Colegio de Postgraduados

Evaluation of protein sources in snail (*Helix aspersa* Müller) diets on the antioxidant bioactivity of peptides in meat and slime

Hidalgo-Tovar, Nadia[1]; Tamayo-Galván, Victoria E.[2], Pérez-Ruíz, Rigoberto V.[1]; García-Garibay, Mariano[1,3]; Aguilar-Toalá, José E.[1]; Rosas-Espejel, Monserrat[1]; Arce-Vazquez, Maria B.[1*]

[1] Universidad Autónoma Metropolitana, Unidad Lerma. Departamento de Ciencias de la Alimentación, Av. De las Garzas #10, Col. El Panteón, C. P. 52005, Lerma de Villada, Estado de México.
[2] Universidad Autónoma Metropolitana Unidad Cuajimalpa.Departamento de Procesos y Tecnológia, División de Ciencias Naturales e Ingeniería, Vasco de Quiroga 4871, Contadero, C.P. 05348, Cuajimalpa de Morelos, CDMX.
3 Universidad Autónoma Metropolitana, Unidad Iztapalapa. Departamento de Biotecnología, San Rafael Atlixco #186, Col. Vicentina, C. P. 09340, Iztapalapa, CDMX.
* Correspondence: m.arce@correo.ler.uam.mx

ABSTRACT

Objective: This work evaluates the effect of a dietary supply of amaranth, oats and lentils as a protein source on anthropometric measurements, the chemical composition in meat, as well as antioxidant activity in meat peptides and secretion of the snail (*Helix aspersa* Múller).

Design/methodology/approach: We worked with three groups of snails of 36 individuals and a control group fed with the same diet varying the protein source: amaranth, oats and lentils. A sample was taken every seven days and the shell's weight, width and length were measured. Five individuals from each group were sacrificed and the meat from which they were sacrificed was extracted: weight, moisture and protein. The hydrolysis soluble proteins in meat and slime were obtained and the antioxidant activity was measured using the reducing radicals DPPH• and ABTS•.

Results: Snail meat was obtained with an increase of more than double in weight when 10% of Am was supplied as a protein source.

Likewise, the dimensions of the shell will increase by 5%-11%. In FSM, it was obtained up to 79.8% moisture, 11.2% protein, 1.2% fat and 2.5% collagen. When obtaining snail meat flour, it was reduced to 12±1.9% humidity with up to 24.53 μg/g of soluble protein. When hydrolyzing the proteins, it was observed that the peptides obtained presented the IC_{50} of DPPH scavenging activity of 21.58±2.7, 5.45±1.8, 12.69±1.7 and IC_{50} of ABTS removal activity 8.86±0.9, 1.62±0.04, 10.84±1.0, for HFSM, HSMF and SS samples, respectively.

Limitations on study/implications: It is necessary to carry out other studies on the functionality of snail meat proteins and thus propose their implementation in food formulations to maximize their commercialization.

Findings/conclusions: Feeding snails with amaranth helps to increase the quality of protein in fresh meat and flour. Likewise, requests for soluble proteins from beef, flour and secretion are alternatives for preparing functional foods.

Keywords: snail meat, snail slime, antioxidant peptides, functional foods.

Citation: Hidalgo-Tovar, N., Tamayo-Galván, V. E., Pérez-Ruíz, R. V., García-Garibay, M., Aguilar-Toalá, J. E., Rosas-Espejel, M., & Arce-Vazquez, M. B. (2023). Evaluation of protein sources in snail (*Helix aspersa* Müller) diets on the antioxidant bioactivity of peptides in meat and slime. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i12.2783

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: September 16, 2023.
Accepted: December 14, 2023.
Published on-line: January 03, 2024.

Agro Productividad, 16(12). December. 2023. pp: 161-169.

