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Original article

Physico-chemical characterisation of a non-conventional food protein source from earthworms and sensory impact in *arepas*

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Summary This study aimed to characterise a non-conventional protein source: a powder made from earthworms, and to evaluate its potential use as human food. The way it was prepared led to low solubility and wide particle size distribution. Sensory analysis was used to assess the acceptability and the organoleptic properties of maize-based pan-cakes fortified with this novel protein powder. Satisfying products were obtained with a substitution level of 5.5% (w/w) earthworm powder in pan-cakes. GC-MS allowed the identification of more than seventy volatile compounds that may be responsible for the off-odour of the powder. The most abundant chemical groups found in the volatile fraction were ketones with undecan-2-one, alcohols with pentan-1-ol, and aldehydes with hexanal. Partial delipidation was tested as a way to improve the organoleptic quality of the powder. It clearly led to less intensely coloured samples but delipidation must be optimised to have an impact on odour.

Keywords Biomass utilisation, flavour biogenesis, food fortification, non-conventional proteins, physico-chemical properties, sensory evaluation.

Introduction

The importance of proteins in people's diet is well established. Nowadays, the overall demand for protein is increasing with 5 billion people wanting more animal protein in their diet.

In many countries, traditional diets are based on cereals and must be completed by other protein sources. On the basis of their protein digestibility corrected amino acid score (PD-CAAS), the nutritional value of animal proteins is far higher than that of vegetable proteins (Mosha & Bennik, 2005). For example, in Venezuela, the staple diet is based on maize which has a PD-CAAS lower than 0.6, i.e. lower than the minimum value recommended by the FAO/WHO/UNU (WHO, 2007). The addition of red or black beans to the diet compensates for the essential amino acids that are missing in maize. Nevertheless, the nutritional score of beans is quite low and is, moreover, insufficient for a correct diet for children. As there is mounting pressure on the earth's land and water resources to produce livestock, poultry, and fish feed, non-conventional animal protein sources must be explored to increase world food protein production. In the present paper, the potential of *Eisenia foetida* earthworms as protein source is presented.

Several authors (Marconi *et al.*, 2002; Araujo & Beserra, 2007) reported that some indigenous people in Amazonia are used to eat various species of invertebrates such as earthworms. Additionally, different studies conducted on *Eisenia foetida* earthworm, a species of domesticated earthworm, reported that the content of essential amino acids was higher than that recommended by the FAO, except for tyrosine (Vielma-Rondon *et al.*, 2003). *Eisenia foetida,* brings together many advantages including a high rate of protein synthesis (35.7% protein conversion efficiency; Garg *et al.*, 2005) and a high protein content (62% dry weight; Vielma-Rondon *et al.*, 2003). Different authors underlined the great potential of this protein source as biomass for breeding aquarium fish (Kostecka & Paczka, 2006), or catfish (Ghosh, 2004), and for diet formulations for livestock (Edwards, 1983; Ibáñez *et al.*, 1993). Moreover, Zhenjun *et al.* (1997) suggested that

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earthworm could be of a great nutritional interest for human food. In the present study, the use of this non-conventional protein source is being tested for fortification of a traditional Venezuelan food: maize-based *arepas*, a sort of thick maize-based pancake. Calculations on protein content were done considering that minimum recommended intake is 15 g J^{-1} for a 4-year-old child of 17 kg to 27 g J^{-1} for a 10-year-old child of 31 kg (WHO, 2007). We considered that the average weight of an *arepa* was 50 g and that one *arepa* should bring ¼ of the minimal protein intake. As the average content of protein was 7% and 70% for maize flour and for the non-conventional protein powder respectively, our nutritional objective was to supplement maize flour with protein powder of *Eisenia foetida* from 2% to 12.4%. Parameters linked with food safety were measured on this non-conventional protein source. Medina *et al*. (2003) reported that this protein source does not contain significant levels of heavy metals such as lead or mercury, and that *Eisenia foetida* proteins were not toxic to a human cell line at low concentrations. The absence of pathogens was also checked in the samples.

The aim of the present study was to characterise this novel source of protein and to evaluate its potential as a human food. For this purpose, different standard methods were used to develop a physicochemical description of the earthworm powder. These physicochemical characteristics, and especially the solubility of the powder, were linked to its potential use in food formulation. The low solubility measured in this study indicated that this powder had no functional properties and could not be used to make foams, gels or emulsions. We thus chose to use it in a dry mixture with maize flour for the preparation of a pancake type food (*arepa*). Sensory analysis was then used to assess the acceptability and organoleptic properties of the fortified *arepas*. The objectives of the sensory analyses were more precisely to determine the acceptable threshold of fortification of maize-based *arepas* with regular dried protein powder, and to verify if the acceptability could be improved by the use of delipidated dried protein powder. In fact, phenomena such as oxidation and enzymatic degradation of lipids may be responsible for the appearance of off-flavours and have been reported for various products. For example, many volatile flavour compounds in commercial oil-free soybean lecithin are attributed to the autoxidative decomposition products of unsaturated fatty acids of phospholipids (Kim *et al*., 1984). In fish flesh, Sérot *et al*. (2002) reported that aliphatic aldehydes commonly derive from lipid oxidation. They underlined that autoxidation of unsaturated fatty acids can be initiated by a physical catalyst such as light, or by enzymes or microorganisms present in fish flesh. In an extensive study on volatile aldehydes in smoked fish, Varlet *et al*. (2007) reported that the large quantities of *n*-alkanals found in smoked fish flesh (heptanal and hexadecanal, for example) could be related to the large amounts of their lipidic precursors found in unsmoked fish flesh. In the same study, the aroma descriptor 'fishy' was found for heptanal and E-2-nonenal. In the present study, particular attention was paid to the identification of the volatile compounds extracted from the protein powder in order to identify the origin of rejection.

