An Acad Bras Cienc (2024) 96(Suppl. 1): e20231375 DOI 10.1590/0001-3765202320231375
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Bioactive peptides from *Tenebrio molitor*: physicochemical and antioxidant properties and antimicrobial capacity

JÉSSICA CRISTINA V. VILLANOVA, ALEXANDRA PRETTO, EVANDER M. PENCHEL, SERGIO DOMINGOS S. SERRA, CARLOS FREDERICO C. LANES, VANESSA B. RIBEIRO, CAROLINE S. SPERONI, ANA BETINE B. BENDER & FERNANDA R.G. FERRIGOLO

Abstract: Recent research has demonstrated the increasing interest in using insects for the extraction of bioactive compounds, particularly peptides. These compounds offer a spectrum of beneficial physiological effects. The aim of this study was to standardize a methodology for obtaining bioactive peptides from *Tenebrio molitor* and evaluate its physicochemical characterization, antioxidant, and antimicrobial potential. Six assays were carried out to hydrolyse larvae protein, with variations in Alcalase concentration (0.04 to 0.08%) and reaction time (3 to 8 h). The results indicated that the process applied to defatted mealworm flour was effective in reducing lipids by 82.5%. Consequently, it was an observed increase of 38.4% in protein content. Additionally, an increase in glycogen content was found in defatted mealworm flour (177 µmol glucose g^{-1} sample) and peptides (152.81 µmol glucose g^{-1} sample). The degree of hydrolysis was higher in assays with longer hydrolysis durations (8.14 - 8.38%). The antioxidant capacity was 12 to 14% lower in assays with an incubation time of 8h. In this sense, the methodology proposed in the present study proved to be efficient in obtaining bioactive peptides from *T. molitor*.

Key words: functional ingredient, enzymatic hydrolysis, mealworm, edible insects.

INTRODUCTION

Aquaculture is not only important for generating financial resources and jobs. It is also recognized to contribute to food security and social development in many regions and countries, such as Brazil (Chan et al. 2024, Esteban 2012).

For aquaculture becoming a competitive activity and achieve high production rates, intensification is necessary. Consequently, several factors, including overcrowding, management practices, unfavorable temperature, poor nutrition, and water quality, contribute to stress in the animals (Stanivuk et al. 2024, Awad & Awaad 2017). The immune system of fish is directly influenced by factors in the rearing environment. Disruption of the body's homeostasis promotes a stress situation, leading physiological changes and rendering the animal susceptible to diseases (Urbinati et al. 2014). In this condition, the production of free radicals and/or reactive oxygen species (atoms or molecules highly reactive with cellular constituents) can increase, surpassing the natural antioxidant defense capacity of cells, which leads to the undesirable state of oxidative stress (Lushchak 2011). So, all these biochemical and physiological changes are capable of

disturbing the immune system of fish, thereby favoring the development of diseases.

As a way of preventing and controlling pathogens, the use of antibiotics in intensive production systems has been adopted. However, these antimicrobials are responsible for several negative effects, including the development of resistance in zoonotic pathogenic bacterial strains (Acunha et al. 2023) accumulating residues in edible tissues and the environment can also compromise the effectiveness of antibiotics and weaken the immune system (Khanzadeh et al. 2024, Gufe et al. 2024, Hoseinifar et al. 2015). As a result, ecologically sound alternatives to the use of antibiotics are being sought, offering promising sustainability for disease prevention and /or control in aquaculture (Awad & Awaad 2017, Hoseinifar et al. 2015, Mohammadi et al. 2020, Yousefi et al. 2020).

In this context, bioactive compounds are considered the future of the aquaculture industry. Through preventive health management via feeding practices, aquatic animals can allocate more energy towards growth, reducing biological energy reserves required for combating diseases or stress resistance (Salomón et al. 2020).

Studies have revealed that biologically active peptides obtained from plant and animal protein sources demonstrate antioxidant, immunomodulatory, anti-inflammatory, growth-promoting, and antibacterial properties for humans (Lalani et al. 2024, Sarker 2022, Li-Chan 2015).

These functional bioactive compounds have shown satisfactory effects in the diet of aquatic animals, such as improving nutrient use efficiency by increasing the absorption of amino acids and minerals, promoting protein synthesis and digestive enzyme activity, consequently enhancing growth. This has been observed in specimens of turbot (*Scophthalmus maximus*) (Hao et al. 2020). In addition to benefiting fish growth, the inclusion of low molecular weight nitrogenous compounds also promotes several health benefits for the animal. For instance, the antioxidant properties of protein hydrolysates enable them to act against free radicals present in cells and minimize oxidation in macromolecules. In addition, other properties presented by protein and peptide hydrolysates include prevention of human diseases such as hypertension (Dai et al. 2013) antimicrobial, cytomodulatory, prebiotic, and hypocholesterolemic activity (Halim et al. 2016, Sarker 2022). In addition to presenting anti-inflammatory and inhibitory actions on enzymes such as lipase and angiotensin-converting enzyme (Jakubczyk et al. 2020), it also exhibits antioxidant potential (Riolo et al. 2023).

According to Samaranayaka & Li-Chan (2011), hydrolysis through the use of enzymes has been the primary method for obtaining peptides (molecules containing from 2 to 20 amino acids) from proteins. According to Quah et al. (2023), the bioactive peptide activity is influenced by the composition and sequence of amino acids. Various raw materials such as fish, milk, eggs, soy, wheat, fungi, marine macroalgae, among other protein sources, have been widely studied as potential ingredients for the production of peptides (Sarker 2022). Among the promising potential sources for obtaining peptides, insects such as *Tenebrio molitor* (Linnaeus 1758) deserve to be highlighted due to the abundance of this raw material, which is rich in proteins and has high levels of essential amino acids such as lysine. Considered the main limiting amino acid in agricultural by-products used in the manufacture of commercial feed, lysine plays a crucial role in ensuring optimal protein synthesis and growth in aquaculture species (Montes-Girao & Fracalossi 2006).

