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SHORT PAPER

Shrimp Hepatopancreatic Crude Enzymes as Aids in Rice Bran Hydrolysis: Potential Contributors to Sustainable Aquaculture

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Abstract

Enzyme-pretreated rice bran is commonly used in biofloc aquaculture. In this work, crude enzymes from discards of *P. vannamei* were used for enzymatic pre-treatment of rice bran. *P. vannamei* crude enzyme extract is rich in proteases and amylases that hydrolyze casein and rice bran starch, respectively, at a rate comparable to commercial enzymes. In seawater media, *P. vannamei* midgut gland amylase activity is significantly higher than to commercial enzymes. Data on the use of enzymatic extracts obtained from shrimp discards is presented, contributing to future sustainable use of agriculture, aquaculture, and fisheries resources by probing an application of such by-products.

Introduction

Rice is one of the most important crops worldwide, accounting for 8% of the global crop production (FAO, 2020); during the milling process, several by-products are generated, with rice bran representing around 12% of the total kernel weight (Spaggiari *et al*., 2021). Rice bran contains approximately 33-42% carbohydrates, 11–16% protein, 15-30% dietary fibres, up to 20% of its weight in fats, and some bioactive compounds such as antioxidant and functional components (Spaggiari *et al*., 2021). However, rice bran´s major nutrients are not readily available and contain high amounts of fibre and some antinutritional components (Spaggiari *et al.*, 2021).

Agricultural by-products can be bioprocessed through fermentation with specific microorganisms or by enzymatic treatment to induce plant cell wall disruption; hence the proteins and carbohydrates become available, increasing the nutritional value of these by-products (Berlowska *et al.*, 2018). Bioprocessed rice bran can be suitable for numerous applications (*e.g.* animal feed) after appropriate evaluation of digestibility, feeding value, feasibility, safety, as well as nutritional and antinutritional factors (Ajila *et al.*, 2012).

In aquaculture, rice bran is commonly used as a carbon source in biofloc technology, an aquaculture innovation with less environmental impact since a minimum water exchange is achieved by introducing microbial communities into the culture ponds (Romano *et al.*, 2018). In principle, after introducing an exogenous source of carbon, an optimal carbon: nitrogen ratio is achieved, promoting the development of a symbiotic community of diverse microorganisms that form agglomerates and can degrade animal wastes, preventing the accumulation of toxic substances in water ponds (El-Sayed, 2021). Several studies have found that bioprocessed rice bran in biofloc improves shrimp and fish culture efficiency and biological performance (Romano et al., 2018; Vasconcelos de Andrade et al., 2021).

The carbon source for biofloc tanks is inoculated with a probiotic bacteria, *Bacillus* spp. is commonly applied in aquaculture since it improves animal growth performance and resistance to diseases (Akhter *et al.*, 2015). For example, inoculating rice bran with *Bacillus* sp. decreased the crude fibre content and increased the total soluble sugar; when the media was added to fish biofloc tanks, a general improvement in growth was observed (Romano *et al*., 2018). The bioavailability of nutrients after inoculation is attributed to the presence of endogenous bacterial enzymes, including amylases, xylanasaes or peptidases. Although, the availability of rice bran simple sugars and protein can be further facilitated by adding exogenous enzymes (Fabian & Ju, 2011).

Enzymatic hydrolysis is an important step in the treatment of rice bran for biofloc formation. Hydrolytic enzymes promote the availability of nutrients (mainly simple sugars and amino acids) in forms that are easily metabolized by microorganisms (Vallabha *et al.*, 2015). For example, Hou *et al*. (2010) determined that enzyme-assisted fermentation of rice bran leads to a more balanced amino acid profile, richer in lysine and valine content. Exogenous enzymes used in biofloc technology are generally of bacterial origin and can include amylases, peptidases, phytases, xylanases, and cellulases (Phongthai *et al.*, 2017).