Materials and methods

Preparation of protein powder: from the compost to the dried earthworm powder

Samples were obtained from the species *Eisenia foetida* at the adult stage of development (3 months), with 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. A single batch of earthworm powder was prepared as follows and used all along the study. Before drying and further uses, the earthworms were thoroughly washed and were subsequently stored for 12 h in a water bath with bubbling air. The previously washed earthworms were put in boiling water for 1 min to kill them. The earthworms were removed from the boiling water with a sieve and dried in an oven at 60 °C for 4 h. The dried earthworms were finally ground with a mechanical crusher. The obtained dried powder rich in protein was placed in closed plastic bags and stored at 4 °C until further analyses. The process was chosen on economic criteria (as cheap as possible) and for the simplicity of the required equipment.

Chemicals

Solvents (diethyl ether, ethanol, hexane), series of alkanes, potassium iodide, propyl gallate sodium thiosulfate, malonaldehyde, thiobarbituric acid, acetic acid, 1,1,3,3-tetramethoxypropane, potassium hydroxide, phosphate buffer, sodium chloride and anhydrous sodium sulphate were all purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Dichloromethane (≥99.8%) from Carlo Erba Reagents (Val de Reuil, France) was distilled before use.

Chemical analyses

The following analyses were done using validated methods (AOAC, 1999): Water content was determined as the loss of mass after drying in an oven (104 °C) until

constant weight was obtained. Ashes were weighed after incineration of samples at 600 °C until white ashes were obtained. Crude protein content was obtained from the Kjeldahl method using a conversion factor of 6.25. This factor was calculated according to the total proportion of each amino acid (Vielma-Rondon *et al.*, 2003). This value of 6.25 is also used to calculate the protein content in meat. Crude fat content was measured with the Soxhlet method by weighting fat after diethyl-ether extraction. All chemical analyses were done in triplicate.

Peroxide index and free fatty acid value are the most frequently determined quality parameters during oil production and storage (Saad *et al.*, 2006). It was used to check the quality of lipids extracted from the dried earthworm powder. The peroxide index of the fat extracted from the earthworm powder was determined by adding an excess of potassium iodide (KI) [American Oil Chemist's Society (AOCS) Official Method Cd-8-53, 1989; AFNOR method ISO 3961, 1996]. The peroxide index was measured on the fat extracted from the earthworm powder using Soxhlet extraction with antioxidant addition (propyl gallate). The peroxide index was also evaluated directly by dispersing 0.5 g of earthworm powder in a mixture of chloroform, pure acetic acid and a saturated aqueous solution of potassium iodide (2/2/1 in volume). In the presence of peroxide, iodide ions (I^-) are transformed first into iodine and then into iodine ions ($I_3^- = I_2 + I^-$), which make complexes with amylose and produce a blue colour. The iodine ions are measured out by sodium thiosulfate. In order to confirm the results, the possible peroxidation of the powder was evaluated using malonaldehyde. After co-distillation, an aliquot of the distillate is added to thiobarbituric acid. The presence of aldehyde, the end product of the reaction of unsaturated lipid peroxidation (Frankel & Neff, 1983), gives a purple colour. The quantity was evaluated by spectrophotometry at 538 nm following calibration with 1,1,3,3-tetramethoxyproprane, which is a malonaldehyde precursor (Sørensen & Jørgensen, 1996). The free acid value of fat was determined by titration in ethanol with KOH (AOCS method Ca 5a-40, 1997; Barthet *et al.*, 2008).

Particle size of the dried earthworm powder

Particle size was measured by sifting and, for the finest particles, using a laser scattering device. A representative sample of 5 g of dried powder was put in the sifting device (AS200 basic, Retsch, Haan, Germany) constituted of six sieves whose pore size ranged from 3150 μm to 200 μm. The dried powder was sifted for 5 min with vibrations at 60 movements/min. The fraction collected for each sieve was weighed. Estimates of particle size distribution of the finest particles of the dried powder collected from sifting (diameter < 200 μm) was obtained using a Mastersizer MS20 – Hydro2000G laser scattering device (Malvern Instruments, Malvern, Worcestershire, UK). The powder was dispersed in a 0.4 M phosphate buffer at pH 6.5 with 0.1 M NaCl. The suspension was stirred throughout the measurement. The suspended particles were measured at ambient temperature with obscuration set at 1–10% to calculate the particle size distribution. The particle sizes were estimated using the Fraunhofer theory. At first, the volume median diameter [d (v, 0.5)] (the diameter where 50% of the distribution is above and 50% is below) was used to characterise the particle size of the powder, then the equivalent surface area mean diameter or the Sauter mean diameter of particles, d 3,2, was calculated according to the Sauter mean given by Equation 1:

