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Odour-active compounds of an *Eisenia foetida* protein powder. Identification and effect of delipidation on the odour profile

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ABSTRACT

The odour-active compounds (OAC) of an *Eisenia foetida* (Ef) protein powder were determined using a detection frequency gas chromatography–olfactometry method. They were isolated by a Solvent-Assisted Flavour Evaporation Extraction (SAFE) method. Sixty-eight compounds were designated as OAC, 41 of which were identified. Fifty-one percent of the identified compounds came from lipid oxidation. A Soxhlet delipidation method, with hexane, and an ultrasound extraction method, with a chloroform/methanol mixture, were used in order to dearomatize the Ef protein powder. The effect of delipidation on the OAC was studied. The Soxhlet and the ultrasound methods eliminated 70.5% and 97.7% of the OAC, respectively. The Ef protein powder was characterised by animal, chemical, earthy, floral, fruity and vegetable attributes. Soxhlet extraction reduced the vegetable and the herbaceous attributes, whereas ultrasound extraction reduced the chemical, earthy, floral, fruity and Maillard attributes.

1. Introduction

Concentrated protein powder, obtained from *Eisenia foetida* (Ef) earthworms, is a potential source of food protein. In fact, Ef protein powder contains about 62% of protein and presents a good composition of essential amino acids (Velásquez, Herrera, & Ibañez, 1986). The cited authors also reported that processed worm meat presents no microbiological risk to humans. Medina et al. (2003) demonstrated that the Ef proteins were not toxic to human cell lines at low concentrations: they reported that, at 0.75 μg/well of Ef protein, 96% viability was observed. They determined the toxic metal content of the Ef protein powder and showed that the levels of lead and mercury were low and comparable to those of fish.

Cayot et al. (2009) prepared maize-based pancakes fortified with this novel protein powder and showed that, above a substitution level of 5.5% (w/w), the pan-cakes were rejected by the panellists because of the bad odour of the Ef protein powder. This substitution level is below the nutritional target (13%) chosen in the study of Cayot et al. (2009). This amount corresponds to 1/4 of the minimal protein intake recommended by the WHO for a 10 year-old child, taking into consideration that a child eats one 50 gram pancake per day. In order to reach the nutritional target, the organoleptic quality of the raw protein powder must be improved by reducing the off-flavour. Off-flavours present in food may be formed during storage due to microbiological deterioration. They may arise from the animal feed or may be formed by the animal's metabolism. They may also come from environmental pollution or from food processing (Belitz, Grosch, & Schieberle, 2009).

Gas chromatography–olfactometry is a technique based on sensory evaluation of the eluate from a chromatographic column. It has been used to identify key aroma compounds and off-flavours in several food products (Grosch, 1994). Three main methods can be used to characterise the odour activity of the volatile compounds: detection frequency, dilution to threshold and direct intensity (Delahunty, Eyres, & Dufour, 2006). In comparison with the other methods, frequency detection presents the following advantages: (i) simplicity, since qualified evaluators are not required (ii) repeatability (iii) the least time consuming (Plutowska & Wardencki, 2008).

The chemical or enzymatic degradation of lipids is the main origin of off-flavours in food. In meat, lipids act as solvents for the volatile compounds that develop during production, handling, and thermal processing (Moody, 1983). It has been reported that levels of fat above 7.3% in meat may have a negative effect on flavour perception and acceptability (Calkins & Hodgen, 2007).

In this work, a detection frequency GC–olfactometry method was used to identify the key aroma compounds in Ef protein powder. Delipidation was used to dearomatize the powder, and the effect of delipidation on the aroma-active compounds was studied.

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2. Materials and methods

2.1. Chemicals

Hexane (⩾96.5%) was purchased from Sigma–Aldrich (Saint–Quentin Fallavier, France), chloroform (>99%) from Aldrich (Saint–Quentin Fallavier, France) and methanol (>99.9%) from Carlo Erba Reagents (Val De Reuil, France). Dichloromethane (>99.8%), from Carlo Erba (Val De Reuil, France), was distilled before use. The chromatographic internal standard, 2-methylpropyl 3-methylbutanoate (⩾98.0%), was purchased from Sigma–Aldrich (Saint–Quentin Fallavier, France). Deionised water was obtained from a Milli-Q RG, Millipore apparatus (Millipore SAS, Molsheim, France).

2.2. Preparation of the earthworm protein powder

The earthworm protein powder was kindly donated by Professor Ana Luisa Medina (*Los Andes* University, Merida–Venezuela). Samples were obtained from the species *E. foetida* at an adult stage of development (3 months), with an average length and weight of 8.5 cm and 0.45 g, respectively (earthworm cultures from "Luis Ruiz Terán" Herbarium at the Faculty of Pharmacy, University of the Andes, Merida-Venezuela). The earthworms were fed on a diet of organic waste compost, obtained from a university canteen in the region. In order to guarantee optimum growth conditions, the temperature, moisture and pH of the compost were kept under control. The earthworms were thoroughly washed and were subsequently stored for 12 h in an air suffused water container. The previously washed earthworms were put in boiling water for 1 min to kill them. Excess water was then removed and the earthworms were dried in an oven at 60 °C for four hours and finally ground to a homogeneous flour. The dried earthworm protein powder obtained (regular protein powder = RPP) was placed in closed plastic bags and stored at 4 °C prior to further analyses.

