Journal of Food and Nutrition Research, 2016, Vol. 4, No. 6, 343-348
Available online at http://pubs.sciepub.com/jfnr/4/6/1

DOI:10.12691/jfnr-4-6-1

Nutrient Composition and Fatty Acid Profiles of Oven-dried and Freeze-dried Earthworm *Eisenia foetida*

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Abstract ***Background:*** *Eisenia foetida* are used as a feed source. However, information on nutrient composition and fatty acid profiles of earthworm *E.foetida* is limited, making it complex to include them into feed formulation. ***Objective:*** The aim of this study was to determine the nutritive value of freeze-dried and oven-dried earthworms, *E. foetida.* ***Design:*** The earthworms were oven- or freeze-dried, then analysed for nutrient composition (protein, fat, moisture and minerals) according to AOAC method and fatty acids using gas chromatography. ***Results***: Protein content was higher in freeze-dried earthworms while fat content of earthworms was not influenced by drying methods used. Most minerals (macro and micro) of *E. foetida* were significantly different except for calcium ($P < 0.05$) with freeze-dried *E. foetida* having the predominant minerals than oven-dried earthworms. Most of essential fatty acids were significantly higher in oven-dried *E. foetida* than in freeze-dried earthworms. No significant differences ($P > 0.05$) were observed on Margaric, Vaccenic, Arachidic, Tricosanoic, omega-3, SFA, MUFA, n-3, PUFA: SFA and PUFA/MUFA between oven-dried and freeze-dried samples. ***Conclusions***: The study revealed that freeze-dried *E. foetida* can serve as a better source of nutrients than oven-dried earthworms whereas fatty acids were better in oven-dried *E. foetida* than freeze-dried *E. foetida*. These results indicated that the effects of drying methods on *E. foetida* nutritive value were different.

Keywords: *chemical composition, drying methods, Eisenia foetida, fatty acids*

Cite This Article: Busisiwe Gunya, Patrick Julius Masika, Arno Hugo, and Voster Muchenje, "Nutrient Composition and Fatty Acid Profiles of Oven-dried and Freeze-dried Earthworm *Eisenia foetida*." *Journal of Food and Nutrition Research*, vol. 4, no. 6 (2016): 343-348. doi: 10.12691/jfnr-4-6-1.

1. Introduction

Earthworms *E. foetida* are commonly used as a protein source to feed chickens, pigs, rabbit and fish [1,2] and it has been found to be a substitute for fishmeal and soya bean in animal diets [3]. Moreover, they are a good source of essential amino acids, calcium, iron and fatty acids [4].

Earthworms are fed to animals in dried form. Frequently, they are dried using oven-drying and freeze drying methods [5]. Freeze-drying is a method of removing water by sublimation of ice crystals from frozen material [6]. The advantage of using freeze-drying is that there are minimal losses in volatile chemicals and heat-sensitive nutrients [6]. Moreover it ensures long shelf-life of the protein product [7]. Oven-drying is the commonly used method. Despite its frequent use, it does not enhance nutrient stability and bioavailability [8].

The value of earthworms as supplement in animal dietary formulation had been rated high by nutritionist [9]. Therefore it is a necessity to determine the chemical composition and fatty acid profile of *E. foetida*, which could help in the formulation of diets according to nutrient requirements of chickens. Information on the nutrient composition of earthworm *E. foetida* is limited, making it complex to include them into feed formulation by nutritionists and farmers. Furthermore, determination of the fatty acid profile of a diet is of importance to know the quality of the fat fraction of the diet [10] and may also be indicative of the absorbability of the lipids. Previous studies focused on nutrient composition of *E. foetida* [2,9,11,12,13] however, these studies did not provide in-depth comparison of fatty acid and nutrient composition of freeze-dried vs. oven-dried *E. Foetida*. Therefore the objective of this study was to determine the effects of oven drying and freeze drying on nutrient composition and fatty acid profile of *E. foetida*.

2. Materials and Methods

2.1. Study Site

Earthworms were obtained from Soil Science Department, University of Fort Hare, Alice. The worms were reared at the Fort Hare Research Farm, Alice, South Africa. The farm lies along longitude 32°78'E and latitude 26°85'S at an altitude of 450-500m above sea level. The farm is located in the False Thorn veld which is characterised by mean annual rainfall of 480mm and mean annual temperature of 18.7°C.

2.2. Ethics and Consent

The procedures followed in this study were approved by University of Fort Hare Ethics Committee (Certificate reference number: MAS021SGUN01).

