

MOL231: Development and Optimization of Chicken Viscera Hydrolysis for Pet Food Formulations



UNIVERSITETET
I BERGEN

Emma Andreassen Berge¹, Øivind Larsen², Gro Elin Kjæreng Bjerga^{1,2}, Tejaswini Rama Bangalore Ramakrishna²
1: Department of Molecular Biology, University of Bergen
2: NORCE Norwegian Research Centre, Bergen, Norway



Introduction

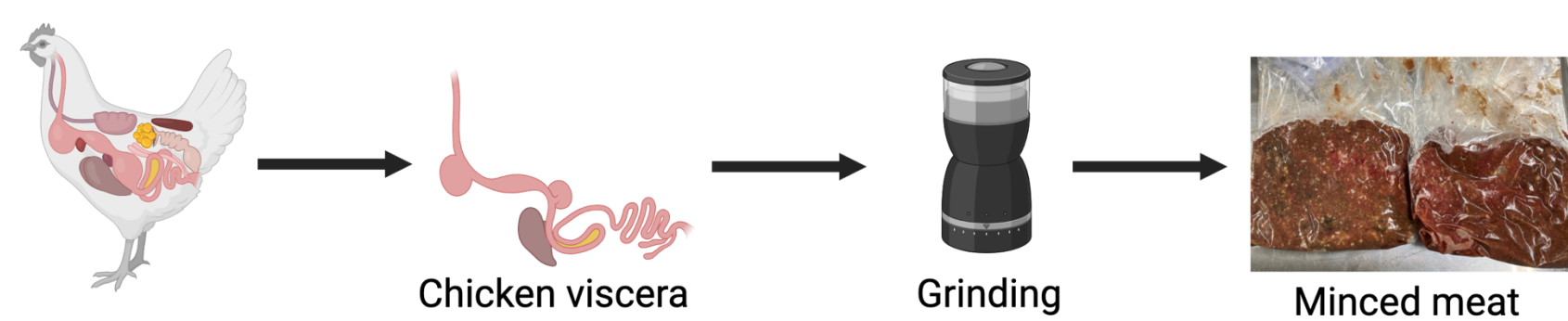
Chicken production is massive worldwide, and in 2022, Norway alone produced ~100,000 metric tons of chicken meat. These industries generate by-products such as feathers, feet, head, viscera and blood, among which viscera constitutes 30% of total by-products (1,2). Chicken viscera is rich in essential peptides and can be used as a potential feed ingredient in pet food formulation (3). However, current literature shows limited studies on chicken viscera hydrolysis process using a wide range of industrial enzymes targeted to pet food market.

In this project, we will explore a broad spectrum of industrial enzymes to tailor the enzymatic hydrolysis process. Additionally, we will explore the effect of chicken blood in the hydrolysis of chicken viscera. Traditionally, hydrolysis process requires significant amounts of water. However, substituting water with chicken blood presents an approach that could reduce water consumption, lower production costs, and minimize waste. Our aim is to achieve higher yield from enzymatic hydrolysis process by analysing its dry matter, protein content and peptide distribution to assess the quality of protein hydrolysates.

