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PROXIMATE, MICROBIOLOGICAL ANALYSIS AND DIGESTIBILITY OF PEPSIN IN *Atta sexdens* FLOUR, FROM THE REGION OF RIOJA, PERÚ

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ABSTRACT

The consumption of insects, also known as entomophagy, is not a new eating habit; on the contrary, the insects are eaten in many countries worldwide, where insects are served as the main dish in many of them. In Peru, insect consumption is limited to regions of the central jungle where locals consume ants of the "*Atta sexdens*" variety in the form of toasted snacks with salt. Analysis of insects has shown a wide variation in macronutrient content relative to most wild vertebrate meats, encompassing a wide range of protein, fat and carbohydrate concentrations. In that sense, in terms of their proportional macronutrient composition, insects could serve as equivalents not only to wild meat, but also to other foods, such as seafood, nuts, legumes, vegetables or fruits. The aim of this study was to assess nutritional quality of "*Atta sexdens*" ant flour and determine its physical and chemical characteristics. The ants were obtained from the department of San Martín, province and district of Rioja and underwent a rigorous cleaning process to eliminate impurities such as wings, leaves and soil which are attached to the ants. The ants were dried using a Biobase oven, model BOV-T30C, and then ground in an electric grinder and finally passed through a sieve. The final product was measured for physicochemical properties such as pH, % acidity in aqueous extract and % acidity in ethanolic extract, resulting in 6.57, 0.21, and 0.29, respectively. The nutritional composition assessment was based on the AOAC standard method where the fat content of the meal was 35.40%; protein, 35.51%; carbohydrate, 16.12%; ash, 5.39%; and moisture, 7.58%. For the assessment of pepsin digestibility, the AOAC 971.09 test method was used, resulting in 99.77% activity. In regard to techno-functional properties, it was found that swelling capacity (SC) and water absorption capacity (WAC) resulted in 2.91 WAC g water/g m and 1.86 SC ml/g, respectively. On the other hand, the microbiological results were within the permissible limits for health; therefore, we can consider this ant meal a safe and innocuous product as well as an excellent food source with exceptional nutritional characteristics.

Key words: *Atta sexdens*, flour, Entomophagy, Protein, Digestibility, Microbiology, Physicochemical properties, Proximate

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INTRODUCTION

Atta sexdens belongs to the genus Atta of the order *Hymenoptera*, family *Formicidae* and subfamily *Myrmicinae* [1, 2]. *Atta sexdens* is a brown or dark brown ant; adults are considered workers or soldiers [3]. Ants are considered predators, herbivorous, and burrowing insects and are species that have existed for a long time [1]. Leaf-cutting ants are known in Peru as hormigas culonas or big-bottomed ants and; in countries such as Brazil, they are called *Saúvas* or *Quenquéns* [4]. Insect "meat" is similar in composition to the meat of vertebrates such as pork, chicken, and fish [5]. Table 1 shows the comparison of the proximate analysis of the following species: *Atta sp*, *Atta mexicana*, *Atta cephalotes, Carebara vidua Smith* and *Rhychonphorus Palmarum* [6, 7, 8]. The order *Hymenoptera* has between 10% and 62% protein content [9]. Authors such as Ramos E. et al 2002, reported protein values between 42 - 44% in their nutritional study on *Atta sp., Atta mexicana* and *Atta cephalotes* ant species. The genus Orthoptera such as *Acheta domesticus* and *Gryllus peruviensis* have values between 27% and 76% protein content. In the case of *Gryllus peruviensis*, it has 66.9% [10]. Insects are also rich in fat, sodium, potassium, zinc, magnesium, iron, copper and calcium [11]. Species such as *Atta sp*, *Atta mexicana* and *Atta cephalotes* show ash content values of 2.49 %, 3.40 % and 4.03 %, respectively [6]. Insects show high values for calcium and phosphorus, and some of them could have a higher iron content than meat; hence, their consumption would be an alternative for providing minerals such as iron and zinc [6].

Entomophagy has been reviewed in several countries such as the United States, Canada, Japan, China, Peru, Colombia, Venezuela, among others [12, 13, 14]. The consumption of insects in Peru is scarce and found only in regions of the central jungle the most reported insects as a food source are *Coleoptera* of the *Rhynchophorus palmarum* species. For example, "*Suri*" and ants of the genus *Atta sp.* known as "*Mamako*" or "*Siqui Sapa*" are consumed in these regions, where the latter is highly valued due to its pleasant taste [15]. Thus, insect protein has a lower impact on the environment than animal protein (cattle, poultry) [16].
In Indonesia, the diversification of the diet is due to the consumption of worms called sago [17]. In Holland, pasta based on durum wheat semolina was elaborated with the addition of cricket powder (CP), which contains proteins, unsaturated fatty acids, minerals, and vitamins. The product obtained firmness and was very well accepted, resembling conventional products [18]. The objective of this study was to assess the flour based on *Atta Sexdens* ants by analyzing it physicochemically, nutritionally and microbiologically and to determine the digestibility of its protein. This study will provide information on the nutritional

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characteristics of *Atta sexdens* ant flour in order to consider it in the diet of the future.