In aquaculture, mealworm larvae meal has already been included in the diet of various

species such as gilthead seabream (*Sparus aurata*, Linnaeus 1758), sea bass (*Dicentrarchus labrax*, Linnaeus 1758), rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), African catfish (*Clarias gariepinus*, Burchell 1822), meagre (*Argyrosomus regius*), as a potential substitute for fish meal (Coutinho et al. 2021, Henry et al. 2015). Additionally, in diets for Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758), grass carp (*Ctenopharyngodon idellus*), and mirror carp (*Cyprinus carpio* var. specularis), mealworm meal have been used to replace soybean meal (Li et al. 2022, 2023, Zhang et al. 2023). However, studies on bioactive peptides obtained from *T. molitor* are scarce.

In this context, the main aim of the present study was to apply methodologies for obtaining bioactive peptides from *T. molitor* and to evaluate the physicochemical properties, as well as the antioxidant and antimicrobial activities, with the intention of exploring their potential for use in fish diets.

MATERIALS AND METHODS

Cultivation and physicochemical characterization of *T. molitor* larvae

Tenebrio molitor larvae were cultivated at Animal Biodiversity Laboratory (Universidade Federal do Pampa, Uruguaiana City, Rio Grande do Sul state, Brazil) and fed a commercial poultry feed (Presence, Brazil) (Bordiean et al. 2022, Romero-Lorente et al. 2022) composed of ground corn, corn gluten meal, soybean meal and rice meal (proximate composition: moisture 10.64 ± 0.97, crude protein 19.96 ± 0.70%, fat 3.83 ± 0.13%, ash 10.28 ± 0.98%, acid detergent fiber 37.05 ± 1.39, and carbohydrates 18.68%). The larvae were cultivated until reaching 2 cm in size (± 80 days). *T. molitor* larvae were partially dried (55 °C for 48 h), ground and evaluated by proximate composition: dry matter (method 925.45b), ash (method 923.03), crude protein (method 960.52) (AOAC 1995), lipids (Bligh & Dyer 1959), and acid detergent fiber (Van Soest et al. 1991). Carbohydrates were obtained by difference using the formula: 100 - (moisture + crude protein + ash + lipids + acid detergent fiber).

Glycogen analysis

The glycogen levels of mealworm flour, defatted mealworm flour, and bioactive peptides were determined according to Bidinotto et al. (1997). Sample (50 mg) was homogenized with KOH and ethanol for hydrolysis and glycogen precipitation. The glucose was estimated using a hydrolytic method with phenol-sulfuric acid. The results were calculated based on the standard curve of glucose solution (50 to 200 µM) and expressed as µmol glucose g^{-1} of sample.

Production of bioactive peptides

The larvae were ground to obtain flour. Then, the lipids were extracted with hexane (1:2, w:v) as described by Goulart et al. (2013). The defatted mealworm flour was homogenized and pre-incubated in distilled water (1:10, w:v) at 55 °C for 30 min. After pre-incubation, the following assays were conducted using Alcalase® (density 1.17 g mL^{-1}):

Assay 1: 0.04% enzyme for 3 h;
Assay 2: 0.08% enzyme for 3 h;
Assay 3: 0.04% enzyme for 4 h;
Assay 4: 0.08% enzyme for 4 h;
Assay 5: 0.04% enzyme for 8 h;
Assay 6: 0.08% enzyme for 8 h.

All tests were conducted at 55°C, and the pH was adjusted to 7.0 with NaOH 0.1 N. After the hydrolysis step, the enzyme was inactivated by heating (95 °C for 15 min), and the mixture was cooled to 20-25 °C in an ice bath. Hydrolyzed samples were centrifuged (9300 g/20 min) to separate the insoluble materials from soluble

peptides. The supernatant was collected and stored at -20 °C until analysis. The methodology employed was described by Tang et al. (2018).

Degree of hydrolysis

After hydrolysis, 20% trichloroacetic acid (TCA) solution was added to the supernatant, and the mixture was centrifuged at 9300 g for 20 min. The nitrogen content of the fresh supernatant (10% TCA solution) was determined by the micro-Kjeldahl method, as described by Tang et al. (2018), with some modifications. The degree of hydrolysis was calculated as following:

Enzymatic hydrolysis (%) = (N (nitrogen) total of sample) / (N of 10% TCA solution) x 100

Total antioxidant capacity of bioactive peptides

The antioxidant capacity was evaluated by the Ferric Reduction Antioxidant Power (FRAP) assay as described by Pulido et al. (2000) with some modifications. Briefly, *T. molitor* peptides samples were diluted in distilled water (1:25 v:v). The FRAP reagent was prepared by combining 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM ferric chloride aqueous solution (10:1:1 v:v). For the assay, 90 µL of diluted sample was transferred to test tubes, in a dark environment, followed by the addition of 270 µL of distilled water and 2.7 mL of the FRAP reagent. The tubes were mixed and incubated in a water bath at 37°C for 30 min. The absorbance was measured using a spectrophotometer at 595 nm. The results were calculated based on the standard curve of ferrous sulfate hexahydrate (500 to 2000 µM) and expressed as µmol Fe (II) equivalent g^{-1} of sample.

Antimicrobial capacity of bioactive peptides

Initially, the inoculums of the following bacterial cultures were standardized: *Pseudomonas* sp., *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella* sp., *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus* sp. The inoculums were spiked in PCA (Plate Count Agar) 24 h before testing. Bacterial suspensions were diluted in 10% saline solution, according to the 0.5 Mc Farland scale (approximately 1.0 x 10^8 CFU mL^{-1}).

The Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution method, which is defined as the lowest concentration of extract (mg mL^{-1}) capable of inhibiting microbial growth. A sequence of serial dilutions was prepared with the peptides from assay 5, as follows: 1:2; 1:5; 1:10; 1:50; 1:100 (v:v, peptides: Mueller Hinton broth). This assay was used due to its higher degree of hydrolysis and lower enzyme requirement. Subsequently, 50 µL of each dilution were added to 96-well microdilution plates, followed by 50 µL of bacterial inoculum. The plates were incubated at 37 ºC for 24 h. MIC values were assessed in quadruplicate (Clinical and Laboratory Standards Institute 2013).

Statistical analysis

The data were submitted to a normality test, followed by one-way analysis of variance (ANOVA). Means were compared by Tukey's test ($p<0.05$).

RESULTS

Physicochemical characterization of *T. molitor* larvae

The physicochemical composition of the mealworm larvae used in this study is shown in Table I. The crude protein (CP) content was 45.17%, ash content was 3.22%, and fat content was 34.36%. After the fat extraction process, the CP content of mealworm flour increased by 38.4%. In contrast, the lipid content was reduced by 82.5%.

Table I. Physicochemical characterization of *Tenebrio molitor* larvae.

Parameter	Tenebrium flour	Defatted tenebrium flour
Crude protein (%)	45.17±1.50	62.51±3.20
Dry matter (%)	87.55±0.09	86.65±0.14
Ash (%)	3.22±0.24	4.53±0.03
Lipid (%)	34.36±0.68	6.02±1.17
Carbohydrates (%)	4.8	13.59

Values expressed as mean ± standard deviation (n=3).

Glycogen levels in *T. molitor*

The glycogen levels in *T. molitor* larvae and bioactive peptides are shown in Figure 1. The results (µmol glucose g^{-1} of sample) were numerically higher for the deffated mealworm flour (177.04) and bioactive peptides (152.81) compared to the larvae sample (144.08).

Degree of hydrolysis of bioactive peptides

Table II shows the degree of hydrolysis for each assay used for obtaining the bioactive peptides. Tests 1 to 4, with shorter hydrolysis time (3-4h), exhibited significantly lower degrees of hydrolysis compared to tests 5 and 6.

Total antioxidant capacity of bioactive peptides

The results related to the total antioxidant capacity of the bioactive peptides measured by the Ferric Reduction Antioxidant Power – FRAP – assay, are shown in Table III. The bioactive peptides obtained in assays 1, 3, and 4 have significantly higher antioxidant potential compared to those obtained in assays 2, 5 and 6.

Antimicrobial capacity of bioactive peptides

Figures 2 and 3 demonstrate the effects of the bioactive peptides on the growth of various bacteria. As shown, the bioactive peptides did not exhibit efficacy in inhibiting the growth of the evaluated microorganisms. Bacterial growth was observed in all tested dilutions.

DISCUSSION

Physicochemical characterization of mealworm flour and defatted mealworm flour demonstrated that the chemical process used to extract fat was efficient, leading to a concentration of the protein content. In a review by Hong et al. (2020), it was demonstrated that *T. molitor* larvae contain between 47.0 and 60.2% crude protein and 22.87 to 37.70% fat. It is suggested that these values may vary depending on the

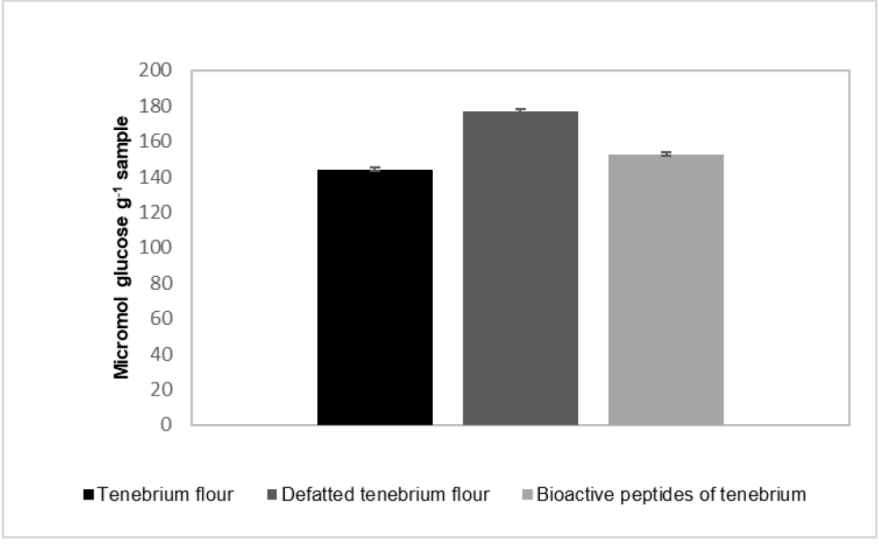

Figure 1. Glycogen levels of *T. molitor* larvae and bioactive peptides.

insect diet. The levels observed in our study are similar to those reported by Hong et al. (2020), which confirms the hypothesis that these larvae are excellent sources of protein and fat, as highlighted by previous studies.

Regarding the composition of defatted mealworm flour, the values found for crude protein (62.51%) are similar to those observed by Tran et al. (2022) (71.1%) and Basto et al. (2023) (71.0%). Furthermore, it is important to highlight that these values are comparable to those of fish meal (67.5%) and higher than levels of soybean meal (on average 49.4%) (Hong et al. 2020). As a result, it is suggested that defatted mealworm flour is a highly sustainable source of protein and an alternative to conventional protein ingredients used in aquaculture, such as fish and soybean meal. Similar values were observed for dry matter and ash contents in raw and defatted flour, demonstrating that the process of removing fat does not affect the mineral content of the ingredient.