On the other hand, *P. vannamei* midgut gland is rich in proteolytic and amylolytic enzymes (Castro *et al.*, 2012; Hernandez-Cortes *et al.*, 1997; Navarrete del Toro *et al.*, 2011); however, shrimp cephalothorax, which accounts for up to 40% of the total weight, is considered a by-product. Marine by-products are often rich in biomolecules, enzymes among them, which can be extracted and utilized in different applications (Suleria *et al*., 2016; Välimaa *et al.*, 2019). The biotechnological potential of enzymes extracted from crustaceans has been proposed by various researchers (Rossano *et al.*, 2011; Shahidi & Kamil, 2001). Nevertheless, this valuable source of enzymes and other molecules, like chitin and carotenoids, are frequently discarded during slaughtering. Therefore, greater effort is needed to promote its utilization. In this work, we explored the use of *P. vannamei* digestive gland crude extract as an alternative source of enzymes for the pre-treatment of rice bran. Further, its safe use in shrimp biofloc tanks was also tested, and the subsequent effects on *P. vannamei* culture water quality and growth were determined.

Material and Methods

P. vannamei Midgut Gland Crude Enzyme Extract

Specimens of *P. vannamei* juveniles were donated by Larvas Gran Mar, SA de CV (La Paz, BCS, Mexico) and transported alive to aquariums at the Biochemistry Lab in CIBNOR. The specimens were sacrificed by ice-water immersion, and the midgut gland was immediately removed and processed for the preparation of enzymatic extracts. Midgut glands obtained from 30 specimens were pooled in a 50 mL sterile tube, weighed, and homogenized in three volumes of 50 mM Tris-HCl buffer, pH 7.5, using an ultrasonic tissue homogenizer (Misonix XL-2000) at five 10-second pulses and medium intensity; the process was carried out on ice. Homogenates were centrifuged at 10,000 *g* for 30 min at 4°C to remove lipids and tissue debris. The aqueous fraction (or crude enzyme extract) was separated into 1 mL batches and stored at −20°C until used. Midgut gland total soluble protein content was determined according to the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

Bacterial Strain

Bacillus subtilis used in this study belong to the Collection of Genomics and Bioinformatics Laboratory at CIBNOR and was identified by 16S rRNA gene sequencing as *Bacillus subtilis* BL-01; this strain is a heterotrophic, nitrifying, halotolerant, aerobic bacteria previously isolated from a mangrove area located near La Paz, BCS, México (unpublished data). The strain was stored at −80°C in 50% glycerol. For the experiments, bacteria were cultured on Tryptic Soy Agar (TSA) supplemented with 2.5% NaCl at 35°C and harvested after 24 h. Finally, the optical density of each strain was adjusted in sterile artificial seawater (SASW) to 1 at 600 nm (DO600 = 1) in a spectrophotometer (Spectroquant 1117, Merck KGaA, Darmstadt, Germany), corresponding to 1.7 x10^7 CFU mL^{-1}.

Proteolytic and Amylolytic Activity in *P. vannamei* Hepatopancreas and Bacillus Crude Extracts

Total proteolytic activity in *P. vannamei* hepatopancreas was determined using azocasein as a substrate. Briefly, 10 µL of shrimp crude enzyme extract (equivalent to 8 µg of total protein) was added to 150 µL of azocasein (0.5%, w/vol) in reaction buffer (50 mM

Tris–HCl at pH 7.5), and the mixture was incubated at 25°C. After 10 min, the reaction was stopped by adding 150 µL of 20% (w/vol) trichloroacetic acid (TCA) and incubated for 5 min on ice. The precipitated protein was removed by centrifugation at 10,000 *g* for 10 min at room temperature. The reaction products in the supernatants were then transferred to a microplate, and the absorbance at 366 nm was read. For blanks, TCA was present before adding the substrate. Enzyme activity was expressed as a change of absorbance per min per mg protein (activity units=Δ Abs_{366}nm per min per mg of protein). Activity assays were run in triplicate.

The same procedure was used for the determination of proteolytic activity in *B. subtilis* cell-free extract. The crude enzymes produced by *B. subtilis* isolates were freshly obtained after inoculation in 100 mL of TSB media supplemented with 2.5% NaCl and incubated at 37°C for 24 h (average OD_{600nm} = 0.99). The proteolytic and amylolytic activity was assayed in the cell-free supernatant containing crude enzymes, which was collected by centrifugation of the bacterial culture at 4,500 *g* for 15 min at 4°C, and was stored at -80 until analyzed.

