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Characterization of potent odorants in male giant water bug (*Lethocerus indicus* Lep. and Serv.), an important edible insect of Southeast Asia

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ABSTRACT

Potent odorants in frozen fresh (FFB) and salted boiled (SBB) male giant water bugs (*Lethocerus indicus*), or 'Maengdana' in Thai, were characterized by application of direct solvent extraction/solvent-assisted flavour evaporation (SAFE), gas chromatography–mass spectrometry (GC–MS), gas chromatography-olfactometry (GC-O), aroma extract dilution analysis (AEDA) and stable isotope dilution assays (SIDA). Twenty and 27 potent odorants were detected in FFB and SBB, respectively. Most odorants were lipid-derived compounds, including the two most abundant volatile components (*E*)-2-hexenyl acetate and (*E*)-2-hexenyl butanoate, which contributed banana-like odours. 2-Acetyl-1-pyrroline and 2-acetyl-2-thiazoline, responsible for popcorn-like odours, were detected in SBB only. An aroma reconstitution model of SBB was constructed in an oil-in-water emulsion matrix using 12 selected potent odorants based on the results of AEDA, accurate compound quantification and the calculated odour-activity values (OAV). Omission studies were carried out to verify the significance of esters, particularly (*E*)-2-hexenyl acetate was determined to be an important character-impact odorant in male giant water bug aroma.

1. Introduction

Lethocerus indicus (Lep. and Serv.), or giant water bug, is a popular edible insect in the northern and north-eastern parts of Thailand and in other parts of Southeast Asia, including Laos, Vietnam and Cambodia. Its popularity stems from the unique and desirable odour it imparts to food and food products. Only the male giant water bugs have this special odour, which is contained in its abdominal reservoir or scent gland. Currently there are two popular forms of male giant water bugs available in the local market; frozen fresh (FFB) and salted boiled (SBB), but occasionally live specimens are also available. Traditionally, the male giant water bug has been used as an essential 'flavour' component of Thai chili paste, or nam prik, and as an ingredient in some types of fish sauce. Hence, the added odour or scent of male bugs is important for consumer acceptance in those food products.

Nowadays, wild-caught giant water bugs are scarce due to habitat decline and water pollution. Meanwhile, their demand is increasing. Unfortunately, commercial farming of giant water bugs has not been successful. For this reason most of the giant water bugs available in the market are either trapped from their natural environment in Thailand or imported from neighbouring countries, such as Cambodia and Laos. To help meet demand, efforts have been made to produce artificial flavourings which mimic the odour of the giant water bug. Unfortunately, these flavourings are not very similar to the natural odour, and differences among brands are obvious to consumers. Knowledge of the key aroma compounds, responsible for the scent of the male giant water bugs, will help in the creation of superior flavourings for use in Thai cuisine and food industry.

The giant water bug has been widely studied with respect to the biochemical properties of its fibrillar flight muscle (Martin et al., 2011). Studies concerning its use as a food or food ingredient, however, are limited to nutritional quality and consumer acceptability studies (DeFoliart, 1999). Studies have been conducted on the pheromones of male giant water bug. Devakul and Maarse (1964) reported that the scent glands of male giant water bug contained mainly (*E*)-2-hexenyl acetate and (*E*)-2-hexenyl butanoate, the predominance of these two compounds was later confirmed by Mahattanatawee and Rouseff (2010) who employed headspace solid-phase microextraction (SPME), gas chromatography-olfactometry (GC-O), and GC–mass spectrometry (GC–MS) to identify the odorants, consisting mainly of aldehydes, esters and acids, in frozen whole male giant water bugs. In addition to the compounds reported in the above mentioned study, 1-undecen-3-one, 3-mercaptohexyl acetate, and 3-mercapto-1-hexanol were also

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indicated as potent odorants in the scent glands of male giant water bugs (Kiatbenjakul, Intarapichet, & Cadwallader, 2014).

Results of GC–MS and GC-O might not provide sufficient information to verify the significant aroma-active compounds in a food product. Additional studies, such as the creation and sensory analysis of aroma models, should be considered. Therefore, the objectives of this study were to apply GC-O, AEDA, and sensory evaluation techniques to characterize the chemical nature of aroma-active compounds of FFB and SBB. Potent odorants in SBB were verified by the sensory analysis of an aroma reconstitution model. FFB was not considered for aroma validation studies because it rapidly oxidized and elicited green and rancid odours after being blended, thus leading to unacceptable aroma.