Related to glycogen concentration, samples of bioactive peptides showed higher values than *T. molitor* larvae. In insects, glycogen is associated with a non-reducing disaccharide composed of two glucose units known as trehalose. This compound is found in the hemolymph of insects and serves as a source of energy, particularly during muscle contraction, such as during flight. The synthesis of trehalose in insects occurs in the fat body, where it is also stored for utilization by the hemolymph when required (Lopes & Villela 1972). Some authors suggest that trehalose protects cells during stressful conditions, such as high temperatures,

Table II. Degree of enzymatic hydrolysis of defatted *Tenebrio molitor* with Alcalase®.

Sample	Enzyme (%)	Incubation time (h)	Degree of hydrolysis (%)
Assay 1	0.04	3	52.68±0.15[a]
Assay 2	0.08	3	62.43±0.03[b]
Assay 3	0.04	4	63.60±0.30[c]
Assay 4	0.08	4	68.09±0.10[d]
Assay 5	0.04	8	72.13±0.13[e]
Assay 6	0.08	8	74.28±0.14[f]

Lower case letters indicate statistical differences among tests by Tukey's test (p<0.05).

Table III. Total antioxidant capacity of bioactive peptides from *Tenebrio molitor*.

Sample	Enzyme (%)	Incubation time (h)	Antioxidant capacity (FRAP)*
Assay 1	0.04	3	276.41±6.41[b]
Assay 2	0.08	3	275.13±8.00[b]
Assay 3	0.04	4	265.15±7.00[b]
Assay 4	0.08	4	235.09±6.00[a]
Assay 5	0.04	8	237.31±7.00[a]
Assay 6	0.08	8	239.89±0.89[a]

]Lower case letters indicate statistical differences among tests by Tukey's test (p<0.05). *Ferric Reduction Antioxidant Power (µmol Fe (II) equivalent/g of sample).

osmotic shock, ethanol toxicity, and dehydration (Alcarde & Basso 1997).

Alcarde & Basso (1997) observed an increase in the trehalose content of yeasts with the application of thermal treatment (increase in temperature). These results corroborate with our findings, as higher levels of glycogen were observed in bioactive peptides that underwent heat treatment at the end of the hydrolysis process to inactivate the enzyme. Furthermore, it is suggested that trehalose may interfere with the drying process of peptides obtained from *T. molitor*, as observed in our study. Initially, we attempted to use lyophilized samples. However, through this drying method (results not included), we did not obtain viable samples suitable for study or use in fish feed, due to its viscous and adherent appearance. This behavior can be attributed to the high levels of glycogen (trehalose) present in both the raw samples and peptides. Thus, we prioritized studying the wet sample instead of the dry one, which will consequently facilitate the homogenization of the peptides in fish feed.

In the present study, the hydrolysis time and the concentration of Alcalase enzyme influenced the degree of hydrolysis. Consistent with our findings, Centenaro et al. (2009) also observed an increase in the degree of hydrolysis with higher concentrations of Alcalase. According to Kristinsson & Rasco (2000), the increase in protease concentration is associated with a corresponding increase in the degree of hydrolysis. However, the authors highlighted that the greater the amount of enzyme used, the higher the cost of obtaining the peptides. The degree of hydrolysis in defatted mealworm flour, using Alcalase, ranged from 52.68 ± 0.15% to 74.28 ± 0.14%, higher than those values found for protein hydrolysis in other products previously studied, such as chicken leg (18.62 to 38.79%), chicken breast (20.93% to 57.42%) (Schmidt & Salas-Mellado 2009), and croaker (12.2 ±0.11 to 43.7 ±0.33%) (Centenaro et al. 2009). Despite this, the study of obtaining bioactive peptides from insects is relatively recent (Rivero-Pino et al. 2020). Further research is necessary to comprehend the biology of mealworm and,

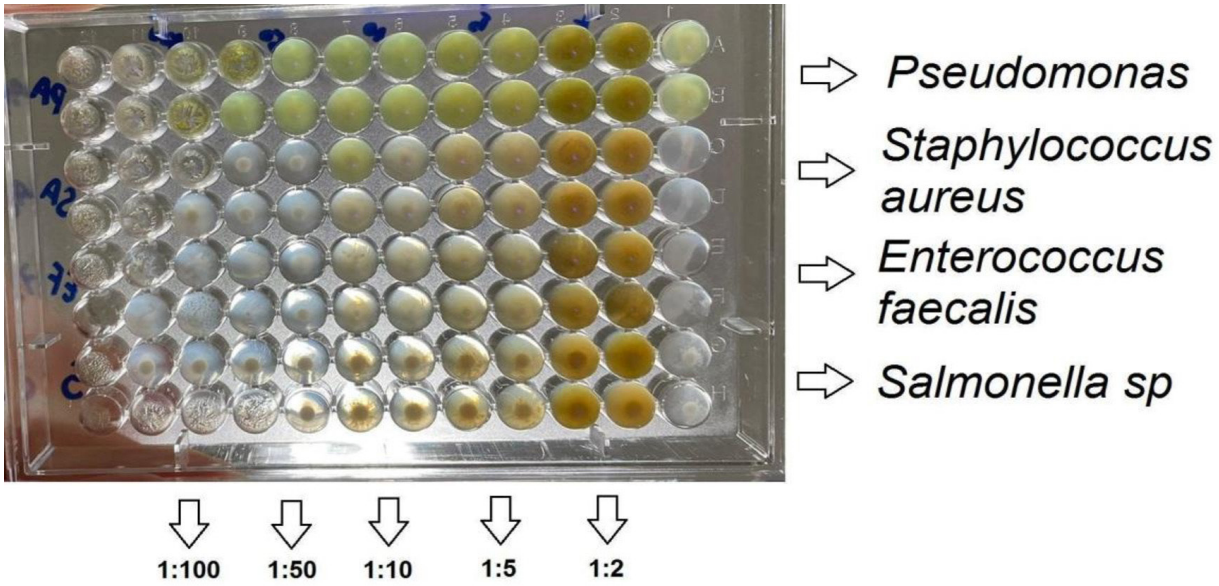

Figure 2. Growth of *Pseudomonas* sp., *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella* sp. in broth with serial dilution of *T. molitor* peptides.

consequently, efficiently explore and optimize the production of peptides.