A quantitative amylolytic activity assay was performed by measuring the amount of reducing sugars released from soluble starch according to the method described by Bernfeld (Bernfeld, 1955) with modifications. Starch 1% (w/v) in 40 mM sodium phosphate buffer (pH 7.0) was used as a substrate, and the hydrolysis was determined by measuring the increase of the concentration of reducing sugars in the reaction tube using dinitrosalicylic acid (DNS). In brief, 50 µL of 1% starch was treated with 10 µL of *P. vannamei* hepatopancreatic crude enzyme extract (equivalent to 8 µg of total protein) and incubated at 25±1°C. After 30 minutes, 300 µL of 3,5-dinitrosalicylic acid (DNS) solution was added, and the mixture was heated at 95°C in a thermo-block for 5 min to stop the reaction. Samples were transferred to a 96-well microplate reader (Synergy 4, Biotek), and the absorbance was recorded at 540 nm. Blanks were similarly prepared, except that 40 mM phosphate buffer (pH 7.0) replaced the enzyme extract. All assays were carried out in triplicate. The same procedure was used for the determination of amylolytic activity in *B. subtilis* cell-free extract.

A reducing sugars calibration curve was established using glucose (0.01 to 1 mg/mL dissolved in water) to convert the colourimeter readings into milligrams of glucose. One unit of amylase activity was expressed as mg of glucose released at 25±1°C per min per mg of protein.

The total proteolytic and amylolytic activity was also determined for locally produced commercial enzymes of bacterial origin used as a reference in the experiment described in the shrimp bioassay section, using the same reaction conditions described above. The commercial enzymes used as control are a two-component presentation; the enzymatic activity was determined for each enzyme in the kit.

Degree of Protein Hydrolysis

The degree of hydrolysis of rice bran in the presence of shrimp enzymatic extract or the commercial enzyme 2 (positive for proteolytic activity) was measured using the pH-stat method (Ezquerra-Brauer *et al.*, 1997). In brief: 0.813 g of pulverized rice bran (containing 0.08 g of protein) was solubilized in 9 mL of distilled water. Then NaOH 1.0 N was added while stirring until the pH was stabilized near 8.0 (approximately 2 hours). The hydrolysis reaction started by adding the equivalent volume containing 3 Activity Units (3 U) of the corresponding enzyme. The final reaction volume was 10 mL (Rice bran + water + enzyme). The reaction was kept at pH 8.0 using a pH-stat device (718 STAT Tritino, Metrohm) by adding NaOH 0.1 N and recording the amount of base added during 60 min. Casein degree of hydrolysis was also determined as a control (a single protein substrate).

The degree of hydrolysis (DH%) was calculated according to Lemos *et al.* (2004) using the following equation:

$$DH\%=100\,[(B\times N_B)\,(1/\alpha)]\,[1/Mp)\,(1/h_{tot})]$$

Where:

B=Consumed 0.1 N NaOH (mL).

N_B=0.1 (normality of the base).

$1/\alpha$=1.45 (calibration factor at 28°C)

h_{tot}=7.4 or 8.2 (amino meq/g of rice bran protein and casein, respectively) (Adler-Nissen, 1986; Suphat Phongthai *et al*., 2017).

All determinations were run in triplicate.

Additionally, the progress of rice bran protein and casein hydrolysis by shrimp crude extract and commercial enzymes was observed in 12% SDS-PAGE. At 0, 5, 20, 40 and 60 min, 0.2 mL subsamples were taken from the reaction vessel and boiled for 5 min to stop the reaction, then mixed with SDS sample buffer and separated in an SDS-PAGE at 4°C. The protein bands were stained with Coomassie blue solution and digitalized in an image analyzer (EZ-Doc, Bio-Rad).

