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Earthworm (*Alma millsoni*) Powder's Nutritional and Microbial Qualities Significantly Affected by Processing Protocols

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Abstract: Earthworm powder is an earthworm-derived product that has been successfully used as therapeutic medicine and livestock feed supplement. A number of earthworm powder processing protocols are randomly used, but there is inadequate information on the comparative effects of these protocols on powder quality. In this study, earthworm powders were prepared from gut-voided earthworms by sun-drying, oven-drying, and freeze-drying protocols. The nutritional and microbial qualities of the processed powders were assessed using standard procedures. Results showed that the macronutrient concentrations of sun-dried, oven-dried, and freeze-dried earthworm powders were significantly different from one another (p<0.05). Freeze-dried earthworm powder had the highest percentage of fat (14.20±0.07%), protein (66.60±0.06%), and fibre (0.18±0.01%), while sun-dried powder had the least. Relatedly, freeze-dried earthworm powder had the highest concentrations of almost all the amino acids. Sun-dried earthworm powder recorded the highest significant (p<0.01) total viable counts of bacteria and fungi. The significant differences observed in nutritional and microbial qualities among the earthworm powders are instructive. It is not just enough to produce earthworm powder, consideration should be given to the appropriate protocol that would yield the right quality and reduce microbial contamination. Where processing duration, equipment availability, and ease of processing are no challenge, freeze-drying protocol is best recommended. The high nutrient content of all the earthworm powders reinforces the potential opportunity presented by earthworms and earthworm powders for use as rich sources of quality nutrition.

1. Introduction

Apart from their roles in promoting soil fertility, plant growth, and crop yield, earthworms and earthworm-derived products have continued to gain increasing applications in medicine, environmental health management, and livestock feed supplement. Earthworm-derived products include vermicasts, vermicompost, vermifluid, earthworm powder. Earthworm powder, also referred to as earthworm

paste, is obtained when fresh earthworms are processed into powder or paste by drying out their moisture content. Earthworm powders from different species have been used in medicine and agriculture, with good results. For example, oral preparations of *Lumbricus rubellus* powder is reportedly used in the Bali province of Indonesia, to treat health conditions, including diabetes, stroke, joint pain, hypertension. In addition, laboratory study showed that *L. rubellus* powder can be used as a natural antioxidant source, to treat conditions associated with inflammation and oxidative stress (Dewi *et al*., 2017). *Perionyx excavatus* powder possesses hapatoprotective and antioxidant properties (Prakash *et al*., 2008). *Lampito mauritii*, *Eudrilus euginae* powders have been found to possess antimicrobial and antifungal properties (Vasanthi *et al*., 2013; Bhorgin and Uma, 2014). Bansal *et al*. (2015) observed that *P. excavatus* and *Pheretima posthuma* powders inhibited pathogenic bacteria growth, and are therefore potential sources of drugs to suppress antibiotic-resistant bacteria.

Similarly, earthworm powders are now receiving attention for use as livestock feed supplements, owing to increasing cost of conventional sources of proteins, such as fishmeal. Zang *et al*. (2018) in their study found that dietary inclusions of broiler feed with 1%–5% earthworm powder safely improved feed conversion ratios by up to 12.64% and 22.45%. Agnes and Thirumathal (2018) reported improved growth performance and gonad weight in freshwater male fish (Common carp) when fish feed was supplemented with earthworm powder, at levels of 5-7.5 %. Sogbesan *et al*. (2007) in their study, included tropical earthworm (*Hyperiodrilus euryaulos*) powder in fish feed and concluded that the substitution of earthworm powder (7.5 to 25%) for fishmeal lowered the cost of diet, without a reduction in the specific growth rate. Hesami *et al*. (2020) reported that 1% and 1.5% levels of dietary inclusions of earthworm (*Eisenia fetida*) powder significantly improved the growth and reproductive performance of Japanese breeder quails, respectively. Nalunga *et al*. (2021) equally reported that *Eisenia fetida*-supplemented feed improved the meat quality of broiler chickens.