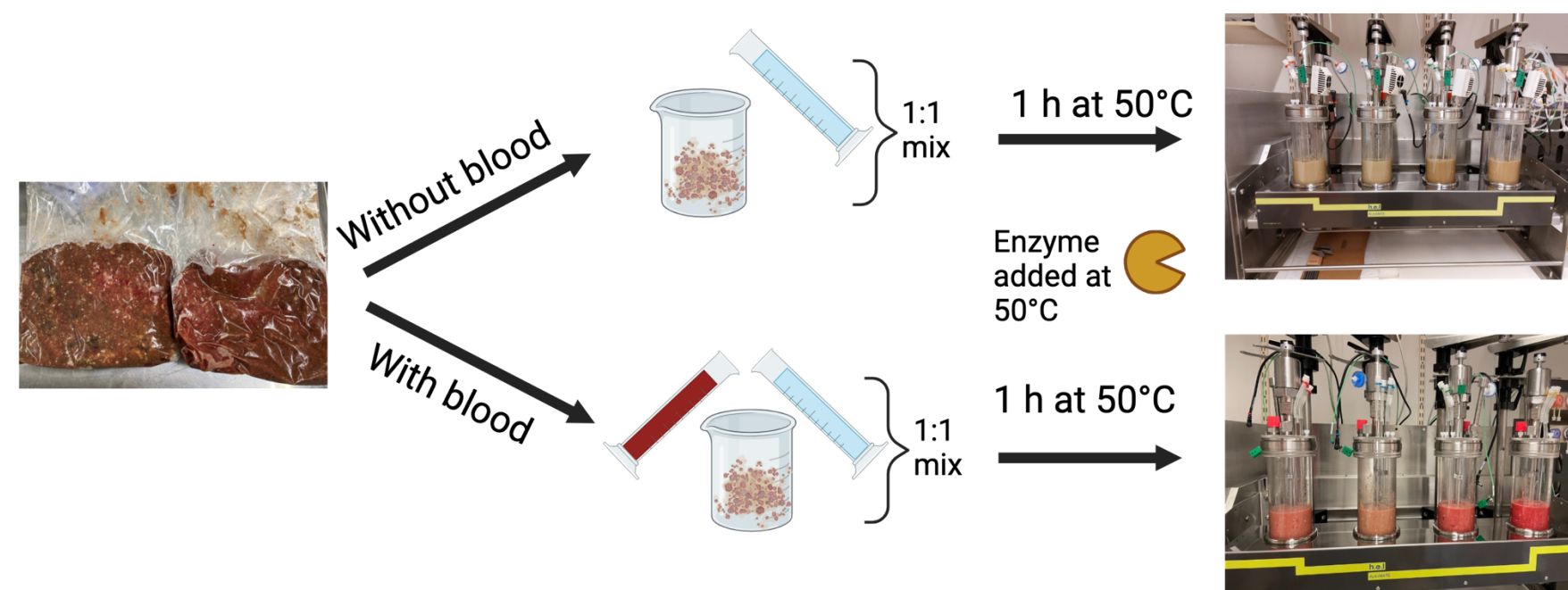
Research question: Can industrial enzymes increase dry matter yield in chicken viscera hydrolysis, and can chicken blood effectively replace water in this process?