Enzymatic Saccharification of Rice Bran in Seawater

Rice bran was purchased in a local market and sieved at 450 µm before use. The release of soluble reducing sugars from rice bran polysaccharides because of enzymatic treatment using shrimp, bacterial, and commercial amylases was determined. Rice bran (0.3 g) was mixed in 10 mL of UV-treated reconstituted seawater (Sigma-Aldrich, Sea salts 3.5%), and the corresponding enzyme was added (3 IU/g of the substrate). The glucose concentration was determined for each rice bran treatment at 0, 10, 20, 40, 60 and 90 min. The amount of reducing sugars released by the enzymatic hydrolysis was estimated by the dinitrosalicylic acid (DNS) method following the procedures described above.

Shrimp Bioassay

Juvenile Pacific white shrimps were obtained from Larvas Gran Mar, SA de CV, maintained under indoor laboratory conditions (27±0.5°C, >5 mg L–1 DO and 37.5‰ salinity) during three weeks for acclimatization before exposure to experimental conditions. Shrimps with an average weight of 3.45 ± 0.46 g were randomly distributed in 12 polyethene round tanks of 250 L capacity and 0.70 m diameter at 20 shrimps per unit. An air diffuser grid was installed at the bottom of each tank to maintain dissolved oxygen (DO) concentration at 6–8 mg L^{-1}. A daily water exchange ratio of 5 % was performed using seawater filtered through a 1-µm mesh and passed by UV light.

Water quality parameters, temperature (°C), dissolved oxygen (ppm), pH, salinity (ppt), and ammonia (ppm of NH3/NH4+) were monitored daily during the bioassay. The API® Ammonia Test Kit was used to measure the dissolved ammonia, following the manufacturer's instructions.

Bioprocessed rice bran as a carbon source was poured into experimental shrimp tanks. Based on the manufacturer's recommendation, equivalent enzyme units were added for enzymatic pre-treatment of rice bran, 28 mU of protease activity units, and 33 mU amylase activity units per mL of rice bran (6 L at 1:30 w/v in UV-sterilized seawater) using *P. vannamei* midgut gland and commercial enzymes. Four experimental treatments evaluated in triplicate were conducted as follows: **1)** 6 L per treatment of rice bran (1:30 w/v in sterile seawater) treated for 60 min with *P. vannamei* midgut gland enzymes and inoculated with *Bacillus subtilis* strains at 1.7 x10^7 CFU mL^{-1} (PV); **2)** 6 L per treatment of rice bran treated with commercial enzymes and *Bacillus subtilis* at 1.7 x10^7 CFU mL^{-1} (CE); **3)** 6 L per treatment of rice bran without enzymatic pre-treatment and inoculated with *Bacillus subtilis* at 1.7 x10^7 CFU mL^{-1} (BO) and; **4)** Control treatment without rice bran nor bacteria and enzymes.

Rice bran was incubated for 24 h and directly added to each shrimp tank every two days for 18 days. Shrimps were fed *ad libitum* starting at day 1 with 4% of total biomass per tank, divided into two equal parts; feed was provided at 09:00 and 14:00 h.

Determination of Growth Parameters

Eighteen days after the trial started, shrimp performance was determined in terms of final weight (FW), and the following parameters were calculated:

Average weight gain per week. WG = (final weight−initial weight)/number of weeks).

Specific growth rate. SGR (% day−1) = 100 × (ln final weight−ln initial weight)/number of days) (Ricker, 1975).

Survival (%) = final number of shrimp/initial number shrimp × 100).

Statistical Analysis

The effect of the treatments on shrimp performance was tested for significance using a one-way ANOVA test, the normal distribution was determined using a Shapiro-Wilk test, and a Tukey Post-Hoc Test was applied to determine differences among means at P>0.05, statistical analyses were run using the software R (R Core, 2020).

Results and Discussion

Despite the proven advantages of biofloc in aquaculture, the application of technologies based on the introduction of microorganisms into culture tanks is still scarce in the region where the present study was conducted (Northwest Mexico) (Betanzo-Torres *et al.*, 2020), perhaps due to insufficient information that leads to indifference from producers. The results presented in this work provide further evidence of the advantages of alternative technologies in shrimp farming and of the potential utilization of the resulting aquaculture discards through extraction and applications of shrimp digestive enzymes found in the discarded organs.