INTRODUCTION

Mollusks represent an exceptionally diverse group, giving rise to the gastropod class (Aouji *et al.*, 2023), which includes, slugs and snails, encompassing approximately 35,000 terrestrial gastropods. At present, the commercially significant species belong to the genera *Achatina* and *Helix* (Corzas & Silvia, 2021). Heliciculture is an activity dedicated to the extensive and intensive production of snails, common in countries such as France, Italy, Spain and the United States (Colmenares-Flores & Alcántara-Gutierrez, 2021). Although snail farming, or heliciculture, has limited participation in Mexico, it is gradually expanding due to increased demand from the pharmaceutical, cosmetology and food sectors seeking bioactive compounds for the development of innovative formulations (Dhiman & Pant, 2021. The consumption of snails in Mexico has historical roots dating back to pre-Hispanic times, and their preparation methods have evolved, ranging from roasting and steaming to inclusion in traditional dishes like tamales (Cruz & Gómez, 2021). In contemporary times, snails are highly sought after in gourmet cuisine, commanding a market price of $14.00 MXN/kg (Romero-Díaz *et al.*, 2022). The nutritional composition of the snail meat positions it as an excellent alternative for human consumption, given its high protein content, low fat content and contributions of vitamins and minerals (Figure 1). On the other hand, the secretion produced by snails, known as "snail slime" contains proteins, polypeptides, glycans, and phenolic compounds. This diverse array of bioactive components adds to the potential applications of snails in various industries.

Recent studies have revealed that protein peptides in meat exhibit various functionalities, including antioxidant activity, antioxidant activity, ACE-Inhibitory activity, α-Amylase inhibitory activity and α-glucosidase inhibitory activity. Additionally, Aouji *et al.*, (2023) reported the presence of antimycobacterial, antioxidant, antitumor, antiinflammatory and anticancer compounds in snail slime. The occurrence of these functional compounds is directly influenced by the diet and living conditions of the snails. In the realm of farm animal production, switching protein sources is a common practice employed to enhance the nutritional value of the final product (Rygaÿo-Galewska *et al.*, 2022). The primary

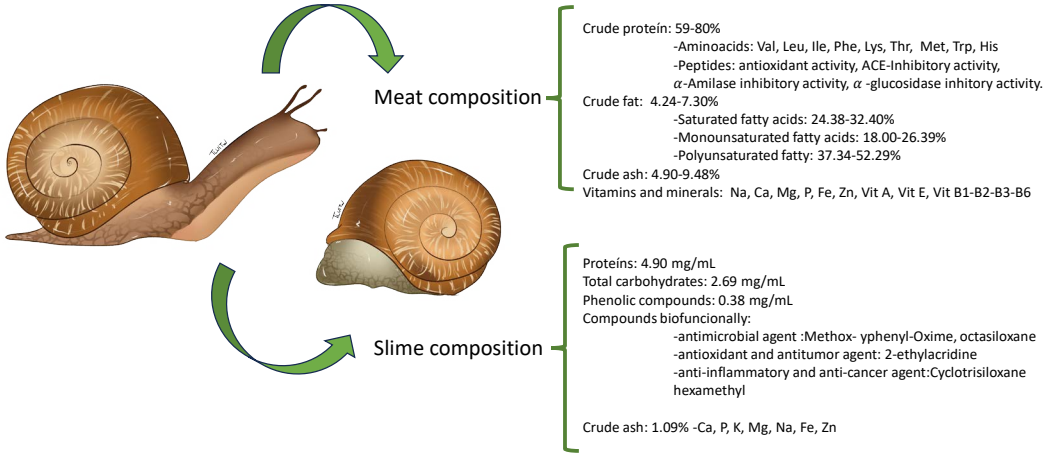


Figure 1. Molecules in meat and slime of nails *Helix aspersa*.

objective of the current study is to investigate the impact of diets incorporating amaranth, lentil and oat proteins on snail weight and size. Additionally, the study aims to assess the antioxidant activity of meat protein hydrolysates and snail slime.

MATERIALS AND METHODS

Helix aspersa Müller snails, sourced from heliciculturists in the municipality of Lerma, State of Mexico, were utilized in this study. All reagents were of analytical grade.