Methods

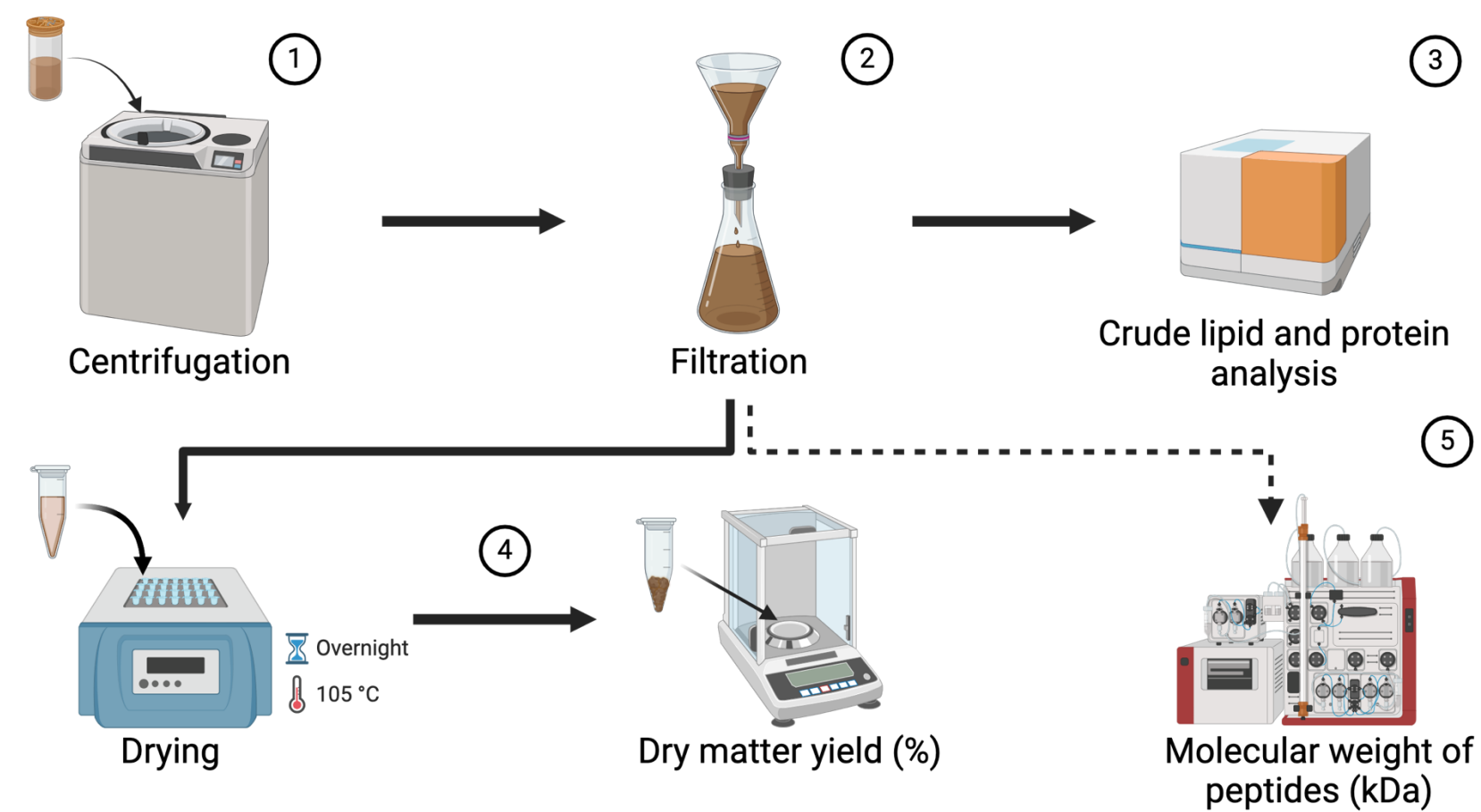
Step 1: Pre-treatment



Step 2: Hydrolysis



Step 3: Downstream processing and analysis



Scheme 1: Chicken viscera hydrolysis process and downstream processing

Figure created with BioRender.com

Acknowledgements

I thank the Research Council of Norway for funding the project under SFI-Industrial Biotechnology (project code – 309558) and providing us the necessary equipment. Also, our industry collaborators, Norilia and Biocatalyst, for supplying the biomass and enzymes for testing.



Results

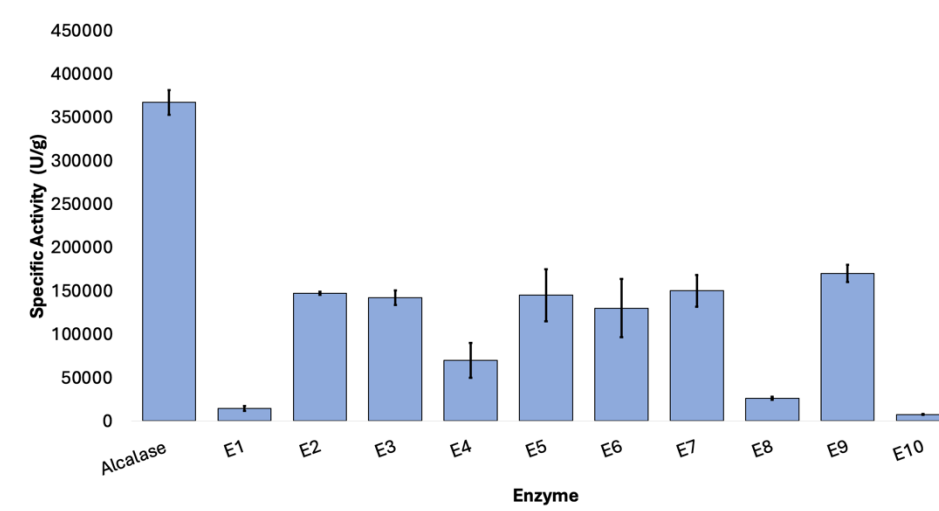


Fig. 1: Normalizing the specific activity of the proteases against casein substrate. Specific activity was measured in 25 mM phosphate buffer pH 6 at 50°C for 10 min. Alcalase was used as a reference enzyme, E1–E10 were test enzymes used in this study.

