

Food Protein Powder from *Eisenia foetida*: Dearomatization Using Food Grade Solvents and Controlled Storage Conditions

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Abstract

Delipidation was used to dearomatize protein powder of *Eisenia foetida*. The remaining volatile fraction after delipidation was studied over a period of three months. Volatile fraction dramatically increased between the first and the second month of storage. Four volatile compounds, chosen as tracers, were studied, namely: benzaldehyde, 2-pentyl furan, o-xylene, and limonene. Controlled conditions of storage are very efficient to limit volatile compound increase: 10% or less for three among the four volatile compounds chosen as tracers. The main parameter to control is temperature. To obtain a food grade protein powder, delipidation was done using ethyl acetate/ethanol instead of chloroform/methanol mixture. The remaining volatile fraction after delipidation ranged from 6% to 18%. Dearomatization was improved using an additional drying after delipidation. Solvent residues amounted to a few mg/g and conformed to EU regulations concerning solvent residues in food stuffs and food ingredients.

Keywords: Protein powder; Dearomatization; Delipidation; Off-flavors; Solvent residues

Introduction

Delipidation was studied as a way to dearomatize a non-conventional protein powder obtained from *Eisenia foetida* earthworms by Romero et al. [1]. These authors showed that an ultrasound extraction method using a chloroform/methanol mixture could be used to extract lipids efficiently and, at the same time, to extract a wide range of volatile compounds. In fact, the volatile compounds detected in the headspace of the delipidated powder represented less than ¼ of the volatile compounds detected in the headspace of the non-delipidated powder (named regular powder).

Nevertheless, some lipids still remain in the powder, and can evolve during storage to produce off-flavors. If the final goal is to use the delipidated protein powder for food supplementation, the efficiency of dearomatization must be checked during storage in order to guarantee protein powder with a flavor as neutral as possible for future uses.

Another point is the use of organic solvent in the delipidation step in comparison with solvent-free techniques such as the supercritical CO₂ extraction. This latter method is, however, costly threatening the economic viability of the powder. Thus the chosen process must use simple equipment with reasonable overall cost and delipidation must be improved by choosing a more environment-friendly solvent. From that point of view, ultrasound-assisted leaching is interesting as it allows the extraction of a wide variety of compounds, whatever their polarity, because it can be used with any solvent [2].

Finally, to be able to conform to regulations and consumer demand for safer products, the residual amount of solvent must be checked.

Thus, the present study was done to answer to these three questions:

- What are the best storage conditions to maintain dearomatization? For that purpose an experimental design was done to check the impact of oxygen, light, and temperature on the evolution of the volatile fraction during a three month storage period.
- Could the chloroform/methanol be replaced by safer solvents for delipidation/dearomatization using ultrasound assisted extraction? For that purpose, ethyl acetate/ethanol mixtures were tested. The polarity of the mixture is similar to the one of chloroform/methanol: polarity of ethyl acetate, ethanol,

chloroform, and methanol are respectively 4.4, 5.2, 4.1, and 5.1 [3]. Moreover, EU regulations mention that ethyl acetate and ethanol can be used as 'extraction solvents in compliance with good manufacturing practice for all uses' i.e. the presence of residues in technically unavoidable quantities is acceptable [4,5].

- What is the amount of solvent residues in the delipidated powder? For that purpose, the standard addition method was used to quantify solvent residues in delipidated powders.

Materials and Methods

Samples and reagents

Earthworm protein powder obtained from *E. foetida* was kindly donated by Professor Ana Luisa Medina of Los Andes University, Merida (Venezuela). The protein powder was obtained from earthworms fed a diet of organic waste compost. The earthworms were washed with abundant water before they were killed with boiling water. They were then dried at 60°C for 4 hours and ground to homogeneous powder designated as raw protein powder, RPP. The detailed procedure is described in a previous paper [1].

Chloroform (>99%) and ethyl acetate (≥99.9%) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Dichloromethane (≥99.8%), methanol (>99.9%) and ethanol (≥99.8%) were bought from Carlo Erba Reagents (Val De Reuil, France).

