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Research Article

NUTRITIONAL AND ANTIOXIDANT EVALUATION OF EARTHWORM POWDER *(Eudrillus euginae)*

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ABSTRACT

Studies have been made to understand the nutritional, aminoacids and antioxidant concentrations in the dried powder of *Eudrillus euginae,* an African soil dweller. The nutritional factors such as carbohydrate, glucose, total free aminoacids, protein, vitamin A, and total phenol were determined. The individual aminoacids includes Histidine, Serine, Arginine, Methionine, Proline, Tryptophan, and Lysine was also quantified. The enzymatic antioxidants, such as Glutathione S transferase (GST), Glutathione reductase (GR), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) and the non-enzymatic antioxidant like Reduced Glutathione (GSH), Vitamin C and Vitamin E were assayed. The findings of the present study reveals that the earthworm is rich in protein, carbohydrate, fat and rich antioxidants suggest that this earthworm can be used as animal protein with good source of antioxidants.

KEY WORDS: Earthworm powder, *Eudrillus euginae,* antioxidants, nutritional, aminoacids, Protein.

INTRODUCTION

Although Wallace (1853, 1889) reported more than 100 years ago the inclusion of earthworms in diets of Amerindian populations little is known about the nutritive value of these edible invertebrates[1]. Animal protein in the feeds of animal and food of man is essential for growth and health. Earthworm meal (vermin meal) is as good as Peruvian fishmeal in terms of quality and has been found to be an efficient substitute for fish meal in domestic animal diets. The study several authors[2&3] revealed that earthworms are an excellent source of protein. Furthermore studies have shown that not only could earthworms serve as a rich protein source, but also as a source of essential amino acids, especially lysine which, is limiting in many basic foodstuffs[4]. The content of lysine in earthworm flour is significant representing the daily requirement for children between the age of 2 and 5 years[5]. Earthworm meal was shown to have an amino acid composition very similar to that of fishmeal and potentially superior to meat meal and the protein was shown to contain such essential amino acids as phenylalanine, leucine, lysine, methionine and valine[6]. Against these backdrops and the prohibitive market cost of animal meal due to increasing demand for high quality protein for use in intensive animal feed industries and ethical issues, earthworm could fit into this mold.

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Free radicals are produced in normal and/or pathological cell metabolism[7]. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing[8].

Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase and catalase or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenolic compounds and glutathione[9]. However, these systems are frequently insufficient to totally prevent the damage, resulting in diseases and accelerated ageing. Natural products with antioxidant activity may be used to help the human body to reduce oxidative damage. Many fruits, vegetables, herbs, cereals, sprouts, seeds and edible mushrooms have been investigated for their antioxidant activities in the last years[10]. The antioxidants present in earthworms are of great interest as protective agents to help the human body reduces oxidative damage without any interference. They are recognized as functional foods and as source of physiologically beneficial components[11]. Thus our present study was focus to determine the nutritional and antioxidant levels in earthworm powder of *Eudrillus euginae.*

MATERIALS AND METHODS

Collection of earthworm

The earthworm namely *Eudrillus euginae* was collected from Aarthi farms, Kondegoundampalaym Village, Pollachi Taluk, Coimbatore District, Tamil Nadu, India. The species were cultured in Kongunadu Arts and Science College Premises, Coimbatore - 641 029, Tamil Nadu, India, for further use. The earthworms were harvested whenever needed.

Extraction and preparation of the sample

The earthworms were washed with running tap water to remove any dirt from body surface. The earthworm was kept in 0.65% NaCl at room temperature for 1-2h with few changes of solution until their digestive systems were clean. Animals were taken out of the solution and minced with scissors. Three grams of earthworm tissue were homogenized in 40ml of chloroform-methanol (v/v) solution and left overnight at 4°C. The following day, 16 ml of distilled water was added to the homogenate. It was mixed and centrifuged at 2460 X g for 10 min. Three clearly visible layers were obtained. The upper, water/methanol layer was taken out by pipette and evaporated on a rotary evaporator until methanol was left. An opalescent fluid, pH 7, was obtained. It was freeze dried earthworm powder (EWP) was kept at 4°C until use[12].