Organism rearing: Three groups and a control of *Helix aspersa* Müller snails of 3-4 g comprising 36 individuals, were acclimated one week prior to the study. They were housed in separate mesh containers with a pre-sterilized 1.5 cm soil bed and a container for water and food. Daily cleaning involved the removal of excreta and leftover food. Water was sprayed twice a day, and environmental conditions were maintained at a constant temperature (20±1 °C), humidity (80±5% RH) and a photoperiod (14:10 light-dark) were kept constant (Çelik *et al*., 2022).

Experimental design: The study was conducted in the Food Science laboratory of the Universidad Autónoma Metropolitana Lerma campus, held from March 7 to April 11, 2023. Three protein sources were tested: Amaranth (Am), Oatmeal (Oa), Lentil (Le). Diets were formulated following the guidelines of Ademolu *et al.* (2004), with slight modifications. Each group's diet included lettuce (35%), nopal (35%), calcium carbonate (16%), vitamins (4%), and the respective protein source 10% Am, Os and Le. Weekly samples were collected from six individuals over a span of five weeks. Snail weight and morphometry were documented by measuring the length and width of the shell with a Vernier caliper. Slaughter was carried out according to NOM-033-ZOO-1995, for the humane slaughter of domestic and wild animals. The meat was dissected, deveined, washed, and its weight recorded. On the other hand, the volume of snail slime (SS) obtained was measured using a pipette and stored at −4 °C.

Snail meat meal: Fresh snail meat (FSM) samples were arranged in a tray with a 2 cm separation subjected to a drying oven at 100 °C for 8 hours, cooled, and milled using a MOLINOX mil. The resulting snail meat flour (SMF) particles were sieved until achieving a particle size of 240 μ. Chemical analysis: The protein, fat, moisture and collagen content of SMF were determined using a FoodScanTM-lab meat analyzer. The equipment was calibrated prior to use, and 180 g of the sample was placed in the holder to prevent the formation of bubbles. Moisture in SMF samples from each week was determined using the oven method (method No. 14004). Soluble protein was determined using the Bradford method (1976) with Bovine Serum Albumin standard (10-100 μL in 10mM Tris-HCL buffer pH 7.3), and absorbance was measured at 595 nm.

Protein hydrolysis: Hydrolysis in FSM, SMF and SS was performed following the method of Hamid (2015) with some modifications. A 50 g sample (FSM, SMF) was mixed with 130 mL of 0.5 M phosphate buffer, pH 7.5. SS was taken directly after extraction. In both, native enzymes were inactivated by heating at 90 °C for 10 min, followed by an ice bath for 10 minutes. The samples were placed in a shaking incubator at 50 °C, and 85 μL of Alcalase® enzyme (Sigma Aldrich, 165 mUA) was added. The reaction was stopped by adding 120 μL of phenylmethylsulfonyl fluoride in ethanol (2 mg/ml).

Antioxidant activity of snail protein peptides: The antioxidant capacity of snail meat protein hy-drolysates (H-SMF) and snail slime hydrolysates (H-SS) was determined by their ability to reduce DPPH- and ABTS- radicals, according to Brand-Williams (1995) and Re-Pellegrin (1988), respectively. The IC_{50}, representing the amount of sample needed to inhibit 50% of each radical, was recorded.

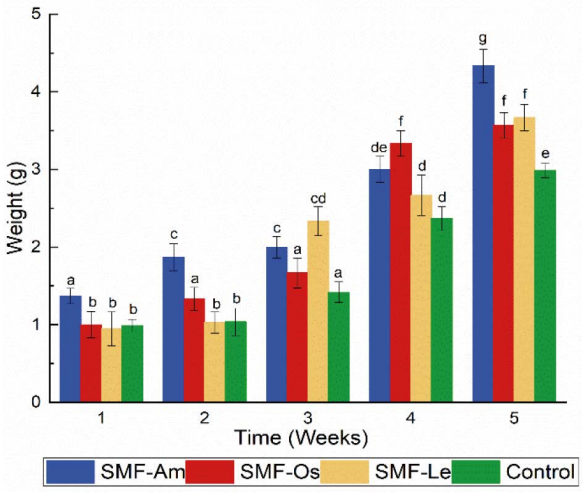
Statistical Analyses: Multiple assays were performed in triplicate, and the statistical analysis for sample comparison was conducted using NCSS software.