Delipidation of the protein powder

The delipidation of the protein powder was done by ultrasound extraction using a US bath Branson Mod 3210 (Branson Europe

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Received August 24, 2015; Accepted September 03, 2015; Published September 11, 2015

Citation: Bou-Maroun E, Cayot N (2015) Food Protein Powder from *Eisenia foetida*: Dearomatization Using Food Grade Solvents and Controlled Storage Conditions. J Food Process Technol 6: 501. doi:10.4172/2157-7110.1000501

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B.V.) with an ultrasound fixed-frequency of 47 kHz ± 6%. In a typical experiment, 30 g of RPP was extracted with 150 ml of solvent for 3 minutes in an ultrasound bath. After the first extraction step, the delipidated protein powder (DPP) was filtered off. This process was repeated twice more using the DPP obtained from the previous step of extraction. The three solvent fractions were gathered, the solvent was evaporated under reduced pressure at 45°C using a rotary evaporator and the extracted lipids were weighed.

Different solvents were used for delipidation:

- I. Chloroform/methanol mixture as published previously [1]. In this case, the residual solvent of DPP was evaporated overnight at room temperature. The obtained powder was designated DPPa1-8 and stored in different conditions before analysis of the volatile fraction (Table 1).
- II. Ethyl acetate/ethanol mixture was used in order to replace chloroform/methanol: solvent proportions were 67/33 or 87/13; powder/solvent ratio was 2/5 or 1/5. Extracted lipids and volatile fraction were determined in the samples obtained with ethyl acetate/ethanol, designated as DPPb1-4. The best results were obtained with DPPb3 (Table 2). Two ways of solvent elimination were tested on this sample: powder dried overnight under a hood, or powder dried overnight in an oven at 60°C. Additionally, DPPb3 was also washed using ethanol (1 hour with a powder/ethanol ratio 1/5 (w/v)) before being oven dried. Solvent residues were determined in these samples.

Study of the volatile fractions using headspace solid phase micro extraction, gas chromatography-mass spectrometry (HS-SPME-GC-MS)

For the HS-SPME analysis, 1 g of each sample was transferred into a 10 ml headspace vial which was immediately sealed with a Teflon-lined septum and screw cap. After an equilibration time of 24 h at 4°C in obscurity incubation was operated at 30°C for 30 minutes in a thermostated water bath. The headspace volatiles were then extracted using an SPME fiber (2 cm–50/30 µm DVB/Carboxen/PDMS/StableFlex, Supelco, USA) for 60 minutes at 30°C. The fiber was desorbed in an injector of a 5973 Hewlett–Packard, Palo Alto, CA, USA chromatograph in splitless mode. It was 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 and W Scientific, USA). Helium

was used as the carrier gas with a constant flow of 1.5 ml/min. The initial oven temperature was maintained for 3 minutes at 40°C. Then, it was increased from 40 to 180°C at a rate of 4°C/min and from 180 to 240°C at a rate of 10°C/min. The final temperature was maintained for 10 minutes. Mass spectrometry was taken in the electron ionization mode at 70 eV and the scan range was 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 (WILEY138, NIST, and INRA database) and by the calculation and comparison of the GC retention index of a series of alkanes (C8–C30) with the retention index from published data calculated under the same conditions. The quantitative data were obtained by electronic integration of the TIC peak areas with the ChemStation program.

Impact of storage conditions on the evolution of the volatile fraction of DPPa samples during storage

For this experiment, delipidation was done using the chloroform/methanol mixture, i.e. DPPa samples. After delipidation, 1 g of DPPa was transferred to 25 ml hermetic Hungate tubes. The tubes were immediately closed with open top screw caps having a rubber septum. Then, samples were stored under various conditions of temperature, light, and oxygen. A 2³ factorial experimental design (Table 1) was carried out to map the effects of three qualitative factors on the percentage of the volatile fraction of the DPPa sample as compared to the total volatile fraction of RPP and to determine the optimal conditions for the storage of the delipidated powder. The selected factors were considered at two levels: light (obscurity or day light), gas storage (nitrogen or air) and temperature (4°C or ambient). Storage was at room temperature or at 4°C in a refrigerator. The tubes were put in an opaque box for the experiments done in the dark. The refrigerator used had a glass door enabling light to enter. In order to have a low-oxygen atmosphere, the tubes were placed for 10 minutes in a nitrogen glove-box before their storage.

The quantitative observed variable (percentage of volatile fraction) was calculated from results obtained after HS-SPME-GC-MS analyses. The main effects and interactions were calculated on variables as described by Box et al. [6].