Shrimp Midgut Gland Proteolytic and Amylolytic Activity

Total proteolytic and amylolytic activity in *P. vannamei* midgut gland and *B. subtilis* crude extract is shown in Table 1; the corresponding activities calculated from commercial enzymes of bacterial origin are shown for comparative purposes. The proteolytic activity was determined using 0.5% azocasein at pH 7.5, and the amylolytic activity was determined using 1% starch at pH 7.0. As observed in Table 1, activity against both protein and carbohydrate substrates is present at considerably high concentrations in crude extracts of *P. vannamei* midgut gland.

This effect is also reflected in the degree of hydrolysis (DH%) determination. Rice bran DH% using

Table 1. Proteolytic and amylolytic activity units in *P. vannamei* midgut gland and *B. subtilis* cell-free extract, commercial enzyme mix used as control were analyzed as reference.

	P. vannamei hepatopancreas	*B. subtilis*	Commercial enzyme 1	Commercial enzyme 2
Proteolytic activity (U/mg)[1]	2.76±0.18	0.51±0.009	0.079±0.03	31.19±1.92
Amylolytic activity (U/mg)[2]	3.05±0.04	24.79±1.10	7.1±0.41	3.43±0.23

[1]Activity Unit = Δ Abs_{366}nm per min per mg of protein. Substrate, azocasein 0.5% pH 7.5

[2]Activity Unit = mg of glucose released per min per mg of protein. Substrate, starch 1% pH 7.0

shrimp enzymes was 2.79% (±0.13), while the DH% using the commercial enzyme reached 4.16% (±0.18), meaning commercial enzymes are 33% more efficient than shrimp enzymes hydrolyzing rice bran proteins. However, this outcome is different when casein is used as a substrate since the DH% was 8.7% (±0.7) and 7.6% (±0.4) for shrimp and commercial enzymes, respectively, meaning shrimp enzymes are 12.5% more efficient in degrading casein. This is reflected in SDS-PAGE analysis; the amount of rice bran proteins of high molecular weight (>97 kDa), diminishes after 5 min of enzymatic treatment using commercial enzymes (Figure 1B), while this effect is not as evident when treating with shrimp hepatopancreatic enzymes (Figure 1A). On the other hand, when using casein as substrate, the prominent protein bands (~40 kDa) are fully degraded after 5 min using both commercial and shrimp enzymes.

In *P. vannamei* (and all Decapoda species), the midgut gland is the main metabolic organ. Its functions include the synthesis of digestive enzymes, absorption and metabolization of nutrients, storage of energy reserves, immune defence and detoxification, among others (Vogt, 2019). Gene expression and activity of peptidases and amylases in the midgut gland of *P. vannamei* have been widely described (Castro et al., 2012; Fan et al., 2016; Klein et al., 1996; Navarrete del Toro et al., 2011). The serine peptidases, trypsins and chymotrypsins have been described as the main proteolytic enzymes responsible for food digestion (Navarrete-Del-Toro *et al.*, 2015; Sainz *et al*., 2004); these enzymes are active in the neutral to alkaline Ph range, up to 60°C , and are highly stable at storage conditions *(Navarrete del Toro et al.*, 2011). Amylolytic enzymes are also widely represented in the digestive tissues of *P. vannamei* (Castro *et al*., 2012), alpha-amylases are the best studied, and they are considered to account for up to 1% of total proteins in juvenile and adult shrimp midgut gland (Wei *et al.*, 2014; Wormhoudt & Sellos, 1996).

