

## ESSAY 2

Answers must be written out in paragraph form. Outline form is not acceptable. Labeled diagrams may be used to supplement discussion, but a diagram without a written explanation will not receive credit. You must cite the source of all outside information you include. Include the page number of information from the course textbook or the web address of information found online.

Cheese whey, a byproduct of the cheese-making industry, is discarded in many countries in the environment, causing pollution. This byproduct contains high-quality proteins containing encrypted biologically active peptides. In an investigation evaluating the suitability of using this waste to produce antioxidants by enzymatic hydrolysis with a digestive enzyme, cheese whey from white cheese (panela cheese) was hydrolyzed with trypsin (Figure 1), a protease found in the digestive systems of many vertebrates (Martín-del-Campo et al., 2019). Antioxidants are compounds that protect cells from harmful molecules known as free radicals. Eating foods rich in antioxidants may improve heart health and lower risks of infection and certain types of cancer.

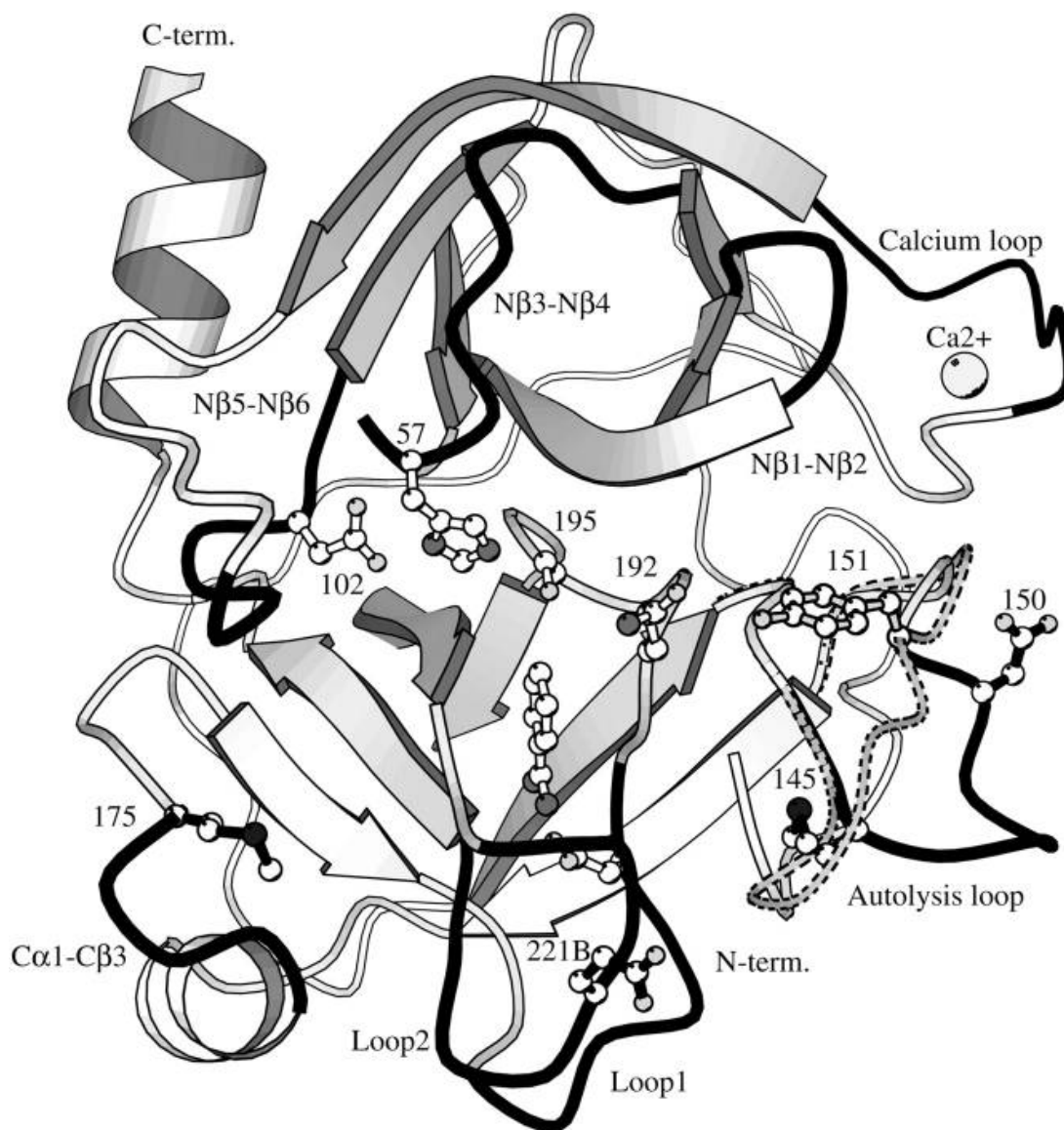


Figure 1. Ribbon diagram defining the secondary and tertiary structural elements of trypsin (Leiros et al., 2004). Cylindrical spirals represent  $\alpha$ -helices and arrows show the direction and twists of  $\beta$ -sheets.