There are three major procedures used to process fresh earthworms to earthworm powder, of which the difference lies mainly in the method of moisture removal. These are oven-drying, freeze-drying, and sun-drying methods. In the oven-drying method, gut-voided earthworms are dried in an oven at a temperature of between 40-80^{o}C for 3-12 hours, after which the dried earthworms are homogenized (Sogbesan *et al*., 2007; Dedeke *et al*. 2009; Bhorgin and Uma, 2014; Dewi *et al*., 2017). In the freeze-drying method, fresh gut-voided earthworms are homogenized and frozen at low temperature (< -5^{o}C). The temperature of the homogenate is then increased stepwise from -10^{o}C to 90^{o}C. The suspension is then vacuum-dried under a vacuum of 100 mmHg for 5-60 hours. This results in a sterile earthworm powder (Prakash *et al*., 2008). In the sun-drying method, gut-voided earthworms are kept in plastic containers, covered tightly with transparent polythene sheets, and exposed to sunlight for about three days. A brown paste or powder that is formed is thereafter homogenized (Vasanthi *et al*., 2013; Bansal *et al*., 2015). Though these three methods are randomly used in research, information on the comparative effects of all the protocols on powder quality is limited. This study therefore aimed at comparing the qualities of earthworm powders processed by oven-drying, freeze-drying, and sun-drying methods.

2. Methodology

2.1 Collection and processing of earthworms to powder

Alma millsoni, a tropical wetland earthworm species, was collected from within the main campus of the University of Lagos, Nigeria. Earthworm collection was done by digging and hand sorting. The harvested earthworms were put in dechlorinated tap water for about two hours, to void their gut (Dada

et al., 2021). Gut-voided earthworms (Fig. 1) were processed to earthworm powders using three protocols: (1) oven-drying, (2) freeze-drying, (3) sun-drying.

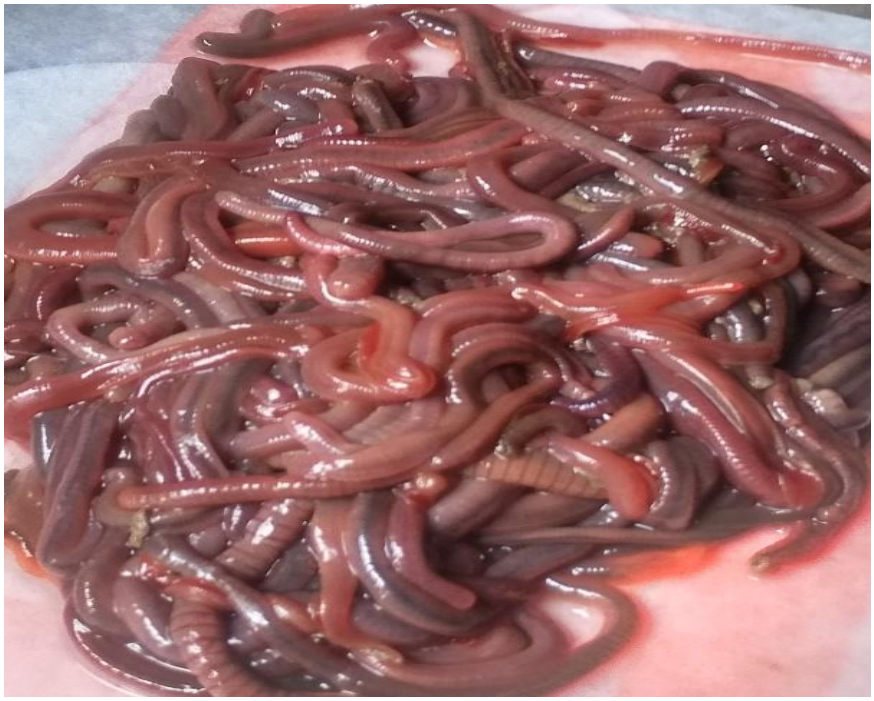

Fig. 1. Fresh, gut-voided earthworm (*Alma millsonia*)

To prepare earthworm powder using the oven-drying protocol, gut-voided worms weighing 500 g were collected in a petri dish and kept in an oven at 70°C for 24 hours. The dried worms were thereafter homogenized into powder (Bhorgin and Uma, 2014).