MATERIALS AND METHODS

Obtaining *Atta sexdens* ants

The *Atta sexdens* ants were brought from Rioja, department of San Martín, and were carefully selected by a distributor in the same area. The cleaning consisted in the elimination of wings, soldiers, and some of their own impurities (leaves and branches). To preserve them in good condition, the ants were subjected to a salting process to prevent the proliferation of microorganisms and the inhibition of bacteria. The salting process consisted in adding sodium chloride (NaCl), commonly known as table salt, directly to the ants in a quantity of 1/5 (NaCl/Ant). Then, they were vacuum-packed, thus, maximizing the preservation of the product. The correctly packed ants were stored at a temperature of 1-5ºC until the moment of processing

Preparation of ant flour

The selected and sanitized ants were placed in a heat oven at 65 ºC for 220 minutes, and the weight was controlled every 30 minutes in constant movement to prevent the sample from burning and also to ensure that the water is eliminated homogeneously. The final product was placed inside an electric grinder until the flour was obtained. To avoid lumps and large particles, the material was passed through a 60-micron diameter stainless steel sieve to obtain a homogeneous product. (Figure 1).

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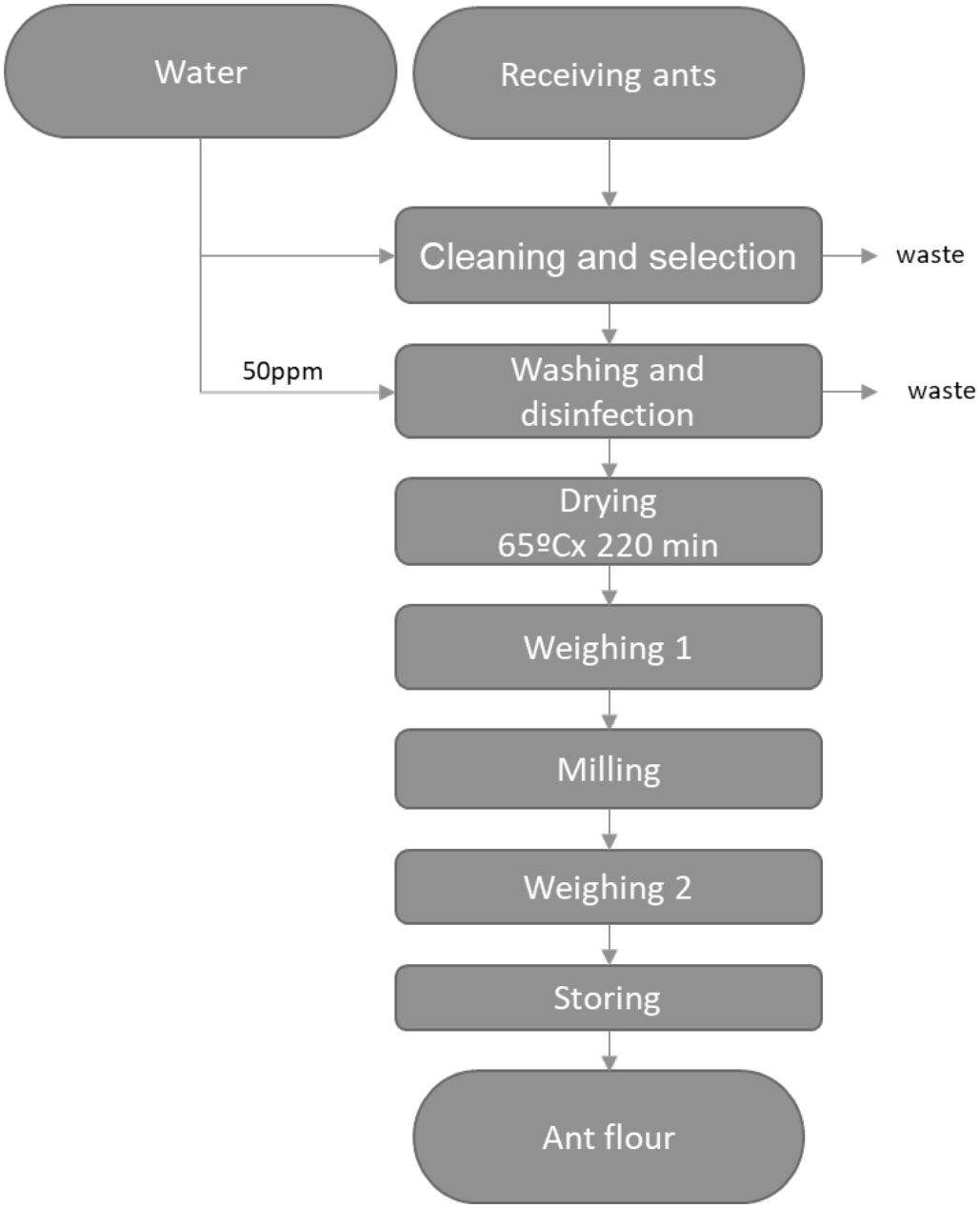