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

n_i is the number of particles with d_i diameter

Functional properties of the earthworm protein powder

Water solubility index of the dried earthworm powder

The water solubility index (WSI) of the dried earthworm powder was measured using the method reported by Singh *et al.* (2005) and corresponded to the maximum capacity of powder dispersion. Aqueous suspensions of 4% (w/v) dried powder in 0.4 M phosphate buffer at different pH (6.0, 6.5 and 7.0) and with different NaCl contents (0 and 0.1 M) were stirred at room temperature for 15 min. Samples was then centrifuged at a relative centrifuge force of 700 ***g*** for 10 min in a centrifuge (Sorvall SS-34 rotor and Sorvall RC6 plus centrifuge; Thermo Scientific, Waltham, MA, USA). An aliquot of the supernatant was dried for 48 h in an oven at 105 °C and left to cool in a dessiccator before weighting. WSI was reported as the ratio [(dry matter of the supernatant)/(dry matter of the suspension)] × 100. WSI was calculated using three replications of the experiment.

The ability to remain dispersed in a viscous aqueous phase (Singh *et al.*, 2005) was observed using suspensions of the earthworm powder (20 g L^{-1}) in phosphate buffer (0.4 M; pH 6.5) with 0.1 M of sodium chloride and at different xanthan concentrations: 0%, 0.05%, 0.1%, 0.2%, 0.4% and 0.5% (w/v). Sodium azide (0.01%) was added to ensure the preservation of the sample and allow the visual observation of the suspensions during 48 h at 20 °C. Centrifugation (1000 ***g***, 3 min) was also performed to check the stability of the suspensions. The optical density (500 nm) was recorded after 48 h of storage or after centrifugation to measure the turbidity. A stability index (SI) was calculated as followed using three replications of the experiment: $SI = OD_{48\ h}/OD_i$ or $SI = OD_{1000\ g}/OD_i$, with OD_i the optical density just after mixing powder and xanthan solution.

Solubility of proteins
The solubility of proteins was compared for the dried earthworm powder and for the finest fraction of this powder (diameter < 200 μm) assuming that the finest fraction could be more soluble. Aqueous suspensions of 40 g of dried earthworm powder in 1 L of 0.4 M phosphate buffer at pH 6.5 and (with or without 0.1 M NaCl) were stirred at room temperature for 1 h to ensure complete dispersion before analysis (Linden & Lorient, 1994). Samples were then centrifuged at a relative centrifugal force of 700 ***g*** for 10 min in a centrifuge. Protein content in the supernatants was measured by the Kjeldahl method using three replications of the experiment.

Delipidation of the protein powder
Delipidation was done using a hexane method as following: a sample of 250 g was added into 300 mL of hexane and vortex for 15 min. After that, the sample was filtered through Buchner's funnel. Immediately, the powder was dried at 60 °C in oven for 6 h. The dried delipidated protein powder obtained was placed in closed plastic bags and stored at 4 °C until sensory analyses.

Extraction of volatile compounds from the dried protein powder and GC-MS analysis

Solvent assisted flavour evaporation extraction
About 20 g of sample (dried earthworm powder) was mixed with 150 mL of Milli-Q water. The mix was introduced into the solvent assisted flavour evaporation extraction (SAFE) apparatus (Engel *et al*., 1999) and vacuum distillation (10^{-2} Pa) was performed for 2 h at 30 °C. The water phase containing the volatile compounds was stirred three times with 15 mL of distilled dichloromethane. After liquid-liquid separation, the organic phase was collected, filtered through glass wool, and dried over anhydrous sodium sulphate. The extract was concentrated to 222 μL using a Kuderna-Danish apparatus in a 70 °C water bath.

GC-MS analysis
The extract (1 μL) was injected in a splitless mode of an Agilent 6890 series chromatograph (Agilent Technologies, Massy, France). The temperature of the injector was 240 °C. A fused-silica capillary column (30 m × 0.32 mm ID, 0.5 μm film thickness) coated with a stationary phase DB-Wax (J & W Scientific, Folsom, CA, USA) was used. Helium was used as the carrier gas and the chromatographic temperature was programmed from 40 °C to 240 °C at a rate of 4 °C min^{-1}, with a final isotherm of 20 min. Mass spectrometry was performed on a mass selective detector model 5973 (Agilent Technologies) operated on the electron ionisation mode. Mass spectrometry was taken at 70 eV and a scan range between 29 and 350 amu. Ion source was set at 230 °C and transfer line at 240 °C. Compounds were identified by comparison with mass spectra libraries (WILLEY138, NIST, and INRA database) and by the calculation and comparison of the GC retention index of a series of alkanes (C_8–C_{30}) with the retention index from published data calculated under the same conditions.

Table 1 Composition* of the fortified *arepas* for a 300 g powder mix

Sample labels	A	B	C	D	E	F	G
% of fortification	2	2.8	3.9	5.5	7.7	10.8	15.1
Earthworm powder (g)	6	8	12	17	23	32	45
Whole maize flour (g)	294	292	288	282	277	268	255

Product C, D and E (grey columns) were made either with regular protein powder or with partially delipidated protein powder.
*Water was added to dough up to obtain the desired texture.

