

Impact of Preparation Process on the Protein Structure and on the Volatile Compounds in *Eisenia foetida* Protein Powders

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ABSTRACT

Protein powders from *Eisenia foetida* were prepared using different drying processes and fractionation. Differential scanning calorimetry was used to show that heat denaturation occurred during the drying process above 42°C. Protein solubility was also studied. The addition of dissociating reagents allowed concluding that solubility was decreased during oven drying due to thermo denaturation including hydrogen bonds. The volatile compounds of the different powders were extracted by solid phase micro-extraction and identified by mass spectrometry. Volatile compounds were related to lipid oxidation and Maillard reactions occurring during the preparation of the powders. High drying temperatures led to more volatile compounds resulting from Maillard reactions. In the protein powder preparation process, a fractionation step led to a “pulp fraction” and a “juice fraction” of earthworms. The “pulp fraction” contained less odorant volatile compounds resulting from Maillard reactions than the “juice fraction” did.

Keywords: *Eisenia foetida* Protein Powder; Drying Process; Protein Structure; Volatile Compounds; HS-SPME/GC-MS

1. Introduction

Some authors have pointed out the great potential of *Eisenia foetida* as a non-conventional protein source [1]. They determined that earthworms had a high protein content of about 62% dry weight [2] and that the content of essential amino acids, except for tyrosine [1], was higher than that recommended by the FAO. Earthworms could then have many different nutritional applications as animal feed and as an ingredient in food products for humans. They have already been used in an experimental diet for rainbow trout [3] and as chicken feed [4].

For practical reasons, earthworms were often dried and used as a powder in the different applications. We conducted former studies on oven-dried powders. Two main problems were encountered when these earthworm protein powders were used in food products. One was related to the odor properties, and the other to protein denaturation.

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Concerning the odor properties, off-flavors can make the powder unacceptable to humans. In fact, the Solvent-Assisted-Flavor-Evaporation (SAFE) method was used in a previous study [5] to obtain the volatile fraction from the oven-dried protein powder. Gas chromatography coupled with mass spectrometry (GC-MS) was then used to analyze this volatile fraction and allow the identification of more than 70 volatile compounds. Among these volatile compounds, some might come from the raw material (*i.e.* the earthworm itself, its feed and its environment such as earth, water...); others might be due to microbiological modifications or to lipid peroxidation. Additionally, in the case of protein powder, drying is a crucial step because it may concentrate off-flavors and/or create new odorant compounds. The most abundant chemical groups found in the volatile fraction of the earthworm powder were ketones (29%) including undecan-2-one, alcohols (21%) including pentan-1-ol, and

aldehydes (15%) including hexanal. Pyrazines (10%) were also a major chemical group and could be considered a product generated during drying as a result of thermally induced reactions. In fact, Maillard reactions are known to give alkylpyrazines, or benzene acetaldehyde among many other volatile compounds [6]. Some of the aldehydes and acids found in the volatile fraction may come from the degradation of lipids. In fact, the first step of lipid oxidation generates hydroperoxides, the decomposition of which leads to the formation of aldehydes, acids, esters and hydrocarbons. For example, model experiments of the autoxidation of oleic, linoleic and linolenic acids gave pentanal, hexanal, heptanal, E hex-2-enal [7]. This mixture of volatile compounds extracted from the earthworm powder gave it a strong animal odor, which was described by Cayot *et al.* [5] as “dried fish”. This odor may limit the use of this protein powder in food products.

Nevertheless, dehydrated proteins offer a longer shelf life, and lower storage and transports costs. The traditional methods used to transform protein suspensions into dry powders are spray drying, solar drying, convective hot air drying and freeze drying. A number of effects are observed during the heat-drying (spray, solar and convective hot air drying) of protein-containing product, the main effect being the thermal denaturation of protein. Heat causes rapid thermal motion of the atoms leading to rupture of the hydrogen bonds that link them together. Under suitable conditions, thermal denaturation leads to coagulation. During the process, peptide chains are propagated and form agglomerates. The denatured protein is insoluble [8]. As a low temperature process, freeze-drying is a known method to ensure the long-term stability of high-value or therapeutic proteins that are not stable enough in aqueous solutions and that are very sensitive to heat. However, some proteins are also sensitive to the stresses imposed by freeze drying and can be degraded or decomposed during the process [9]. The low temperature of freeze drying does not guarantee protein stability because some proteins also experience cold denaturation. Moreover, freeze drying is costly in terms of both time and energy, and can therefore only be used to dry high value proteins.

