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Study of Different Conditions of Enzymatic Hydrolysis in Earthworms

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***Abstract*— The aim of this paper was to study different conditions of enzymatic hydrolysis in earthworms. As to methodology, 25 g of earthworms of the Eisenia andrei species were frozen and crushed. After, the samples were divided into two different procedures (A and B). In the first one, pH level of the earthworms was adjusted to 8.6 with a 0.5 mol/L NaOH solution prior to the addition of a buffer solution (H3BO3.KCl - NaOH). The second procedure consisted of adding the same buffer solution without pH modification. Both methods were submitted to the addition of the alkaline enzyme. Samples were agitated with a shaker equipment (280 rpm at 54.2 ºC) and tested at time intervals between 0 and 10 hours. The Lowry method for protein quantitation was used to analyze the soluble protein content. A difference of 22 times in the better performance time of the two methodologies was noted. Procedure B presented the best efficiency, with the time interval of 6 hours. This work may contribute to the development of new products containing high levels of hydrolysates, which facilitate their absorption into livestock.**

***Index Terms*— bioproduct, *Eisenia andrei*, hydrolisys.**

I. Introduction

A trend of resource depletion due to the indiscriminate exploitation of natural resources is currently observed. One of the main reason for this is the exponential population growth. Due to this, the lack of essential elements for human sustenance, in particular food and fuel, is predictable [1]. Research on different alternatives that meet the needs of animal production has been stimulated, due to the high price of cereal grains and protein supplementation used in animal feed [2].

For its great biological value and market availability, soybean meal is the main protein food used in Brazil and other countries in monogastric rations. However, the increases of soybeans on the market and in human consumption has brought attention to new alternative protein sources studies with the aim of replacing soybean meal in diets [3].

High protein values can be found in unprocessed earthworms as flour (from 68 to 82%), and they appear to be pathogen free. Its application may be in natura or as flour, being accepted by various animals such as frogs, fish and chickens [4]. When utilized for the diet of birds and pigs, it may yield better or equally satisfactory results than the traditionally used protein sources of animal origin [5].

Enzymatic and chemical modifications are implemented to favor the functional properties of proteins [6]. However, superior advantages and benefits are found in the enzymatic hydrolysis towards chemical change, because it contains the specificity of the enzyme in relation to the substrate, and the possibility of undesirable reactions resulting from the formation of toxic products is low [7].

Since 1940, protein hydrolysates have been used in medicinal applications, in patients unable to digest proteins, to maintain their nutritional status, as well as in specific diets for enteral feeding of infants.

The methods of preparation and the use of protein hydrolyzates grew significantly in the 70's, both for clinical and nutritional purposes and for the improvement of functional properties of protein-based foods and proteins [8].

Hydrolyzates are proteins separated by peptides of various sizes by chemical or enzymatic action, which can be used in various food products, such as flavor enhancers, milk substitutes, protein supplements and beverage stabilizers. The use of specific enzymes is not a unique factor that affects the peptide profile in the final product. The kinetics of the enzymatic reactions can be affected by environmental factors such as temperature and pH, having a different effect for each enzyme [9].

Thus, it is interesting to evaluate the previously established conditions, but increasing the reaction time and adjustment in the pH before the buffer solution, to verify if there will be a change in the hydrolyzate. In spite of this, the objective of this work was to evaluate different pH and time conditions for obtaining hydrolysis in Eisenia andrei type worms, with the alkaline enzyme.

According to Pereira [10], earthworms belong to the annular phylum and are segmented animals, composed of divisions called metamer, which look like rings - due to this

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the origin of the name. Its body is lumbriform, tapering at the extremities and flattened in the posterior region. The sexual maturity of the worms is indicated by the clitelo, of white-yellowish coloration.

The class to which they belong is *Oligochaeta*, and can be found in variable sizes from 0.5 mm up to 3.30 m in length (*Megascoloides australis*). There are five families in which they can belong: *Lumbricidae, Megascolicidae, Monoligastridae, Glossoscolici Jae* and *Eudrilidae*.

Because it contains reproductive, digestive, excretory, circulatory, nervous and muscular devices much more developed than a worm, the earthworm should not be considered one, contrary to what one thinks. Being placed at a much higher level than the worms on the biological scale of living beings [10].

They are photophobic animals, that is, they do not support the ultraviolet light of the sun. When exposed to sunlight, they may suffer from dehydration, leading to death. A humid environment is extremely important for their survival [10], but they do not support environments with a lot of water, because their respiration is cutaneous [11].

The temperature of your body may vary depending on the environment in which they are inserted. They are petiolethermos, such as fish and amphibians, considered cold-blooded animals [10]. Because they are hermaphroditic beings, they have female and male reproductive organs, and there are few species that are self-productive, so they need two individuals for reproduction [12]. His sense of direction is not very sharp, because they have neither eyes nor ears. The most extended senses are tact and taste, which help them in finding a partner, finding food and escaping from possible predators. Its movement is controlled by cells affected by light, located on its skin [11].

Earthworms live inside the soil, rustling and fertilizing it, as well as other living things. From their food sources, they use about 30% to 40% for own consumption, the rest return in the form of humus.

The species California Red (*Eisenia fetida* Savigny) is the most used in the world for the manufacture of humus. In Brazil, more than 240 species of earthworms are known, most of them being native worms. In the world, it is believed that 8,000 different species are found [11].