Freeze-drying protocol was adapted from Prakash *et al*. (2008), with a slight modification**.** Gut-voided earthworms weighing 500 g were wet-ground and then frozen immediately at low temperature (< -5°C). The frozen homogenate was then vacuum-dried under a vacuum of 100 mmHg for 48 hours.

In the sun-drying method, gut-voided earthworms were spread in a plastic container, covered tightly with transparent polythene sheet, and exposed to sunlight for 3 days. The earthworms were homogenized, forming a brown paste or powder (Vasanthi *et al*., 2013; Bansal *et al*., 2015).

The processed earthworm powders were collected in airtight containers and refrigerated at < - 4^0C until further analyses.

2.2 Determination of nutritional composition of earthworm powder samples

The percentage composition of protein (crude protein), fat (crude fat), fibre (crude fibre), ash, total carbohydrates, and moisture was determined according to the procedures outlined in the Official Methods of Analysis by AOAC (Horwitz and Latimer, 2005).

2.3 Determination of amino-acid profiles in earthworm powders

To determine the amino acid profile of earthworm powders, samples of earthworm powder were first extracted following the standard procedure described by Kaji *et al.* (1964). Two grams (2.0 g) of each sample was weighed into a 250 ml beaker. Thereafter, 20 ml of 0.2M phosphate buffer solution, pH 7.0, was added to the beaker. The beaker content was stirred for about three minutes and the resulting mixture was centrifuged at 2,000 rpm for 10 minutes. The supernatant was shaken three times

with 10 ml portion of petroleum ether, to remove the organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acids was retained. Protein was precipitated from the aqueous phase by adding 5.0 ml of 10 % trichloroacetic acid (TCA) to 5.0 ml extracts. The mixture was shaken and kept in a freezer for 10 minutes. The precipitate formed was removed by centrifugation (2,000 rpm), and the filtrate was used for the amino acids profiling.

The amino acids content in the extracted earthworm powder were separated by thin layer chromatography method. Aliquots of 50 µl of the extract were spotted in Avical microcrystalline cellulose (Whatman analytical plates) thin-layer plates, along with 20 µl of reference standard mixture. The reference mixture contained lysine, histidine, phenylanaline, methionine, glycine, cysteine, proline, leucine, Iso-leucine, threonine, tyrosine, valine, arganine, tryptophan, and glutamic acid (BDH and Sigma Chemical), each present at a concentration of 0.1 % w/vol. One-dimensional ascending chromatography was done. The solvent system employed for the separation was n-buternol-glacial acetic acid-water, at a ratio of 4:1:2 v/v. After 4 hours of separation, the chromatograms were air-dried and the amino acids were located by spraying with locating reagent of 0.2 % w/v of Ninhydrin in ethanol. The sprayed chromatograms were air-dried and later oven-dried at 100˚C for 5 minutes, for the spots to be clearly identified. The separated amino acids were identified using the reference standard spotted analysis.

The quantitative estimation or determination of the amino acids (profiles) was done through the colorimetric method of Rosen (1957). First, the developed thin-layer chromatography plates used in locating the positions of amino acids were cut out and eluted with 5 ml distilled water at 70˚C, for two hours. The cellulose powder was removed by centrifugation at 5.00 rpm for 5 minutes. The supernatants were decanted and kept for the colorimetric analysis of amino-acid profile.

For colorimetric analysis of amino acid profile, to 1ml of the diluted extracts of each amino-acid, 0.5ml cyanide-acetate buffer (pH 5.4) and 0.5ml of 3% ninhydrin in methylcellosolve were added. The mixture was heated in a boiling water-bath at 100˚C for 15 minutes. Immediately the mixture was removed from the water bath, 5.0 ml of Iso-propyl alchohol-water mixture (ratio 1:1) was added as diluents, and the mixture was shaken vigorously, and then cooled to room temperature (25˚C). The amino acid profiles were estimated by determining the optical density at 570nm wavelength, using Pre-Unicam UV-visibile spectrophotometer (Model: 5623 uv/vis). The blank was given the same treatment as the sample and used as the control to set the absorbance to zero (diluted water). The amount of each amino-acids (profile) content was calculated from the standard curve of known concentration of leucine (100 mg/ml) as indicated in **Eqn. 1** below:

$$Amino\ acid\ profile\ (mg/100g) = \frac{Absorbance\ of\ sample}{Slope}\ X\ \frac{1}{w}\ X\ \frac{100\ x\ df}{1} \qquad \textbf{Eqn. 1}$$