For these reasons, different processes to kill the earthworms, to crush them and to dry the different fractions obtained were tested in the present study. Two main types of drying process were used, oven drying (60°C during 4 h) and, for reference in terms of protein structure preservation, freeze drying. The volatile compounds were extracted from the protein powder by Headspace Solid Phase Micro Extraction HS-SPME, and were further analyzed by GC-MS. Protein denaturation was checked by Differential Scanning Calorimetry (DSC) and

solubility measurements.

The aim of the study was to understand the impact of preparation process on volatile composition and on protein denaturation, in order to obtain fractions as odor-free as possible and with proteins as little denatured as possible.

2. Materials and Methods

2.1. 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 throughout the study. Different preparation processes were then applied as summarized in **Figure 1** and the dried protein-rich powders obtained were stored until further analyses. The data we referred to in the introduction part were corresponding to ODEW sample of the present study.

2.2. Composition of the *Eisenia foetida* Powders

2.2.1. Water Content

The moisture content of each powder was determined in triplicate using volumetric Karl Fisher titrimetry. This chemical method allowed the determination of the water content with a reproducibility of 0.5%. The titration was checked with a water standard at 1% (Epura, Merk). A Karl Fisher titration apparatus from Mettler Toledo (DL38, Inc., Columbus, OH, USA) was used.

2.2.2. Protein Content

The Kjeldahl procedure [10] was used to determine the nitrogen content in earthworm powders (0.1 g), or suspensions (5 mL). Samples were weighed and transferred into the Kjeldahl digestion flask containing 10 mL of concentrated H₂SO₄ and a catalyst. After 4 h of digestion in a unit with electrical heat and fume removal (Labonco, Kansas City, MO, USA) and cooling to room temperature, 5 mL of distilled water was added to each flask. By distillation, ammonia was trapped as ammonium borate in a 3% boric acid solution (3 g of boric acid in 100 mL deionized water (w/v)). Total nitrogen was determined by titration with standardized HCl to recover

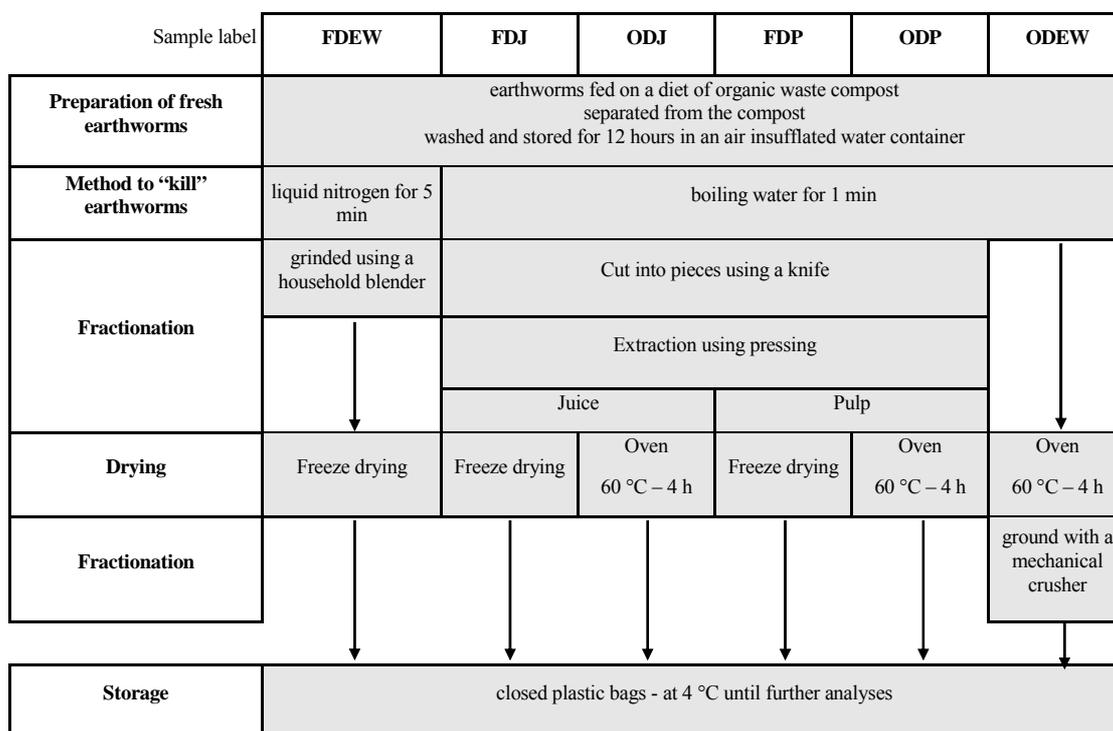


Figure 1. Process for the preparation of earthworm protein powders.

the initial boric acid pH (pH 4.6). The conversion factor from nitrogen content to protein content was 6.25.