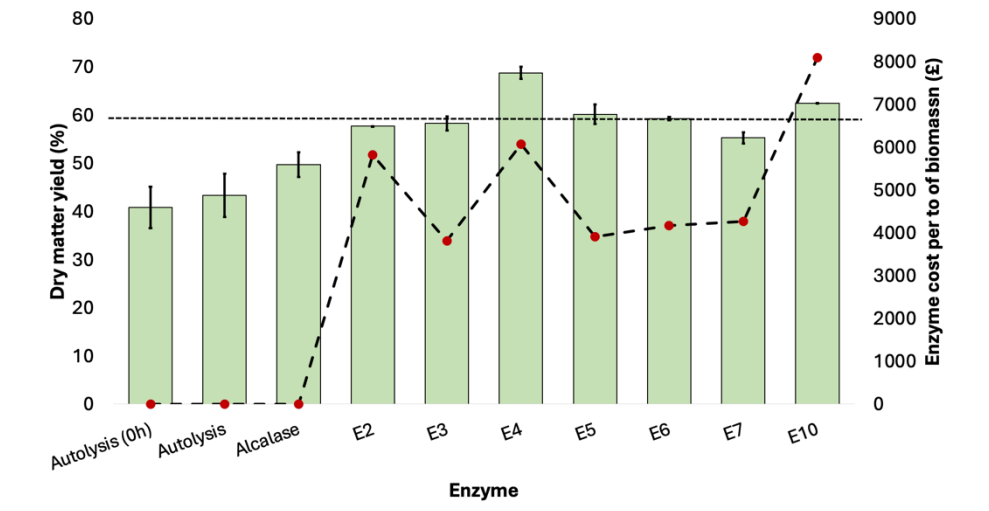


Fig. 2: Dry matter yield and cost per ton of protein recovery across different proteases. Hydrolysis was performed by adding same units of enzyme per substrate (U/S). The cost of Alcalase in bulk volume is unknown.

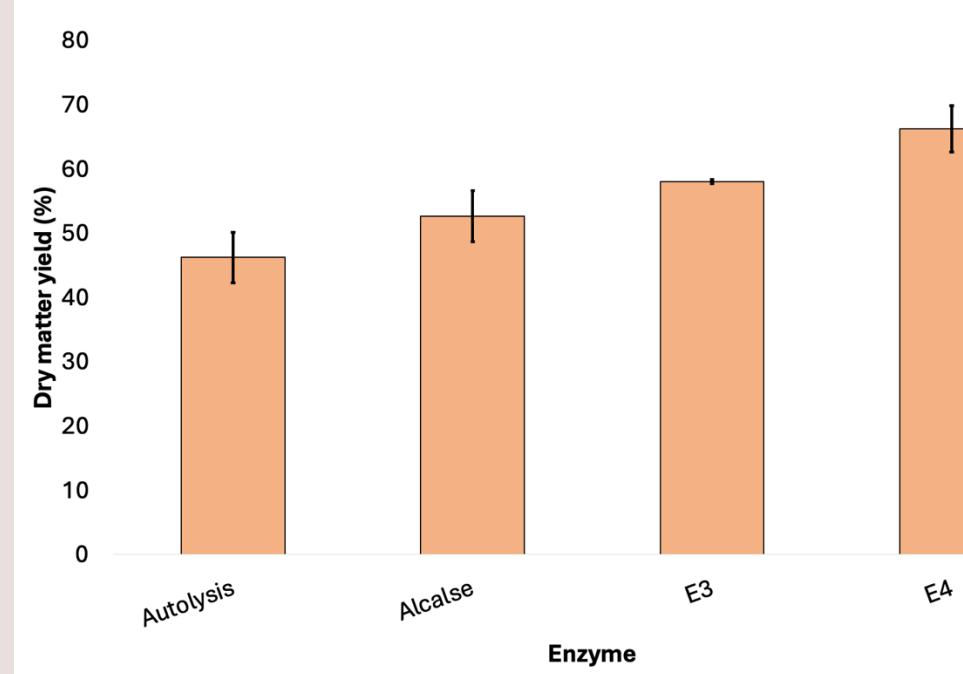


Fig. 3: Effect of proteases in the hydrolysis of a new batch of chicken viscera. The dry matter yield obtained for the new batch of chicken viscera hydrolysates followed a similar profile to that observed in the previous biomass batch (Fig. 2).