RESULTS AND DISCUSSION

Snail growth

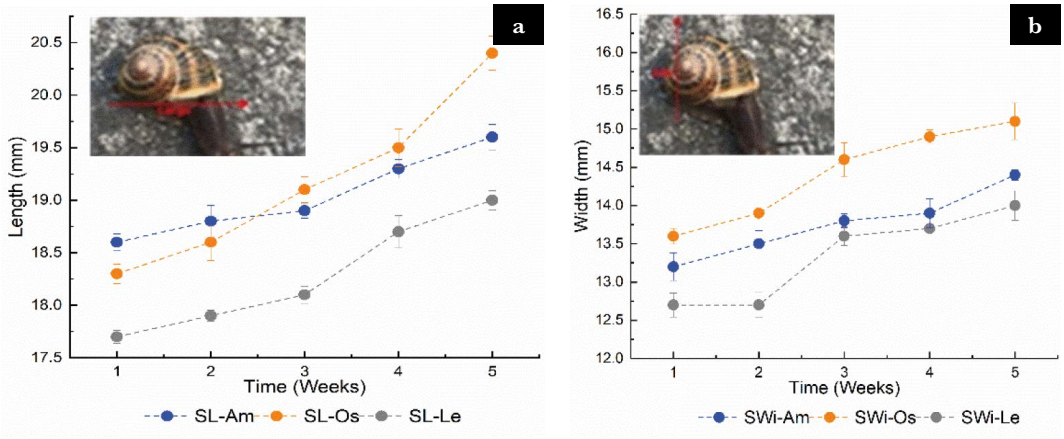
The weight gain in SMF is illustrated in Figure 2, indicating a 48% increase in SMF-Am, 28% in SMF-Os and 36% in SMF-Le compared to the control. SMF-Am samples are notably higher than others, while there is no significant difference between SMF-Os and SMF-Le, In comparison to the control, SMF-Am shows a 20% higher weight.

The growth effect in SMF is similar observed for snails fed with compound feed to accelerate growth, as reported by García *et al*., (2005). When compared to a vegetable diet, a 25% growth inhibition is noted, attributed to a dwarfing phenomenon related to feeding. Numerous studies have indicated that the nutritional value can vary between species base don the supplied diet, as well as factors such as the place of collection, season, and sexual condition. Diets enriched with amaranth, oat and lentil flour are found to be favorable nutritional supplements for weight gain in SMF. Morphometric records are presented in Figure 3, revealing a growth of 5.37% in length and 9.09% in width for SL-Am, 11% in both length and width for SL-Os, and 7.34% in length and 10.23% in width for SL-Le, compared to the control. This growth shows no significant differences regardless of the diets fed.



SMF-Am: amaranth-fed snail meat flour, SMF-Os: oat-fed snail meat flour, SMF-Le: lentil-fed snail meat flour.

Figure 2. Weight control of fresh snail meat fed with Amaranth, Oats and Lentils. Different letters indicate statistically significant differences at $p<0.05$.



SL-Am: Amaranth-fed snail shell length, SL-Os: Oat-fed snail shell length, SL-Le: Lentil-fed snail shell length
SWi-Am: Amaranth-fed snail shell width, SWi-Os: Oat-fed snail shell width, SWi-Le: Lentil-fed snail shell width

Figure 3. Record of carapace measurements a) carapace length, b) carapace width, in snails with. Amaranth, Oat and lentil diets.