2.3. Evaluation of Protein Denaturation

2.3.1. Protein Solubility

To determine protein solubility, protein powders were suspended in water at 40 g·L⁻¹ and stirred overnight at 4°C. The pH of the suspension was adjusted to pH 6 at the end of the stirring step. The suspensions were centrifuged at 700 g for 30 minutes and the Kjeldahl procedure was used to analyze the protein content as previously described. Some samples of ODEW were suspended at 40 g·L⁻¹ but in the presence of different reagents: 1% w/v of sodium dodecyl sulfate (SDS), 1 mol·L⁻¹ of dithio-reitol (DTT), or 6 mol·L⁻¹ of urea were added before centrifugation. Biochemical grade urea, SDS and DTT were purchased from Sigma.

2.3.2. Differential Scanning Calorimetry

A microcalorimeter DSC III (Setaram Instrumentation, France) was used to determine the denaturation temperature of the protein suspension. The range of temperature chosen for this study was 30°C to 110°C, with a temperature gradient of 0.5°C·min⁻¹, and a sample weight of approximately 600 mg (exact weighting) placed in a hermetically closed cell "batch". Water was used as the reference. The temperature profile of the thermogram

was done using the software of the apparatus (Setsoft, Setaram Instrumentation, France).

2.4. Extraction of Volatile Compounds by Headspace Solid Phase Micro-Extraction (HS-SPME)

For the HS-SPME analysis, 1 g of each protein powder was transferred into a 10 mL vial, which was immediately sealed with a Teflon-lined septum and a screw cap. The equilibration time was 24 h at room temperature. The powder was incubated at 30°C for 90 min with an agitation of 250 rpm at intervals of 20 s using an auto sampler (Gerstel MPS2). After incubation, the headspace volatiles were extracted using an SPME fiber (2 cm-50/30 µm DVB/Carboxen/PDMS/StableFlex, Supelco, USA) for 60 min at 30°C. Finally, for the injection, the fiber was inserted into the GC injection port and desorbed for 3 min in splitless mode. The fiber was cleaned at 240°C for 12 min after each injection. The relative standard deviation of this method was calculated by analyzing the ODEW sample five times and was equal to 2.17%. The other samples were analyzed twice.

2.5. Gas Chromatography Mass Spectrometry Analysis (GC-MS)

Volatile compounds were analyzed on a 5973 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA)

equipped with a fused-silica capillary column (30 m × 0.32 mm ID, 0.5 μm film thickness) coated with a DB-Wax stationary phase (J & W Scientific, USA). The instrument was equipped with an injection port operating at 240°C. 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, with a final isotherm of 10 min. Mass spectrometry was taken in the electron ionization mode at 70 eV and the scan range between 29 and 350 amu. The ion source was set at 230°C and the transfer line at 240°C. Compounds were identified by comparison with mass spectra libraries (WILLEY138, NIST, and INRA database) and by calculating and comparing the GC retention index of a series of alkanes (C8 to C30) with the retention index from published data calculated under the same conditions.

2.6. Statistical Data Analysis

The absolute area values of the four compounds chosen as related with heating process (hexanal, 2-pentyl furan, 2,6-dimethylpyrazine and benzaldehyde) were first centred and reduced in order to attribute the same importance to the four variables (four compounds). Standardized data were submitted to a hierarchical clustering analysis using Euclidean distances and Ward's aggregation method in order to identify homogeneous subgroups of powders. Then, the subgroups were verified by k-means clustering, calculated on the standardized data and using the un-scaled squared Euclidean distances. In fact, three k-means algorithms were made in parallel in order to verify the stability of the subgroups. Data analysis was done using the Statistica software (version 10).