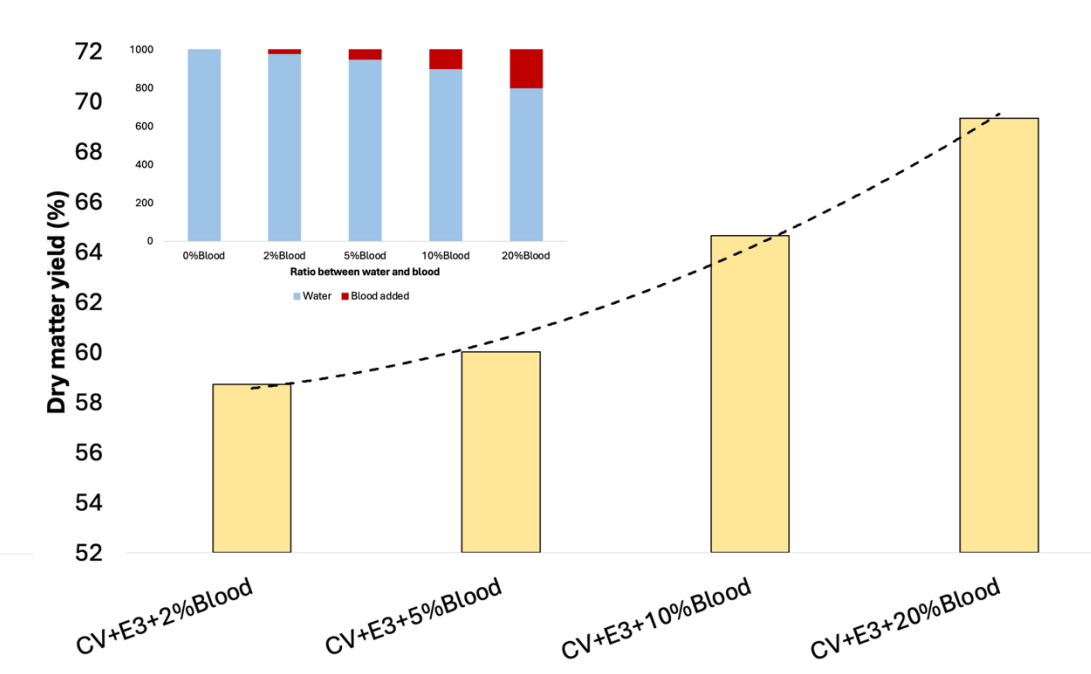


Fig. 4: Effect of chicken blood replacing water in the hydrolysis of chicken viscera. E3 was less expensive than E4 and was used for optimizing the hydrolysis process with varying concentration of blood. Dry matter yield increased exponentially with increase in blood. The supplementary figure displays the ratio between water and blood.

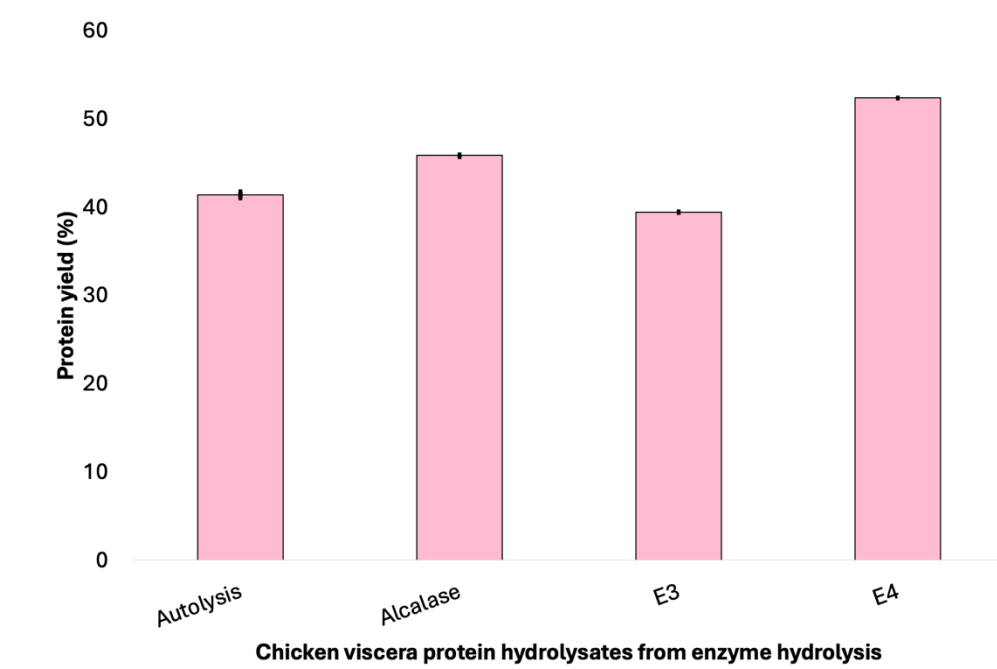


Fig. 5: Protein yield of chicken viscera hydrolysates using selected proteases. Protein yield increase observed in the hydrolysates from proteases was as follows: E4 > Alcalase > E3 > Autolysis.