Extraction and quantification of the residual solvent by the standard addition method

For this experiment, delipidation was done using the ethylacetate/

Sample labels	Factors			Observed variables: Remaining volatile fraction, RVF (% of the cumulative peak areas obtained for DPPa to the one obtained for RPP by HS-SPME-GC-MS)			
	Light	Gas	Temperature	Storage period (days)			
				13	33	58	93
DPPa1	- (obscurity)	- (N2)	- (4°C)	3.5	3.7	14.8	7.5
DPPa2	+ (day light)	- (N2)	- (4°C)	3.5	2.7	16.8	13.6
DPPa3	- (obscurity)	+ (air)	- (4°C)	3.1	2.1	5.4	5.9
DPPa4	+ (day light)	+ (air)	- (4°C)	3.0	2.6	5.2	6.3
DPPa5	- (obscurity)	- (N2)	+ (ambient)	5.6	9.7	17.1	19.7
DPPa6	+ (day light)	- (N2)	+ (ambient)	6.9	2.4	18.9	18.1
DPPa7	- (obscurity)	+ (air)	+ (ambient)	5.8	10.0	20.9	15.5
DPPa8	+ (day light)	+ (air)	+ (ambient)	6.7	10.0	20.6	19.5
Calculated effects (in bold if significant)							
Light				0.3 ± 0.3	-1.0 ± 0.9	0.4 ± 0.8	1.1 ± 0.9
Gas				-0.1 ± 0.3	0.8 ± 0.9	-1.9 ± 0.8	-1.5 ± 0.9
Temperature				1.5 ± 0.3	2.6 ± 0.9	4.4 ± 0.8	4.9 ± 0.9

Table 1: Experimental conditions, response values and main effects of the 2³ experimental design used to study the impact of storage on the volatile fraction.

ethanol mixture, i.e. DPPb samples. Using a micro syringe, 0,1 or 2 μ l of ethylacetate or ethanol were added to a 10 ml headspace vial containing 0.4 g of a delipidated protein powder. The vials were closed using an aluminum capsule with a rubber septum coated with PTFE and then subjected to ultrasonic vibrations for five minutes. After an equilibration period of one night at room temperature, the headspace was exposed to a SPME fiber (DVB/carboxen/PDMS) for 30 minutes at room temperature. The fiber was then desorbed in the GC-MS apparatus equipped with a fused-silica capillary column (30 m \times 0.32 mm ID, 0.5 μ m film thickness) coated with a DB-Wax stationary phase. Helium was used as the carrier gas at a constant flow of 1.5 ml/min. The initial oven temperature was maintained for 2 minutes at 35°C. Then, it was increased to 60°C at a rate of 2°C/min and from 60 to 240°C at a rate of 10°C/min. The final temperature was maintained for 10 minutes. All analyses were duplicated.

Results and Discussion

Impact of storage conditions on the evolution of the volatile fraction of DPPa samples during storage

Factors of the 2³ factorial experiment and corresponding percentages of volatile fraction are reported in Table 1. Tested factors were chosen for their known effect on the oxidation phenomenon and other degradation reactions: light, O₂, and temperature. The observed variable is the volatile fraction of the samples. The remaining volatile fraction recovery (RVF) was calculated as the percentage of the cumulative peak areas obtained for a given sample of delipidated protein powder (DPPa) compared to the cumulative peak areas obtained for the raw protein powder (RPP). RVF was measured during a three-month storage period with measurements at 13, 33, 58, and 93 days of storage. RVF ranged from 2.1% to 20.9% with mean values of 4.7, 5.4, 15.0, and 13.3, for 13, 33, 58, and 93 days of storage respectively. As hypothesized, the volatile fraction continues to evolve after delipidation. Whatever the conditions of storage, RVF dramatically increased between the first and second month of storage. After 2 months, it seemed quite stable.

The main effects of the different factors of the experimental design on RVF were calculated and are given in Table 1. Main effects showing contrasts higher than the experimental error were thus significant and reported in bold letters in the table. At 13 days of storage, only temperature had had an effect on RVF. As time went by, more and more factors became significant. Finally, at the end of three months storage, all the factors had a significant impact. Throughout storage, temperature had a positive effect: an increase of storage temperature induced an increase of RVF.

Surprisingly, in the presence of air, RVF decreased significantly at storage of two months and beyond. Despite this, the effect of air on RVF during storage remains low: -1.9 ± 0.8 after 58 days storage and -1.5 ± 0.9 after 93 days storage.