Preparation of arepas
Integral maize flour and salt were purchased at a local market and were used to make the *arepas*. A given percentage of maize flour was replaced by protein powder as indicated in Table 1. Powders were mixed together manually. As much water as necessary was then added to obtain the required texture i.e. to obtain homogeneous soft dough. Dough was kneaded with hands to form a ball. Dough was divided into balls of about 50 g, each of which was then flattened. The pancakes were cooked in a lightly greased pan until golden. Each *arepa* was divided into four parts and one quarter of each type of *arepa* was immediately served to the sensory panel. A new batch of pan-cake dough was used for each sensory session.

Sensory analyses
For the sensory panel, twenty students and staff (mean age = 26.2 years, five men and fifteen women) were recruited from ULA, Merida, Venezuela. All of them were used to eating *arepas*. The participants were volunteers and they were informed that the aim of the study was to taste *arepas* that may contain earthworm protein powder. Sessions of 30 min were organised before lunch time in a sensory laboratory room fulfilling the general requirements of the International Standard Organization (ISO 8589 1998), in individual booths, at the Faculty of Medicine of ULA.

The samples, approximately 12 g each, were presented to the assessors in three-digit coded plastic plates (diameter 10 cm) in random order. With the samples, the panellists received a cup of room-temperature spring water to cleanse their palates.

Seven samples of *arepas* fortified with various regular earthworm powder (log progression), corresponding to samples A–G in Table 1, were evaluated in the first session. Samples were classified from the worst to the best preferred. The ranks sum was calculated and compared

with the least significant difference. Statistical significance was established using the theoretical Chi-square table at 5% (O'Mahony, 1986). The acceptable threshold was determined from the results of the first session.

A second session was then organised to determine the impact of delipidation on the acceptability of the *arepas* fortified with earthworm powder. The fortification levels tested were chosen around the acceptable threshold previously determined. Six samples of *arepas* fortified with regular or delipidated earthworm powder, corresponding to samples C–E in Table 1, were classified following the same procedure as for session I. Assessors were then asked to score each sample on scales structured in five categories from 'not intense enough' (score = 1) to 'too intense' (score = 5) for taste, odour, colour, hardness and crunchiness. Results were analysed on the basis of the mean score obtained for each descriptor.

Results and discussion

Chemical analyses and particle size of the dried earthworm powder

Moisture, ashes, crude protein and crude fat contents were reported in Table 2 for regular dried protein powder and delipidated protein powder. The results obtained for the regular powder were consistent with already published data (Vielma *et al.*, 2003; Vielma-Rondon & Medina, 2006).

It is noticeable that delipidation was done using a rapid method and that a significant amount of lipids remained in the 'delipidated' protein powder. Such a result is not surprising and was already reported in the case of delipidation of milk powder, for example (Clarke & Augustin, 2005). The extracted lipid fraction was characterised by its free fatty acid index and its peroxide index. With a peroxide value of 634 μg g^{-1} of active oxygen (79 meq kg^{-1}), the earthworm powder appeared to be peroxidised as compared to other values reported in the literature (fresh fats gave a peroxide value inferior at 13 meq kg^{-1}; Saad *et al.*, 2006). As example, fresh sunflower oil gave a peroxide index of 117 μg g^{-1} (14.6 meq kg^{-1}), and 5-year-old sunflower oil (kept at room temperature without light) gave a peroxide index of 1443 μg g^{-1} (180 meq kg^{-1}). The malonaldehyde test confirmed the low peroxidation of the powder: 7 nM g^{-1} of powder (less than one with fresh oil, nearly twenty with old oil). Moreover, the presence of free fatty acid was low: 5.9 ± 0.9 mg of KOH per gram of the oil extracted from earthworm powder. The analysis of fatty acids reported by Vielma *et al.* (2003) showed that saturated, monounsaturated and polyunsaturated fatty acid residues represented respectively 5.11, 3.14, and 0.92 g/100 g of the regular protein powder. The relatively low proportion of polyunsaturated fatty acid residues (10% (w/w)) explains the low sensitivity of earthworm flesh to peroxidation during drying.

Considering particle size distribution shown in Figs 1 and 2, the powder appears to be quite heterogeneous. Eighty percent of the particles were found to be equal or below 500 μm and about 25% were below 200 μm. For the finest fraction, laser scattering showed a monomodal distribution centred on mean diameter of 250 μm. The mean diameter should have been below 200 μm but the sieve did not perfectly stop the particles that were a little bigger than 200 μm probably because they were not spherical. Additionally, the results obtained with laser scattering were calculated with an algorithm adapted to spherical particles and, thus, giving overrated mean diameter in the present case (Mühlenweg & Hirleman, 1998). These characteristics of particle size distribution correspond to a heterogeneous non-caking powder that is sensitive to segregation and contains dust.

Table 2 Proximal analysis of regular and partially delipidated dried protein powder (mean values of three replicates ± standard deviation except for peroxide index)

	Regular dried protein powder	Delipidated dried protein powder
Water content (% of wet weight)	5.36 ± 0.31	6.88 ± 0.40
Ashes (% of wet weight)	3.60 ± 0.12	3.94 ± 0.04
Crude protein (% of wet weight)	65.55 ± 0.38	63.68 ± 0.49
Crude fat (% of wet weight)	10.52 ± 0.61	4.99 ± 0.14
Free fatty acid value (milligram of KOH per gram of extracted lipids)	5.9 ± 0.9	nd
Peroxide index (active O_2 in μg g^{-1} of extracted lipids)	634	nd

nd = not determined.