3. Results and Discussion

3.1. Impact of the Preparation Process on the Protein Structure and Solubility

Differential scanning calorimetry was used to determine the impact of the preparation process on protein structure for the traditional powder (ODEW) and for a powder prepared without any heating step, neither during “killing” of earthworms, nor during drying (FDEW). **Figure 2** presents typical thermograms obtained by DSC on suspensions of freeze dried (FDEW) and oven dried (ODEW) protein powders. It was not possible to observe any endothermic peak on samples that had been dried in the oven at 60°C as ODEW. The thermogram obtained for samples which had been freeze dried (FDEW) exhibited an endothermic peak at 42°C, indicating that a denaturation process of non-denatured proteins had occurred during DSC at this temperature. As a consequence, FDEW can be considered a reference for earthworm powder as non-denatured protein and the powders that

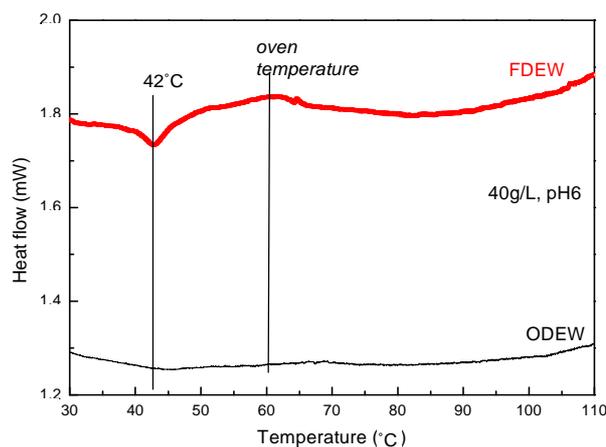


Figure 2. Thermograms obtained by differential scanning calorimetry of oven dried earthworm powder (ODEW, black line) and freeze dried earthworm powder (FDEW, bold line) (exo up).

had been prepared at temperatures above 42°C thus presented flat thermograms.

Preliminary studies on solubility were done from pH 2 to pH 8 on FDEW and on ODEW, and showed that maximum solubility was obtained for pH 6 and above (data not shown). As a pH value compatible with food products, pH 6 was selected to continue the study. Solubility at this pH value was 43% ± 22% for ODEW and 117% ± 27% for FDEW (value above 100% was due to the precision of the method). FDEW was thus less denatured than ODEW but also far more soluble.

It is well established that the functionality of proteins is strongly influenced by the processing conditions. The lack of solubility can be due to protein aggregation via covalent or non-covalent bonding. For example, heating whey proteins leads to denaturation of the whey proteins and beta-lactoglobulin, which modifies the availability of some free thiol groups. These free thiol groups can react with disulphide bonds, or can react with another thiol group to form a disulphide bond [11]. Non-covalent bonding can include the modification of dipole-dipole interactions, hydrogen bonds, or hydrophobic interactions.

To study which phenomena were involved in the loss of solubility of earthworm proteins, the solubility of ODEW at pH 6 was measured in the presence of different dissociating compounds, such as DTT, SDS and urea. In fact, hydrogen bonds and hydrophobic interactions in proteins can be destabilized by urea [12]. Urea denatures a protein molecule through preferential adsorption with charged protein solutes. This mechanism dehydrates the molecules and causes repulsion between proteins, which in turn stabilizes the unfolded form [13]. SDS allows the breaking of hydrophobic bonds [14]. DTT is often used to reduce the disulphide bonds of proteins and to prevent intra- and intermolecular disulphide bonds from forming

between cysteine residues [15].

In our experiments, the addition of DTT did not change the solubility of ODEW (36% ± 14%). In the presence of SDS, the solubility increased to 64% ± 19%, and finally, the addition of urea led to 100% ± 19% solubility. It can then be concluded that the loss of solubility of earthworm protein due to heat denaturation involved hydrogen bonds and probably also hydrophobic interactions.

From these experiments on protein structure and solu-

bility, FDEW rather than ODEW should be considered the reference for the preparation of earthworm powder. It was thus important to check the impact of the preparation process also on the volatile composition of these two powders.

The volatile compounds were identified for the whole powders, for two fractions of earthworms and for the two drying processes and were reported in **Table 1**. Among the identified compounds, some compounds are strongly

Table 1. Volatile compounds identified by GC-MS in an HS-SPME extract from protein powders and their corresponding peak area.

