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Chemical compositions and muddy flavour/odour of protein hydrolysate from Nile tilapia and broadhead catfish mince and protein isolate

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ABSTRACT

Chemical compositions and muddy compounds in dorsal and ventral muscles of Nile tilapia and broadhead catfish were comparatively studied. On a dry weight basis, Nile tilapia was rich in protein (93.1–93.8%), whilst broadhead catfish contained protein (55.2–59.5%) and lipid (36.6–42.4%) as the major constituents. Ventral portion had higher lipid or phospholipid contents with coincidentally higher geosmin and/or 2-methylisoborneol (2-MIB) contents. Geosmin was found in mince of Nile tilapia and broadhead catfish at levels of 1.5 and 3.2 μg/kg, respectively. Broadhead catfish mince had 2-MIB at level of 0.8 μg/kg, but no 2-MIB was detected in Nile tilapia counterpart. When pre-washing and alkaline solubilisation were applied for preparing protein isolate (PI), lipid and phospholipid contents were lowered with concomitant decrease in geosmin and 2-MIB contents. Protein hydrolysate produced from PI had a lighter colour and a lower amount of muddy compounds, compared with that prepared from mince. Therefore, PI from both Nile tilapia and broadhead catfish could serve as the promising proteinaceous material, yielding protein hydrolysate with the negligible muddy odour and flavour.

1. Introduction

Nowadays, aquaculture provides approximately one-third of the world's fishery products. Muddy flavour and odour are amongst the most severe problems encountered in aquaculture and cause unacceptability by consumer as well as the reduction of market value of product. Two chemical compounds associated with muddy taints are known as geosmin and 2-methylisoborneol (2-MIB). The former compound renders an earthy pond-bottom taste, whilst the latter one is associated with a musty taste (Robin, Cravedi, Hillenweck, Deshayes, & Vallod, 2006). These metabolites produced by cyanobacteria, actinomycetes and certain fungi, are excreted into the environment (Jensen et al., 1994; Lovell & Sackey, 1973). Typically, fish readily absorbs these compounds through the gills, then transfer through the digestive tract, and finally accumulate in lipid rich tissue. Although both compounds are present at an extremely low level in fish tissue, they can cause the muddy flavour due to their low threshold values. Sensory threshold of geosmin in rainbow trout was estimated to be 0.9 μg/kg (Rohrlack, Christoffersen, & Friberg-Jensen, 2005). This is slightly higher than that of channel catfish (0.7 μg/kg) (Dionigi, Johnsen, & Vinyard, 2000; Johnsen & Kelly, 1990). Threshold value of 2-MIB in rainbow trout was 0.6 μg/kg (Persson, 1980).

To reduce muddy flavour, several strategies have been proposed. Rohani and Yunus (1994) reported that soaking of gutted tilapia in salt solution (5% w/v) for 30 min prior to deboning was able to lower muddy off-flavour of surimi to some degree as reflected by the higher hedonic score. Additionally, soaking, dipping or washing the farm-fish with tamarind pulp, lemon juice, lemon grass and banana leaf ash can also minimise muddy taint associated with fish tissue (Bakar & Hamzah, 1997; Mohsin, Bakar, & Selamat, 1999). Recently, acid- and alkaline-aided solubilisation has shown significant potential to reduce lipids and phospholipids in fish muscle. DeWitt et al. (2007) reported that 2-MIB and gesosmin spiked in channel catfish (*Ictalurus punctatus*) were effectively removed by acid and alkaline solubilisation. Due to their lipophilic nature, these muddy compounds might be localised in the lipid rich portion in fish muscle. However, little information regarding the distribution of muddy compounds in Nile tilapia and broadhead catfish muscle has been reported. Additionally, the development of muddy flavour and odour associated with hydrolysate and its reduction has not been reported. The objective of this study was to investigate the effect of muscle position and pretreatment on muddy flavour and odour associated with the muscle and protein hydrolysate produced from Nile tilapia and broadhead catfish.

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2. Materials and methods

2.1. Chemicals and reagents

Geosmin, 2-methylisoborneol (2-MIB) and isobornyl acetate with purity more than 95% were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Methanol, hexane and water were HPLC grade, and obtained from Lab-Scan (Bangkok, Thailand). Alcalase 2.4L (E.C. 3.4.21.62) with the activity of 2.4 unit/g was obtained from Novozyme (Bagsvaerd, Denmark).

2.2. Collection and preparation of fish

Fresh Nile tilapia (*Oreochromis niloticus*) and broadhead catfish (*Clarias macrocephalus*) with the age of 4-months and the body weight of 0.5–0.8 kg/fish were collected from a farm with earth ponds in Ranote, Songkhla province. Fish were transported in ice with a fish/ice ratio of 1:2 (w/w) to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand within 1 h.

Upon arrival, fish were washed with tap water and the flesh was separated manually into dorsal and ventral portions. The dark meat located at the lateral line was discarded. The portions were then minced to uniformity using a Moulinex AY46 blender (Group SEB, Lyon, France) in a walk-in-cold room (4 °C). The mince samples from both portions were placed in polyethylene bags and kept in ice not longer than 2 h before analyses.

2.3. Analyses

2.3.1. Proximate analysis

All samples were subjected to proximate analysis, including moisture, protein, lipid and ash contents following the AOAC (2000) methods with analytical No. of 950.46, 928.08, 960.39 and 920.153, respectively.

