

Natural v/s Commercial trypsin isolated from liver waste of *Labeo rohita*

Charu Batav* and Ragini Gothalwal

Department of Biotechnology, Barkatullah University, Bhopal

*Corresponding author

Charu Batav, Department of Biotechnology, Barkatullah University, Bhopal

Submitted: 26 Feb 2022; Accepted: 03 Mar 2022; Published: 10 Mar 2022

Citation: Charu Batav and Ragini Gothalwal (2022). Natural v/s Commercial trypsin isolated from liver waste of *Labeo rohita* *J Textile Eng & Fash Tech* 4(1): 16-20.

Abstract

The mounting human population and the massive amount of waste generated from the same is receiving particular attention towards valorisation of waste. According to the annual report of FAO, the human consumption of fish protein has reached 87% in 2016 from 67% during 1960s [1]. Aquaculture only has contributed to 5.8% annual growth rate among food sectors in the past decade. In this milieu, disposal of fish visceral waste is becoming a major menace to fishery industries exerting a great economic and environmental impact. Being perishable in nature, the organic portion of the waste decomposes rapidly and acts as a breeding ground for microbes. Moreover, the hefty and indiscriminate use of antibiotics and disinfectants in farmed animals is developing resistant strains, thus raising environment and ecological concerns. In order to solve such problem, the present investigation focussed upon employing the visceral trypsin as a cell dissociating agent. The efficacy of trypsin obtained from viscera of *Labeo rohita* upon KB cell line (Doubling time 50 hrs.) was assessed in terms of cell viability. The cytotoxic effect of the visceral trypsin at 0.01%, 0.1% and 1% concentration were investigated at three time points (10 sec, 15 sec and 20 sec). Commercial (bovine) trypsin was considered as control.

A time dependent decrease in cell viability upon gradually increasing the concentration was observed in all groups of treatment. The lowest reduction in cell viability (2%) was observed with 1% concentration at 15 sec and 20sec. Although, commercial trypsin was found more efficient than trypsin isolated from waste during this study but the potency of visceral trypsin observed cannot be ruled out. Thus, the application of this enzyme as a cell-dissociating agent suggested it as a comparable candidate with commercial trypsin.

Keywords: Fish Visceral Waste, Trypsin, Protein Purification, Sds-Page, Trypsinisation

Introduction

Fishery sector is contributing towards the global economy since time immemorial so as to sustain the livelihood and maintain the nutritional status. Moreover, Idowu *et al.*, have highlighted over the nutritional contents in fishes (protein, omega-3, vitamins, fatty acids, minerals etc). Fundamental for human health. According to the annual report of FAO, the human consumption of fish protein has reached 87% in 2016 from 67% during 1960s, and the annual waste discard is around 9.1 million tons FAO [1-3]. The consumption will continue to rise as fish and fishery by-products are easily accepted by major religions such as Jews, Buddhists, Muslims, Christians and Hindus Nawaz *et al.* [4]. Moreover, prevailing pandemic conditions have aroused people's seriousness towards protein-rich diet. However, this sector has several challenges which includes improved recovery and up gradation of waste. "Fish waste" is in itself a broad term that includes dead/damaged fish, irrelevant species, fish trimmings, tissues, blood, bones etc. While the former denotes by-catch, the remaining represents discards generated during processing. This includes viscera, head, bones, skin, fins, blood, liver frame, gonads, gut etc. Ahuja *et al.* [5]. Both these side-stream biomass

accounts for around 25-70% of the global fish processing Ucak *et al.* [6]. Being perishable in nature the organic portion of fish waste is more prone to bacterial decomposition and undergoes rapid autolysis and lipid oxidation. Moreover, Lulijwa *et al.*, have highlighted over the indiscriminate practise of antibiotics, disinfectants and chemotherapeutic drugs against pathogens in farmed animals leading to development of resistant microorganisms. Such species further raising ecological and economic impressions [7]. Using technical advancements, this waste can be valorised to obtain industrially important products, thereby lessening the waste burden over the environment. Among the various value-added products, enzymes are the most attractive and promising one. Thus, the premise of present study was to focus on cost effective ways to obtain trypsin from visceral waste of widely consumed freshwater carp- *Labeo rohita*, which was purified, characterized and applied as a tissue dissociating agent.