Where;
Slope= from standard curve
W= weight of the sample
df = dilution factor

2.4 Microbiological analysis of earthworm powders

Earthworm powder was analysed for total heterotrophic bacteria and fungi population in colony forming unit per gram (CFU/g), using the 'standard pour plate technique', as described by Dubey and Maheshwari (2014). One gram (1g) of earthworm powder sample was suspended in 9 ml of distilled water and mixed well. The suspension was serially diluted; dilutions 10^{-1} to 10^{-3} were chosen for

inoculation. One hundred microlitre (100 µl) of the diluted samples were inoculated into sterile Petri dishes using the pour plate method with appropriate media. The total bacterial plate count was isolated on Nutrient agar (NA), Coliform on MacConkey agar, *Escherichia coli* on Eosin Methylene Blue (EMB) agar, *Salmonella* spp. on Salmonella Shigella (SS) agar, *Staphylococcus* spp. on Mannitol salt agar, *Clostridium* spp. on Reinforced Clostridial agar (RCA), *Vibrio* spp. on Thiosulfate citrate bile salts sucrose (TCBS) agar, Actinomycetes on Starch Casein agar (SCA). Fungi isolation was carried out using Potato dextrose agar (PDA), supplemented with streptomycin (0.14gkw/v). All media were prepared according to manufacturer protocol. The bacteria plates were incubated at 37°C for 24 hours and fungi plates were incubated at room temperature (28°C) for 3 to 5 days. The colonies that developed were counted in duplicates using colony counter and expressed in colony forming unit per gram (CFU/g).

2.5 Statistical analysis

The data generated from the nutritional and microbial analyses of earthworm powders were subjected to descriptive analysis using the Analysis of Variance (ANOVA). The mean differences were separated using Duncan Multiple Range Test at 5% level of significance ($p < 0.05$). All statistical analyses were performed using IBM SPSS v 26 (IBM Corporation, New York).

3. Results and discussion

3.1 Nutritional composition of earthworm powders

The result of proximate analysis of earthworm powders, as presented in Table 1, showed that the macronutrient compositions of sun-dried, oven-dried, and freeze-dried powders were significantly different from one another ($p < 0.05$), except for crude fibre which percentage composition in oven-dried and sun-dried powders were not significantly different ($p > 0.05$). Freeze-dried earthworm powder had the highest percentage of fat (14.20±0.07 %), protein (66.60±0.06 %), and crude fibre (0.18±0.01 %). Sun-dried earthworm powder had the least percentage ash (4.90±0.07 %), fat (10.93±0.04 %), protein (59.92±0.05 %), and fibre (0.14±0.01 %), but the highest percentage carbohydrate (11.86±0.48%) and moisture (12.27±0.33 %).

Table 1. Nutritional composition of earthworm powders

Earthworm powder type	Proximate composition					
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fibre (%)	Total carbohydrate (%)
Oven-dried	9.07±0.02^{c}	5.93±0.04^{a}	12.70±0.07^{b}	61.93±0.15^{b}	0.17±0.01^{a}	10.22±0.21^{b}
Sun-dried	12.27±0.33^{a}	4.90±0.07^{c}	10.93±0.04^{c}	59.92±0.05^{c}	0.14±0.01^{b}	11.86±0.48^{a}
Freeze-dried	10.90±0.57^{b}	5.58±0.04^{b}	14.20±0.07^{a}	66.60±0.06^{a}	0.18±0.01^{a}	2.66±0.39^{c}
F	35.90**	217.17*	1433.44**	2389.54**	17.33*	338.40**

Data (mean ± standard deviation, n = 3) in the same column with different superscripts are significantly different (**$P < 0.01$, *$P < 0.05$).