Protein hydrolysis can be performed through various methods, including the use of acids, alkalis, or enzymes. Among the procedures, enzymatic hydrolysis has been widely recommended due to several advantages, such as the modification of functional, physicochemical, and sensory properties of the original proteins. In addition, enzymatic hydrolysis allows greater control over the process and enables the retention of beneficial properties in the resulting product (Schmidt & Salas-Mellado 2009). According to Fennema et al. (2018), the functional properties of protein hydrolysates are influenced by the type of enzyme used in their production. In this sense, the Alcalase derived from *Bacillus licheniformis* is the main commercial enzyme used and recommended for the production of protein hydrolysates due to its non-specific specificity and efficiency (Schmidt & Salas-Mellado 2009). The study carried out by Tang et al. (2018), which evaluated the enzymatic hydrolysis of *T. molitor* protein using different enzymes (Alcalase and Flavourzyme) and enzymatic combinations, found that the highest protein hydrolysis yield was achieved with Alcalase, ranging from 10.0% to 38.7%. Based on these findings, it is suggested that further studies could be conducted to explore the combination of different physico-enzymatic methods to enhance the degree of hydrolysis of *T. molitor* protein while minimizing hydrolysis time and enzyme concentration.

The total antioxidant capacity of the bioactive peptides varied among the tests applied. Higher values were found after applying shorter hydrolysis time. In contrast, a study carried out by Wu et al. (2003) reported that hydrolysates with greater antioxidant activity were obtained with longer durations of hydrolysis. The authors suggest that a longer duration of enzyme-substrate contact leads to greater antioxidant capacity, which contradicts the findings of our study. In the same point, Tang et al. (2018) also observed high antioxidant capacity of bioactive peptides obtained from *T. molitor* using Alcalase.

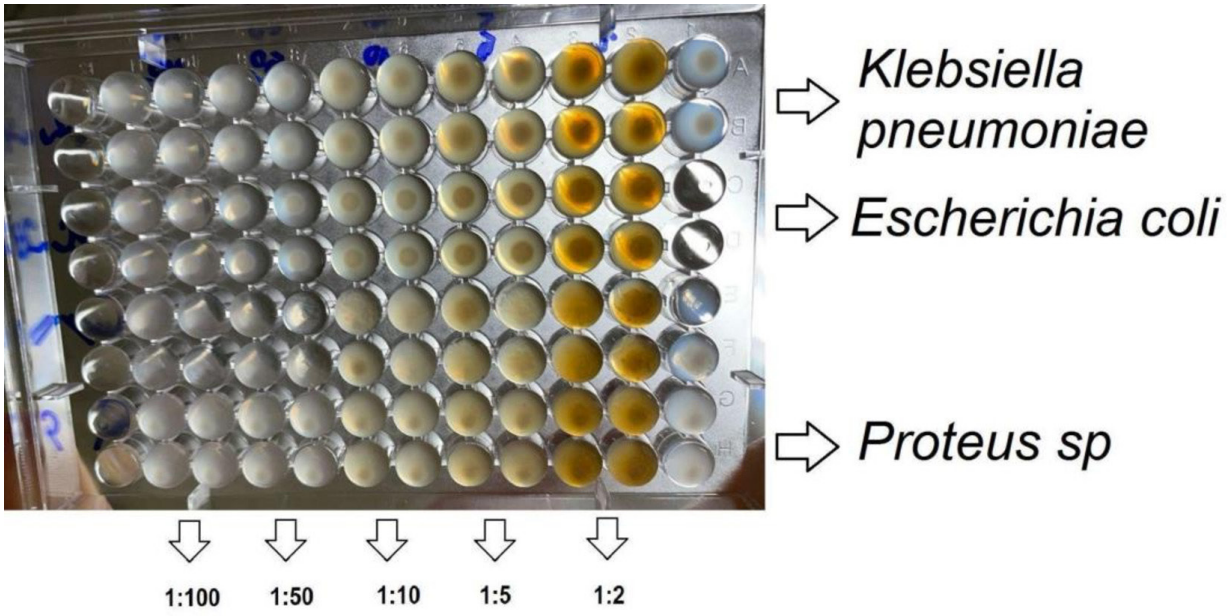

Figure 3. Growth of *Klebsiella pneumoniae, Escherichia coli, and Proteus* sp. in broth with serial dilution of *T. molitor* peptides.

Several methodologies have been developed and applied with the aim of evaluating the antioxidant capacity of different ingredients. Due to the diversity of techniques and the absence of a universal method capable of measuring the antioxidant capacity, it is difficult to make numerical comparisons between the results obtained in different studies (Morais et al. 2013). However, when compared with cerrado fruits, our results (μM ferrous sulfate/g) were higher than those reported for *Solanum lycocarpum* seed (fruta-do-lobo) (167.11±0.219), *Byrsonima verbascifolia pulp* (murici) (148.42±0.047), and *Cipocereus minensis* peduncle (quiabo da lapa) (151.67±0.043) (Morais et al. 2013). Therefore, regardless of the values found in the different tests, all hydrolysates obtained in the present study demonstrated antioxidant capacity. As potential sources for inclusion in fish diets, exerting an effective protective action against oxidative processes occuring in the animal's organism and aiding in disease prevention. Antioxidant molecules have the ability to decrease the rate of oxidation by inhibiting free radicals or removing metals that damage cells (Kabel 2014). According to Chi et al. (2014), peptides with lower average molecular weight, particularly those consisting of shorter and more active peptides, act as electron donors and react with free radicals, transforming them into more stable substances that interrupt chain reactions.