2.3.2. Determination of phospholipid content

Prior to analysis, lipid was extracted according to the method of Lee, Trevino, and Chaiyawat (1996) with a slight modification. One gram of sample was homogenised at a speed of 13,500 rpm for 2 min in 11 ml of chloroform/methanol (2:1, v/v) mixture using an IKA Labortechnik homogeniser (Selangor, Malaysia). Homogenate was then filtered using a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Two millilitres of 0.5% NaCl were added to the filtrate, vortexed and then centrifuged at 3000*g* for 3 min to separate into two phases. The chloroform phase was collected and mixed with 0.5 g of anhydrous sodium sulphate. The solvent was then liberated by nitrogen flushing until dryness.

Phospholipid content was measured based on the direct spectrophotometric measurement of complex formation between phospholipids and ammonium ferrothiocyanate as described by Stewart (1980). A standard curve was prepared using phosphatidylcholine (0–50 ppm). The phospholipid content was expressed as mg/100 g sample.

2.3.3. Determination of geosmin and 2-MIB

Geosmin and 2-MIB were extracted using vacuum distillation/liquid–liquid extraction following the method of Tanchotikul and Hsieh (1990) with a slight modification.

2.3.3.1. Vacuum distillation. Ground samples (50 g) were weighed in a round bottom flask and mixed with 12.5 ml of HPLC grade water. To the mixture, 2.5 ml of methanol containing 50 ppb isobornyl acetate were added. Flask was then equipped with vacuum distillation apparatus. The flask containing sample was heated in an EYELA water bath OSB-2000 (Tokyo, Japan) by increasing the temperature from 50 to 90 °C with heating rate of 2 °C/min. Vacuum distillation was conducted at 0.073 MPa for 45 min. Two collection tubes submerged in ice bath (0 °C) were used to trap the condensed distillate. The distillate obtained (~20 ml) was then subjected to liquid–liquid extraction.

2.3.3.2. Liquid–liquid extraction. Liquid–liquid extraction was performed to separate the tested compounds. The distillate (20 ml) was extracted with 3 ml of hexane in a separatory funnel. The extraction process was repeated for three times. The hexane layer was transferred to a 25 ml Erlenmeyer flask containing 2–3 g of anhydrous sodium sulphate and shaken well. The mixture was then filtered using a Whatman No.4 filter paper to remove sodium sulphate. The solvent in the filtrate was removed by flushing nitrogen until dryness. Volume of analytes was made up to 200 μl using hexane prior to GC–MS analysis.

2.3.3.3. Gas chromatography–mass spectrometry. Compounds were separated on a TRACE TR-WAXMS capillary column (Thermo Scientific, San Jose, CA, USA) (30 m × 0.25 mm ID, with film thickness of 0.25 μm) equipped with gas chromatography (GC) (Trace GC Ultra/ISQMS, Thermo Scientific Inc., San Jose, CA, USA). The GC conditions were operated as per the method of Yarnpakdee, Benjakul, Nalinanon, and Kristinsson (2012c). The mass spectrometers were run in the selected ion monitoring mode. Ions at m/z 95, 135 and 168 were monitored for 2-MIB, whilst 112, 126 and 182 were monitored for geosmin. The selected ion at m/z 93, 95, 121 and 136 were used to monitor for isobornyl acetate.

For semi-quantification, the calibration curve was prepared using a mixed solution of standard geosmin and 2-MIB at concentrations ranging from 25 to 400 ppb, in which isobornyl acetate (50 ppb) was used as an internal standard. A linear correlation was observed between the peak area and concentrations. The linear equation obtained was used to convert peak areas to concentrations of target compounds in the samples. The limit detection was 5 ppb for each compound.

2.4. Effect of pretreatment on chemical compositions and sensory properties of protein hydrolysate derived from the muscle of Nile tilapia and broadhead catfish

2.4.1. Preparation of fish mince and protein isolate (PI)

Flesh of Nile tilapia and broadhead catfish without lateral line was minced to uniformity using a blender. To prepare PI, the prepared mince was pre-washed and subjected to membrane separation as previously described by Yarnpakdee, Benjakul, and Kristinsson (2012a). Fish mince was homogenised with five volumes of cold distilled water (2–4 °C) at a speed of 11,000 rpm for 1 min. The homogenate was adjusted to pH 11 using 2 M NaOH and placed on ice for 60 min with a continuous stirring. The mixture was then centrifuged at 5000*g* for 10 min at 4 °C. The alkaline soluble fraction was collected and adjusted to pH 5.5 to precipitate the proteins. The mixture was then centrifuged at 10,000*g* for 20 min. The pellet obtained was referred to as 'PI'. The mince and PI were subjected to determination of total lipid, phospholipid, geosmin and 2-MIB contents as described above.

2.4.2. Production of protein hydrolysate

Mince and PI from Nile tilapia and broadhead catfish were mixed with distilled water to obtain a final protein concentration of 2% (w/v). The mixtures were then adjusted to pH 8 using 2 M NaOH. All mixtures were pre-incubated at 50 °C for 10 min prior to enzymatic hydrolysis using Alcalase at 1.1–1.3% (w/w) for Nile tilapia, and 3.8–4.3% (w/w) for broadhead catfish to obtain DH of 30%, following the method of Benjakul and Morrissey (1997). After 2 h of hydrolysis, the reactions were terminated by heating the