Materials And Methods

Collection of samples

The fish waste was procured in an ice-box from local fish market of Shahpura, Bhopal and immediately transferred to Molecular

Biology Laboratory, Department of Biotechnology, Barkatullah University, and Bhopal. The fish was identified as *Labeo rohita* using an online database, Fish Base. 100g liver (Axis LC/GC

weighing balance) was excised, washed with 0.8% saline to remove blood and debris, labelled and stored at -20°C for further use.



Figure 1: *Labeo rohita* with liver waste

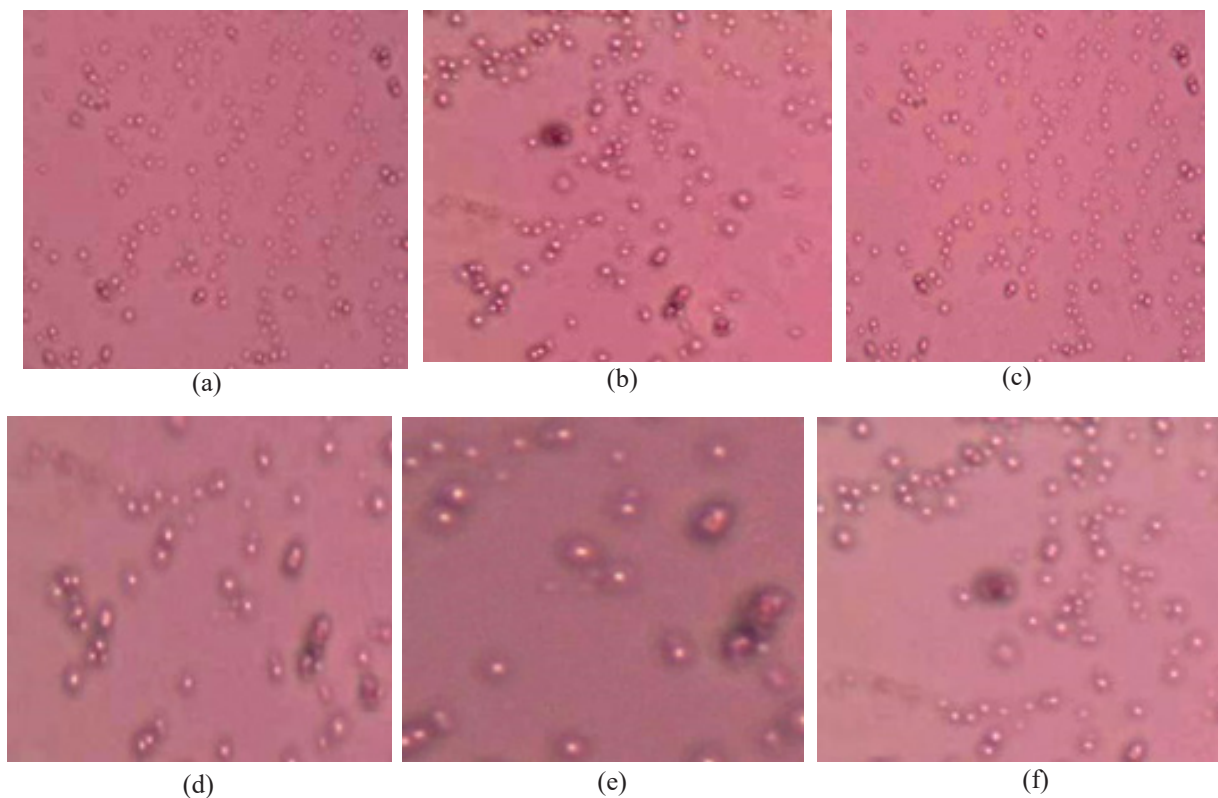


Plate 1: Microphotograph of (a) Control for 10sec, (b) 15sec, (c) 20sec, (d) sample for 10sec, (e) 15sec, and (f) 20sec (40X)

Isolation of trypsin

According to the method of Simpson and Haard, crude enzyme extract was prepared, clarified, purified and characterised as trypsin as described by Batav and Gothalwal, [8-9].