Figure 1: *Atta sexdens* flour flow chart

Physicochemical properties

pH measurement of the flour

The pH of the flour was calculated by mixing 0.5 g of the sample with 10 ml of distilled water. A digital pH meter of the HANNA HI9126 brand was used, and the mixture of flour and distilled water was kept in constant movement with a magnetic stirrer. All measurements were performed in triplicate.

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Acidity in flour

To determine the titratable acidity index in distilled water, 10 g of the sample were weighed and homogenized for 1 hour with depression every 10 minutes. It was then filtered, and 50 ml of supernatant was taken, which was titrated with NaOH (sodium hydroxide) at 0.1 normality with phenolphthalein indicator. For the determination of ethanolic extracts, the same process was carried out considering the dilution in ethanol for the analysis. All measurements were made in triplicate.

Evaluation of nutritional composition

Fat, moisture, protein, carbohydrates, and ash of the ant flour were obtained through AOAC standard methods. Moisture was determined through NTP 205.037:1975 method; fat, through NTP 206.017:1981; ash, through NTP 205.038:1975; protein, through CORPLAP-AL-001(COVENIN 1218-80 Rev.01). Carbohydrates were calculated by subtracting the percentage of the sum of fat, moisture, protein, and ash from 100%.

Pepsin digestibility

The pepsin digestibility analysis was carried out through the ALS-Perú S.A.C. accredited by INACAL – DA with registration No LE - 029. The laboratory used the AOAC 971.09 (2012) test method. Pepsin digestibility of animal protein feeds/ Filtration method. A sample of *Atta sexdens* flour was submitted for the tests in triplicate.

Techno-functional Properties

Techno-functional properties were evaluated in the previously defatted flour, using the method by Valencia G, 2006 [19].

Swelling capacity (SC)

A total of 0.5 g of the sample of flour was weighed in a centrifuge tube, 10 ml of distilled water were added, and the occupied volume was measured, and it was agitated with the help of a vortex for 5 min. It was left to rest for 24 hours at room temperature. Subsequently, the final volume of the samples was measured. The SC was expressed as ml water/g sample.

Water absorption capacity (WAC)

First, 0,3 g of each sample of flour was weighed and 10 ml of distilled water was added, maintaining in manually agitation during 10 minutes. Thereafter, the samples were left for 24 hours at room temperature and centrifuged at 3000 rpm for 10 minutes. The supernatant was immediately removed, and the sediment was weighed. The WAC was expressed as g water/g sample.

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RESULTS AND DISCUSSION

Physicochemical properties

Table 2 shows the obtained pH and acidity values in aqueous and ethanolic extract. The acidity in flour allows us to determine the presence of some mineral acids, organic acids, salts of strong acids and weak bases; it is an indicator of the oxidative proliferation of the fat, and in this study its evaluation is necessary due to the high fat content of the sample.

It was found that the pH was 6, 50 and the percentage of acidity was 0.21 for the extraction from aqueous medium and 0.29% in ethanolic extracts, which were acceptable values. Thus, this flour could be used as an ingredient in instant food, considering that the final acidity should be less than 0.40% [20]. Regarding flour based on grains and protein enriched extruded food products, a maximum of 0.15% is required. The values obtained were higher. For bakery products such as sliced bread, there is a maximum of 0.50 [21], which is within the permitted values; higher values could modify the physical, chemical, and rheological properties of doughs in the case of bakery products.

Protein digestibility

Regarding protein digestibility, (see Table 2), the obtained value of protein digestibility was 99.77%, which could be compared with what was reported in the study called Nutritional and sensory quality of edible insects. In that investigation, a range between 76 and 96% was extended for digestibility of insect proteins. These results are comparable with egg protein (95%) and beef protein (98%), which had values close to those obtained, while vegetable protein had lower values [22].