3.2 Amino acid profile of earthworm powders processed by different methods

Of the seventeen amino acids found in the earthworm powder samples, glutamic acid occurred in significantly highest ($p < 0.05$) concentrations of 7.00±0.04 mg/g, 7.17±0.04 mg/g, and 7.57±0.08 mg/g

in oven-dried, sun-dried, and freeze-dried samples, respectively (Table 2). Next to glutamic acid in concentrations, were iso-leucine (oven dried: 2.92±0.00 mg/g, sun-dried: 3.06±0.04 mg/g, freeze-dried: 2.52±0.95 mg/g), and aspartic acid (oven-dried: 2.81±0.00 mg/g, sun-dried powder: 2.92±0.08 mg/g, freeze-dried: 3.06±0.04 mg/g). Earthworm powder prepared by freeze-drying method had the highest concentrations of almost all the amino acids (valine, phenylalanine, methionine, leucine, histidine, aspartic acid, glutamic acid, serine, proline).

Table 2. Amino acid profile of earthworm powders processed by different methods

S/N	Types of amino acids	Amino acids concentrations (mg/g) in earthworm powder types			
		Oven-dried	Sun-dried	Freeze-dried	F
	Essential				
1	Lysine	1.43±0.04^{a}	1.38±0.27^{a}	1.71±0.04^{a}	2.43
2	Valine	1.32±0.04^{b}	1.38±0.04^{ab}	1.49±0.04^{a}	8.63
3	Phenylalanine	1.06±0.04^{b}	1.14±0.08^{ab}	1.27±0.04^{a}	7.88
4	Methionine	1.65±0.04^{b}	1.78±0.00^{a}	1.87±0.04^{a}	23.07*
5	Iso-leucine	2.92±0.00^{a}	3.06±0.04^{a}	2.52±0.95^{a}	0.52
6	Leucine	1.11±0.42^{b}	1.19±0.07^{ab}	1.32±0.04^{a}	7.84
7	Histidine	2.84±0.04^{b}	2.94±0.04^{b}	3.11±0.04^{a}	23.83*
8	Threonine	1.29±0.07^{a}	1.32±0.04^{a}	1.44±0.04^{a}	4.37
9	Arganine	1.24±0.07^{a}	1.30±0.08^{a}	1.46±0.07^{a}	4.90
	Non-essential				
10	Tyrosine	0.64±0.40^{a}	0.95±0.04^{a}	1.16±0.10^{a}	2.38
11	Cysteine	0.41±0.04^{a}	0.54±0.07^{a}	0.46±0.04^{a}	3.44
12	Aspartic acid	2.81±0.00^{b}	2.92±0.08^{ab}	3.06±0.04^{a}	12.42*
13	Glutamic acid	7.00±0.04^{b}	7.17±0.04^{b}	7.57±0.08^{a}	55.65*
14	Serine	2.41±0.04^{b}	2.49±0.08^{ab}	2.62±0.04^{a}	7.79
15	Alanine	0.89±0.04^{a}	1.00±0.04^{a}	0.95±0.04^{a}	3.74
16	Proline	1.81±0.04^{b}	1.87±0.04^{b}	2.03±0.04^{a}	16.22*
17	Glycine	0.12±0.08^{a}	0.07±0.01^{a}	0.10±0.01^{a}	0.62

Data (mean ± standard deviation, n = 3) in the same row with different superscripts are significantly different; *$P < 0.05$.

3.3 Microbial counts of earthworm powders processed by different methods

The total viable count was significantly highest ($p < 0.01$) in sun-dried earthworm powder (88.67x10³ ± 3.06 CFU/g), and least in freeze-dried powder (56.00x10³ ± 2.65 CFU/g). Freeze dried powder also had the least counts for *Clostridium* spp. (14.33x10³ ± 1.53 CFU/g) and Fungi (2.00 x10³ ± 1.00 CFU/g). Sun-dried earthworm powder also recorded the highest counts for Coliform: 38.67 x10³ CFU/g; *Staphylococcus* spp.: 26.33 x10³ CFU/g; *Clostridium* spp: 30.67 x10³ CFU/g; *Vibrio* spp.: 6.00 x10³ CFU/g; Fungi: 7.00 x10³ CFU/g; Actinomycetes: 17.00 x10³ CFU/g. Oven-dried earthworm powder had the least count for Coliform (28.00 x10³ CFU/g), *Vibrio* spp. (2.00 x10³ CFU/g), and Actinomycetes (6.67 x10³ CFU/g). There was no growth recorded for *E. coli* and *Salmonella* spp in all the samples of earthworm powder (Table 3).