The shell width data align closely with findings by Kathyuska *et al.*, (2016) in snails fed with soy-bean, pea and wheat. For shell length, the values are slightly below those reported by the same author. These results suggest that the calcium supplied during this growth phase is adequate, covering the nutritional requirements for shell deposition. A deficiency in calcium colud otherwise lead to reduced growth and eventual mortality, as noted by Mayoral *et al.*, (2004).

The shell is a by-product of this process, can serve as a calcium source, containing over 80% calcium carbonate with 25-35% fractional absorption bioavailability (García *et al.*, 2011).

Partial characterization of FSM

Observations indicate a protein content of 11.2±0.17%, twice that of *Limicolaria* FSM and comparable to *Achatina* FSM as reported by Babalola and Akinsoyinu (2009). Snail meat emerges as a non-conventional source of high-quality protein, complementary to cereal sources due to its lysine content. The determined fat content is 1.2±0.02%, similar to that reported for *Limicolaria* and half fat content of *Achatina*, a commercial species in Nigeria. Cholesterol levels are comparable to those found in *Achatina fulica* and *A. Limicolaria* snails (Zarai, 2012), offering consumers the Benefit of reducing the risk of cardiovascular diseases, such as heart attacks, hypertension and cerebrovascular events. Moisture was 78.8±3.52%, falling within the reported range for various species between 84.91% and 73.67%. Moisture content is indicative of the freshness of meat. Values above 70% typically indicate fresh meat, while below 40% may suggest that the snail meat is the process of deterioration. Collagen recorded for FSM is 2.5±0.26%, twice that reported in soy-fed *Lupinus* snails (Gogas *et al.*, 2021). Collagen, a high molecular weight protein, holds significance in the cosmetological and pharmaceutical industry.

Soluble protein and moisture content in SMF

The moisture content of the SMF samples ranged from 12-13.9%, irrespective of the diet fed. Soluble protein (P) content is depicted in Figure 4. P-Am samples exhibited the highest increase in soluble protein in their SMF (62%), while P-Le and control samples maintained the increase of 44.2-43.6% and P-Os had the lowest increase (29.6%).

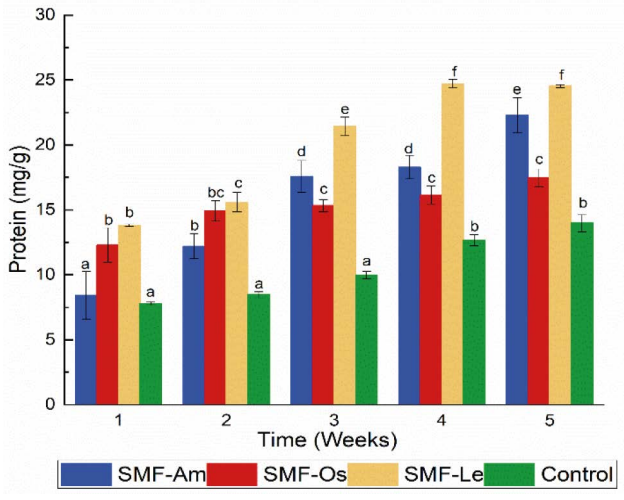
P-Am, P-Os and P-Le simples were significantly higher at the end of the treatments compared to week 1. Notably, P-Le achieved approximately twice the soluble protein content as the control, followed by P-Am with 37.35%, and P-Os with only a 20% increase over the control.

The results suggest a direct correlation between soluble protein and the supplied dietindicating a notable protein efficiency rate for all cases. This could be attributed to modifications in the oral mass and, primarily, in the randula, allowing snails to adapt various available food sources (Aguilera, 1996). Hydrolyzed SMF proteins may contain peptides of 3, 5 and 10 Kda (Saallah *et al.*, 2020).

Antioxidant activity of fresh meat and snail slime protein hydrolysates

Antioxidant activity, assessed by the reduction of DPPH• and ABTS• radicals was determined in hydrolysates (H) of FSM, SMF and SS, as presented in Table 1. HFSM-Am, HSMF-Am and HSS-Am exhibited the lowest inhibition IC_{50} for both radicals, indicating superior antioxidant activity compared to the other samples from the Os and Le groups. Evaluation of the antioxidant activity is complex and system-dependent, hence the preference for assessment in multiple systems.