In the present experiments, light had little effect on RVF. After 93 days storage, a slight effect of light appears (1.1 0.9) indicating that this factor is not the most important one to be controlled in the storage of the delipidated protein powders. However, it should be noted that a part of UV light, especially UVB, was stopped by the refrigerator glass door. UVA ($315 < \lambda < 400$ nm) are able to induce lipid peroxidation [7].

Temperature increase can accelerate all types of reactions. For example, Maillard reactions, which occur slowly under normal conditions, can be accelerated by a temperature increase. A relative humidity (RH%) around 60 to 70% is optimum for these reactions

which produce furfurals and aldehydes. As the water content of samples is quite low (under 7% w/w [8], RH% is low too and does not correspond to the optimum for Maillard reactions. On the contrary, this low RH% is known to favor oxidation reactions. Some volatile compounds coming from lipid oxidation (aldehydes and ketones) are able to interact with amines and give a similar effect to reducing sugars in Maillard reactions [9]. Oxidation reactions and Maillard reactions are thus closely linked and detected volatile compounds can come from both types of reactions. Some volatile compounds chosen as tracers were studied more closely. Their relative quantities determined in DPPa1 (sample stored in the dark, at low temperature, and under nitrogen: the storage conditions supposedly the most favorable to low volatile fraction) and DPPa8 (sample stored in the light, at ambient temperature, and in presence of oxygen: the storage conditions supposedly the less favorable to low volatile fraction) are reported in Figures 1a and 1b respectively. At first sight, it seemed that amounts of volatile compounds found in the DPPa1 sample were far lower than the ones found in DPPa8, which was consistent with hypotheses. For each volatile compound, amounts increased as storage went on. This means that degradation reactions were not totally stopped using controlled storage conditions and that, as delipidation was not total, some substrate was still present in the powder for degradation reactions.

Benzaldehyde comes from Strecker degradation and is known to be a Maillard reaction product [10,11]. Amounts found in DPP were lower than 20% of what was found in RPP. In DPPa8, amount of benzaldehyde

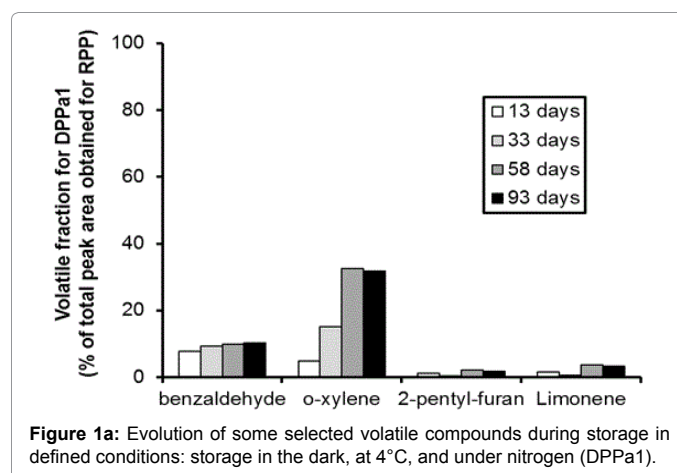


Figure 1a: Evolution of some selected volatile compounds during storage in defined conditions: storage in the dark, at 4°C, and under nitrogen (DPPa1).

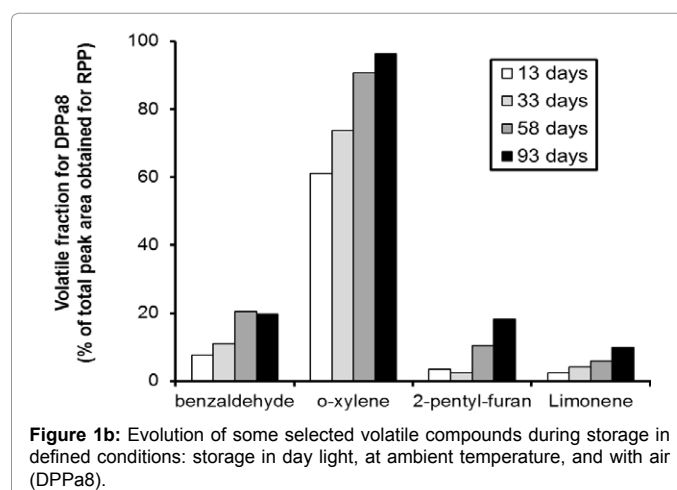


Figure 1b: Evolution of some selected volatile compounds during storage in defined conditions: storage in day light, at ambient temperature, and with air (DPPa8).

increased as storage went on, but seemed to be stable after 63 days of storage. The increase was feeble for DPPa1. This Maillard product was clearly favored by temperature increase and this is consistent with literature. Hexanal has been reported to be the most abundant compound resulting from lipid oxidation [12]. Unfortunately, in the present experiments, it was not detected as it was eluted at the same time as the residual solvent in the protein powder. Thus, it could not be selected as tracer of lipid oxidation, but other compounds linked with lipids degradation could be observed.