When processing captured or cultivated *P. vannamei,* the midgut gland is generally discarded during the removal of the cephalothorax (de-heading). However, the potential recovery and utilization of

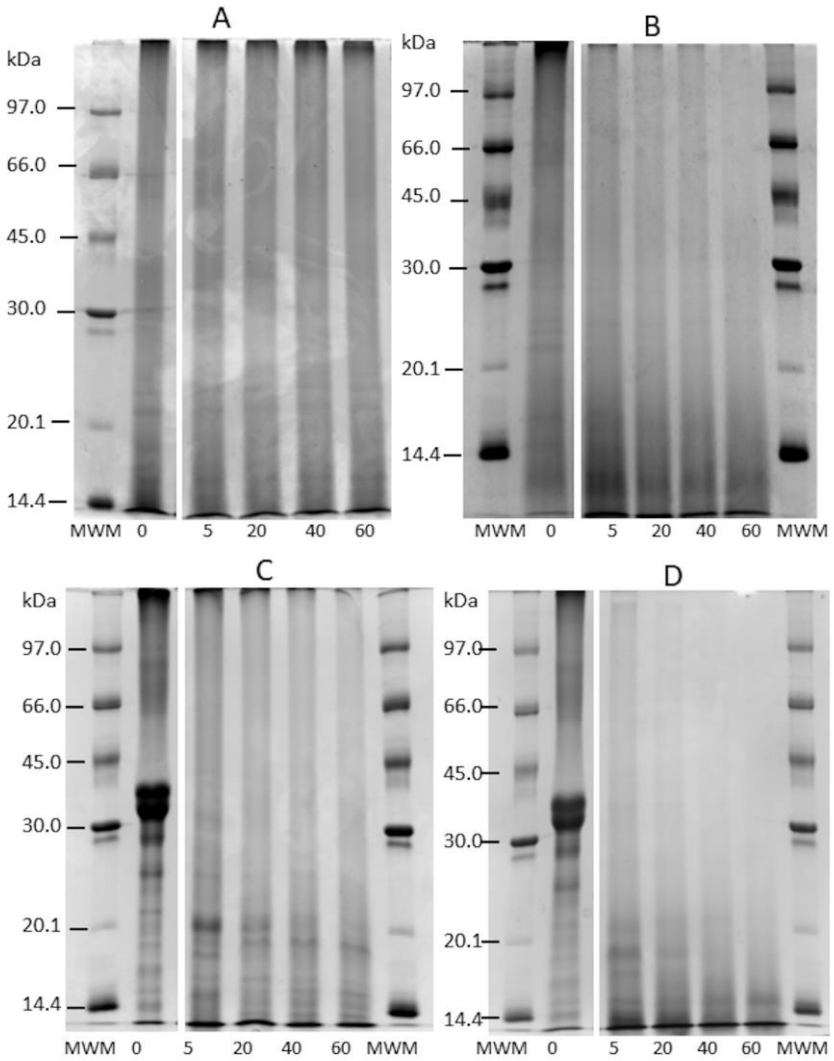

Figure 1. Progress of rice bran and casein proteins hydrolysis products at 5, 20, 40 and 60 min: A) Rice bran treated with *P. vannamei* crude midgut gland extract. B) Rice bran treated with commercial enzyme 2. C) Casein treated with *P. vannamei* crude midgut gland extract, and D) Casein treated with commercial enzyme 2. The protein present in the samples before the addition of enzymes is shown at 0; the numbers at the bottom of each gel indicate the sampling time (min).

bioactive compounds present in shrimp by-products have been suggested (Kandra *et al*., 2012; Mao *et al.*, 2017). Unfortunately, its recovery rate is relatively low since shrimp cephalothorax is highly prone to deterioration due to melanosis and microbial growth (Nirmal *et al*., 2015). Chitin, carotenoid pigments and total proteins are considered the most valuable bioactive compounds in shrimp waste *(*Cahú *et al.*, 2012); however, shrimp cephalothorax is rich in enzymatic activity that, if properly handled, can also be recovered and used in biotechnological applications such as biofuel, food, leather and detergent industries (Rossano *et al.,* 2011). In some biotechnologies, enzyme cost is considered one of the most significant factors defining the final product price (Klein-Marcuschamer *et al.,* 2011); therefore, recovered enzymes from the otherwise discarded material of the fisheries and aquaculture industries, are potentially cost-saving relative to the currently available commercial enzymes.