The California Redworm shows great capacity in humus production, is easy to become captive and reproduces at high speed. For these factors, it is considered the favorite for the production of humus. In an interval of 3 to 7 days, this species produces a cocoon containing 2 to 5 new earthworms, and can consume the equivalent of its weight in organic matter daily [11].

Schmidt and Salas-Mellado [13] tested two enzymes to obtain a hydrolyzate in thigh and chicken breast. Using the temperature and pH conditions of maximum catalytic activity of each enzyme according to the literature. The substrate was homogenized in a 0.2 M phosphate buffer solution and the pH set at 7 for the enzyme flavourzyme and 7.5 for the alkaline enzyme. Results were obtained from 17.22 to 40.47% in chicken breast when and from 8.30 to 22.54% in chicken thigh used the enzyme flavourzyme. And there was variation in chicken breast from 20.93 to 57.42%, and from 18.62 to 38.79% using chicken thigh using the alkaline enzyme.

In the article "Use of mechanically separated meat of chicken for the production of a protein hydrolyzate from microbial enzymes" author

Rossi et al [14], mechanically separated chicken meat was used, where a protein recovery of 89% with alkaline enzyme and 66.5 with flavourzyme was obtained. For the production of the hydrolyzate, the alkaline enzyme was obtained, obtaining a total protein content of 57.90%.

In the article "Protein hydrolyzate of fish obtained by chemical and enzymatic routes from corvina (*Micropogonias furnieri)"* written by Martins et al [15], were used fillet and corvina processing residue (*Micropogonias furnieri*). In the lyophilized residue, 64.26% of proteins, 21.09% of lipids and 8.07% of ashes were obtained. It was observed an increase in the km of protein of 86.94 and 72.34% in the isolates obtained by acid and alkaline chemical extraction. In the enzymatic hydrolysates 50.15% for the hydrolyzate with Flavourzyme and 47.09% for the hydrolyzate with Alcalase. There are few works in the literature on obtaining protein hydrolyzates using earthworms [16], in this way, to evaluate different pH and time conditions, becomes important for the development and new products rich in proteins.

II. MATERIAL AND METHODS

In order to obtain the worm hydrolyzate initially, the worms of the Eisenia andrei species were cultivated in existing minhocks in the biotechnology laboratory of Tecnovates, Univates.

The worms received feed containing coffee grounds, fruit peels and leafy vegetables for two months until they reached adulthood. After that period, around 100 earthworms were collected, soaked for cleaning of their digestive tubes and about 25g of this mass was frozen and crushed, to be mixed with the enzyme. After that, the samples were taken to a shaker (Marconi MA 830) at 280.54 rpm at 54.2 ° C, and then analyzed at different time intervals (every 2 h).

For the procedure, an alkaline pH buffer of H_3BO_3.KCl-NaOH solution was used. The times tested were to 10 h. The hydrolysis procedure was adapted from Rodrigues et al.[16].

To evaluate the different conditions, two different procedures were performed: Procedure "A" by adjusting the pH directly in the earthworm mass by adding 0.5M NaOH to pH 9, and thereafter, the buffer solution was added to maintain that pH value throughout the hydrolysis. And procedure "B" there was no preliminary pH adjustment and the buffer solution was directly added to the earthworm mass.

After the tests, the samples were collected and hydrolysis was performed by the Lowry method following the reference in the Univates manual for protocols and methods of analysis in agrifood biotechnology and human health laboratories. [17]. All the results were statistically analyzed by the ANOVA test, followed by Tukey's test ($p < 0.05$ significance level), using InfoStat software.

III. RESULTS

Table 1: results of procedure "A"

Time	Degree of Hydrolysis
0h	0
1h	0.022 ± 0.003^{c}
2h	0.034 ± 0.004^{b}
4h	0.035 ± 0.001^{b}
6h	0.038 ± 0.002^{b}
8h	0.042 ± 0.002^{a}
10h	0.036 ± 0.003^{b}

Different letters on the same column show significant differences between populations ($p < 0.05$).

Table 2 shows the results of procedure "B".

Table 2: results of procedure "B"

Time	Degree of Hydrolysis
0h	0
1h	$0{,}670 \pm 0{,}088^{b}$
2h	$0{,}650 \pm 0{,}025^{b}$
4h	$0{,}651 \pm 0{,}034^{b}$
6h	$0{,}930 \pm 0{,}010^{a}$
8h	$0{,}665 \pm 0{,}089^{b}$

Different letters on the same column show significant differences between populations ($p < 0.05$).

IV. DISCUSSION AND CONCLUSION

It was concluded that the procedure "B" in the time of 6h, was obtained the most suitable condition for the hydrolyzate of earthworm (22 times better than procedure “A” in 8h). That is, in procedure "A", with preliminary pH adjustment with a strong base, a breakdown in the amino acid linkages may have occurred.

Thus, the most appropriate way was to adjust the pH only with a buffer solution to remain the ideal pH for the action of the alkaline enzyme. It is suggested that in this hydrolyzate, there are more free amino acids, which in the future may be used as a food supplement. Further tests will still be done using other enzymes.

This work may contribute to the development of new products containing high levels of hydrolysates, which facilitate their absorption into livestock.

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