2-pentyl furan was studied originating from the thermal degradation of linoleic acid [13]. In the DPPa1 sample, 2-pentyl furan was found in very small amounts: 1 or 2% of what was found in RPP. In DPPa8, amounts of 2-pentyl furan increased exponentially during storage and were at 18% for 93 days storage. As expected, delipidation was not total and residual amounts of fatty acids can produce degradation products. As foreseen, thermal degradation of linoleic acid was favored by storage at ambient temperature. Nevertheless, controlled storage conditions can limit very efficiently the production of 2-pentyl furan.

Terpenes, such as limonene, are known as compounds that originate from animal feed; they can be directly transferred from grass to animal tissue and can be considered a green forage indicator [14]. Limonene was also reported to be a product generated from enzymatic hydrolysis-mild thermal oxidation of tallow [15]. In the DPPa1 sample, limonene was found in very small amounts: 1 to 4% of what was found in RPP. In DPPa8, amounts of limonene increased regularly during storage and were at 10% for 93 days storage. Limonene production was favored by storage at ambient temperature. So, it can be concluded that part of the limonene detected in *Eisenia foetida* powder comes from fat oxidation. Nevertheless, controlled storage conditions can limit and stabilize very efficiently the production of limonene.

Aromatic hydrocarbons, such as o-xylene, are reported as one of the largest classes of volatile compounds found in cooked meat, and are derived from the thermal degradation of lipid by thermal homolysis or autooxidation of long-chain fatty acid [16]. In the present experiments, o-xylene was found in huge amounts in DPP samples. In DPP8, amounts found after 93 days of storage were almost 100% of what was found in RPP. Nevertheless, controlled storage conditions succeeded in reducing the production of this volatile because it seemed to be stable after 58 days and represented 33% of the amount found in RPP.

Extraction by ethyl acetate/ethanol and quantification of solvent residues by the standard addition method

Experiments described here above showed the efficiency of delipidation using chloroform/methanol to obtain a dearomatized powder. In order to obtain a food grade ingredient, the extraction solvent must be changed for a safer one. For this purpose, an ethyl acetate/ethanol mixture was chosen on the basis of its similar polarity compared to the chloroform/methanol mixture. The amount of extracted lipids and the RVF determined for DPPb samples are reported in Table 2. The amount of extracted lipids depends on

the powder/solvent ratio and on the solvent proportion of the ethyl acetate/ethanol mixture. When the proportion of solvent towards the powder mass increased, the amount of extracted lipids increased. The 67/33 ethyl acetate/ethanol solvent proportion, which is similar to the Folch solvent proportion (a reference in lipid extraction [17]; 2/1, chloroform/methanol), extracted better the protein powder lipids in comparison with the 87/13 ethyl acetate/ethanol solvent mixture.

The amount of extracted lipids significantly varied with experimental conditions and ranged from 8.3% to 9.5%. These values were twice as low as what was obtained with the chloroform/methanol mixture [1]. Associated RVF aligned with the variations of the amount of extracted lipids, and ranged from 6% to 18% which is quite similar to what is obtained with the chloroform/methanol mixture. The sample showing the best results i.e. the highest amount of extracted lipids and the lowest RVF was DPPb3. It was kept for the following experiments.

In order to eliminate solvent residues, samples were dried overnight, either under a hood, or in an oven at 60°C. RVF was found at 4.8% ± 0.2 for DPPb3 under hood and 0.20% ± 0.01 for DPPb3 oven dried. It was then possible to reduce drastically the volatile fraction of the delipidated samples using a safe solvent and using an overnight drying. To ensure the safety of the delipidated powder, solvent residues were determined in DPPb3-oven dried. This was done using a standard addition method. Known amounts of ethyl acetate and ethanol were added to the samples and total peak areas were determined as previously done by HS-SPME-GC-MS.

Resulting curves were presented in Figure 2a for ethyl acetate and Figure 2b for ethanol.

