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Proceeding Paper

Antioxidant and Antihypertensive Properties from Muscle Hydrolysates of Farm Rainbow Trout †

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Abstract: Fish proteins are a promising source for multifunctional bioactive peptide production. Thus, this work aimed to establish the potentiality of rainbow trout muscle protein for the obtention of antioxidant and antihypertensive hydrolysates. Alcalase application produced a hydrolysate with inhibition of angiotensin-converting enzyme equivalent to 56.43 ± 2.05% after one hour. The same hydrolysate exhibited a scavenging and ferric-reducing power of 2.65 ± 0.07 µM Trolox equivalents and 32.12 mM Fe^{2+} equivalents, respectively. Results showed that rainbow trout muscle could be essential for identifying peptides with bifunctional properties.

Keywords: rainbow trout; hydrolysates; antioxidant; antihypertensive

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1. Introduction

Aquaculture has become a potential strategy for worldwide fishery production. Its growth has been slow since 2009, but just in 2018, a historical record was achieved, as production reached 81.1 million tons [1,2]. In this sense, farming finfish has gained such relevance because, in some countries, species are produced by this method instead of by fishing. Additionally, finfish farming represents 90% of total fishery production, where grass and silver carp, such as the Nile tilapia, are the highest farming species [2]. In Mexico, the main farming specie is tilapia. Still, specifically in Hidalgo, a state located in the center of the country with temperate weather, rainbow trout has been considered an emerging farm finfish with human feeding and tourism aims [3].

Fish proteins have been recognized as a valuable source for antioxidant and antihypertensive hydrolysates generation, with salmon, tuna, and tilapia as actual examples. In contrast, trout are freshwater fish, and a short exploration was conducted to produce hydrolysates with these functions [4]. Indeed, research into bioactive peptides obtention from rainbow trout has been mainly oriented towards the use of by-products [5–9]. On the contrary, investigation related to muscle exploitation has been scarce [10].

Cardiovascular diseases are a severe public health problem because they are the most common non-communicable disease in the world. It is estimated that in the year 2030, there will be 22.2 million cases [11]. Nowadays, a novel approach for cardiovascular disease treatment has been proposed, where the application of multifunctional peptides is highly desirable to attend to different pathologies simultaneously [12]. Thus, the objective of the

present work was to determine the potentiality for the obtention of hydrolysates with both antioxidant and antihypertensive activities from rainbow trout muscle.

2. Methods

Sample obtention: Rainbow trout (*Oncorhynchus mykiss*) specimens were acquired from a local Huasca de Ocampo, Hidalgo, Mexico farm. The trout was eviscerated and muscle processed to obtain a homogenized paste, which was used in protein determination and freeze-dried at −43 °C and 286 × 10^{-3} mbar for 24 h. Lyophilized samples were used to generate systems for the enzymatic reaction.

Protein determination: Protein content was realized using the Kjeldahl method [13]. Briefly, 70 mg of dried and fat-free rainbow trout homogenized paste was digested at 370°C for 15 min in the presence of 0.5 g of K_2SO_4 and digestive mixture composed of $CuSO_4 \cdot 5H_2O$ (0.3 g), H_3PO_4 concentrate (5 mL), H_2SO_4 concentrate (43 mL) and distilled water (2 mL). Then, the cooled mixture was added with 1.5 mL of H_2O_2 (30%), and digestion was completed. Afterward, distilled samples were collected on 50 mL of indicator solution. Finally, the previous volume was titrated with HCl 0.01 N to obtain the nitrogen percentage, applying a 6.25 factor to obtain the protein concentration (%). Results were reported on a wet basis.

Enzymatic hydrolysis: Hydrolysis systems were prepared as suspensions at 5% *(w/v)* in Tris-HCl buffer (pH = 9) from lyophilized trout paste. Afterward, protein trout was hydrolyzed at 55 °C with alcalase, adding a mass ratio of 100:10 (soluble protein: enzyme) [14]. Sampling hydrolysis times were 0, 1, and 2 h, stopping the enzymatic reaction with boiling water treatment for 10 min. Respective supernatants were obtained through centrifugation at 10,000 rpm for 10 min at 4 °C. Then, samples were stored at −18 °C until they were used in free amino groups, antioxidant, and antihypertensive determinations.

Hydrolysis degree determination: Free amino groups from rainbow trout hydrolysates were determined with the trinitro benzenesulfonic acid method [15]. In brief, 0.250 mL of each hydrolysate was mixed with 2 mL of phosphate buffer (pH = 8.2) and 2 mL of picryl sulfonic acid solution (0.1%). The reaction was carried out for 1 h at 50 °C and stopped by adding HCl 0.1 N (4 mL). All procedure was realized in the absence of light. Results were expressed as a glycine mM concentration from the calibration curve previously performed (0–300 ppm).

Antioxidant capacity analysis: Radical scavenging and ferric reduction power was determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl)and FRAP (Ferric Reducing antioxidant Power)techniques, respectively [16]. In summary, for DPPH determination, 0.1 mL of each hydrolysis time tested was mixed with 2.9 mL of DPPH solution (0.1 mM in MeOH). The reaction was performed at room temperature for 50 min, and absorbance was recorded at 515 nm. In parallel, a control was realized, in which the sample was replaced by 0.1 mL of MeOH. Then, the %DPPH remanent was calculated from the quotient between sample and control absorbances. Results were expressed as concentrations of Trolox (µM) from a calibration curve, which was accomplished at 0–33 µM. For FRAP analysis, 0.250 mL of hydrolysate was allocated with 1 and 8.75 mL of FRAP reactive and deionized water, respectively. The reaction was carried out at 37 °C for 15 min, and absorbance was recorded at 593 nm. Results were expressed as Fe^{2+} (mM) equivalents from the calibration curve realized with $FeSO_4 \cdot 7H_2O$ at concentrations between 0 and 70 mM.