Table 3. Microbial count of earthworm powders processed by different methods

S/N	Microorganisms	Colony Forming Unit/g (x10³)			
		Oven dried	Sun dried	Freeze dried	F
1	Total viable count	68.67±3.06^{b}	88.67±3.06^{a}	56.00±2.65^{c}	95.12**
2	Coliform	28.00±7.94^{b}	38.67±1.53^{a}	33.67±2.52^{ab}	3.58*
3	*Escherichia coli*	0.0000	0.0000	0.0000	-
4	*Staphylococcus* spp.	10.00±1.00^{b}	26.33±2.52^{a}	8.00±2.00^{b}	80.32**
5	*Clostridium* spp.	22.33±2.08^{b}	30.67±3.06^{a}	14.33±1.53^{c}	37.52**
6	*Salmonella* spp.	0.0000	0.0000	0.0000	-
7	*Vibrio* spp.	2.00±1.00^{c}	6.00±2.00^{a}	3.00±1.00^{b}	6.50*
8	Actinomycetes	6.67±1.55^{c}	17.00±1.00^{a}	12.00±1.00^{b}	70.10**
9	Fungi	5.00±1.00^{b}	7.00±1.00^{a}	2.00±1.00^{c}	19.00*

Data (mean ± standard deviation, n = 3) in the same row with different superscripts are significantly different (**$P < 0.01$, *$P < 0.05$).

The effectiveness or potency of any therapeutic or feed supplement is largely dependent on the quality of its bioactive compounds; in turn, the quality of bioactive compounds can be influenced by the handling and processing procedures. Hence, the need for comparative assessment of available protocols on the quality of a product, as done in this current study. Although studies that directly corroborate the significant variations in nutritional and microbial qualities among earthworm powders processed by the three protocols were not readily available, the high nutritional composition recorded by the earthworm powders agrees with many previous studies (Sogbesan *et al*., 2007; Dedeke *et al*. 2009; Zang *et al*., 2018; Agnes and Thirumathal, 2018).

The findings of the current study show that the methods used in removing moisture during the preparation of earthworm powders had significant impact on both the nutritional quality and microbial safety of the powders. Freeze-drying method produced earthworm powder with the highest nutritional quality (fat, protein, crude fibre and amino acids), relative to the two other methods. This is consistent with the findings of Gunya *et al*. (2016) who compared the nutrients and fatty acids of earthworm (*Eisenia foetida*) powder processed by oven-drying and freeze-drying methods. Bou-Maroun *et al.* (2013) also reported increased protein content in freeze-dried *E. foetida* powder, relative to the oven-dried powder.

The relatively higher microbial counts observed in the sun-dried powder might have resulted from the longer period of handling and other conditions, such as moisture and temperature, that allowed for the propagation of microbial communities. Thus, the least fat, protein, crude fibre and amino acids contents recorded for sun-dried powder may be due to its high microbial activities (as reflected by its high microbial counts) that ultimately resulted in the degradation of organic molecules (Belitz *et al*., 2004). Degradation by microbes may occur by direct secretion of degrading enzymes on the organic substrate or release of enzymes from the cells dead microbes (Belitz *et al*., 2004; Oanh, 2016; Raveendran *et al*., 2018). Lipid oxidation is also a phenomenon that can account for the decreased fat and protein components in the sun-dried powder. Lipid oxidation initiated by light (form the sun, in this case) and free radicals, released at cell death, significantly modify the chemical structure of fats, and by a cascade of reactions, leads to amino acid destruction and decrease in protein solubility (Ahmed *et al*., 2016; Abraha *et al*., 2018; Huang and Ahn, 2019). The implication of these is that, using sun-drying method to process earthworm powder significantly compromises its nutritional value. Similarly,

the decreased fat and protein recorded for oven-dried powder, relative to freeze-dried powder, may be due to lipid oxidation and protein denaturation, occasioned by the heat applied during processing (Abraha *et al*., 2018). On the other hand, the higher nutritional contents recorded for freeze-dried powders, compared to the two other powders, can be attributed to possible suppression of lipid oxidation, microbial activities and enzymatic degradation, by the low processing temperature.