2.3. Worm Management

Earthworms were fed a diet of organic waste compost twice a week specifically shredded paper and cow manure. The feed was spread on top of compost and water was sprinkle on it and then feed was thoroughly mixed with compost. In order to guarantee optimum growth conditions, optimum temperature 12-24°C, moisture 80-90% and pH >5 <9 of the compost were kept under control [7].

2.4. Processing of Earthworms

Earthworms were harvested with hand picking method. The harvested worms were thoroughly rinsed in water and kept in a bowl for 30 min to evacuate their guts [14]. They were killed with boiling water for a minute [7] and after they were dried with oven and freeze drying method.

2.4.1. Oven Drying

Earthworms were evenly spread on trays covered with foil to avoid fixing of earthworms on trays. Earthworms were dried in a conventional laboratory oven (Henaeus, Model no. T5050) at 90 ˚C for four hours [15]. Dried samples were milled into a powder using a mortar. The resultant dried powder was packed in airtight containers and refrigerated until they were analysed.

2.4.2. Freeze Drying

Earthworms were meshed for a minute with a household blender machine (Sunbeam Deluxe Glass Blender, Model no. SGB150). Samples were diluted with distilled water in a ratio of one kg of earthworm in two litres of distilled water [16]. The samples were then filtered with filter paper to remove dirt and soil particles. Earthworm samples were placed in 500ml vacuum bottles and then chilled at -35°C using a chilling machine (Vir Tis Benchtop K, VirTls Co., Gardner, NY, USA) up until they were frozen. Then they were placed in freeze dryer (Vir Tis Benchtop K, VirTls Co., Gardner, NY, USA) for 24 hours. The resultant dried powder was placed in airtight containers and refrigerated until it was analysed.

2.5. Nutritional Composition Determination

The nutrient composition of freeze-dried and oven-dried *E. foetida* was analysed to determine crude protein, calcium, magnesium, potassium, phosphorous, zinc, copper, iron, manganese, selenium and sodium. The proximate composition of earthworms was determined according to the AOAC [17] method. Crude protein was determined by the micro Kjedahl. Five macro (Calcium, Potassium, Sodium, Phosphorus and Magnesium) and four micro minerals (Iron, Zinc, Copper and Manganese) were determined using an Atomic Absorption Spectrophotometer.

2.6. Fatty Acid Profile Determination

A lipid aliquot (20 mg) of earthworm lipid was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Park and Goins, 1994).FAMEs from earthworm were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25mm ID, 0.2 µm film thicknesses). Analysis was performed using an initial isothermic period (40°C for 2 minutes). Thereafter, temperature was increased at a rate of 4°C/minute to 230°C. Finally an isothermic period of 230°C for 10 minutes followed. FAMEs in n-hexane (1µl) were injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

2.7. Statistical Analysis

The effect of drying methods (freeze drying and oven drying) on fatty acid profile and nutrient composition of *E. foetida* were analysed statistically using one way analysis of variance (ANOVA) of SAS (2006) and the differences among mean were tested for significance using Duncan test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Nutrient Composition of Freeze-dried and Oven-dried *Eisenia foetida*

Table 1. Nutrient composition of freeze-dried and oven-dried *Eisenia foetida*

Nutrients	Freeze dried	Oven dried
Crude protein (%)	66.2^{b}±0.63	59.7^{a}±0.63
Calcium (%)	0.82^{a}±0.02	0.82^{a}±0.02
Magnesium (%)	0.3^{b}±0.01	0.1^{a}±0.01
Potassium (%)	2.2^{b}±0.03	0.9^{a}±0.03
Phosphorous (%)	1.2^{b}±0.02	0.9^{a}±0.02
Zinc (mg/Kg)	317.0^{b}±5.02	150.7^{a}±5.02
Copper (mg/Kg)	812.1^{b}±11.23	22.3^{a}±11.3
Manganese (mg/Kg)	116.6^{b}±2.21	26.0^{a}±2.21
Iron (mg/Kg)	1498^{b}±7±23.35	495.3^{a}±23.3
Aluminium (mg/Kg)	1117.7^{b}±31.51	86.0^{a}±31.51

a, b Means in the same row with different superscripts are significantly different ($P < 0.05$).

