptides has also been described as *kokumi* that has translated as "rich thick taste" (Kawajiri, 1999). It is reported that pyrazines and some peptides, generated together in certain foods during the processes of boiling or aging for a long period, form *kokumi* (Ogasawara, 2003). Moreover, Shima, Yamada, Suzuki, and Harada (1998) have identified *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene) alanine in beef broth as a "brothy taste" modifier, whereas glutathione enhanced the flavour characteristics of thickness, mouthfulness and continuity when added to an umami solution (Ueda et al., 1997). Recently, it has been reported that the addition of γ-glutamyl peptides to a savoury matrix, e.g. sodium chloride and monosodium glutamate solutions or chicken broth, significantly decreased the detection thresholds and remarkably enhanced mouthfulness, complexity, and long lastingness of the savoury taste (Dunkel, Köster, & Hofmann, 2007).

Preliminary experiments showed that addition of DHF water-soluble extracts to Japanese noodle soup (JNS) enhanced soup flavour characters, such as thickness, mouthfulness and continuity, and enhancement of these flavour characters increased with drying time (Shah, Tokunaga, Kurihara, & Takahashi, 2008). However, to the best of our knowledge, compounds responsible for the characteristic flavour-enhancing effects of DHF have not been identified. Therefore, this study aimed to identify the flavour-enhancing compounds of DHF and to evaluate their effects on sensory perception.

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2. Materials and methods

2.1. General

Field desorption mass spectra (FD-MS) were determined on a JEOL JMS-SX102A mass spectrometer (JEOL Ltd., Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX-500 NMR spectrometer (Bruker, Rheinstetten, Germany) at 500 and 126 MHz, respectively. Deuterium oxide (D_2O) was used as solvent and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP-d_4) was used as an internal standard.

2.2. Materials and chemicals

DHF was obtained from a fishery processing company, in Hakodate, Japan. Herring (*C. pallasii*) was captured at the coast of Kamchatka Peninsula, Russia, in October 2006, and kept frozen until it was processed. Upon arrival at the factory, herring was thawed, gutted, washed and then filleted for drying. Herring fillets were dried using huge electric fans. Room temperature and relative humidity were maintained at approximately 14 °C and 45%, respectively. After drying, DHF was randomly sampled for analysis. The DHF was comprised of 54.4% crude protein, 39.1% total lipid, and 4.3% ash on a dry weight basis. All chemicals used were of analytical or HPLC grade and were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), Sigma (St. Louis, MO), and Amersham Pharmacia Biotech (Uppsala, Sweden).

2.3. Preparation of water-soluble extracts (WSE)

DHF was cut into small pieces and freeze-dried. The freeze-dried DHF (20 g) was then defatted using *n*-hexane and homogenised with a 10-fold volume of de-ionised water. The homogenate was centrifuged at 10,000 *g* for 20 min at 4 °C and the supernatant was collected. Resulting supernatant was extracted with ethanol (final concentration, 80%) to remove high molecular weight compounds, followed by centrifugation and then filtration. After evaporation and further freeze-drying of the filtrate, lyophilised powder was obtained as the WSE of DHF (1.37 g, yield = 6.85%). In parallel, freeze-dried DHF (20 g) was boiled in a 10-fold volume of de-ionised water for 30 min and then homogenised. The homogenate was centrifuged at 10,000 *g* for 20 min at 4 °C and supernatant was obtained. After ethanol extraction and centrifugation, the obtained clear supernatant was freeze-dried to give the WSE of boiled DHF (1.78 g, yield = 8.90%).

2.4. Fractionation of the WSE of DHF

The WSE of DHF was dissolved in de-ionised water, and then the solution was dialysed against de-ionised water using 1000 and 5000 Da cut-off membranes (Spectrum Laboratories, Inc., California, USA) and finally freeze-dried. Three fractions were obtained, namely fraction I (<MW 1000 Da), fraction II (MW 1000–5000 Da), and fraction III (>MW 5000 Da).