Simple linear regressions were calculated for ethyl acetate and for ethanol from the following equation: $y=ax+b$; where x is the amount of pure compound added, y is the corresponding peak area. Using the regression models, the concentration of both solvents in the delipidated samples (x-intercept) was calculated. The concentration of ethyl acetate was found to be 3.072 mg/g of the sample, and of ethanol 1.164 mg/g.

Acute toxicity is lower for ethyl acetate than for ethanol: LD50 (orally tested on rat) is 7060 mg/kg for ethanol [18] and 11300 mg/kg for ethyl acetate [19], but sub-chronic toxicity of ethyl acetate is higher than that of ethanol. NOAEL values are respectively 900 mg/kg/day and 2400 mg/kg [20,21]. An additional experiment was then done to remove as much ethyl acetate as possible. DPPb3 was washed with ethanol before being oven dried. In this case, the concentration of ethyl acetate measured in the sample was far lower: 0.008 mg/g. As a consequence of ethanol washing, ethanol concentration increased and was found at 4.791 mg/g.

Conclusion

It is possible to obtain a dearomatized protein powder using delipidation and storage under controlled conditions. Storage experiments conducted up to 93 days showed limited volatile compounds evolution: 10% or less for three among the four volatile

Sample labels	Solvent proportion Ethyl acetate/ethanol (v/v)	Powder/solvent ratio(w/v)	Extracted lipids (% w/w of RPP, mean value ± standard deviation)	Remaining volatile fraction, RVF (% of the cumulative peak areas obtained for DPPb to the one obtained for RPP by HS-SPME-GC-MS, mean value ± standard deviation)
DPPb1	67/33	2/5	8.3 ± 0.3	12 ± 2
DPPb2	87/13	2/5	7.8 ± 0.0	18 ± 7
DPPb3	67/33	1/5	9.5 ± 0.0	6 ± 2
DPPb4	87/13	1/5	8.6 ± 0.5	9 ± 3

Table 2: Experimental conditions and response values for lipid extraction and volatile compounds recovery using ethyl acetate/ethanol mixture.

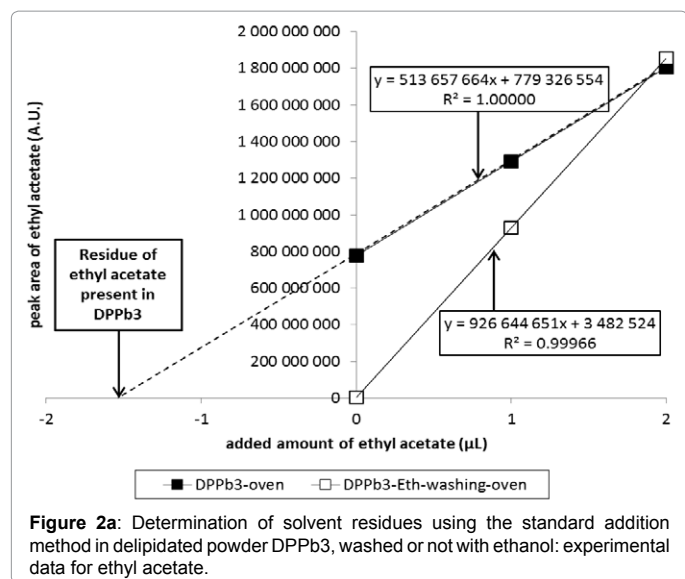


Figure 2a: Determination of solvent residues using the standard addition method in delipidated powder DPPb3, washed or not with ethanol: experimental data for ethyl acetate.

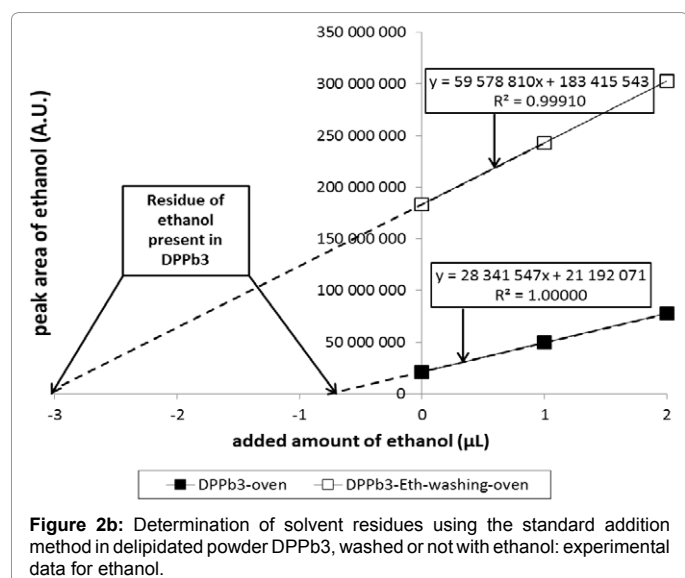


Figure 2b: Determination of solvent residues using the standard addition method in delipidated powder DPPb3, washed or not with ethanol: experimental data for ethanol.