Functional properties of the earthworm protein powder

Functional properties of the regular protein powder were evaluated through WSI and protein solubility. WSI

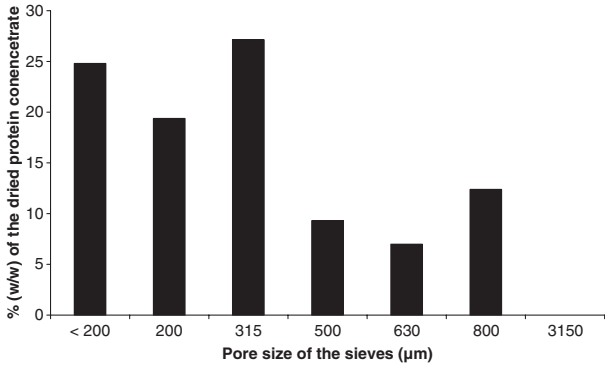

Figure 1 Particle size distribution obtained by sifting for the regular dried protein powder.

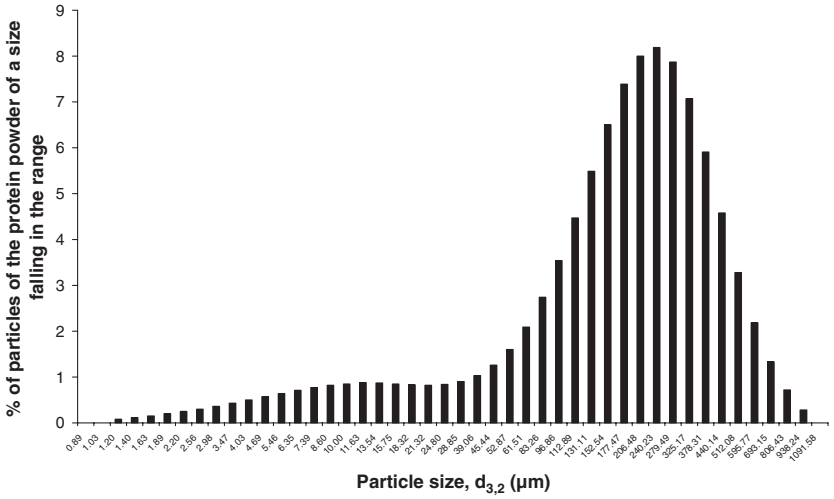

Figure 2 Particle size distribution obtained by laser scattering for the regular dried protein powder.

ranged between 30% and 40% depending on pH and ionic strength. The protein content in the supernatant was low: from 10% to 15% between pH 6 and 7, and from 18% to 33% with 0.1 M NaCl. The solubility of the proteins was not improved for the finest fraction of the protein powder. By comparison, sodium caseinates in neutral buffer with or without sodium chloride (0.1 M) are totally soluble (Strange *et al*., 1994). These results raised the problem of the future uses of the protein powder. If not soluble, the protein could not be used to make gels, foams or emulsions. Because of low solubility, the only possible use is dispersion in viscous food systems. The dispersion of the earthworm powder was stable after 48 h or after centrifugation at 1000 ***g*** (3 min) with a minimum of 0.4% of xanthan (the stability index was near 85% in the two cases). It corresponded to a dynamic viscosity value of 0.6 Pas and indicated that the only possible use in cooking was to mix it in a dough (Linden & Lorient, 1994). Some applications such as fortification of viscous food products by substitution of part of the traditional ingredients may be tested. This was done in the present study for the fortification of *arepas* by partial substitution of maize flour in the dough.

Volatile compounds of the dried protein powder

One of the problems when a given ingredient is substituted by another one is the stability of the sensory properties of the final product. Among the sensory properties, odour is one of the most important characteristics because volatile compounds may reach the olfactory epithelium of the consumer before eating. The volatile fraction of the protein powder was analysed as a first step for odour analysis. The SAFE extraction method, used in this work to obtain the volatile fraction from the regular protein powder, is

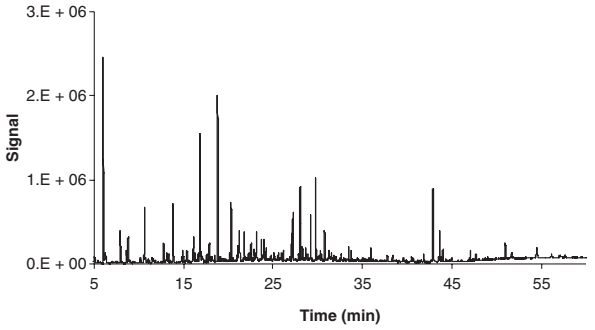

Figure 3 GC-MS chromatogram of a SAFE extract from regular dried protein powder.