RI ^a	Identification ^b	Name	CAS number	FDEW	ODEW	FDJ	ODJ	FDP	ODP
1083	A	Hexanal (<i>m/z</i> = 82)	66-25-1	435603	792038	510838	995617	620397	919452
1165	A	Pent-1-en-3-ol	616-25-1	0	16862807	1571370	0	0	0
1188	A	Heptan-1-al	111-71-7	2957276	4237536	2182076	2973887	2127609	2548139
1200	A	Dodecane	112-40-3	0	0	10271953	6095898	0	0
1202	A	p-mentha-1,8-diene (limonene)	95327-98-3	0	3086859	0	0	4993981	5098878
1235	A	2-pentyl furan	3777-69-3	387000	3492850	306153	1457416	206160	377380
1241	A	6-methyl-heptan-2-one	928-68-7	0	1050938	0	0	0	0
1258	A	Pentan-1-ol	71-41-0	1334302	3499926	0	1879380	0	0
1273	A	1-methyl-4-(1-methylethyl)benzene	99-87-6	1846764	278404	0	0	0	1152006
1289	A	Octan-2-one	111-13-7	263751	1436805	0	0	0	286278
1307	A	Oct-1-en-3-one	4312-99-6	1098471	726976	769269	1252966	590119	652081
1327	A	2,6-dimethylpyrazine	108-50-9	0	3059196	0	4220157	0	1920495
1340	A	Oct-3-en-2-one	18402-82-9	255951	2942391	2465463	248874	68187	689294
1343	A	6-methyl-hept-5-en-2-one	110-93-0	467307	249658	509444	0	0	189492
1362	A	Hexan-1-ol	111-27-3	1004912	2627738	2649907	2068947	623320	1485772
1395	A	Nonan-2-one	821-55-6	0	120005	0	0	0	0
1398	A	Nonan-1-al	124-19-6	528165	446024	1186327	2088480	0	459443
1420	B	5-ethyl-1-formylcyclopentene	-	372893	294482	413625	843813	212376	543645
1434	A	Cyclohex-2-en-1-one	930-68-7	0	2610028	0	3483881	0	1950115
1451	A	Acetic acid	9035-69-2	760807	8190101	753608	32515159	0	641846
1458	A	Oct-1-en-3-ol	3391-86-4	8132508	8788512	6007789	5404839	3453999	5900573
1492	B	3,5,5-trimethyl-hex-2-en	26456-76-8	592147	857455	324731	498399	0	0
1498	A	Decan-2-one	693-54-9	0	426681	0	0	0	0
1521	A	Octa-3,5-dien-2-one (<i>m/z</i> = 95)	30086-02-3	2925190	843142	2027823	962378	1241743	973075
1524	A	Benzaldehyde (<i>m/z</i> = 105 + 106)	100-52-7	1031804	3058667	767255	3816239	484894	1318509
1540	A	Propanoic acid	79-09-4	4535554	1958294	2535796	9546045	661820	1475021
1568	A	Octan-1-ol	111-87-5	0	414594	114375	617447	0	0
1573	B	Octa-3,5-dien-2-one (ui) [*]	-	3625640	2923317	2142919	1064940	1383211	1294623
1604	A	Undecan-2-one (<i>m/z</i> = 170)	112-12-9	0	27665	0	0	0	0
1605	B	2,6-dimethyl Cyclohexanol (<i>m/z</i> = 128)	5337-72-4	0	51268	42104	0	0	34043
1624	A	Oct-2-en-1-ol (E)	18409-18-2	0	780186	592421	1118664	0	0
1630	A	Butanoic acid	107-92-6	0	3092595	39101	0	0	0
1649	B	Undecan-6-ol	23708-56-7	0	0	1392786	1230098	1203617	920051
1665	A	Furfuryl alcohol	98-00-0	0	0	0	3542250	0	0
1710	A	Dodecan-2-one	6175-49-1	0	419726	0	459043	0	381307
1805	A	4-methyl pentanoic acid	646-07-1	0	579506	0	0	0	0
1815	A	Tridecan-2-one	593-08-8	83529	298119	0	241804	0	0
1848	A	Hexanoic acid	142-62-1	706807	1657848	523068	5309713	301330	585619
1914	A	2-phenyl ethanol	60-12-8	581848	917356	1026107	152704	0	0
1943	A	β -Ionone	14901-07-6	63746	99855	117107	0	0	128966
1955	A	Heptanoic acid	111-14-8	1131334	864757	1376250	647697	0	1494944
Sum of absolute areas of identified compounds multiplied by 10 ⁻⁷				3.51	8.41	4.26	9.47	1.82	3.34
Sum of absolute areas of HPIC multiplied by 10 ⁻⁶				1.85	10.40	1.58	14.03	1.31	4.54
Sum of absolute areas of Maillard compounds multiplied by 10 ⁻⁶				1.42	9.61	1.07	13.04	0.69	3.62