Enzymatic Saccharification of Rice Bran in the Presence of Seawater

Commercial rice bran contains a high amount of carbohydrates, mainly in the form of starch (Fabian *et al*., 2011). Amylolytic enzymes like α-Amylase catalyze the hydrolysis of glycosidic bonds in starch and release glucose and dextrose. Therefore, an enzymatic treatment will increase the concentration of free glucose that will be readily available as an energy source if microorganisms are present in the mix.

In this work, the efficiency of *P. vannamei* hepatopancreatic enzyme extract to catalyze the hydrolysis of rice bran starch was tested in the presence of seawater. Commercial microbial enzymes developed specifically for such application were used for comparative purposes. Reducing sugars concentration, a product of rice bran enzyme treatment, was quantified during the reaction as shown in Figure 2; it may be observed that after 90 min, the reducing sugar concentration released by *P. vannamei* enzymes is superior to the one obtained when the rice bran is treated using commercial enzymes, with an increase of ~47% of the initial starch concentration compared to untreated rice bran. This is due mainly to the action of shrimp amylolytic enzymes promoting the liberation of glucose and other fermentable sugars from the rice bran. The superiority of *P. vannamei* enzymes over commercial enzymes of microbial origin is putatively due to its halotolerance nature; hepatopancreatic amylases from a related Penaeid shrimp species can endure NaCl concentrations as high as 3 M (Vega-Villasante *et al*., 1993).

Rice bran processed by enzymatic treatment is widely applied in the food industry due to its recognized improvement in nutritional properties (Spaggiari *et al.*, 2021); but the potential is higher since the production of bioactive compounds from cereal by-products is well documented (Spaggiari *et al.*, 2021). Our results provide input on alternatives for developing rice bran biotechnology, a needed step to upgrade the current uses of this and other cereal by-products. In this work, we demonstrate that amylolytic enzymatic activity can be easily recovered from shrimp industry discards, greatly consisting of the cephalothorax (heads) that encase the midgut gland; furthermore, an application is suggested. A great potential is anticipated for halotolerant enzymes, including in the bioethanol industry since production under seawater-based media is gaining popularity as a sustainable practice to lower the freshwater footprint of some industries.

Shrimp Performance

During the shrimp bioassay, the water quality parameters in all treatments were kept at 5.0-5.9 ppm of dissolved oxygen, 26-28°C, pH of 7.9, and 0.064-0.55 ppm of ammonia (NH3/NH4+).

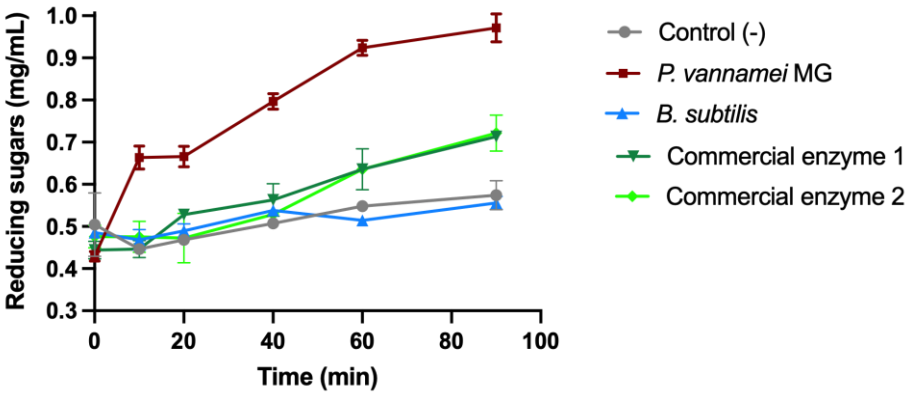

Figure 2. Enzymatic saccharification of rice bran in the presence of seawater. Rice bran (1:30 w/v) solubilized in seawater was treated with *P. vannamei* hepatopancreatic crude enzyme extract; commercial enzymes and *B. subtillis* cell-free extract were used for comparison. An increase in reducing sugars concentration is observed from the first minutes of the reaction and continually increases during the reaction time. The results prove that *P. vannamei* midgut gland enzymes perform significantly better than commercial enzymes when the reaction is carried out at high salt concentrations (seawater). Rice bran without enzyme was used as a control.