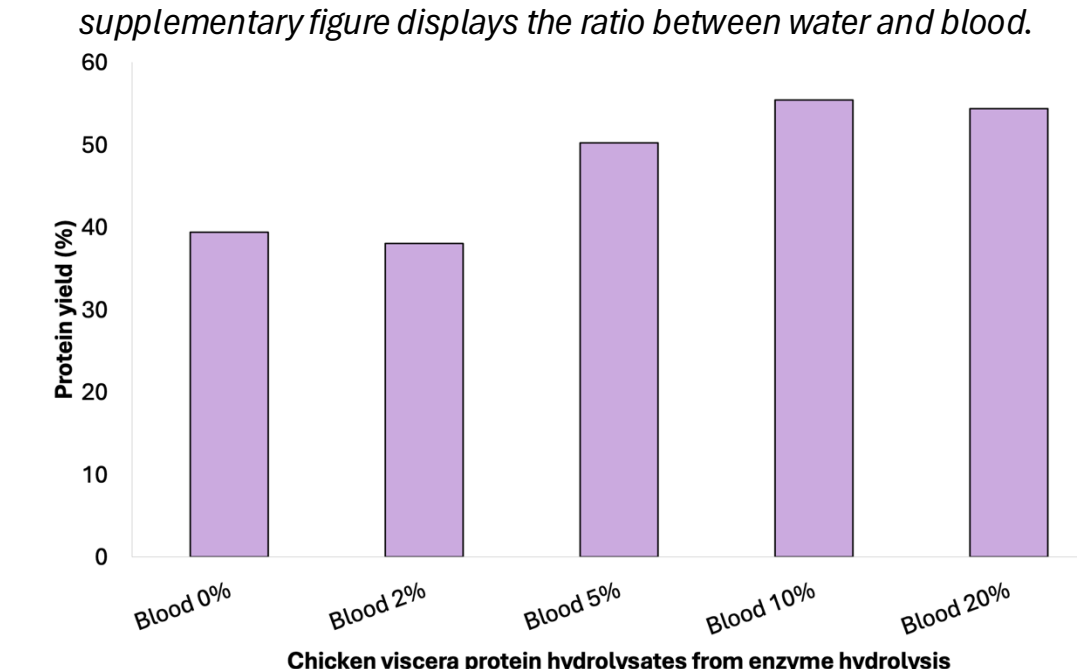


Fig. 6: Protein yield of chicken viscera hydrolysates produced using protease E3 and varied concentration of blood. The increased blood concentration (5–20%) in the hydrolysis shows a positive effect on the protein yield.

Discussion and conclusion

- The chicken viscera hydrolysis process was successfully tested using various industrial enzymes. Pre-liminary testing of blood replacing water in chicken viscera hydrolysis was studied.
- Literature shows that there is a 50% dry matter yield when using the enzyme Alcalase (1), which was consistent with our study. Interestingly, all the test enzymes from E1 – E10 showed dry matter greater than 50%, outperforming both the endogenous enzyme (Autolysis) and Alcalase.
- E4 achieved over 60% dry matter yield, which contradicts its specific activity profile, suggesting that its catalytic efficiency differs between casein and chicken viscera.
- The protein yield (%) was highest for the chicken viscera hydrolysed using E4 followed by Alcalase, Autolysis and E3. E3's high dry matter yield likely reflects higher carbohydrate and ash content in this batch of chicken viscera.
- Lipid yield in all hydrolysates was below 1%, indicating that fat is separated during downstream steps in the hydrolysis.
- Both dry matter and protein yield can be increased by replacing 5 - 20% of water in chicken viscera hydrolysis, with protein yield increasing with increase in blood concentration.

Future work

- The peptide distribution in the protein hydrolysates needs to be analysed to understand the molecular weight of peptides and the degree of hydrolysis.
- The specific activity of proteases should be reanalysed using a substrate more representative of chicken viscera.
- The chicken viscera hydrolysis process will be repeated with a new batch of biomass to evaluate the protease's efficiency in hydrolysing chicken viscera, as the biomass composition can vary from batch to batch.

References

- (1) Lapeña, D., Vuoristo, K. S., Kosa, G., Horn, S. J., & Eijsink, V. G. H. (2018). Comparative Assessment of Enzymatic Hydrolysis for Valorization of Different Protein-Rich Industrial Byproducts. *Journal of agricultural and food chemistry*, 66(37), 9738–9749.
- (2) Lasekan, A., Bakar, F. A., & Hashim, D. (2013). Potential of chicken by-products as sources of useful biological resources. *Waste management*, 33(3), 552–565.
- (3) Five, K. K., Fållun, I., Roland, G. J., Forshaug, D., Helgetand-Rossavik, M. K., Hats, R., ... & Rustad, T. (2024). Enzymatic hydrolysis of chicken viscera and bones: Rest raw material characterization and evaluation of industrially relevant process parameters on product yields. *Process Biochemistry*, 146, 68–80.