Food Chemistry 122 (2010) 1327–1332

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Analysis of free and glycosidically bound compounds of ash gourd (*Benincasa hispida*): Identification of key odorants

Jyoti Sharma, Suchandra Chatterjee, Vivekanand Kumar, Prasad S. Variyar *, Arun Sharma

Food Technology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

ARTICLE INFO

Article history:
Received 9 March 2009
Received in revised form 27 October 2009
Accepted 24 March 2010

Keywords:
Acetoin
Aroma glycosides
Ash gourd
Volatile essential oil
GC–olfactometry

ABSTRACT

Free and glycosidically bound aroma compounds of ash gourd were investigated. Gas chromatography–mass spectrometry (GC/MS) analysis of the free aroma compounds isolated by both steam distillation and high vacuum distillation revealed the presence of acetoin as the major constituent in both the oils. Acetoin was also identified as the major constituent existing as glycosidic conjugate. GC–olfactometry (GC–O) of the volatile oil based on detection frequency (olfactometry global analysis) was further used to characterise odour active compounds of the vegetable. Based on odour activity value (OAV) measurements the potent odorant among these were identified as acetoin (9522.5), octanal (8571) and nonanal (24,000). The region corresponding to the characteristic ash gourd aroma when collected by cryotrapping from a packed GC column and subsequently analysed by GC/MS was found to be mainly comprising of acetoin suggesting the role of this compound as the key odour active compound of this vegetable.

1. Introduction

Ash gourd (*Benincasa hispida*), also known as winter melon, white gourd or wax gourd is a vegetable widely consumed in Indian households. Besides its use as a vegetable it is extensively used in traditional oriental medicine for treatment of various ailments such as gastrointestinal problems, respiratory diseases, heart diseases, vermifuge, diabetes mellitus and urinary diseases (Aslokar, Kakkar, & Chakre, 1992; Sivarajan & Balachandran, 1994). While the intact fresh green vegetable is nearly odourless, a characteristic fatty green and buttery odour is perceived either during cutting or cooking. Despite its wide use in culinary preparations, aroma active constituents of this vegetable have not been extensively investigated. To the best of our knowledge, only one report exists on the nature of volatile aroma compounds in this vegetable (Chung, Shu, Chang, & Chiang, 1987). The major volatile compounds reported by these researchers include (E)-2-hexenal, *n*-hexanal and *n*-hexyl formate. However, the role of the individual compounds in contributing to the characteristic odour of the vegetable was not investigated.

GC–O techniques have in recent years proved to be a powerful tool in correlating chemical information with sensory impression. Based on these techniques odour active compounds in various foods have been investigated and reported (Acree, Barnard, & Cunningham, 1984; Jordan, Tandon, Shaw, & Goodner, 2001; Ullrich & Grosch, 1993). Among the GC–O techniques, the dilution sniffing methods (Aroma Extract Dilution Analysis, AEDA and Combined Hedonic Aroma Response Measurement, CHARM) based on odour detection thresholds have the drawback of reproducibility due to a very high variability within and between panelists (Pollien et al., 1997). The odour specific magnitude estimation (OSME) method based on odour intensity, although more precise than the other methods, requires at least four sensitive assessors who are repeatable and trained in odour intensity. A more recent method based on frequency of detection (olfactometry global analysis) rather than perceived intensity aided in identifying odour active components in a given food stuff. Despite the use of untrained panel and one dilution level, repeatability of the method was demonstrated to be satisfactory and is almost twice as fast as the other methods (Guen, Prost, & Demaimay, 2000). So far, this technique has not been applied to characterise the aroma profile of ash gourd.