At the end of the eighteen-day experiment, the growth performance was assessed; the zootechnical parameters are shown in Table 2. In terms of final weight and weight gain, shrimps from tanks where rice bran was added, performed significantly better than control shrimps. No differences were observed among hydrolyzed rice bran using commercial (CE), shrimp hepatopancreatic enzymes (PV) and untreated rice bran (BO) when is inoculated with *B. subtilis* 24 h before pouring into the experimental tank ($P<0.05$). Our data support previously established ideas on the use of bio-stimulated rice bran as an option to implement innovative methodologies for shrimp farming. Biofloc could lead to a decrease in the amount of food offered and, consequently, a decrease in fishmeal per hectare of shrimp grown under these conditions and ultimately, a decrease in the environmental footprint of shrimp farming (Weldon *et al.*, 2021).

On the other hand, no significant differences in survival among the four experimental conditions were observed. All shrimp groups showed survival rates greater than 83% (Table 2), but a tendency for better survival in the rice bran-treated tanks is observed; perhaps within the time frame of our experiment, the accumulation of ammonia and other toxic compounds did not reach lethal levels.

Although during our experiments, the establishment of biofloc was not determined, we demonstrated that rice bran treated with *P. vannamei* hepatopancreatic enzymes is a potential carbon source for biofloc formation. In extensive and intensive shrimp farming systems, technologies that promote more sustainable practices like aquamimicry or biofloc approaches can be adopted. Such practices are considered environment-friendly alternatives since water exchange is minimized or even eliminated, leading to nutrient recycling and fewer pollutant discharges.

Conclusions

The results presented here are proof of concept experiments on the recovery of shrimp enzymes from culture and fisheries discards and their use as an enzymatic complement (protease and amylase) to support the hydrolysis of grains and grain by-products. A remarkable fact of the assay conditions presented here is the use of crude enzymatic extracts from *P. vannamei* midgut gland, which can be directly applied to grain by-products for enzymatic treatment, decreasing the inconveniences and costs associated with the purification of specific enzymatic activity.

Our approach contributes to the valorization of waste from aquaculture and agriculture industries; in a single step, those by-products are indirectly converted into potentially highly valuable products. The methods presented here can be easily adapted to other applications and eventually contribute to the sustainable production of shrimp.

Ethical Statement

The authors followed all applicable international, national, and institutional guidelines for the care and use of shrimp.

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Author Contribution

LRA, EQG, JHCM, and FLGC conceived the project. JLC and EIPG performed microbiology and shrimp bioassays. LRA, JLC, and JHCM carried out enzyme assays. LRA wrote the manuscript and designed the figures. LRA and EQG reviewed the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2. Growth performance parameters of *P. vannamei* reared for 18 days in the presence of rice bran with and without enzymatic treatment. Means ± standard deviation of three replicate tanks.

	Treatment			
	CE	Pv	Bs	Control
Final weight (g)	7.55±1.44 [a]	6.68±1.50 [a]	7.20±1.45 [a]	5.58±2.07 [b]
Survival (%)	96.66±5.77 [a]	98.33±2.88 [a]	98.33±2.88 [a]	83.33±17.55 [a]
Weight gain	4.10±1.97 [a]	3.24±1.72 [a]	3.76±1.72 [a]	2.13±2.82 [b]
Weight gain / week	1.59±0.76 [a]	1.26±0.67 [a]	1.46±0.66 [a]	0.83±1.10 [b]
SGR	0.22±0.10 [a]	0.18±0.09 [a]	0.21±0.09 [a]	0.12±0.15 [a]

CE= Rice bran pre-treated with commercial enzymes and inoculated with *B. subtilis.*

Pv= Rice bran pre-treated with *P. vannamei* hepatopancreatic crude enzymes and inoculated with *B. subtilis.*

Bs= Untreated rice bran inoculated with *B. subtilis.*

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