^aui = Unidentified isomer. ^bRetention index calculated with a DB-Wax stationary phase using a series of alkanes between C8 and C30. ^cA, compounds identified by MS and GC retention index as compared with those published in the literature using a similar stationary phase [16-18]; B, tentative identification by MS. In bold: Products obtained from a heat induced reaction such as: Maillard reactions, lipid oxidation and lipid degradation. n.d. = not detected.

dependent on thermal treatments: 1) Benzaldehyde comes from Strecker degradation and is known to be a Maillard reaction product [19,20]. 2) Alkylpyrazines generates from two accepted mechanisms: the Strecker degradation and the ammonia/acyloin reaction. In both mechanisms, the temperature is a determinant factor. 2,6-dimethylpyrazine may come from the thermal degradation of serine or threonine [21]. 3) 2-pentyl furan comes from the thermal degradation of linoleic acid [22]. Strecker degradation produced by some lipid oxidation products such as 4,5(E)-epoxy-2(E)-decenal yields 2-pentyl furan [23]. 4) Hexanal is often found to be a major compound in the volatile profile of meat products and is often chosen as an indicator of oxidation in meats, especially during the early oxidative changes [24].

These four compounds were further used to compare the different powders. The term “heat process implicated compounds” (HPIC) will be used here to name these compounds.

3.2. Impact of the Preparation Process on the Volatile Composition of ODEW and FDEW

A global comparison of ODEW and FDEW volatile composition was done by comparing the sum of identified compounds areas. This comparison shows that ODEW contains 2.4 times more volatiles compounds than FDEW (Table 1). Compounds from various chemical classes such as aldehydes, alcohols, benzene derivatives, or acids were encountered in both powders. As previ-

ously reported [25], volatile compounds are originated from lipid oxidation, Maillard reactions, and animal feed. Amino acid catabolism, environmental pollutants and the growth of microorganisms have also been reported as possible origins of volatile compounds detected in earthworm powders [7].

The amount of Maillard reactions products and HPIC was higher in ODEW than in FDEW. 2,6-dimethylpyrazine was found in ODEW but not in FDEW. Killing earthworms in boiling water and drying them in a traditional oven favor Maillard reactions due to the higher temperature during the process and, consequently, lead to the production of Maillard compounds in larger quantities.

As many parameters were different in the preparation processes of these two powders, a deeper study of the impact of the drying step and the fractionation step on volatile compounds production was necessary.

3.3. Impact of the Drying Step on the Volatile Composition of the Powders

Each protein fraction was dried using either a traditional oven (ODJ and ODP) or using freeze drying (FDJ and FDP). In order to study the impact of the drying on the volatile composition of the powders, we compared the powders that differ only by the drying step, *i.e.* the two couples FDJ/ODJ and FDP/ODP.

Benzaldehyde, 2,6-dimethylpyrazine and 2-pentyl-furan were chosen as indicators of Maillard reactions and their relative quantities were reported in Figure 3.