The results for the effects of preparation method on nutrient composition of *E. foetida* are presented in Table 1. Significant impact ($P < 0.05$) on most of the macro elements (Potassium, Phosphorous and Magnesium) was observed, except for calcium ($P > 0.05$) where freeze dried *E. foetida* had the higher values. Similarly, there was a significant difference ($P < 0.05$) on trace elements between the two drying methods, where freeze-dried *E. foetida* had higher values for all micro elements (zinc, copper, manganese, iron and aluminium) measured than oven-dried samples.

3.2. Fatty Acid Composition of Freeze-dried and Oven-dried *E. foetida*

Table 2. Fatty acid composition of freeze-dried and oven-dried

Sample ID	Freeze dried	Oven dried
Proximate analysis (%)		
Fat (%)	10.0^{a}±0.44	9.5^{a}±0.44
Fat free dry matter (%)	36.4^{a}±2.08	79.9^{b}±2.08
Moisture (%)	53.5^{b}±1.66	10.5^{a}±1.66
Fatty acids		
C14:0	2.6^{a}±0.02	3.8^{b}±0.02
C15:0	1.0^{a}±0.01	1.2^{b}±0.01
C15:1c10	1.8^{b}±0.11	1.4^{a}±0.11
C16:0	14.8^{b}±1.03	6.7^{a}±1.03
C17:0	4.1^{a}±0.09	4.6^{a}±0.09
C17:1c10	1.3^{b}±0.23	0.1^{a}±0.23
C18:0	19.9^{b}±0.54	17.9^{a}±0.54
C1:1t9	1.6^{b}±0.01	0.0^{a}±0.01
C18:1c9	3.8^{a}±0.05	4.7^{b}±0.05
C18:1c7	13.4^{a}±0.34	14.5^{a}±0.34
C18:2c9,12(n-6)	6.2^{a}±0.13	10.2^{b}±0.13
C20:0	0.4^{a}±0.02	0.3^{a}±0.02
C18:3c9,12,15(n-3)	1.2^{b}±0.04	0.0^{a}±0.04
C20:2c11,14(n-6)	3.4^{b}±0.24	2.6^{a}±0.24
C22:0	0.4^{a}±0.03	1.3^{b}±0.03
C20:2c11,14(n-6)	2.4^{b}±0.04	1.6^{a}±0.04
C20,3c11,14,17(n-3)	5.3^{b}±0.19	0.0^{a}±0.19
C20:4c5,8,11,14(n-6)	7.3^{a}±0.41	9.3^{b}±0.41
C23:0	0.1^{a}±0.03	0.0^{a}±0.03
C20:5c5,8,11,14,17(n-3)	2.6^{a}±0.77	8.8^{b}±0.77
C24:0	0.4^{b}±0.12	0.0^{a}±0.12
C22:5c7,10,13,16,19(n-3)	0.5^{b}±0.03	0.0^{a}±0.03
Fatty acid ratios		
SFA	48.1^{a}±1.40	45.8^{a}±1.40
MUFA	23.4^{a}±0.36	22.2^{a}±0.36
PUFA	28.5^{a}±1.37	31.9^{b}±1.37
n-6	18.6^{a}±0.59	23.5^{b}±0.59
n-3	9.4^{a}±1.30	8.3^{a}±1.03
PUFA: SFA	1.2^{a}±0.06	1.4^{a}±0.06
PUFA/MUFA	2.0^{a}±0.21	2.9^{a}±0.21

a, b Means in the same row with different superscripts are significantly different ($P < 0.05$).

Results of the fatty acid composition of freeze and oven dried *E. foetida* are summarised in Table 2.Drying methods used on *E. foetida* had no ($P > 0.05$) significant effect on fat percentage of *E. foetida*. Significant differences were observed on moisture content and fat free dry matter of earthworms, with freeze-dried had higher moisture content and lower fat free dry matter than oven-dried *E. foetida*. Almost all fatty acids for freeze-dried and oven-dried *E. foetida* samples were significantly different ($P < 0.05$), except for C17:0, C18:1c7, C20:0, C23:0Margaric, n-3, SFA, MUFA, PUFA: SFA and PUFA/MUFA. Results showed that lipids of freeze-dried earthworms were composed of 48.1% of saturated fatty acids (SFAs), 24.4% of monounsaturated fatty acids (MUFAs) and 28.5% of polyunsaturated fatty acids (PUFAs) while oven-dried earthworms were composed of 45.8% of SFAs, 22.2% of MUFAs and 31.0% of PUFAS. Among the SFAs which occurred in higher proportions in freeze-dried earthworms were C16:0 and C18:0 and the rest were higher in oven-dried earthworms. C18:1c9 was the main fatty acid among MUFAs observed in *E. foetida*, and a higher proportion of C18:1c9was seen in oven dried earthworms. Significant differences ($P < 0.05$) were observed in PUFAs between oven-dried and freeze-dried earthworms. The oven drying method induced an increase of PUFAs between oven-dried and freeze-dried *E. foetida*. A higher level of n-6 was recorded in oven dried, whereas the proportions of n-3 among oven-dried and freeze-dried earthworms were similar.