Water absorption capacity

Water absorption capacity depends on the amount of protein, its structure, and physicochemical properties as hydrophilic-hydrophobic balance of amino acids as well as lipids and carbohydrates [23]. The found value for *Atta sexdens* flour regarding water absorption was 2.91g water per g of sample, on average, slightly higher than the techno-functional properties of legumes that have values of 2 g water/g sample [24]. The swelling capacity of Atta was 1.86 ml of water / g of sample, which is similar to the legumes such as chickpeas and lentils.

Swelling capacity

The swelling capacity of *Atta sexdense* flour turned out to be 1.86ml/g; this indicates the low fiber content of this kind of ant, compared to broccoli and

pumpkin flour, which have values between 6 and 8. This is due to the high fiber content that increases the total mass through higher water absorption [25]. It is essential to establish these parameters because they indicate how to use the new product: as instant food, thickener, for baking, for fermentation, among others, so we can be able to classify it [26]. In general, insect proteins are highly soluble; they can form emulsions and have the capacity to absorb water and to form gels [9].

Nutritional composition of *Atta sexdens* ant flour

Table 3 shows the percentages of nutrients such as fats, proteins, and carbohydrates of ants, which have always been highly appreciated by locals and foreigners of the Amazon. The fat content obtained from the flour was 35.4%, a high value because it is known that the abdomen of these ants has a high fat content, unlike armored crickets *(Canthoplus discoidalis),* which only have 16.8% fat. [27]. It should be noted that insect fat is high in monounsaturated and polyunsaturated fatty acids, including oleic, linoleic and linolenic acid [27]. In addition, it would also sensorially improve food texture, absorption, flavor retention, and provide fat-soluble vitamins. The protein content of the sample was 35.51%, lower than what was reported by other authors [6, 7, 8]. These differences are due to the metabolic variations that exist between species; on the other hand, the protein content obtained shows that the quality is even higher than chicken (23%) and beef (23%) [22].

Regarding total ash, the value obtained was 5.39 %, which is slightly high compared to species such as *Atta sp, Atta mexicana,* and *Atta cephalotes* (Table 1).

Microbiological analysis

One study considered the microbiological changes occurring during refrigerated (4 °C) and ambient (25 °C) storage of insect flour *(A. domesticus)*, achieved by monitoring over 15 days [28]. Insect flour shows levels of cocci, enterococci and pseudomonas, family of bacilli. Yeasts and molds showed undetectable amounts. In general, *A. domesticus* dust showed lower levels of all microbial groups than *T. molitor* dust, except members of the Enterobacteriaceae family [29].

The microbiological results obtained from the flour of *Atta sexdens* ants (molds, yeasts, Salmonella, and Escherichia coli) were within the range permitted by the Peruvian legislation [30]. This indicates adequate processing and that it is suitable to be considered an innocuous and safe food for human consumption.

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CONCLUSION

The results of the nutritional evaluation and protein digestibility of *Atta sexdens* flour show that it can be an excellent source of protein. In addition, protein quality of this flour measured through pepsin digestibility is like protein quality of pork and beef meat. The microbiological analysis concluded that the ant meal complies with the microbiological parameters permitted for human consumption, making it a safe and innocuous product.

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Table 1: Comparison of the proximate analysis of flour of some Atta species and others

	Atta Sexdens	*Atta sp*[6]	*Atta mexicana*[6]	*Atta cephalotes*[6]	*Carebara vidua Smith*[7]	*Rhychonphorus palmarum L*[8]
Protein	38,42	42,95	41,90	44,90	46,95	45,82
Total fat	38,30	32,50	31,20	30,10	31,47	22,15
Ash	5,83	2,49	3,40	4,03	4,21	4,83
Carbohydrate	17,44	22,06	23,42	20,97	21,93	2,20

*Data are expressed on a dry basis**Data collected from [6, 7, 8]

Table 2: Physicochemical and Techno-functional properties

Assay	Result
Pepsin digestibility (%)	99,77
pH	6,57 ± 0,05
Acidity in aqueous extract (%)	0,208 ± 0,02
Acidity in ethanolic extract (%)	0,288 ± 0,04
Water absorption capacity water/g sample	2,91 ± 0.03
Swelling capacity (ml/g)	1,86 ± 0.06

Table 3: Proximate analysis of *Atta sexdens* flour*

Assay	Result	Unit
Fats	35,40	%
Moisture	7,58	%
Protein	35,51	%
Carbohydrates	16,12	%
Ash	5,39	%

*Data are expressed on a wet basis.

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Table 4: Microbiological analysis

Assay	Result	Unit
Mould count	NE1.0x10^1	UFC/g
Detection of Salmonella	Absence	A-P/25 g
Micro-organism count	NE 4.0x10^1	UFC/g
Mesophilic Aerobes		
Escherichia coli enumeration	<3	NMP/g
Yeast count	<1.0x10^1	UFC/g

NE: Estimated count
UFC: Colony forming units
A-P: absence or presence

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