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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 was slow from 2009, but just in 2018, a historical record was achieved, producing 81.1 million tons [1,2]. In this sense, farming finfish has taken such relevance because, in some countries, species are produced highly by this method instead of by fishing. Also, finfish farming represents 90% of total fishery production, where the grass and silver carp, like 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 have been a freshwater fish with short exploration to produce hydrolysates with these functions [4]. Indeed, research in the bioactive peptides obtention from rainbow trout has been mainly oriented to using by-products [5-9]. Contrary, the 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 estimates 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 sample was eviscerated and muscle processed to obtain a homogenized paste, which was used in protein determination and freeze-dried at -43°C and 286X10^{-3} mbar to generate systems for the enzymatic reaction.

Protein determination: Protein content in trout homogenized paste was realized through the Kjeldahl method [13].

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 enzymatic reaction by boiling water treatment for 10 min. Respective supernatants were frozen and used to free amino groups, antioxidant and antihypertensive determinations.

Hydrolysis degree determination: Free amino groups from rainbow trout hydrolysates were determined with the trinitrobenzenesulfonic acid method [15]. Results were expressed as a glycine mM concentration.

Antioxidant capacity analysis: Radical scavenging and ferric reduction power was determined by DPPH and FRAP techniques, respectively [16]. Results were expressed as concentrations of Trolox (μM) and Fe^{2+} (mM) equivalents.

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].

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 a 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 [18].

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. Also, 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].

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	3.31±0.12	24.46±0.08	ND
1	19.76±0.59	2.65±0.07	32.12±0.98	56.43±2.05
2	32.64±0.37	3.68±0.09	33.98±0.75	2.14±0.04

ND: Not detected

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, obtaining 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. Conclusion

Rainbow trout muscle protein showed high potentiality to generate hydrolysates with bifunctional bioactivity, where the best time for the obtention of as antioxidant as antihypertensive capacities was after one hour of hydrolysis. In the same way, the exposed results represent a promising beginning for developing novel alternatives in cardiovascular treatment.

Supplementary Materials: No supplemental material is presented.

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