2. Materials and methods

2.1. Chemicals

4-($^2H_3$-Methyl) phenol was purchased from CDN (Quebec, Canada) and [6,6,6-$^2H_3$]-hexanoic acid was purchased from Isotec (Miamisburg, OH, USA). Florisil was purchased from US Silica Company (Berkeley Springs, WV, USA). (*E*)-2-Hexenyl acetate, (*E*)-2-hexenyl butanoate and (*E*)-2-hexenoic acid were supplied by Bedoukian Research Inc. (Danbury, CT, USA). Methylene chloride (CH_2Cl_2), magnesium oxide, sodium chloride (NaCl), methanol, pentane, hydrochloric acid (HCl), sulphuric acid (H_2SO_4), sodium sulfate (Na_2SO_4, anhydrous), sodium hydroxide (NaOH, anhydrous) and sodium bicarbonate ($NaHCO_3$) were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

The following compounds were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA): diethyl ether, tetrahydrofuran (THF, anhydrous), pyridinium chlorochromate (PCC), 1.0 M of $LiAl^2H_4$, deuterium oxide, chlorotri(triphenylphosphine)rhodium(I) (Wilkinson's catalyst), (carbethoxymethylene)triphenylphosphorane, 0.5 M KOH (ethanolic), ammonium ion standard, potassium permanganate, Tris–maleate buffer, ammonium chloride, triethylamine, acetyl chloride, butyryl chloride, 3-methyl-3-buten-1-ol, 3-butyn-1-ol, 2-hexyn-1-ol, 3-buten-1-ol, 2-heptyn-1-ol, (*E*)-3-hexenoic acid, *p*-cresol, guaiacol, (*E,E*)-2,4-decadienal, hexanoic acid, 4-methylpentanoic acid, 2-acetyl-2-thiazoline, (*E,E*)-2,4-nonadienal, 2-methylbutanoic acid, 3-methylbutanoic acid, butanoic acid, (*E*)-2-nonenal, (*Z*)-2-nonenal, (*E*)-2-hexenol, (*E*)-2-hexenal, 1-octen-3-one, methional, sotolon. Deodorized distilled water was prepared by boiling glass-distilled deionized water to two-thirds of its original volume.

The following compounds were synthesized using the previous published methods: (*E*)-2-heptenyl acetate, 1-undecen-3-one, 3-mercaptohexyl acetate (Kiatbenjakul et al., 2014) and (*E*)-4, 5-epoxy-(*E*)-2-decenal (Lin, Fay, Welti, & Blank, 1999). 2-Acetyl-1-pyrroline was obtained from Dr. M. Fang (University of Illinois at Urbana-Champaign, IL, USA).

2.2. Synthesis of [2,3-$^2H_2$]-(E)-2-hexenol

The synthesis method was performed according to the procedure modified by Lin et al. (1999). In a two-neck side arm flask fitted with a reflux condenser and purged with N_2, 20 ml of 1.0 M of $LiAl^2H_4$ was added, followed by 2-hexyn-1-ol (1.57 g, 16 mmol) dissolved in 5 ml of anhydrous tetrahydrofuran (THF). The mixture was refluxed for 1 h and then stored overnight at room temperature. After cooling in an ice bath, 4.0 ml of deuterium oxide was added dropwise, followed by 20 ml of 4 N H_2SO_4 to dissolve any insoluble residue. The organic phase was separated and the aqueous solution was then extracted with diethyl ether (3 × 20 ml). The ether extract was successively washed with saturated $NaHCO_3$ (2 × 10 ml) and saturated NaCl (2 × 15 ml). The solvent was evaporated off using a Vigreaux column and the product distilled under vacuum to yield the target compound.

[2,3-$^2H_2$]-(*E*)-2-hepten-1-ol was synthesized by following the procedure described above, except for the use of 2-heptyn-1-ol (0.15 g, 1.3 mmol) as the substrate.

Yield of [2,3-$^2H_2$]-(*E*)-2-hexenol: 1.21 g (75.0%). MS-EI: *m/z* (intensity in %) 59 (100), 42 (41), 45 (35), 43 (33), 58 (32), 41 (29), 40 (23), 44 (19), 57 (19), 102 (1, M^+).

Yield of [2,3-$^2H_2$]-(*E*)-2-hepten-1-ol: 0.12 g (77.0%). MS-EI: *m/z* (intensity in %) 59 (100), 41 (43), 42 (40), 58 (40), 43 (39), 45 (38), 56 (37), 55 (28), 44 (25), 116 (2, M^+).