The presence of *Staphylococcus* spp., *Clostridium* spp., *Salmonella* spp., *Vibrio* spp., and fungi in the earthworm powders could be ascribed to the close association of earthworms to soil environment, where these microorganisms are abundant. A previous study has similarly isolated *Clostridium* spp, along with *Bacillus* spp and *Aspergillus* spp, from the vermifluid of *A. millsoni* (Dada *et al.,* 2021). Some of these microorganisms, such as Actinomycetes and Fungi, are useful, as they are known to produce antimicrobials that have been utilized in therapeutic and drug development. Others, like *Staphylococcus* spp., *Clostridium* spp., *Salmonella* spp., *Vibrio* spp could be dangerous indicators, and their presence may require subjecting the earthworm powders to additional post-powdering treatments, to completely eliminate them. Nevertheless, the total viable bacterial and other microbial counts recorded for all the powders ($< 10^4$ CFU/g) were below the acceptable limits ($< 10^5$ CFU/g) for food products (OECD, 2011). Hence, each of the three protocols yielded earthworm powder of acceptable microbial level.

In view of the variations in nutritional and microbial qualities of the earthworm powder types, deciding which protocol to adopt for processing earthworms to powder requires a careful balance of several factors, including the intended use of the powder. If nutrient richness is of paramount importance, then, freeze-drying protocol should be considered. If the earthworm powder is meant for therapeutic use, where sterility is highly desirable, then the choice should be between freeze-drying and oven-drying protocols, preferably, freeze-drying, for its higher nutrient advantage. However, when multiple factors are taken together, including equipment, duration, and ease of processing, then, oven-drying protocol may be a choice of balance. This is because oven-drying protocol required less time (24 hours), yielded earthworm powder of relative lower microbial counts and higher nutritional composition, and at the same time, required less sophisticated equipment. Sun-drying protocol is the least recommended, as it required a longer time, yielded earthworm powder of least nutrient composition, and of highest microbial counts. Where processing duration, equipment availability, and ease of processing are no challenge, freeze-drying protocol is best recommended; it yielded earthworm powder highest in nutritional content and relatively lower microbial counts.

The high crude protein, amino acids, and other nutrients recorded for all the earthworm powder samples in this study reinforces the potential opportunity presented by earthworms and earthworm powders for use as rich sources of human and livestock nutrition. In this study, the protein concentrations in earthworm powders ranged between 59.9 and 66 percent. This compares favourably with protein levels in fishmeal (60-68 %) (Turan *et al*., 2007), and is far higher than protein contents of meat products (22 %) and chicken breast (34 %) (FAO, 2015; Ahmad *et al*., 2018). In addition, the high proportion of essential amino acids, including methionine, cystine, lysine, and threonine make the earthworms and earthworm powders potentially excellent sources of livestock, animal, and human nutrition. It is therefore not surprising that some people in China have, for long, been using earthworms in traditional medicine, and as food in homes and restaurants (Sun and Jiang, 2017; Dada and Balogun, 2023). Although some countries in Western Europe, Southern East, America, Africa have started the production of various food and medicinal products from earthworms and earthworm products (Sun and Jiang, 2017), the inherent potential of earthworms as therapeutic medicine and source of animal and

human nutrition has not been fully exploited. Therefore, research priority need be given to earthworm culture and utilizations in nutrition and medicine.

4.1 Conclusion

Three earthworm powder preparation protocols were assessed for product quality. Freeze-drying protocol yielded earthworm powder of the highest nutritional composition, but took a longer time, and required more sophisticated equipment. Oven-drying protocol generated earthworm powder relatively high in nutritional composition and lower in some microbial counts. Sun-drying protocol produced earthworm powder that is lowest in nutritional composition and highest in microbial counts, hence, the least recommended. Where processing duration, equipment availability, and ease of processing are no challenge, freeze-drying protocol is best recommended due to the high nutritional composition and lower microbial counts of the earthworm powder it yielded. The high protein and other nutrients recorded in all the earthworm powders reinforces the potential opportunity presented by earthworms and earthworm powders for use as rich sources of human and livestock nutrition.

Disclosure statement

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subject that require ethics approval.

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