compounds chosen as tracers. The main parameter to be controlled is temperature. There is no need to use storage under modified atmosphere. Additionally, it is possible to use a solvent mixture compatible with food grade products and conforming to EU regulations concerning solvent residues in food stuffs and food ingredients. De-aromatization was completed using an additional drying after delipidation.

References

- Romero BA (2010) Impact of lipid extraction on the dearomatization of an *Eisenia foetida* protein powder. Food Chemistry 119: 459-466.
- Luque-García JL, Luque de Castro MD (2003) Ultrasound: a powerful tool for leaching. TrAC Trends in Analytical Chemistry 22: 41-47.
- Sadek PC (2002) The HPLC solvent guide (2nd edn.) Wiley Interscience p: 664.
- The European Parliament and Council Directive (2009) Approximation of the laws of the member states on extraction solvents used in the production of food stuffs and food ingredients (2009/32/EC). Official Journal of the European Union.
- The European Parliament and Council Directive (2010) Amending Directive on the approximation of the laws of the member states on extraction solvents

used in the production of food stuffs and food ingredients (2010/59/EU). Official Journal of the European Union.

- Box GEP, Hunter WG, Hunter JS (1978) Statistics for experimenters. Design, data analysis and model building. Wiley and Sons Inc., New York.
- Bose B, Agarwal S, Chatterjee SN (1989) UV-A induced lipid peroxidation in liposomal membrane. Radiation and Environmental Biophysics 28: 59-65.
- Cayot N (2009) Physico-chemical characterisation of a non-conventional food protein source from earthworms and sensory impact in arepas. International Journal of Food Science & Technology 44: 2303-2313.
- Lorient D (1998) Modifications biochimiques des constituants alimentaires. Techniques de l'ingénieur Procédés biochimiques et chimiques en agroalimentaire: F 3400.
- Adamiec J (2001) Minor Strecker degradation products of phenylalanine and phenylglycine. European Food Research and Technology 212: 135-140.
- Mancilla-Margalli NA, López MG (2002) Generation of Maillard Compounds from Inulin during the Thermal Processing of Agave tequilana Weber Var. azul. Journal of Agricultural and Food Chemistry 50: 806-812.
- Bou-Maroun E, Cayot N (2011) Odour-active compounds of an *Eisenia foetida* protein powder. Identification and effect of delipidation on the odour profile. Food Chemistry 124: 889-894.
- Mandin O, Duckham SC, Ames JM (1999) Volatile Compounds from Potato-like Model Systems. Journal of Agricultural and Food Chemistry 47: 2355-2359.
- Vasta V, Priolo A (2006) Ruminant fat volatiles as affected by diet. A review. Meat Science 73: 218-228.
- Shi X (2013) Identification of characteristic flavour precursors from enzymatic hydrolysis-mild thermal oxidation follow by descriptive sensory analysis and gas chromatography-olfactometry and partial least squares regression. Journal of Chromatography B 913-914: 69-76.
- Domínguez R (2014) Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. Meat Science 97: 223-230.
- Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226: 497-509.
- Wiberg GS, Trenholm HL, Coldwell BB (1970) Increased ethanol toxicity in old rats: Changes in LD50, in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicology and Applied Pharmacology 16: 718-727.
- Smyth HF (1962) Range-Finding Toxicity Data: List VI. American Industrial Hygiene Association Journal 23: 95-107.
- US EPA (1986) Rat oral subchronic study with ethyl acetate. United States Environmental Protection Agency, Office of Solid Waste, Washington DC.
- OECD-SIDS (2004) SIDS Initial assessment report Ethanol in SIAM, UNEP Publications, Berlin.

Citation: Bou-Maroun E, Cayot N (2015) Food Protein Powder from *Eisenia foetida*: Dearomatization Using Food Grade Solvents and Controlled Storage Conditions. J Food Process Technol 6: 501. doi:10.4172/2157-7110.1000501