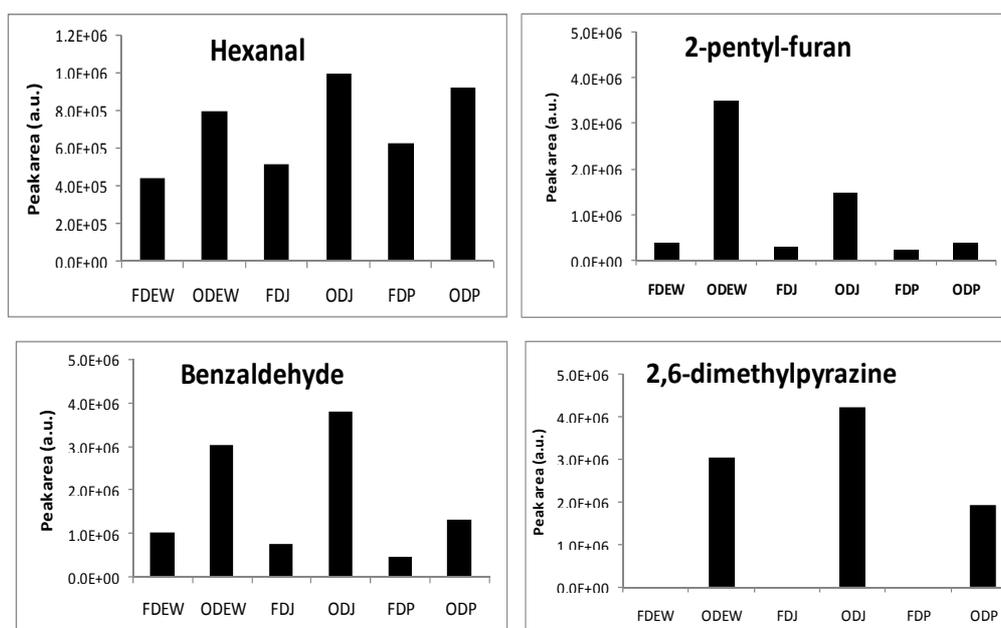


Figure 3. Absolute peak areas in the different powders obtained by HS-SPME-GC-MS. For 2-pentyl-furan and 2,6-dimethylpyrazine, areas were calculated from the TIC (Total Ion Chromatogram). For hexanal, areas were calculated from single ion $m/z = 82$ and for benzaldehyde, areas were calculated from single ions $m/z = 105 + 106$.

Oven dried juice contained 12.2 more Maillard compounds than freeze dried one and oven dried pulp contained 5.2 more Maillard compounds than the freeze dried one. This is clearly linked with the temperature of the drying process, which is higher in the oven than in the freeze dryer. It is noticeable that 2,6-dimethylpyrazine was found in all oven dried powders but not in freeze dried powders.

Hexanal was chosen as an indicator for lipid oxidation. It occurred in all the powders and its relative quantities found in the different powders were reported in **Figure 3**. Here again, oven dried powders contained higher quantities of hexanal than the corresponding freeze dried powders, which is consistent with the fact that lipid oxidation was accelerated by temperature increase. The larger amount of hexanal in oven dried powders in comparison with the freeze dried powders can be related to protein denaturation. Indeed, the oven dried proteins were more denatured than the freeze dried ones. This increases the availability of heme groups which are more efficient catalysts for lipid peroxidation than metal ions are. Belitz *et al.* [7] have already suggested that the activity of a heme protein towards hydroperoxides is influenced by its steric accessibility to fatty acid hydroperoxides.

Water content was higher in FDJ than in ODJ and it was higher in FDP than in ODP (**Table 2**). The Maillard compounds were higher in ODJ than in FDJ and higher in ODP than in FDP. The water content was therefore inversely proportional to the amount of Maillard compounds in these powders. Lu *et al.* [26] have already shown that volatile compounds generated from Maillard reactions increases as water content decreases.

3.4. Impact of the Fractionation Step on the Volatile Composition of the Powders

As detailed in **Figure 1**, different earthworm powders were prepared. ODEW was obtained using traditional drying in an oven. FDEW was our reference for less denatured protein and was done using freeze drying. The soluble fraction (juice) of earthworms was separated from the solid fraction (pulp). Protein content was higher in the pulp fraction than in the juice fraction (**Table 2**). Conversely, sugars are supposed to be mainly contained in the soluble fraction. As a consequence, Maillard reactions should be reduced in the pulp fraction.

In order to study the impact of the fractionation on the volatile composition of the powders, we compared the powders that differed only by the fractionation step, *i.e.* the two couples FDJ/FDP and ODJ/ODP. Even though the separation of the two fractions (juice and pulp) was not perfect, and some reducing sugar might remain in the pulp fraction, the quantity of Maillard reactions compounds (**Table 1**) (Benzaldehyde, 2,6-dimethylpyrazine and 2-pentyl-furan) was found to be 1.6 times lower in the pulp than in the juice for freeze dried powders and 3.6 times lower in the pulp than in the juice for the oven dried powders. This confirmed that reducing sugar was mainly in the juice. Additionally, as the proteins that were oven dried were denatured, NH₂ groups of amino acids might have been more available for reactions with reducing sugar, leading to more Maillard volatile compounds.

Juice and pulp fractions both contained larger quantities of hexanal than did the whole fractions. Fraction separation might favor oxygen contact with the intracellular medium and membranes and thus might have increased lipid oxidation. Oxygen contact could also have an impact on Maillard products. In fact, Fong Lam [27] showed that molecular oxygen can influence bond cleavage, which may lead to greater amounts of benzaldehyde. This can explain the high amount of benzaldehyde found in ODJ powder.