A central composite design was used to find the pH and temperature conditions resulting in the maximum hydrolysis of cheese whey (Figure 2). A higher percent of cheese whey hydrolyzed is associated with higher antioxidant production.

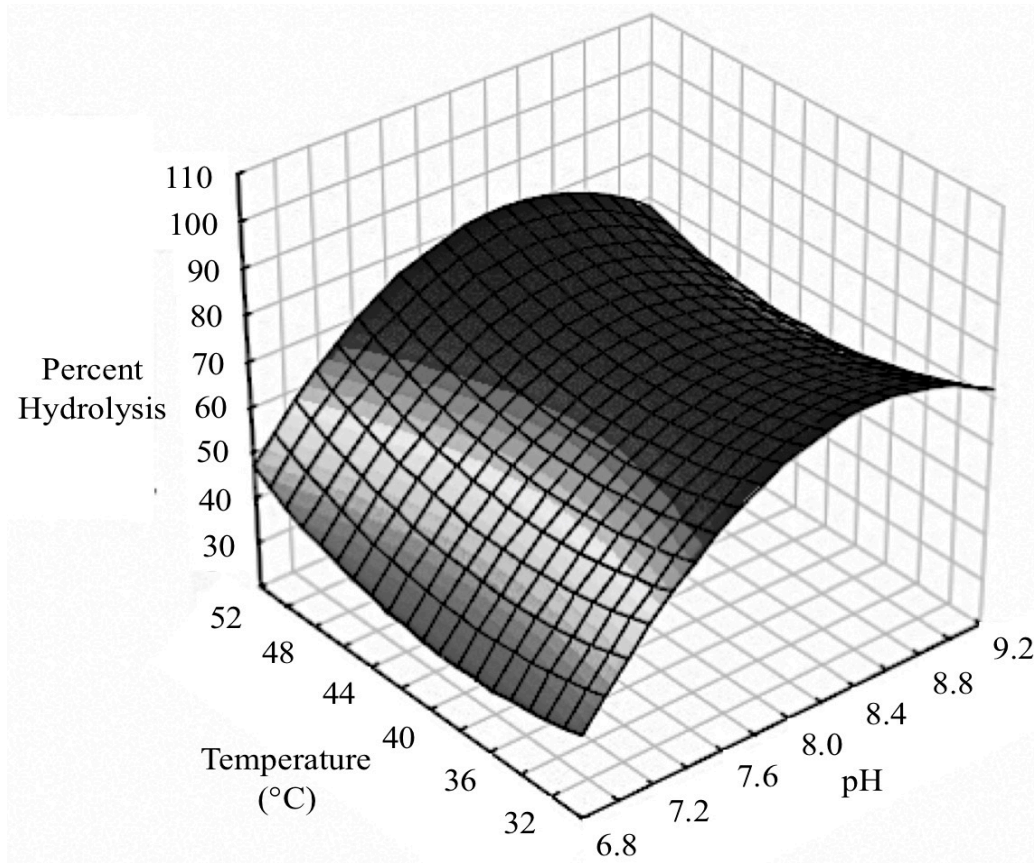


Figure 2. Surface plot of percent hydrolysis ( $y$ -axis) as a function of temperature ( $x$ -axis) and pH ( $z$ -axis).

- Explain** why whey protein hydrolysis increased as the temperature was increased from 40°C to 50°C. A mutation in the gene coding for trypsin results in an alteration in the primary structure of the enzyme. **Explain** why the mutation would likely also lead to changes in the tertiary structure of the enzyme.
- Identify** ONE independent variable AND ONE dependent variable in the experimental design. **Propose** a negative control treatment for the experiment and **explain** how this treatment would confirm that trypsin is necessary for cheese whey hydrolysis.
- Using Figure 2, **identify** the combination of temperature AND pH associated with the maximum antioxidant production.
- Predict** how whey protein hydrolysis would change if the temperature of the reaction was increased from 50°C to 70°C. **Justify** your prediction based on the structure AND function of trypsin.

## References

- Leiros, H. K., Brandsdal, B. O., Andersen, O. A., Os, V., Leiros, I., Helland, R., ... Smalås, A. O. (2004). Trypsin specificity as elucidated by LIE calculations, X-ray structures, and association constant measurements. *Protein Science*, 13(4), 1056–1070.
- Martín-del-Campo, S. T., Martínez-Basilio, P. C., Sepúlveda-Álvarez, J. C., Gutiérrez-Melchor, S. E., Galindo-Peña, K. D., Lara-Domínguez, A. K. & Cardador-Martínez, A. (2019). Production of antioxidant and ACEI peptides from cheese whey discarded from Mexican white cheese production. *Antioxidants*, 8(6), 158.