Biochemical analysis

The carbohydrate[13], glucose[14], total free aminoacids[15], protein[16], vitamin A[17], and total phenol[18] content in

Earthworm Powder (EWP) of *Eudrillus euginae* were estimated.

Estimation of aminoacids

The earthworm powder are subjected to the analysis of individual aminoacids concentrations includes Histidine[19], Serine[20], Arginine[21], Methionine[22], Proline[23] Tryptophan[24], and Lysine[24].

Antioxidant assay

The activities of enzymatic antioxidants, such as Glutathione S transferase[25], Glutathione reductase[26], Glutathione Peroxidase[27], Superoxide Dismutase[28] and Catalase[29] and the non-enzymatic antioxidant like Reduced Glutathione[30], Vitamin C[17], and Vitamin E[31] were assayed.

Statistical Analysis

The result obtained in the present study statistically analyzed and the data were expressed as mean ± S.D from triplicate determination.

RESULTS AND DISCUSSION

The nutritional status of dried powder of *E. euginae* was found in the present study was elucidated in Table I. The findings reveal that the levels of carbohydrate, glucose, total free aminoacids, protein, and vitamin A of EWP showed the higher percentage of protein that account for 32.13% of the dry weight of earthworm, which represents its high nutritional value. By comparing the essential aminoacid content of a sample protein with that of the World Health Organisation (WHO) standard protein, one can calculate the protein's chemical source and identify limiting aminoacids in the nutritional source and found that the earthworm proteins were of high quality. Our study was in accordance with the study of Edwards (1985)[32] who reported that the dry matter of an earthworm's body consist of 60-70% protein, 7-10% fat, 8-20% carbohydrates, 2-3% minerals, and a variety of vitamins.

The potential value of earthworm as a protein source had been established by several authors[33,34&35] who had also suggested that earthworms provide a substantial nutrition to the animals consuming them. Furthermore, the studies of [3] had shown that not only could earthworm serve as a rich protein source but also as a source of essential amino acids, especially lysine which is limiting in many basic foodstuffs and that the amino acid composition of earthworm meal is very similar to that of fishmeal and potentially superior to that of meat meal.

The antioxidants are of great interest as possible protective agents to help the human body reduces oxidative damage without any interference. One etiologic agent implicated in diseased state is stress "oxidative stress" which involves excess generation of prooxidants. In a healthy human body the generation of prooxidants in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are delicately balanced by the antioxidant defenses. The ROS include the superoxide anions, hydroxyl anions and hydrogen peroxide anions, singlet oxygen all oxidants generally influence the redox status, thereby protecting cell against ROS under certain circumstances, while promoting ROS generation in other[36]. To evade the potential damaging effects of ROS, cells have evolved protection mechanisms, including antioxidant enzymes such as Catalase (CAT), Glutathion S transferase (GST), Glutathion reductase (GR), Glutathion peroxidase (GPx), Superoxide dismutase (SOD), and non enzymatic antioxidants like Reduced glutathione (GSH), vitamin E and vitamin C. EWP are recognized as potential source for both enzymatic and nonenzymatic antioxidants. Therefore in the present work we assayed the antioxidant properties of EWP through the estimation of enzymatic and non-enzymatic antioxidants.