2.3. Synthesis of [2,3-$^2H_2$]-(E)-2-hexenyl acetate, [2,3-$^2H_2$]-(E)-2-hexenyl butanoate and [2,3-$^2H_2$]-(E)-2-heptenyl acetate

[2,3-$^2H_2$]-(*E*)-2-hexenol (0.27 g, 2.6 mmol) and triethylamine (0.35 g, 3.5 mmol) dissolved in 10 ml of CH_2Cl_2 were added to a dry 50 ml screw cap test tube equipped with N_2 purge. The solution was stirred and cooled to 0 °C in an ice-water bath, then acetyl chloride (0.28 g, 3.6 mmol) dissolved in 2 ml of CH_2Cl_2 was slowly added and continuously stirred for 2 h. Once the reaction was complete, 10 ml of deodorized distilled water was added and the mixture stirred until all the precipitate was dissolved. The CH_2Cl_2 layer was collected and extracted with diethyl ether (3 × 10 ml). The solvent extract was successively washed with 10% H_2SO_4 solution (2 × 10 ml) and aqueous saturated $NaHCO_3$ (2 × 10 ml) and distilled under vacuum to yield [2,3-$^2H_2$]-(*E*)-2-hexenyl acetate after removal of the solvent.

A similar procedure, as above, was used to synthesize [2,3-$^2H_2$]-(*E*)-2-hexenyl butanoate and [2,3-$^2H_2$]-(*E*)-2-heptenyl acetate. [2,3-$^2H_2$]-(*E*)-2-Hexenol (0.43 g, 4.2 mmol), triethylamine (0.60 g, 5.9 mmol) and butyryl chloride (0.63 g, 5.9 mmol) were used for [2,3-$^2H_2$]-(*E*)-2-hexenyl butanoate synthesis. [2,3-$^2H_2$]-(*E*)-2-Hepten-1-ol (0.12 g, 1.0 mmol) was used instead of [2,3-$^2H_2$]-(*E*)-2-hexenol for [2,3-$^2H_2$]-(*E*)-2-heptenyl acetate synthesis.

Yield of [2,3-$^2H_2$]-(*E*)-2-hexenyl acetate: 0.32 g (84.6%). MS-EI: *m/z* (intensity in %) 43 (100), 84 (14), 56 (13), 69 (13), 68 (12), 41 (10), 40 (9), 57 (9), 59 (9), 102 (8); (M^+ = 144).

Yield of [2,3-$^2H_2$]-(*E*)-2-hexenyl butanoate: 0.69 g (95.2%). MS-EI: *m/z* (intensity in %) 71 (100), 43 (75), 41 (26), 42 (25), 56 (25), 57 (16), 69 (16), 40 (13), 68 (13), 172 (1, M^+).

Yield of [2,3-$^2H_2$]-(*E*)-2-heptenyl acetate: 0.14 g (89.0%). MS-EI: *m/z* (intensity in %) 43 (100), 56 (29), 55 (16), 41 (14), 83 (13), 69 (11), 98 (10), 57 (8), 82 (8), 116 (8); (M^+ = 158).

2.4. Synthesis of [3,4-$^2H_2$]-butanoic acid and [3,4-$^2H_2$]-3-methylbutanoic acid

The compounds were synthesized according to the method described by Steinhaus and Schieberle (2005) with slight modification.

[3,4-$^2H_2$]-butan-1-ol. Chlorotri(triphenylphosphine)rhodium(I) (Wilkinson's catalyst, 0.45 g) and 3-buten-1-ol (2.5 g, 34.7 mmol) were placed in a pressure reactor equipped with stirring bar and rubber septum. The reactor was flushed for 5 min with deuterium gas (40 psi; UHP grade 99.995%; isotopic enrichment 99.7%; Matheson Tri-Gas, Parsippany, NJ, USA) using a needle, which was placed below the solution. The spent catalyst was removed by centrifugation after the reaction was complete. [3,4-$^2H_2$]-Butan-1-ol was obtained after purification by vacuum distillation.

Yield of [3,4-$^2H_2$]-butan-1-ol: 2.1 g (80.0%). MS-EI, *m/z* (intensity in %): 58 (100), 43 (88), 42 (81), 45 (81), 57 (73), 44 (55), 41 (35), 40 (28), 39 (17), 76 (1, M^+).

The labelled alcohol from above (1.14 g, 15 mmol) was added to a vigorously stirred solution of potassium permanganate (3.5 g,