Efficacy of trypsin

The efficacy of purified trypsin in animal cell culture was es-

timated by the method given by Freshney [10]. As cancerous cell line does not exhibit strict growth conditions and contact inhibition, the cell line KB (HeLa derivative, National Centre for Cell Sciences, Pune) was chosen for the study. Varying concentrations (0.01%, 0.1% and 1%) of the enzyme samples was inoculated to cover the cell monolayer. Incubation at room temperature was given for 10sec, 15sec and 20sec intervals for each

set of experiment. After incubation, the solution was removed and cells were suspended into 10ml of growth media. To check cell viability in each case, 10 µl of inoculum (20,000 cells after 48hrs) was incubated with 10 µl trypan blue solution at room temperature for 1 min. Number of cells per ml was calculated using Haemocytometer as,

$$\text{cells per ml} = \text{viable cells} * D * 5000$$

Where, D = dilution factor Viability (%) was calculated as,

$$\text{viability} = (\text{number of live cells} / \text{number of dead cells}) * 100$$

The cell viability was compared with that of commercial trypsin.

Results And Discussions

The efficacy of Labeo trypsin over KB cell line was determined by measuring cell viability at different concentrations (0.01%, 0.1% and 1%) and time intervals (10sec, 15sec and 20sec). Commercial (bovine) trypsin was considered as control. A time-dependent decrease in cell viability was observed with gradual increase in concentration of the enzyme which was in accordance with the observations of Sutradhar et al., [11]. Plate 1 exhibits cell viability at 0.01% concentration of the two trypsinising agents (in lag phase) at different time intervals. From

Figure 2 it is apparent that fish derived trypsin resulted into 82%, 80% and 75% cell viability, viability at 0.1% concentration was found to be 95%, 90% and 97% Figure3 and viability at 1% concentration was 93%, 98% and 98% Figure 4 respectively at three time points. However, the sample showed a variation in reduction of cell viability (13%, 10% and 5% respectively). Further, the sustainability of the source of the enzyme needs to be studied.

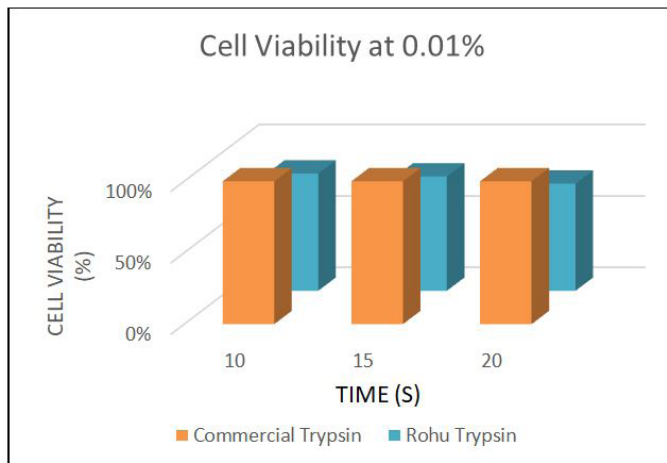


Figure 2: Time dependent trypsinisation on cell viability using 0.01% concentration