2.3. Soxhlet delipidation of the RPP

Soxhlet delipidation was done, following the official method (Horwitz, AOAC, 1980). Thirty grammes of RPP were put into an extraction thimble and covered with cotton to avoid the loss of the sample. The thimble was then inserted into a Soxhlet device, which was filled with 225 ml of hexane. The Soxhlet ran for 8 h. After this time, the Soxhlet-delipidated protein powder (SDPP) was put into a watch glass and the hexane was evaporated overnight at room temperature.

2.4. Ultrasound delipidation of the RPP

The ultrasound extraction experiment was performed using a US bath Bransonic Mod 3210 (Branson Europe B.V.) with an ultrasound fixed-frequency of 47 kHz ± 6%. Thirty grammes of RPP were extracted with 150 ml of a mixture of solvents (chloroform/methanol in a ratio 87/13) for 3 min in an ultrasound bath. After the first extraction step, the delipidated protein powder (DPP) was filtered off under vacuum and the solvent was evaporated at 45 °C under reduced pressure. This process was repeated five times, using the DPP obtained from the previous step of extraction. The powder obtained after five extraction steps was designated ultrasound delipidation protein powder (UDPP).

2.5. Solvent-assisted flavour evaporation extraction (SAFE extraction)

Twenty grammes of sample earthworm powder (RPP, SDPP or UDPP) was mixed with 150 ml of Milli-Q water. The mixture was placed in the SAFE apparatus (Engel, Bahr, & Schieberle, 1999), and vacuum distillation (10^{-2} Pa) was performed for 2 h at 30 °C. The volatile compounds of the aqueous phase were extracted three times with 15 ml of distilled dichloromethane. After the two-phase separation, the organic phase was collected and filtered through glass wool, dried over anhydrous sodium sulphate, and concentrated to 220 μl, using a Kuderna–Danish apparatus in a water bath at 70 °C.

2-Methylpropyl 3-methylbutanoate was dissolved in dichloromethane and was used as the internal standard at a concentration of 2.33 μg/μl. One μl of the internal standard was added to 100 μl of the extract immediately before GC–MS analysis.

2.6. GCO, Gas chromatography–olfactometry

The concentration of the extracts used for GCO analysis (1/200) was chosen in order to ensure correct detection by FID and perception by panellists. One μl of the concentrated extract was injected into a Hewlett–Packard 5890 Series II chromatograph equipped with a split–splitless injector (J&W Scientific Inc., Folsom, CA) and capillary column (30 m × 0.32 mm ID, 0.5 μm film thickness) coated with a stationary phase DB-Wax (J&W Scientific, USA). The column was connected to both a flame ionisation detector and a sniffing port. Helium was used as the carrier gas with a constant flow of 1.5 ml/min, with a linear velocity of 44 cm/s. The injector and detector temperatures were maintained at 240 °C, and the oven temperature was programmed from 40 to 240 °C at 5 °C/min. Humidified air was added via the sniffing port at 100 ml/min. Homemade software COCONUT (R. Almanza and P. Mielle, INRA) was used for data acquisition. GCO frequency analysis was performed, following the methodology described by Charles et al. (1999). A panel of 7 judges (4 women and 3 men) was selected amongst people already experienced in GCO. Each judge repeated the analysis twice. Assessors were asked to smell the effluent of the column (35 min analysis) and to give the start time and a verbal description of each perceived odour, even if they did not recognise the odour (descriptor = unknown). The detection frequency (DF) for an odour with the same retention index and a similar description was calculated (sum of the number of odour detections at a given retention index). A solution of hydrocarbons (C_8–C_{30}) was injected daily, under the same conditions, to calculate retention indices.

2.7. GC–MS. (Gas chromatography–mass spectrometry)

The volatile compounds were identified using a GC–MS (model 5973 Agilent Co., Palo Alto, CA) with the same column and chromatographic conditions as previously described. MS was taken at 70 eV and a scan range between 29 and 350 amu. Compounds were identified by comparison with standard mass spectra (when available) of several libraries (Wiley, NIST, and INRA database) and by the calculation and comparison of the GC retention index with those found in published data calculated under the same conditions (Bianchi, Careri, Mangia, & Musci, 2007; Farkas, Le Quéré, Maarse, & Kovác, 1994; Kondjoyan & Berdagué, 1996). The quantitative data were obtained by electronic integration of the TIC.

3. Results and discussion

3.1. Quantitative and qualitative description of the odour-active compounds

About seventy volatile compounds of the RPP were identified in a previous paper (Cayot et al., 2009) after SAFE extraction. GCO frequency analysis was used in this study in order to identify the odour-active compounds (OAC). The main advantages of this meth-