Our present findings show that EWP was of good source of enzymatic antioxidant with maximum level of CAT (14.43 µmol of H_2O_2 decomposed/min/mg protein). The level of SOD (6.18 inhibition of 50% nitrite formation/min/mg protein) was minimum when compared to other enzymatic antioxidants. Similarly among non-enzymatic antioxidants GSH (7.11 µmol of H_2O_2 decomposed/min/mg protein) was found to be maximum. Earthworms have very intimate contact with the soil that contaminated with various pollutants. Because of exposure to pollutants, earthworms were developed an efficient detoxification system for their survival. The antioxidant protects the organisms from free radicals and retards the progress of many diseases. The normal protector against lipidperoxidation includes SOD and CAT. SOD protects against superoxide radical (O^{2-}), which damages membrane and its biological structure. CAT primarily decompose hydrogen peroxide to H_2O at much faster rate, sharing this function with GPx. GPx may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues[37]. Usually GST activation leads to depletion of the GSH with a simultaneous resynthesis of GSH for the replacement of the normal level. In the cell there is equilibrium between the reduced and oxidized forms of glutathione[38]. Glutathione is a major, non-protein thiol in living organisms which perform a key role in innate antioxidant defense mechanisms[39]. It is involved in the maintenance of normal structure and function of cells, probably by its redox and detoxification reactions[40]. GSH also scavenges reactive oxygen species (ROS) non-enzymatically or in a reaction catalysed by GPx through the oxidation of two molecules of GSH to a molecule of GSSG. Thus as with GPx activity, GST activities also cause a reduction in the GSH level and a decrease in the cellular antioxidant status. GPx uses GSH as a hydrogen donor to eliminate H_2O_2 and to convert the organic hydroperoxides into alcohols. Next, the product of this reaction, GSSG, is reduced by GR in the presence of NADPH, and in this way the GSH level is restored in the cell[41]. GPx is an important antioxidant enzyme present in virtually all tissues. The enzyme limits the generation of lipid peroxides and utilizes glutathione as its cofactor to convert lipid peroxides into relatively harmless hydroxylated fatty acids, water and glutathione disulfide[42]. The levels of vitamin E and C were found to be 1.64/ µ/mg protein, and 0.94±0.025 µ/mg protein respectively. Vitamin E and C play important roles in numerous biological conditions and the major beneficial actions of these vitamins are due to their antioxidant properties that scavenge reactive oxygen species in biological fluid[43] and membranes[44]. The interaction between the two vitamins occurs at the membrane–cytosol interface[45], and vitamin C functions as a reducing agent of the membrane-bound oxidized vitamin E.

CONCLUSION

The nutritional analyses of earthworm powder *(Eudrillus euginae)* provide the quantative and qualitative evidence to support the assertion that this earthworm is capable of satisfying the daily requirements for protein in general and essential aminoacids in particular. Hence EWP could be very well recommended as good source of carbohydrate, fat and protein. Our results also showed that EWP *(Eudrillus euginae)* possess potential enzymatic and non-enzymatic antioxidants which may be used for minimizing or preventing

lipid oxidation in pharmaceutical products, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals.

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Table I. NUTRITIONAL PROFILE OF EARTHWORM POWDER OF *Eudrillus euginae*

S.No	Parameters	Dried earthworm powder (mg/g)
1.	Carbohydrate	04.34±0.010
2.	Glucose	02.33±0.020
3.	Total free fattyacids	31.97±0.050
4.	Protein	05.21±0.015
5.	Vitamin A	01.61±0.152
6.	Total phenol	44.36±0.070

The values were expressed as mean ± S.D from triplicate determination.

Table II. AMINOACID COMPOSITION IN *Eudrillus euginae* DRIED POWDER

S.No	Parameters	Dried earthworm powder (%)	% of aminoacids (WHO ideal protein)
1.	Histidine	2.40± 0.100	1.9
2.	Serine	9.32±0.190	7.4
3.	Arginine	5.41±0.035	4.9
4.	Methionine	2.53±0.020	2.5
5.	Proline	2.40±0.020	-
6.	Tryptophan	4.03±0.010	1.1
7.	Lysine	6.04±0.017	5.8

The values were expressed as mean ± S.D from triplicate determination.

Table III ANTIOXIDANT PROFILE OF EARTHWORM POWDER (EWP) OF *Eudrillus euginae*

S.No	Parameters	Earthworm powder (EWP)
Enzymatic antioxidants		
1.	CAT[a]	14.43±0.020
2.	GST[b]	12.59±0.020
3.	GR[c]	9.02±0.020
4.	GPx[d]	6.89±0.010
5.	SOD[e]	6.18±0.006
Non enzymatic antioxidants		
6.	GSH[f]	7.11±0.020
7.	Vitamin E[g]	1.64±0.011
8.	Vitamin C[g]	0.94±0.025

The values were expressed as mean ± S.D from triplicate determination.
a - µmol of H_2O_2 decomposed/min/mg protein
b - µmol of CDNB conjugated formed/min/mg protein
c - µmol of NADPH oxidized/ min/mg protein
d - µmol of glutathione consumed/min/mg protein
e - inhibition of 50% nitrite formation/min/mg protein
f - µmol of H2O2 decomposed/min/mg prn,
g - µ/mg protein

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