A hierarchical clustering analysis and k-means clusterings were done on the standardized areas of the “heat process implicated compounds” (HPIC) extracted from the different powders. The resulting dendrogram (**Figure 4**) shows three subgroups, which were verified by k-means clusterings: a first subgroup containing FDEW, FDJ and FDP, a second subgroup containing ODEW and ODJ and a final subgroup containing ODP alone. This dendrogram indicates that the three freeze-dried powders FDEW, FDJ and FDP are close together with a distance of aggregation below 13% and far from the oven dried powders. This result shows, as cited above, the importance of reducing the temperature in the preparation process of the powders in order to obtain earthworm powders containing low amounts of HPIC. On the dendrogram, the FDJ powder is closer to FDEW than FDP and ODJ is closer to ODEW than ODP. It clearly appears that the pulp fractions are different from the other powders. In agreement with this observation, the pulp fractions contain, for each thermal treatment, less volatile

Table 2. Water and protein content of the earthworm powders.

Sample	ODEW	FDEW	FDJ	ODJ	FDP	ODP
Water content (%)	5.24 ± 0.60	6.05 ± 0.15	5.38 ± 0.14	1.14 ± 0.51	5.04 ± 0.23	3.99 ± 0.24
Protein content (% w/w wb)	63.78 ± 3.90	66.16 ± 1.50	53.63 ± 1.01	53.73 ± 1.14	71.30 ± 0.42	61.68 ± 1.01
Protein content (% w/w db)	67.31 ± 3.88	70.42 ± 1.50	56.68 ± 1.01	54.35 ± 1.13	75.08 ± 0.42	64.24 ± 1.01

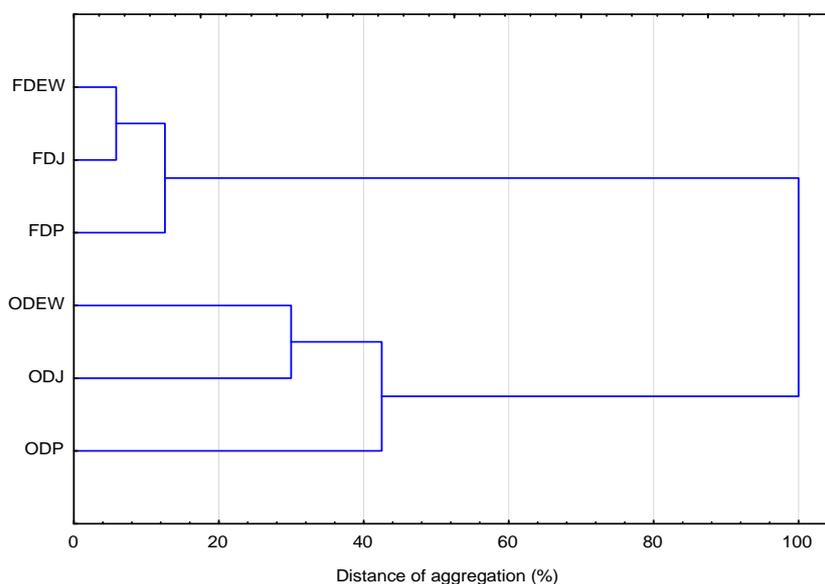


Figure 4. Dendrogram obtained from the hierarchical cluster analysis of the “heat process implicated compounds” HPIC analysed by HS-SPME-GC-MS in earthworm powder samples.

compounds than the other powders (**Table 1**). The difference between the pulp fractions and the other powders is mainly due to the volatile compounds from Maillard reactions than those from lipid oxidation (**Figure 3**).

In this work, differential scanning calorimetry showed that a drying process using temperature above 42°C led to protein denaturation. This thermo-denaturation led to a drastic decrease in solubility. This loss of solubility could be due to protein denaturation involving changes in hydrogen bonding and hydrophobic interactions. Finally, the FDEW powder was 3 times more soluble than the ODEW powder. Moreover, the odor properties were also modified by the drying process, and these were assessed through the analysis of volatile compounds. The amount of Maillard reactions compounds and lipid oxidation compounds was higher in powders that were obtained using oven drying rather than freeze drying. Fractionation was a determinant step in the preparation process of the earthworm powders. For the same heat treatment, the pulp fraction contained less volatile compounds resulting from Maillard reactions than the juice fraction did. It was possible to produce a more neutral protein by using fractionation and freeze drying: freeze dried pulp (FDP).

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