known to provide a good representation of the volatile composition. This method does not create new compounds by heating the sample (Engel *et al*., 1999; Pozo-Bayón *et al*., 2006). GC-MS was then used to analyse this volatile fraction (Fig. 3) and allowed the identification of more than seventy volatile compounds (Table 3). Among these volatile compounds, some might come from the raw material (i.e. the earthworm itself, its food and its environment such as earth, water), others might be due to microbiological modifications or lipid peroxidation. Additionally, in the case of protein powder, drying is a crucial step because it may concentrate off-flavours and/or create new odorant compounds. The most abundant chemical groups found in the volatile fraction of the earthworm powder were ketones (29%) among which undecan-2-one, alcohols (21%) among which pentan-1-ol, and aldehydes (15%) among which hexanal. Pyrazines (10%) also constituted a major chemical group and could be considered to have been produced during drying as a result of thermally induced reactions. In fact, Maillard

Table 3 Volatile compounds identified by GC-MS in a SAFE extract from regular dried protein powder

Calculated RI[f]	Reference RI	Identification	Compounds	Formula	CAS
<1000	977[a]	+	Pentanal	$C_5H_{10}O$	110-62-3
1043	1040[a]	++	Ethyl butanoate	$C_6H_{12}O_2$	105-54-4
1060	1054[d]	++	Pentane-2,3-dione	$C_5H_8O_2$	600-14-6
1076	1075[a]	++	Dimethyl disulfide	$C_6H_6S_2$	624-92-0
1086	1080[a]	++	Hexanal	$C_6H_{12}O$	66-25-1
1102	1101[d]	++	2-methylbut-2-enal	C_5H_8O	1115-11-3
1149	1152[a]	++	Butan-1-ol	$C_4H_{10}O$	71-36-3
1154	1148[d]	++	*(δ)* 3-carene	$C_{10}H_{16}$	13466-78-9
1164	1169[c]	++	Pent-1-en-3-ol	$C_5H_{10}O$	616-25-1
1191	1186[c]	++	Heptan-2-one	$C_7H_{14}O$	110-43-0
1191	1189[c]	++	Heptanal	$C_7H_{14}O$	111-71-7
1200	1200[d]	++	Dodecane	$C_{12}H_{26}$	112-40-3
1205	1200[c]	++	α-Limonene	$C_{10}H_{16}$	138-86-3
1214	1215[a]	++	3-methylbutan-1-ol	$C_5H_{12}O$	123-51-3
1224	1225[a]	++	Hex-2-enal *(e)*	$C_6H_{10}O$	6728-26-3
1236	1240[a]	++	2-pentylfuran	$C_9H_{14}O$	3777-69-3
1239	1238[a]	++	Ethyl hexanoate	$C_8H_{16}O_2$	123-66-0
1243	1221[b]	++	6-methyl-hepta-2-one	$C_8H_{16}O$	928-68-7
1246	1230[d]	++	Hept-4-enal *(z)*	$C_7H_{12}O$	6728-31-0
1257	1256[a]	++	Pentan-1-ol	$C_5H_{12}O$	71-41-0
1268	1266[a]	++	Dihydro-2-methyl(*2h*)furan-3-one	$C_5H_8O_2$	3188-00-9
1273	1264[a]	++	Methylpyrazine	$C_5H_6N_2$	109-08-0
1276	1266[a]	++	1-methyl-4-(1-methylethyl)benzene	$C_{10}H_{14}$	99-87-6
1286		+	1,3,5-trimethylbenzene	$C_9H_{12}O$	108-67-8
1290	1289[a]	++	3-hydroxy-butan-2-one	$C_4H_8O_2$	513-86-0
1290	1280[a]	++	Octan-2-one	$C_8H_{16}O$	111-13-7
1294	1293[c]	++	Octanal	$C_8H_{16}O$	124-13-0
1307	1307[c]	++	Oct-1-en-3-one	$C_8H_{14}O$	4312-99-6
1319	1321[d]	++	Pent-2-en-1-ol *(e)*	$C_5H_{10}O$	1576-96-1
1322	1327[c]	++	4-methylpentan-1-ol	$C_6H_{14}O$	626-89-1
1330	1318[a]	++	2,5-dimethylpyrazine	$C_6H_8N_2$	123-32-0
1337	1325[a]	++	2,6-dimethylpyrazine	$C_6H_8N_2$	108-50-9
1340	1388[d]	++	Oct-3-en-2-one	$C_8H_{14}O$	18402-82-9
1342	1340[a]	++	6-methyl-hept-5-en-2-one	$C_8H_{14}O$	110-93-0
1354	1345[a]	++	2,3-dimethylpyrazine	$C_6H_8N_2$	5910-89-4
1360	1354[a]	++	Hexan-1-ol	$C_6H_{14}O$	111-27-3
1368	1343[b]	++	4-hydroxy-4-methyl-pentan-2-one	$C_6H_{12}O_2$	123-42-2
1393	1406[a]	++	2-ethyl-5-methylpyrazine	$C_7H_{10}N_2$	13360-64-0
1398	1396[a]	++	Nonanal	$C_9H_{18}O$	124-19-6
1438	1425[e]	++	Cyclohex-2-en-1-one	C_6H_8O	930-68-7
1449	1426[b]	++	Linalool oxide	$C_{10}H_{18}O_2$	5989-33-3
1453	1451[c]	++	3-ethyl-2,5-dimethylpyrazine	$C_8H_{12}N_2$	13360-65-1
1456	1456[a]	++	Oct-1-en-3-ol	$C_8H_{16}O$	3391-86-4
1464	1465[c]	++	Decan-3-one	$C_{10}H_{20}O$	928-80-3
1483	1474[a]	++	Tetramethylpyrazine	$C_8H_{12}N_2$	1124-11-4
1490		+	3,5,5-trimethyl-hex-2-en	C_9H_{18}	26456-76-8
1496	1498[c]	++	Decan-2-one	$C_{10}H_{20}O$	693-54-9
1525	1521[a]	++	Octa-3,5-diene-2-one *(e,e)*	$C_8H_{12}O$	30086-02-3
1525	1528[a]	++	Benzaldehyde	C_7H_6O	100-52-7
1564	1561[a]	++	Octan-1-ol	$C_8H_{18}O$	111-87-5
1575		+	Octa-3,5-diene-2-one *(ui)**	$C_8H_{12}O$	
1603	1606[a]	++	Undecan-2-one	$C_{11}H_{22}O$	112-12-9
1608		+	2,6-dimethyl-cyclohexanol	$C_8H_{16}O$	5337-72-4
1622	1626[c]	++	Oct-2-en-1-ol *(e)*	$C_8H_{16}O$	18409-17-1
1630	1640[e]	++	Dihydro(*3h*)furan-2-one	$C_4H_6O_2$	96-48-0
1643	1640[c]	++	Phenylacetaldehyde	C_8H_8O	122-78-1