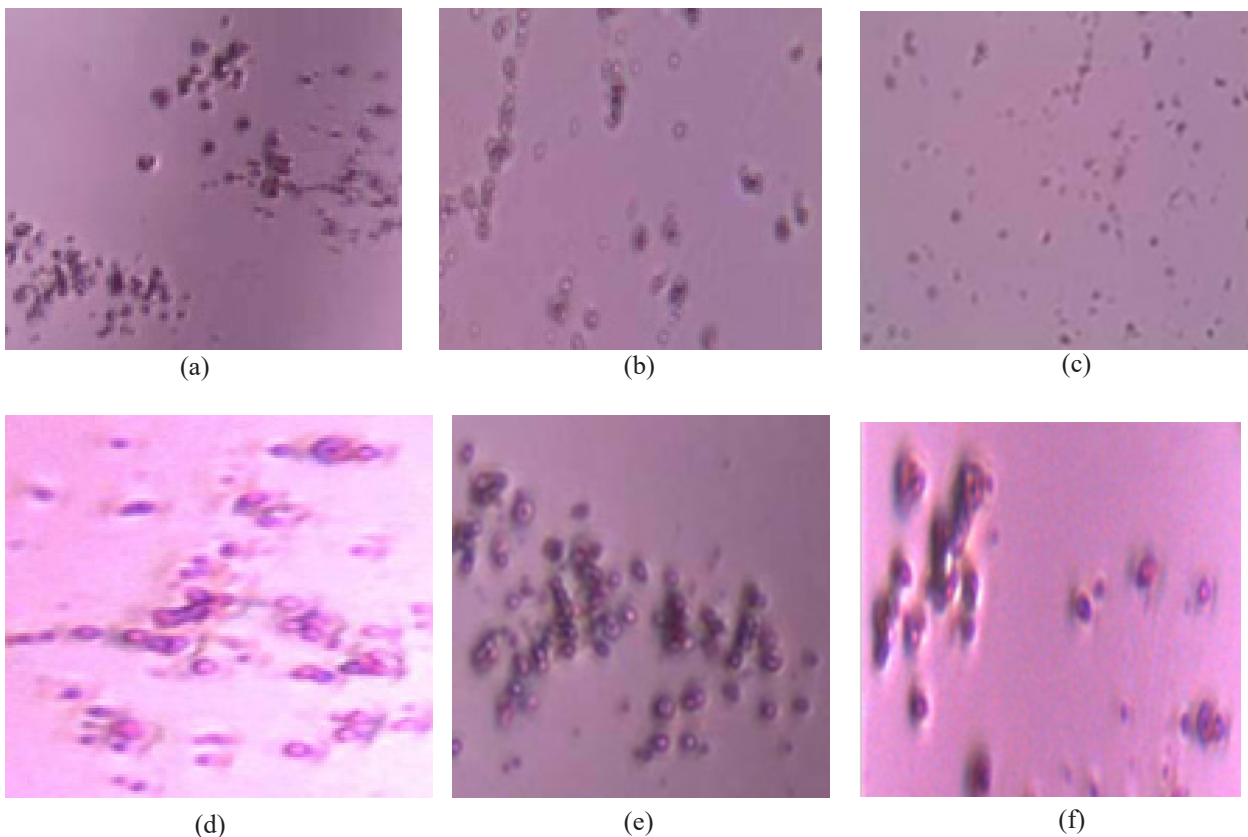


Plate 2: Microphotograph of (a) Standard for 10sec, (b) Standard for 15sec, (c) Standard for 20sec, (d) Sample for 10sec, (e) Sample for 15sec, and (f) Sample for 20sec (40X)

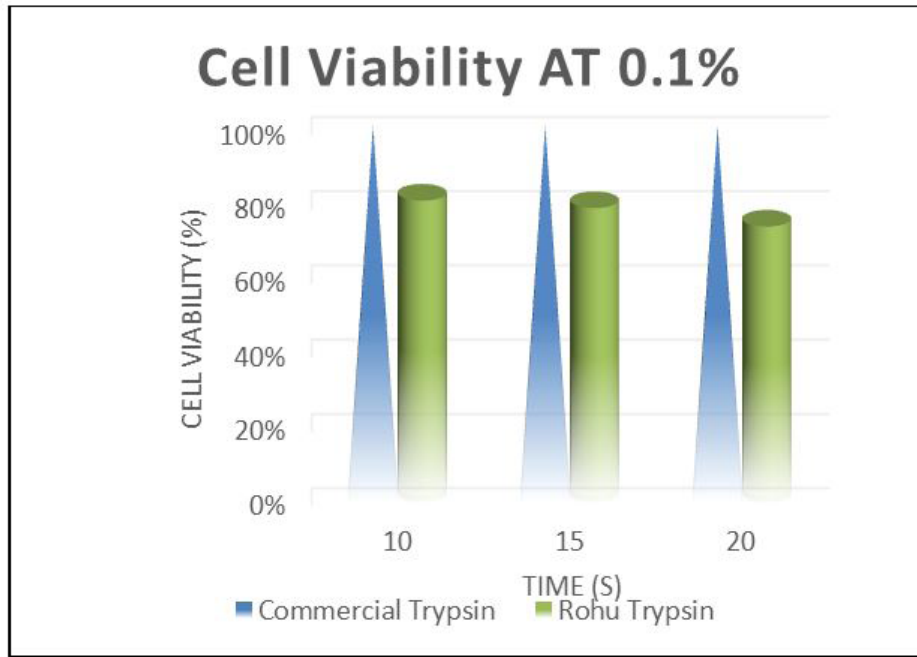


Figure 3: Time dependent trypsinisation on cell viability using 0.1% concentration