4. Discussion

The potential of *E. foetida* as a protein source for animal feed has previously been reported by many researchers [2,9,18,19]. The results of this study established that protein content of *E. foetida* is influenced by the processing method used where freeze-dried *E. foetida* had higher protein content than oven-dried earthworms. The findings of the current study are consistent with the report by [7] who found that protein content of *E. foetida* decreased during oven drying as compared to freeze drying. The high protein content of freeze-dried *E. foetida* may be influenced by low temperatures used in freeze drying that ensure long-term stability of high-value protein [7]. The diminishing protein content of oven-dried *E. foetida* was expected due to the thermal denaturation of earthworm protein. In addition, the loss of protein could be attributed to the heating effect associated with oven drying condition which results in the unzipping of hydrophobic forces leading to a partial distribution of structure of the protein molecule [20]. Nevertheless, the current results contradict the findings on *Sternoceraorrissa* by [8] who found that oven drying increased protein content. This deviation may be due to the temperature variation of these studies. In the current study the temperature used was 90°C and in a study by [8] a temperature of 66˚C was used. *Eisenia foetida* has been known to have high protein content that ranges between 58 % and 71 % dry weight [4,13] and growing chickens require only 20% of crude protein for their growth [21]. The crude protein supplied by *E. foetida* is greater than the protein requirement of chickens thus making it to be a good protein supplement.

The methods used to prepare and preserve food and feed may affect the concentration and availability of minerals and other essential compounds [22]. Out of two preparation methods used, freeze-dried *E. foetida* retained most of the macro minerals (magnesium, potassium and

phosphorous). This may be due to the fact that freeze drying method is able to keep hold of most of nutrients since during freeze drying, external influences are minimum and oxidizable substances are well protected under vacuum conditions [6]. Oven drying reduced the nutrient value of *E. foetida* either through chemical modification or direct loss of minerals by direct heating through inducing biochemical and nutritional variation in earthworm composition. Calcium content between oven-dried and freeze-dried *E. foetida* was not different. This similarity of calcium content between oven-dried and freeze-dried *E. foetida* may be attributed to the fact that calcium can be used as a drying agent [23] hence no differences were found between the two drying methods. The current results are in accordance with finding on injera from pre-fermented flour by [24] who found no differences on calcium content of freeze and oven dried injera from pre-fermented flour. Calcium and phosphorus are essential for the formation and maintenance of the skeleton. Most of the calcium in the diet of the growing bird is used for bone formation, whereas in the mature laying fowl most dietary calcium is used for egg shell formation [21]. Magnesium is found in natural feed of poultry; hence deficiency is rare. Although it has been reported that once newly hatched chicks fed a diet totally devoid of magnesium live only a few days [25]. Potassium is important to control blood pressure, anxiety and stress, enhance muscle strength, water balance, electrolytic function and nervous system [26].

The earthworm *E. foetida* is also capable of providing substantial quantities of trace elements that are essential for chickens, in particular iron, zinc and copper. Freeze-dried *E. foetida* contained peak concentration levels of all trace elements. This is in line with the report by [27] who reported that freeze drying avoids losses of trace element. The high levels of trace elements of freeze-dried earthworms may be due to the low temperature used in freeze drying process which prevented most of microbial reactions with the final product having excellent quality [28]. Decreases in the amount of trace elements in oven-dried *E. foetida* may be due to the heat applied during the process, by inducing biochemical and nutritional variations in earthworm composition. Zinc is important for the immune system, sexual maturity and reproductive capacity [29]. In addition, Zinc is well known for its anti-viral, anti-bacteria; anti-fungal and anti-cancer properties [30]. Iron is a constituent of haemoglobin and myoglobin for oxygen transport and is a component of many enzymes containing protein [29]. It is also needed for normal functioning of the central nervous system and in oxidation of carbohydrates, protein and fats [31]. Copper is a necessary component of a number of enzymes, which function in increased structural strength, elasticity of connective tissues and blood vessels [29]. Moreover, copper is required for antibody development and lymphocyte replication [32].