Antihypertensive evaluation: The in vitro assay for the Angiotensin Converting Enzyme inhibition was used to evaluate antihypertensive properties, measuring hippuric acid produced by spectrophotometry [17]. In brief, three systems were prepared with the following composition. Positive control (A_{100}): 80 µL of saline buffer (Tris-HCl 0.05 M, 0.3 M NaCl, pH = 8.3), 200 µL of hippuryl-histidyl-leucine as substrate (5 mM in saline buffer), and 20 µL of angiotensin-converting enzyme from rabbit lung (0.1 U/mL in saline buffer). Negative control (A_0): 100 µL of buffer and 200 µL of the substrate. Inhibition system (A_i): 80 µL of hydrolysate standardized at 100 ppm of soluble protein, 200 µL of the substrate, and 20 µL of angiotensin-converting enzyme. All systems were maintained

at 37 °C for 80 min. Afterward, 250 µL of HCl 0.1 M was added to stop the reaction, and 1.7 mL of ethyl acetate was incorporated to extract the hippuric acid. Then, 800 µL of the organic layer was separated and evaporated at 80°C. Finally, the product was reconstituted with 1 mL of deionized water, and the absorbance for each system was recorded at 228 nm. Results were expressed as the inhibition percentage, calculated from the following equation.

$$\text{ACE inhibition } (\%) = \frac{(A_{100} - A_i)}{(A_{100} - A_0)} \times 100$$

Statistical analysis: All experiments were performed in duplicate and analyzed through one-way analysis of variance (ANOVA). Significant differences were determined with Tukey's contrast at a 0.05 significance level. Statistical analysis was carried out using the Minitab 18 package.

3. Results and Discussion

Protein content in homogenized muscle paste: The paste analysis obtained from rainbow trout muscle showed a 17.87 ± 0.31% protein content. The last value was found within the range found by other studies [13,18]. Specifically, the protein determined in this work was equivalent to that reported by Craft et al. [18], where trout specimens were fed with a novel formulation composed mainly of poultry by-products, wheat flour, and menhaden fish oil. In contrast, Cano-Estrada et al. [13] found protein content of 19.46 ± 0.78% for rainbow trout from the exact location of the specimens used in this study. Differences could be linked to the feeding source because Cano-Estrada et al. [13] reported that their samples were fed with commercial fish food, suggesting that the trout diet is not the same in the farms from Huasca de Ocampo. Nonetheless, protein content did not fluctuate highly and, in all cases, could be used as a potential source for bioactive peptide obtention.

Hydrolysis degree, antioxidant, and antihypertensive properties: As observed in Table 1, free amino groups increased with higher hydrolysis time, as expected. Hydrolysis shown at the beginning of the performance is associated with the normal autolysis carried out by fish muscle. Indeed, that procedure has also been tested as an alternative for bioactive peptide production from trout by-products [19].

Table 1. Hydrolysis degree and bioactive properties from trout muscle hydrolysates .

Hydrolysis Time (h)	Hydrolysis Degree (mM Glycine Equivalents)	Radical Scavenging Properties (µM Trolox Equivalents)	Ferric-Reducing Power (mM Fe^{2+})	Angiotensin-Converting Enzyme Inhibition (%)
0	16.18 ± 0.30 ^{c}	3.31 ± 0.12 ^{a}	24.46 ± 0.08 ^{b}	ND
1	19.76 ± 0.59 ^{b}	2.65 ± 0.07 ^{a}	32.12 ± 0.98 ^{a}	56.43 ± 2.05 ^{a}
2	32.64 ± 0.37 ^{a}	3.68 ± 0.09 ^{a}	33.98 ± 0.75 ^{a}	2.14 ± 0.04 ^{b}

ND: Not detected; different lowercase letters show significant differences at $\alpha = 0.05$.

On the other hand, antioxidant properties were found in the control time, but antihypertensive activity was not detected. The last property was identified until one hour after hydrolysis and decreased with an additional hour. Additionally, the results showed that while antihypertensive activity is deleted by prolonged hydrolysis times, ferric-reducing power is increased, especially during the first hour of hydrolysis. In the case of scavenging activity, it was lost within the first hour but recovered and increased during the second hydrolysis hour. Results are linked with the alcalase specificity for cutting action, which hydrolyzes preferentially peptide bonds containing aromatic amino acid residues [20].

As the novel investigation is focused on the search for multifunctional peptides [12], in this work, the first hydrolysis hour was the best time to achieve that aim, demonstrating both antioxidant and antihypertensive activities. Comparing the activities found with

other works [6,10], the values obtained here are highly competitive, but with the additional benefit that the hydrolysate obtained showed bifunctional activity.

4. Conclusions

Rainbow trout muscle protein showed high potential to generate hydrolysates with bifunctional bioactivity. The sample with the highest antioxidant and antihypertensive capacities was obtained after one hour of hydrolysis. In the same way, the exposed results represent a promising beginning for developing novel alternatives in the research concerning bioactive peptides for cardiovascular treatment, especially from sources with scarce investigation. Additionally, future studies should be focused on optimizing the hydrolysis process used to obtain multifunctional bioactive peptides, even sequencing to determine the peptidic chain responsible for both antioxidant and antihypertensive properties.

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