In HFSM samples, significant differences in antioxidant activity were observed, irrespective of the radical used, with HFSM-Am and HFSM-Os requiring 3 times less for inhibition. Lower IC_{50} values were obtained in HFSF compared to HFSM simples



P-Am: SMF Soluble Protein Fed with amaranth, P-Os: SMF Soluble Protein Fed with oat, P-Le: SMF Soluble Protein Fed with lentils.

Figure 4. Soluble protein in snail meat meal fed with alternate protein sources. Different letters indicate statistically significant differences at $p<0.05$.

Table 1. Evaluation of the DPPH• and ABTS• radical reduction capacity of snail protein hydrolysates.

Sample	IC_{50} radical inhibition DPPH• (g/mL)	IC_{50} radical inhibition ABTS• (g/mL)
HFSM-Control	61.261 ± 3.3^{a}	25.088 ± 1.5^{a}
HFSM-Am	21.587 ± 2.7^{b}	8.866 ± 0.9^{b}
HFSM-Os	27.077 ± 1.9^{c}	14.491 ± 1.1^{c}
HFSM-Le	45.005 ± 1.9^{d}	14.088 ± 1.7^{d}
HSMF-Control	20.058 ± 2.6^{b}	11.686 ± 0.1^{e}
HSMF-Am	5.451 ± 1.8^{e}	1.629 ± 0.04^{a}
HSMF-Os	12.571 ± 2.5^{f}	1.244 ± 0.07^{e}
HSMF-Le	16.804 ± 3.6^{g}	5.608 ± 0.4^{f}
HSS-Control	45.483 ± 4.1^{d}	15.796 ± 0.8^{ag}
HSS-Am	12.692 ± 1.7^{f}	10.846 ± 1.0^{ag}
HSS-Os	10.866 ± 1.9^{f}	10.552 ± 1.3^{a}
HSS-Le	17.592 ± 2.2^{g}	11.473 ± 0.8^{g}

Equal letters are not statistically different ($p \leq 0.05$).
Different letters are statistically different ($p \leq 0.05$).

due to concentration, and HSMF-Am exhibited the lowest IC_{50} in inhibiting both radicals. The volume of slime extracted was 3.6-4.4 mL/1 snail. SS samples displayed antioxidant activity, especifially in HSS-Am and HSS-Os with 3 times less IC_{50} of DPPH• radical and approximately 50% less IC_{50} for ABTS• radical compared to the control. These findings align with Aouji *et al.*, 2023, where hydrolyzed pep-tides with antioxidant activity on the DPPH• radical were obtained, particularly when featuring amino acid sequences HTYHEVTKH and WPVLAYHFT. The ability to reduce the ABTS• radical may be atributed to amino acids containing hydrophobic groups (Petsantad *et al.*, 2020).

SS peptides exhibit various bioactivities. Hayes and Mora (2021) derived peptides from *Phylum mollusca*, showing functionality on angiotensin converting enzyme ACE-1 up to 95% *in silico* assays, identifying peptides from 628 to 2343 Da.

Peptides from mollusks, l have been increasingly utilized in pharmaceuticals, cosmeceuticals and nutraceuticals, and functional foods, due to their diverse biological properties (Ovchinnikova, 2019).

CONCLUSIONS

The protein sources Am, Os and Le supplied to *Helix aspersa* Müller snails have a positive effect on the growth of the individual as well as in obtaining soluble proteins and slime with a functional potential due to their antioxidant activity. Am the protein source that presented the greatest effect on snail weight and size as well as the highest antioxidant activity in protein hydrolysates. Due to its composition, fresh snail meat is an alternative

that competes nutritionally in the meat market. Additionally, snail protein hydrolysates hold promise as a potential raw material for the development of functional foods.

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