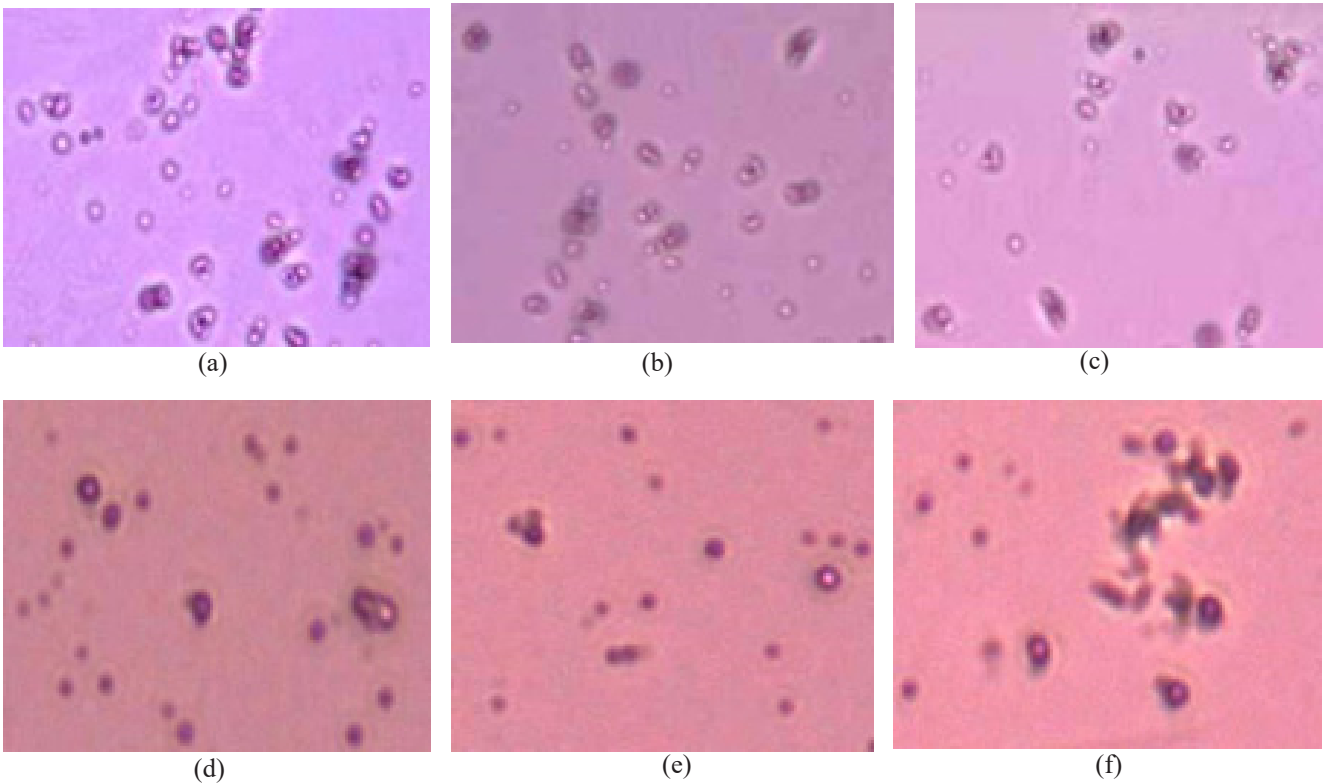


Plate 3: Microphotograph of (a) Standard for 10sec, (b) Standard for 15sec, (c) Standard for 20sec, (d) Sample for 10sec, (e) Sample for 15sec, and (f) Sample for 20sec (40X)