Moisture content of *E. foetida* was influenced by drying methods used in this study where freeze-dried *E. foetida* had higher moisture than oven-dried *E. foetida*. The high moisture content of freeze-dried *E. foetida* may be attributed to the fact that freeze-dried products can be easily rehydrated much more quickly as the process leaves microscopic pores hence; freeze-dried worms had higher moisture content than oven-dried *E .foetida*.

The present findings revealed that fat content of *E. foetida* is not influenced by drying method used. This could be attributed to the temperature (90°C) used in this study for oven drying that did not cause decrease of the fat content hence there were no variation between the two drying methods. The results of the current study are comparable with previous studies, which indicated that the fat content of *E. foetida* ranges between 9 % and 10 % [11]. Fats are required in poultry diets to absorb fat-soluble vitamins (vitamin A, D, E and K) [33]. Furthermore, fats are added in poultry diets to improve palatability of the feed [10]. However, the current findings are different to the report by [8] who found that the oven drying increased the fat content of African metallic wood boring beetle.

Oven-dried *E. foetida* had higher amount of n-6 as compared to freeze dried. The increase of n-6 in oven-dried earthworms may be attributed to the fact that hot air from oven drying induces an increase of PUFAs [34], such as n-6. Hence oven dried *E. foetida* have higher n-6as compared to freeze-dried *E. foetida*. Findings of this study are in line with the report [35] and [36] who reported that earthworms are rich in n-6. In poultry, n-6 is very important for brain and heart function and in growth and development [38].

The unsaturated omega-3 fatty acids were higher in the freeze dried samples. This may be attributed to milder freeze drying process. Furthermore, oven drying is more aggressive process with the samples exposed to air that may lead to oxidation and destruction of long chain unsaturated omega-3 fatty acids like C18:3c9,12,15(n-3) and C20: 3c11, 14, 17 (n-3). The exposure to heat in oven drying may enhance dehydration of earthworms and cause considerable loss of fatty acids [39]. Similarly results were seen on chicken sausage by [40]who found that unsaturated fatty acids decreased significantly in oven drying due to lipid oxidation that initiated by the presence of oxygen, which attack the double bond of unsaturated fats.

Even though, there are different fatty acids present in poultry diets, chickens have a specific requirement for one fatty acid which is C18,2c9,12,15(n-3) [41]. The findings of the current study are in agreement with the report by [42] who found that *E. foetida* had high proportions of essential fatty acid including C18,2c9,12,15(n-6) and C18:3c9,12,15(n-3). C18, 2c9, 12,(n-6) was found to be higher in oven-dried samples than in the freeze-dried in this study. This may be due to the fact that oven drying minimally affects the fatty acids [39]. This can be related to the lower dehydration produced in oven drying [43].C18:2c9, 12 (n-6) is regarded as of particular importance in poultry diets [44]. Once chickens have a deficiency in C18:2c9, 12 (n-6), it causes retarded growth, increased water consumption, reduced resistance to disease, an enlarged liver with increased lipid content and elevated concentrations of eicosattienoic [41]. Hence, diets for poultry should contain adequate amount of C18:2c9, 12 (n-6).

As expected, freeze-dried *E. foetida* contained most of the fatty acids such as C13:0, C15:1c10, C16:0, C17:1c10, C18:0, C18:1t9, C24:0, C22:5c7,10,13,16,19(n-3)though retained non essential fatty acids. The predominance of some fatty acids in freeze-dried *E. foetida* may be due to the minimal changes that occur during the process because

the growth of microbes and enzyme effects cannot be exerted under low temperatures [6]. Moreover, this may be due to the non-existence of water and low temperature employed during freeze drying process, which stops most microbial reactions, with the final product having an excellent quality [28].

5. Conclusion

The study shows that effects of two drying methods (freeze drying and oven drying) had significant differences on nutrient composition and fatty acid profile of *E. foetida*. Freeze drying method recorded higher concentrations of nutrients and fatty acids than oven drying method. Therefore, freeze drying could be preferred drying method for *E. foetida*.

Conflict of Interest and Funding

No conflict of interests was reported by the authors.

Acknowledgements

Authors are grateful for the support provided by the Agriculture and Rural Development Research Institute and the Department of Livestock and Pasture, while this work was being conducted. This work was financially supported by the [NRF Scarce Skills under Grant (97955)]; and [DAFF Zero Hunger under Grant (P271)].

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