Besides free aroma, the potential glycosidically bound aroma compounds have been reported to occur in several food stuffs (Sarry & Gunata, 2004). Release of these compounds during storage or processing can generate modification or enhancement in aroma. Glycoconjugates thus play a crucial role in the overall food quality. No reports exist till date on glycosidically bound aroma compounds of ash gourd. This work thus aims at isolation and identification of free and bound aroma constituents of ash gourd. An attempt was made to identify aroma active constituents based on frequency response (olfactometry global analysis) as well as key odorants based on OAV as well as GC–O in combination with GC and GC/MS.

---

* Corresponding author. Tel.: +91 22 25592531; fax: +91 22 25505150/222198.
E-mail address: prasadpsv@rediffmail.com (P.S. Variyar).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.03.099

2. Materials and methods

2.1. Materials

Commercial samples of ash gourd were procured from a local market. All the standard compounds were obtained from Sigma/Aldrich chemical company, USA. Solvents (analytical reagent grade) were redistilled before use.

2.2. Methods

2.2.1. Isolation of free aroma compounds

2.2.1.1. Steam distillation. Ash gourd (200 gm) was cut into small pieces and subjected to simultaneous distillation extraction (SDE) technique (Nickerson & Likens, 1966) for 2 h using peroxide free diethyl ether (AR Grade, S.D. Fine-Chem. Ltd., Mumbai, India) as extracting solvent. The solvent was then removed by passing a slow stream of nitrogen to obtain the volatile oil.

2.2.1.2. High vacuum distillation (HVD). High vacuum distillation was carried out according to the procedure reported in literature (Bandyopadhyay, Gholap, & Sreenivasan, 1973; Gholap & Bandyopadhyay, 1977). Cut ash gourd (200 g) was frozen in liquid nitrogen before use. The sample was placed in a glass tube (5 cm i.d. × 25 cm length) and then connected to a distillation unit maintained under vacuum (1×10^{-3} torr). The distillate collected in a receiving tube (3 cm i.d. × 20 cm length) was maintained at low temperatures under liquid nitrogen. The isolate (50 ml) was extracted with diethyl ether (3 × 5 ml). Solvent was removed by a slow stream of nitrogen to obtain oil that was subjected to GC/MS analysis as described below.

2.2.2. Analysis of free aroma compounds

2.2.2.1. GC–MS analysis. The aroma concentrates obtained from SDE and HVD were subjected to GC–MS analysis on a Shimadzu GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph and provided with a DB-5 (J&W Scientific, California, USA) capillary column ((5%-phenyl)-methylpolysiloxane, length, 30 m; i.d., 0.25 mm and film thickness, 0.25 μm). The operating conditions were: column temperature programmed from 60 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min. and further to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min; Injector and interface temperatures, 210 and 280 °C, respectively; carrier gas helium (flow rate, 0.9 ml/min); ionisation voltage, 70 eV; electron multiplier voltage, 1 kV. Peaks were tentatively identified by comparing its mass fragmentation pattern with that of standard compounds wherever available, from standard spectra available in the spectral library (Wiley/NIST Libraries) of the instrument as well as by comparing RI (retention index) values of the compounds with literature data. Amounts of the identified compounds in the essential oil obtained from various extraction methods were estimated from a standard curve ($R^2 = 0.99$) of concentration versus peak area prepared using different concentrations of standard acetoin (0.01–20 μg/μl) and expressed as μg/kg of ash gourd.

2.2.3. Isolation and characterisation of major volatile oil constituent

Preparative TLC of all the SDE isolates was carried out on silica gel G plates (20 cm × 20 cm × 0.5 mm thickness) using hexane: ethyl acetate (80:20) as developing solvent system. After development, the plates were dried in air at room temperature. The band at R_f 0.28, having ash gourd aroma was scrapped and eluted with diethyl ether. Solvent was then removed by passing a slow stream of nitrogen and the pure compound was identified as acetoin by GC/MS and other spectral analyses (UV, IR, NMR) as detailed below.