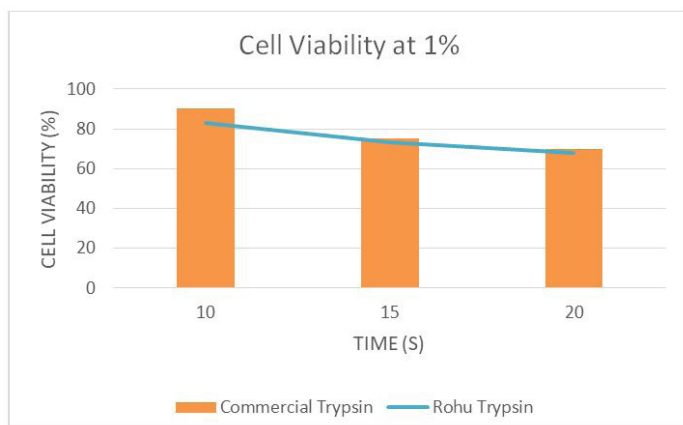


Figure 4: Time dependent trypsinisation on cell viability using 1% concentration

Although, commercial trypsin was found more efficient than trypsin isolated from waste during this study but the source of enzyme obtained during this study cannot be ruled out. The visceral trypsin studied showed similar kinetic properties and structural resemblance with other fish trypsin and commercial (bovine) trypsin. The enzyme was also found capable as a cell-dissociating agent.

Conclusion

The enzyme trypsin isolated from fish visceral waste was found equally effective cell dissociating agent as commercial trypsin. It is anticipated to be more economic as compared to commercial trypsin. On the other hand, the processing of viscera into trypsin could be a practical alternative of waste management. Moreover, fish processing industries could endorse this enzyme by setting up a unit in the vicinity of fish catch and landings to immediately collect the waste generated.

Authors' contributions

The author Dr. Charu Batav has conducted the study and prepared the manuscript under the guidance of Prof. Ragini Gothwal, and is the corresponding author as well.

Acknowledgements

The authors would like to express their gratitude to the Depart-

ment of Biotechnology, Barkatullah University, Bhopal, for providing laboratory facility.

References

1. FAO (2018). The state of world fisheries and aquaculture 2018. Meeting the sustainable development goals. Rome. pp: 80-82. <https://www.fao.org/documents/card/en/c/I9540EN/>.
2. Idowu, A. T., Benjakul, S., Sinthusamran, S., Sookchoo, P., & Kishimura, H. (2019). Protein hydrolysate from salmon frames: Production, characteristics and antioxidative activity. *Journal of Food Biochemistry*, 43(2), e12734.
3. FAO (2019). A third assessment of global marine fisheries discards. Rome 2019. pp: 76-90.
4. Nawaz, A., Li, E., Irshad, S., Xiong, Z., Xiong, H., Shahbaz, H. M., & Siddique, F. (2020). Valorization of fisheries by-products: Challenges and technical concerns to food industry. *Trends in Food Science & Technology*, 99, 34-43.
5. Ahuja, I., Dauksas, E., Remme, J. F., Richardsen, R., & Løes, A. K. (2020). Fish and fish waste-based fertilizers in organic farming—With status in Norway: A review. *Waste Management*, 115, 95-112.
6. Ucak, I., Afreen, M., Montesano, D., Carrillo, C., Tomasevic, I., Simal-Gandara, J., & Barba, F. J. (2021). Functional and bioactive properties of peptides derived from marine side streams. *Marine Drugs*, 19(2), 71.
7. Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*, 12(2), 640-663.
8. Simpson, B. K., & Haard, N. F. (1985). Characterization of the trypsin fraction from cunner (*Tautoglabrus adspersus*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 80(3), 475-480.
9. Batav, C., & Gothwal, R. (2020). Optimization and characterization of trypsin of *Labeo rohita* from its visceral waste. *GSC Advanced Research and Reviews*, 3(2), 039-047.
10. Freshney, R. I., & Vunjak-Novakovic, G. (Eds.). (2006). *Culture of cells for tissue engineering*. John Wiley & Sons.
11. Sutradhar, B. C., Park, J., Hong, G., Choi, S. H., & Kim, G. (2010). Effects of trypsinization on viability of equine chondrocytes in cell culture. *Pak Vet J*, 30(4), 232-238.

Copyright: ©2022 Charu Batav. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.