Table 3 (Continued)

Calculated RI[f]	Reference RI	Identification	Compounds	Formula	CAS
1651	1660[a]	++	1-phenylethanone	C_8H_8O	98-86-2
1672		+	2-butyloct-2-enal	$C_{12}H_{22}O$	13019-16-4
1675	1680[e]	++	α-Humulene	$C_{15}H_{24}$	6753-98-6
1690		+	Cycloocta-1,3-diene	C_8H_{12}	1700-10-3
1708	1698[e]	++	Dodecan-2-one	$C_{12}H_{24}O$	6175-49-1
1740	1721[b]	++	Pentanoic acid	$C_5H_{10}O_2$	109-52-4
1761	1725[b]	++	5-ethyl(*5h*)furan-2-one	$C_6H_8O_2$	2407-43-4
1814	1815[c]	++	Tridecan-2-one	$C_{13}H_{26}O$	593-08-8
1848	1846[e]	++	Hexanoic acid	$C_6H_{12}O_2$	142-62-1
1871	1840[e]	++	Undecanol	$C_{11}H_{24}O$	112-42-5
1881	1837[b]	++	Benzyl alcohol	C_7H_8O	100-51-6
1917	1918[c]	++	2-phenylethanol	$C_8H_{10}O$	60-12-8
1945	1941[c]	++	β-Ionone	$C_{13}H_{20}O$	14901-07-6
1953	1965[e]	++	2-ethyl hexanoic acid	$C_8H_{16}O_2$	149-57-5
1953	1904[b]	++	Heptanoic acid	$C_7H_{14}O_2$	111-14-8
1974	1976[c]	++	2-acetylpyrrole	C_6H_7NO	1072-83-9
2032	2024[e]	++	5-pentyl-dihydro(*3h*)furan-2-one	$C_9H_{16}O_2$	104-61-0
2061	2083[d]	++	Octanoic acid	$C_8H_{16}O_2$	124-07-02

*Unidentified isomer.

[a]Retention index published from a Supelcowax-10 stationary phase (Bianchi *et al.*, 2007). [b]Retention index published from a CW20M stationary phase (Kondjoyan & Berdagué, 1996). [c]Retention index published using a DB-Wax stationary phase (Farkas *et al.*, 1994).

[d]Retention index published from a C20M stationary phase (Flavornet database, http://www.flavornet.org, Acree, 2004).

[e]Retention index published from a DB-Wax stationary phase or a similar polarity phase (The Pherobase: Database of Insect Pheromones and Semiochemicals, http://www.pherobase.net, El-Sayed, 2008).

[f]Retention index calculated with a DB-Wax stationary phase using a series of alkanes between C_8 and C_{30}.

+ ⇒ Identification by comparison with mass spectrum database.

+ + ⇒ Identification by comparison with mass spectrum and retention index database.

reactions are known to give alkylpyrazines, or benzene acetaldehyde (Mottram, 1985). Some of the aldehydes and acids found in the volatile fraction may come from the degradation of lipids. In fact, in the first step, lipid oxidation generates hydroperoxides, the decomposition of which leads to the formation of aldehydes, acids, esters and hydrocarbons. For example, model experiments of autoxidation of oleic, linoleic and linolenic acids gave pentanal, hexanal, heptanal, E-hex-2-enal (Belitz *et al.*, 2004). This mixture of volatile compounds extracted from the earthworm powder gave it a strong animal odour, which was described by the sensory panel as 'dried fish'. This odour may limit the use of this protein powder in food products.

Sensory analysis of arepas fortified with non-conventional protein powder

Sensory analyses were done on *arepas* fortified with the non-conventional protein powder. The aim was to determine an acceptable threshold value for fortification in *arepas* and to check if this threshold could be increased using the partially delipidated protein powder produced as described in the Material and methods section. We hypothesised that the delipidation of the dried protein powder may lower the odour of the protein powder.