UV/Vis spectrum was taken on a UV spectrophotometer (Unicam Heλios UVA, Cambridge, UK) in methanol, λ_{max}: 218 nm. IR spectrum was recorded with a JASCO FTIR 4100 spectrophotometer (Jasco Corporation, Tokyo, Japan). ν_{max} (KBr): 3404, 2987, 2939, 2884, 1714, 1644, 1452, 1380, 1187, 1116, 1024, 939 cm^{-1}. The NMR spectra were recorded with a Bruker AC-200 MHz FT NMR spectrometer (Bruker, Fallanden, Switzerland). The usual abbreviations employed are: t = triplet, q = quartet, J = coupling constant (in Hz), δ = chemical shift in ppm. ^{1}H NMR ($CDCl_3$, 200 MHz): δ ppm, 1.37 (3H, d, J = 5.8) 2.22 (3H, s) 4.42 (1H, q). ^{13}C NMR ($CDCl_3$, 200 MHz): δ ppm, 18, 72, 107, 210. EIMS was recorded using DI probe facility in the GC/MS equipment described above. m/z (%, rel. int.) 88(M^+, 1.4), 73.05(33.41), 61(30.62), 45(100), 44.05(21.33), 43(93.43), 42.05(8.86).

2.2.4. Isolation of aroma glycosides

Ash gourd (1 kg) was cut in small pieces and soaked in aqueous methanol overnight. After filtration the remaining ash gourd was crushed in omni mixer and extracted twice with aqueous methanol. These extracts were pooled and concentrated under vacuum (760 mm Hg) on a rotary evaporator (35 °C) to obtain an aqueous solution. The aqueous solution so obtained was washed with diethyl ether to remove traces of lipophilic substances including free aroma compounds and subsequently passed through an Amberlite XAD-2 column according to the procedure reported earlier (Gunata, Bayonove, Baumes, & Cordonnier, 1985). The bound aroma precursors were desorbed from the resin with methanol as eluting solvent, evaporated to dryness under vacuum and the residue was made to 2% in methanol (w/v).

2.2.5. Separation and estimation of aroma glycosides

2.2.5.1. TLC analysis. Analytical TLC was carried out on ammonium sulphate impregnated silica gel G plate (10 cm × 25 cm × 0.25 mm thickness) using toluene:ethanol:formic acid (60:35:5, v/v/v) as the developing solvent system. The separated spots were visualised by heating the plate in oven at 120 °C for 20 min. Preparative TLC was carried out on silica gel plates (20 cm × 20 cm × 0.5 mm thickness) using the same solvent system as above. Five distinctly resolved bands were scrapped and eluted with methanol. The eluate was evaporated to dryness to make 1% solution (w/v).

2.2.5.2. Estimation by densitometry. Density of the individual spots/bands was determined on a dual wavelength flying spot scanning densitometer Shimadzu CS-9301PC (Shimadzu, Kyoto, Japan). The density of the spots was determined in the reflectance mode at a wavelength of 528 nm. Acetoin glucoside was used as external standard. Aliquots of suitably diluted sample were spotted on the plate in increasing concentration ranging from 0.05 to 6 mg/ml. The amount of different bands was estimated from a standard curve ($R^2 = 0.99$) of spot density versus concentration and expressed as mg/kg of ash gourd.

2.2.6. Analysis of bound aroma compounds

The XAD extract was subjected to acid hydrolysis (1 N HCl, 1 h, 80 °C). The hydrolysate was extracted with diethyl ether (3 × 20 ml) and made free of acid by repeated washing with distilled water. The organic layer was dried over sodium sulphate and then concentrated for subsequent identification of the aglycone moiety on GC/MS. The five bands isolated from preparative TLC as described above were also subjected to acid hydrolysis. The free volatiles obtained after subsequent workup of each band were also analysed by GC/MS. The remaining aqueous solution from the total XAD eluate as well as individual bands was neutralised with 1 N KOH, dried under vacuum and the residue dissolved in methanol. This methanol solution was subjected to TLC in order to identify the sugar residue. The sugar residue obtained from the