2.5. Gel filtration chromatography (GFC)

The fraction II (MW 1000–5000 Da) was dissolved in de-ionised water (100 mg/ml) and, then applied onto the top of a water-cooled glass column (2.2 × 85.0 cm, Amicon Corporation, Lexington, MA, England) filled with slurry of Sephadex G-25 (Amersham Pharmacia Biotech, Uppsala, Sweden). The elution was achieved at a flow rate of 0.3 ml/min at 4 °C with de-ionised water as the mobile phase to allow sensory evaluation of the recovered fractions. The eluting solution was collected in 4 ml fractions. The UV absorbance of each fraction was measured at 220 nm using a Hitachi U-2000 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The fractions were combined into five fractions F1–F5 on the basis of elution profile. The individual fractions were freeze-dried and stored at −50 °C prior to use.

2.6. Separation by high-performance liquid chromatography (HPLC)

Fraction F3, thus obtained, was analysed on a Hitachi 655A HPLC system (Hitachi Ltd., Tokyo, Japan). Chromatographic separation was performed with a TSK-GEL G2000SW semi-preparative column (10 μm; 7.5 × 300 mm, Tosoh Corp., Tokyo, Japan). The mobile phase consisted of an aqueous solution of 80% acetonitrile containing 0.1% trifluoroacetic acid. Elution was conducted at a flow rate of 1.0 ml/min and at room temperature. The eluting solution was monitored by UV absorbance at 214 nm. The obtained fractions were then freeze-dried and stored at −50 °C until used for sensory evaluation and chemical analysis.

2.7. Identification of the flavour enhancers in fraction F3-2

A fraction thus obtained by rechromatography, F3-2, was further rechromatographed by a RP-HPLC system using an LC-10AT*vp* Shimadzu liquid chromatograph (Shimadzu Corp., Japan). Chromatographic separations were performed with an ODS column (Mightysil RP-18, Kanto Chem. Co., Tokyo, Japan), either on analytical (5 μm; 4.6 × 250 mm, flow rate 0.4 ml/min) or semi-preparative scale (5 μm; 10 × 250 mm, flow rate 1.0 ml/min). A linear gradient of acetonitrile, from 10% to 20% for 20 min in water, was used as mobile phase. Fractions F3-2-A and F3-2-B were isolated as flavour enhancers. Subsequent MS and NMR experiments of the isolates of RP-HPLC fractions F3-2-A and F3-2-B led to the identification of the *kokumi* components as creatine and creatinine, respectively.

F3-2-A (Creatine): HRFDMS, *m/z* 132.0785 (MH^+, calcd for $C_4H_{10}N_3O_2$, 132.0774); ^{1}H NMR (500 MHz, D_2O), δ 3.98 (2H, s), 3.05 (3H, s); ^{13}C NMR (126 MHz, D_2O), δ 177.0, 160.0, 56.4, 39.8.

F3-2-B (Creatinine): HRFDMS, *m/z* 114.0673 (MH^+, calcd for $C_4H_8N_3O$, 114.0668); ^{1}H NMR (500 MHz, D_2O), δ 4.06 (2H, s), 3.05 (3H, s); ^{13}C NMR (126 MHz, D_2O), δ 191.9, 172.2, 59.2, 33.0.

2.8. Sensory evaluation

Sensory evaluation was carried out by adding WSE and all other fractions (obtained by various fractionation steps) to JNS, following the method of Ueda et al. (1997) with slight modification. JNS was prepared according to Shah, Tokunaga, Kurihara, and Takahashi (2009). It was diluted with six volumes of distilled water and then subjected to sensory evaluation. The WSEs of DHF and boiled DHF, and the three dialysed fractions obtained from the WSE of DHF were dissolved in JNS at a concentration of 0.10%. The lyophilised GFC and HPLC fractions were dissolved in JNS at concentrations of 0.05% and 0.01%, respectively. After addition of test samples to JNS, the solution was heated to 60 °C in a water bath. About 50 ml of test and control solutions were served in opaque disposable plastic cups at the same time. Panel members were instructed to put an adequate volume in the mouth, and then to expectorate. The panellists were asked to judge the intensities of the test samples using a scale of 1–7, where three points were assigned to the control solution. Scoring was done on the basis of saltiness, umami, thickness, mouthfulness and continuity. Sensory evaluation was performed in the separated sensory booths. The panel was composed of three to five trained assessors (ages between 26 and 37 years) from the Food Research and Development Laboratory, Kirin Kyowa Food Co. Ltd., Ibaraki, Japan. All the panellists were trained in sensory experiments with various concentrations of