The sensory panel classified seven samples containing different amounts of non-conventional protein powder going from 2.0% to 15.1% (samples A–G in Table 1). Samples up to 5.5% of protein powder were not significantly different (Table 4a) and were thus considered to be acceptable by the panel. For higher amounts of non-conventional protein powder, *arepas* were judged to be significantly different. The threshold of acceptability was then fixed at 5.5% of fortification for the second session.

In this second session, the sensory panel had to classify six samples; three of them were made with the regular protein powder and three were made with the partially delipidated protein powder (Table 2). The amounts of protein powder added were chosen around the acceptable threshold determined during the first session (between 3.9% and 7.7%). Rank sums were reported in Table 4b and showed that 7.7% fortified samples were significantly different from the others. It can then be concluded that partial delipidation did not increase the acceptance threshold.

In the second session, the sensory panel was also asked to score general descriptors giving thus a draft

Table 4 Results of hedonic classification tests from sensory analyses of fortified *arepas*

% of fortification ⇩	⇨ Ranks sum	15.1	10.8	7.7	3.9	5.5	2.8	2.0
a: Samples fortified with regular protein powder								
15.1	35	0						
10.8	35	0	0					
7.7	56	21	21	0				
3.9	86	51	51	30	0			
5.5	90	55	55	34	4	0		
2.8	106	71	71	50	20	16	0	
2.0	111	76	76	55	25	21	5	0

% of fortification ⇩	⇨ Rank sums	7.7 reg.	7.7 delip.	5.5 delip.	3.9 delip.	5.5 reg.	3.9 reg.
b: Samples fortified with either regular (reg.) or partially delipidated (delip.) dried protein powder							
7.7 reg.	46	0					
7.7 delip.	46	0	0				
5.5 delip.	74	28	28	0			
3.9 delip.	79	33	33	5	0		
5.5 reg.	86	40	40	12	7	0	
3.9 reg.	88	42	42	14	9	2	0

$\kappa^2 = 12.59$, significance level: $P < 0.05$.
Cells in grey show significantly different products (differences among rank sums are bigger than least significant differences).
$\kappa^2 = 11.07$, significance level: $P < 0.05$.
Cells in grey show significantly different products (differences among rank sums are bigger than least significant differences).

sensory profile of each sample. The mean score obtained for each sensory descriptor is reported in Fig. 4 for 3.9% and 5.5% samples, which were judged to be acceptable samples. It is noticeable that mean scores of all descriptors are comprised between 2.18 and 3.67, confirming that these samples were acceptable, that is to say descriptors were neither too weak nor too intense. The most discriminating descriptor was colour. Partial delipidation clearly led to less intensely coloured samples. Texture (firmness and crunchiness) of samples increased with the amount of protein powder. Concerning taste, samples fortified with 5.5% of partially delipidated powder seemed quite different from the other ones and gave too intensely flavoured *arepas*. Surprisingly, odour was only slightly influenced by the amount of protein powder or by partial delipidation.

Conclusion

The dried protein powder prepared from *Eisenia foetida* was used for the fortification of traditionally maize-based pancakes named *arepas*. From a sensory point of view, satisfactory products were obtained with a substitution level of 5.5% protein powder, which is interesting but below the nutritional target. Recipes must then be improved to allow increased amount of earthworms in fortified food products.

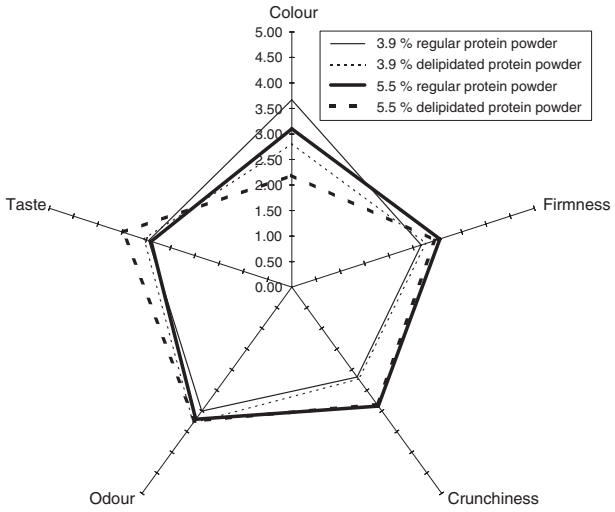

Figure 4 Effect of delipidation on the sensory descriptors of fortified *arepas*. Sensory profiles for 3.9% and 5.5% fortified *arepas*.

Measurements done on functional properties (solubility and granulometry) and on the volatile fraction of the protein powder showed that work needs to be done on the production process of this powder: future aims could be to have a more homogeneous particle size, and less denatured proteins leading to increased solubility. The technology for drying and crushing the earthworms should be revised.

Moreover, a partial delipidation was found to have an impact on colour but was insufficient to improve the odour quality of the fortified *arepas*. Delipidation could be enhanced in order to decrease the amount of volatile compounds coming from lipid oxidation. More generally, further experiments must be done to better understand and control the biogenesis of the volatile compounds identified in the protein powder.

To conclude, the use of earthworms biomass in human food is promising but the technology to prepare the earthworm powder must be considered to obtain an efficient use of this novel protein source in manufactured food products.

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