Regarding the antimicrobial capacity of the bioactive peptides, any effective action was observed to inhibit the growth of the evaluated bacteria. Our results contradict studies that emphasize the antimicrobial potential of insect peptides, including those derived from mealworm larvae (Azmiera et al. 2022). The findings presented here are relevant, especially considering that there is only one study (Chae et al. 2012) focusing on evaluating the antimicrobial capacity of peptides obtained from mealworm has been identified, as reported in the review by Azmiera et al. (2022). Despite the absence of direct *in vitro* inhibitory effects on pathogenic microorganisms, peptides may still confer indirect positive effects. When animals receive these additives through the diet, improvements in the immune system may occur, leading to an enhanced ability to combat pathogens.

CONCLUSIONS

The hydrolysis process applied to defatted mealworm flour, using Alcalase, exhibited effectiveness particularly with longer enzymatic treatment periods. The obtained peptides demonstrated bioactive potential, as evidenced by the high antioxidant capacity observed across all enzymatic treatments tested. Additionally, this study presents promising and innovative findings that allow for further exploration as alternative sources to be used in aquaculture in future research.

Acknowledgments

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for supporting the project (protocol 21/2551-0000605-6) and provision of a scientific initiation scholarship for Jéssica Cristina Verus Villanova.

REFERENCES

ACUNHA RMG, SANCHES DS, ALMEIDA RGS, OLIVEIRA FC, SOARES MP, DAVALO MRS, SILVA MEVM, OLIVEIRA KKC & CAMPOS CM. 2023. O uso de imunomoduladores na alimentação de peixes: Uma revisão. Res Soc Dev 12: 1-14.

ALCARDE AE & BASSO LC. 1997. Efeito da Trealose na manutenção da viabilidade de células de levedura desidratadas por liofilização. Sci Agric 54(3): 3.

AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1995. Official Methods of Analysis, 16th ed, Washington, Association of Official Analytical Chemists, 1141 p.

AZMIERA N, KRASILNIKOVA A, SAHUDIN S, AL-TALIB H & HEO CC. 2022. Antimicrobial peptides isolated from insects and their potential applications. J Asia Pac Entomol 25: 101892.

AWAD E & AWAAD A. 2017. Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol 67: 40-54.

BASTO A, MARQUES A, SILVA A, SÁ T, SOUSA V, OLIVEIRA MBPP, AIRES T & VALENTE LMP. 2023. Nutritional, organoleptic and sensory quality of market-sized European sea bass (*Dicentrarchus labrax*) fed deffated *Tenebrio molitor* larvae meal as main protein source. Aquaculture 566: 739210.

BIDINOTTO PM, MORAES G & SOUZA RHS. 1997. Hepatic glycogen and glucose in eight tropical freshwater teleost fish: A procedure for field determinations of micro samples. Bol Tec CEPTA 10: 53-60.

BLIGH EG & DYER WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

BORDIEAN A, KRZYŻANIAK M & STOLARSKI MJ. 2022. Bioconversion potential of agro-industrial byproducts by *Tenebrio molitor* - Long-term results. Insects 13: 810.

CENTENARO GS, PRENTICE-HERNÁNDEZ C & SALAS-MELLADO M. 2009. Efeito da concentração de enzima e de substrato no grau de hidrólise e nas propriedades funcionais de hidrolisados proteicos de corvina (*Micropogonias furnieri*). Quim Nova 32: 1792-1798.

CHAE JH, KUROKAWA K, SO YI, HWANG HO, KIM MS, PARK JW, JO YH, LEE YS & LEE BL. 2012. Purification and characterization of tenecin 4, a new anti-Gram-negative bacterial peptide, from the beetle *Tenebrio molitor*. Dev Comp Immunol 36: 540-546.

CHAN HL, CAI J & LEUNG PS. 2024. Aquaculture production and diversification: What causes what? Aquaculture 583: 740626.

CHI C-F, CAO Z-H, WANG B, HU F-Y, LI Z-R & ZHANG B. 2014. Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. Molecules 19: 11211-11230.

CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2013. Performance standards for antimicrobial susceptibility testing. Twenty-third informational supplement. M100S23. Wayne, PA: CLSI.

COUTINHO F ET AL. 2021. Mealworm larvae meal in diets for meagre juveniles: Growth, nutrient digestibility and digestive enzymes activity. Aquaculture 535: 736362.

DAI C, MA H, LUO L & YIN X. 2013. Angiotensin I-converting enzyme (ACE) inhibitory peptide derived from *Tenebrio molitor* (L.) larva protein hydrolysate. Eur Food Res Technol 236: 681-689.

ESTEBAN MA. 2012. An Overview of the Immunological Defenses in Fish Skin. Int Schol Res Network Immunol 12: 1-29.

FENNEMA OR, DAMODARAN S & PARKIN KL. 2018. Introdução à química de alimentos. In: Damodaran S & Parkin KL (Eds), Química de Alimentos de Fennema, Porto Alegre: Artmed, Porto Alegre, Brazil, p. 1-16.

GOULART FR, SPERONI CS, LOVATTO NM, LOUREIRO BB, CORRÊIA V, RADÜNZ NETO J & SILVA LP. 2013. Atividade de enzimas digestivas e parâmetros de crescimento de juvenis de jundiá (*Rhamdia quelen*) alimentados com farelo de linhaça *in natura* e demucilada. Semin Cienc Agrar 34: 3069-3080.

GUFE C, MERRIFIELD DL, HOSEINIFAR SH, RATTANAROJPONG T, KHUNRAE P & ABDEL-TAWWAB M. 2024. Prebiotic effects of dietary xylooligosaccharides on fish gut microbiota, growth, and immunological parameters – a review. Ann Anim Sci 24: 331-347.

HALIM NRA, YUSOF HM & SARBON NM. 2016. Functional and bioactive properties of fish protein hydrolysates and peptides: A comprehensive review. Trends Food Sci Technol 51: 24e33.

HAO YT, GUO R, JIA GW, ZHANG Y, XIA H & HE LX. 2020. Effects of enzymatic hydrolysates from poultry by-products (EHPB) as an alternative source of fish meal on growth performance, hepatic proteome and gut microbiota of turbot (*Scophthalmus maximus*). Aquac Nutr 26: 1994-2006.

HENRY M, GASCO L, PICCOLO G & FOUNTOULAKI E. 2015. Review on the use of insects in the diet of farmed fish: Past and future. Anim Feed Sci Technol 203: 1-22.

HONG J, HAN T & KIM YY. 2020. Mealworm (*Tenebrio molitor* Larvae) as an Alternative Protein Source for Monogastric Animal: A Review. Animals 10: 2068.

HOSEINIFAR SH, KHALILI M, RUFCHAEI R, RAEISI M, ATTAR M, CORDERO H & ESTEBAN MA. 2015. Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. Fish Shellfish Immunol 47: 706-711.

JAKUBCZYK A, KARAŚ M, RYBCZYŃSKA-TKACZYK K, ZIELIŃSKA E & ZIELIŃSKI D. 2020. Current Trends of Bioactive Peptides—New Sources and Therapeutic Effect. Foods 9: 846.

KABEL AM. 2014. Free radicals and antioxidants: role of enzymes and nutrition. World J Nutr Health 2: 35-38.

KHANZADEH M, HOSEINIFAR SH & BEIKZADEH B. 2024. Investigating the effect of hydroalcoholic extract of red algae (*Laurencia caspica*) on growth performance, mucosal immunity, digestive enzyme activity and resistance to *Streptococcus iniae* and *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). Aquac Rep 35: 101984.

KRISTINSSON HG & RASCO BA. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40: 43-81.

LALANI N, TIVARI S, JAIN V & JADEJA Y. 2024. Review on therapeutic potential of peptides: Advancements in synthesis methods, linear and cyclic peptides, and strategies for overcoming challenges. Pept Sci 2024: e24343.

LI H, HU Z, LIU S, SUN J & JI H. 2022. Influence of dietary soybean meal replacement with yellow mealworm (*Tenebrio molitor*) on growth performance, antioxidant capacity, skin color, and flesh quality of mirror carp (*Cyprinus carpio* var. specularis). Aquaculture 561: 738686.

LI H, XUE R, SUAN J & JI H. 2023. Improving flesh quality of grass carp (*Ctenopharyngodon idellus*) by completely replacing dietary soybean meal with yellow mealworm (*Tenebrio molitor*). Anim Nutr 12: 375-387.

LI-CHAN ECY. 2015. Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. Curr Opin Food Sci 1: 28-37.

LOPES CP & VILLELA GG. 1972. Trealose e Trealose em *Tenebrio molitor* L. Mem Inst Oswaldo Cruz 70: 574-583.

LUSHCHAK V. 2011. Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101: 13-30.

MOHAMMADI G, RAFIEE G, BASUINI MFE, ABDEL-LATIF HMR & DAWOOD MAO. 2020. The growth performance, antioxidant capacity, immunological responses, and the resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) fed *Pistacia vera* hulls derived polysaccharide. Fish Shellfish Immunol 106: 36-43.

MONTES-GIRAO P & FRACALOSSI DM. 2006. Dietary Lysine Requirement as Basis to Estimate the Essential Dietary Amino Acid Profile for Jundia, *Rhamdia quelen*. J World Aquacult Soc 37: 388-396.

MORAIS ML, SILVA ACR, ARAÚJO CRR, ESTEVES EA & DESSIMONI-PINTO NAV. 2013. Determinação do potencial antioxidante in vitro de frutos do cerrado brasileiro. Rev Bras Frutic 35: 355-360.

PULIDO R, BRAVO L & SAURA-CALIXTO F. 2000. Antioxidant activity of dietary as determined by a modified ferric. J Agric Food Chem 48: 3396-3402.

QUAH Y, TONG SR, BOJARSKA J, GILLER K, TAN SA, ZIORA ZM, ESATBEYOGLU T & CHAI TT. 2023. Bioactive Peptide Discovery from Edible Insects for Potential Applications in Human Health and Agriculture. Molecules 28: 233.

RIOLO K ET AL. 2023. Cytoprotective and Antioxidant Effects of Hydrolysates from Black Soldier Fly (*Hermetia illucens*). Antioxidants 12: 519.

RIVERO-PINO F, ESPEJO-CARPIO R, PÉREZ-GÁLVEZ A & GUADIX E. 2020. Effect of ultrasound pretreatment and sequential hydrolysis on the production of *Tenebrio molitor* antidiabetic peptides. Food Bioprod Process 123: 217-224.

ROMERO-LORENTE MÁ, FABRIKOV D, MONTES J, MOROTE E, BARROSO FG, VARGAS-GARCÍA MC, VARGA ÁT & SÁNCHEZ-MUROS MJ. 2022. Pre-treatment of fish by-products to optimize feeding of Tenebrio molitor *L. larvae*. Insects 13: 125.

SALOMÓN R ET AL. 2020. The growth promoting and immunomodulatory effects of a medicinal plant leaf extract obtained from *Salvia officinalis* and *Lippia citriodora* in gilthead seabream (*Sparus aurata*). Aquaculture 524: 735291.

SAMARANAYAKA AGP & LI-CHAN ECY. 2011. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. J Funct Foods 3: 229-254.

SARKER A. 2022. A review on the application of bioactive peptides as preservatives and functional ingredients in food model systems. J Food Process Preserv 46: e16800.

SCHMIDT CG & SALAS-MELLADO M. 2009. Influência da ação das enzimas alcalase e flavourzyme no grau de hidrólise das proteínas de carne de frango. Quim Nova 32: 1144-1150.

STANIVUK J, BERZI-NAGY L, GYALOG G, ARDO L, VITAL Z, PLAVSA N, KRSTOVIC S, FAZEKAS GL, HORVATH A & LJUBOBRATOVIC U. 2024. The rank of intensification factors strength in intensive pond production of common carp (*Cyprinus carpio L.*). Aquaculture 583: 740584.

TANG Y, DEBNATH T, CHOI E-J, KIM YW, RYU JP, JANG S, CHUNG SU, CHOI Y-J & KIM E-K. 2018. Changes in the amino acid profiles and free radical scavenging activities of *Tenebrio molitor* larvae following enzymatic hydrolysis. PLoS ONE 13: e0196218.

TRAN HQ, PROKESOVÁ M, ZARE M, MATOUSEK J, FERROCINO I, GASCO L & STEJSKAL V. 2022. Production performance, nutrient digestibility, serum biochemistry, fillet composition, intestinal microbiota, and environmental

impacts of European perch (*Perca fluviatilis*) fed defatted mealworm (*Tenebrio molitor*). Aquaculture 547: 737499.

URBINATI EC, ZANUZZO FS & BILLER-TAKAHASHI JD. 2014. Estresse e sistema imune em peixes. In: Baldisserotto B, Cyrino JEP & Urbinati EC (Eds), Biologia e Fisiologia de Peixes Neotropicais de Água Doce. Jaboticabal: FUNEP UNESP, Jaboticabal, SP, Brazil, p. 87-105.

VAN SOEST PJ, ROBERTSON JB & LEWIS BA. 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Methods for Dietary Fiber, Neutral Detergent Fiber and Nonstarch Polysaccharides in Relation to Animal Nutrition. J Dairy Sci 74: 3583-3597.

WU H-C, CHEN H-M & SHIAU C-Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int 36: 949-957.

YOUSEFI S, SHOKRI MM, NOVEIRIAN HA & HOSEINIFAR SH. 2020. Effects of dietary yeast cell wall on biochemical indices, serum and skin mucus immune responses, oxidative status and resistance against *Aeromonas hydrophila* in juvenile Persian sturgeon (*Acipenser persicus*). Fish Shellfish Immunol 106: 464-472.

ZHANG L, WU H-X, LI W-J, QIAO F, ZHANG W-B, DU Z-Y & ZHANG M-L. 2023. Partial replacement of soybean meal by yellow mealworm (*Tenebrio molitor*) meal influences the flesh quality of Nile tilapia (*Oreochromis niloticus*). Anim Nutr 12: 108-115.

How to cite

VILLANOVA JCV, PRETTO A, PENCHEL EM, SERRA SDS, LANES CFC, RIBEIRO VB, SPERONI CS, BENDER ABB & FERRIGOLO FRG. 2024. Bioactive peptides from *Tenebrio molitor*: physicochemical and antioxidant properties and antimicrobial capacity. An Acad Bras Cienc 96: e20231375. DOI 10.1590/0001-3765202320231375.

Manuscript received on December 19, 2023; accepted for publication on June 15, 2024

JÉSSICA CRISTINA V. VILLANOVA[1]
https://orcid.org/0000-0002-2967-2447

ALEXANDRA PRETTO[1]
https://orcid.org/0009-0007-8773-0768

EVANDER M. PENCHEL[1]
https://orcid.org/0009-0005-4944-5355

SERGIO DOMINGOS S. SERRA[1]
https://orcid.org/0009-0000-6777-9778

CARLOS FREDERICO C. LANES[1]
https://orcid.org/0000-0003-3221-2131

VANESSA B. RIBEIRO[2]
https://orcid.org/0000-0002-8640-5142

CAROLINE S. SPERONI[3]
https://orcid.org/0000-0002-5263-2099

ANA BETINE B. BENDER[4]
https://orcid.org/0000-0001-6973-9127

FERNANDA R.G. FERRIGOLO[1]
https://orcid.org/0000-0001-6096-0132

[1]Universidade Federal do Pampa (UNIPAMPA), Curso de Engenharia de Aquicultura, BR 472, Km 585, 97501-970 Uruguaiana, RS, Brazil

[2]Universidade Federal do Pampa (UNIPAMPA), Curso de Farmácia, BR 472, Km 585, 97501-970 Uruguaiana, RS, Brazil

[3]Universidade Federal do Pampa (UNIPAMPA), Curso de Licenciatura em Ciências da Natureza, BR 472, Km 585, 97501-970 Uruguaiana, RS, Brazil

[4]Universidade Federal de Santa Maria (UFSM), Departamento de Tecnologia e Ciência dos Alimentos, Avenida Roraima, 1000, Camobi, 97105-900 Santa Maria, RS, Brazil

Correspondence to: **Fernanda Rodrigues Goulart Ferrigolo**
E-mail: fernandaferrigolo@unipampa.edu.br

Author contributions

JÉSSICA CRISTINA V. VILLANOVA: writing original draft, methodology, formal analysis, investigation. ALEXANDRA PRETTO: writing original draft, review and editing, validation, formal analysis and investigation. EVANDER M. PENCHEL and SERGIO DOMINGOS S. SERRA: formal analysis and investigation. CARLOS FREDERICO C. LANES: writing, review and editing, resources and funding acquisition. VANESSA B. RIBEIRO: review and editing, conceptualization, methodology, validation and investigation. CAROLINE S. SPERONI: review and editing, conceptualization and investigation. ANA BETINE B. BENDER: review and editing and investigation. FERNANDA R.G. FERRIGOLO: writing, review and editing, resources, funding acquisition, supervision and project administration.