Response to Reviewer 2 Comments

The article (beverages-3658908) titled "Enhancing the Thermostability of a New Tannase Through Rational Design and Site-Directed Mutagenesis: A Quality Improvement Strategy for Green Tea Infusion" presents comprehensive research on the innovative thermophilic tanase, TanPL1, which is produced by expressing it heterologously in Yarrowia lipolytica. This represents a significant step towards the industrial application of enzymes in the food industry.

The results of planned design and changes to genes (mutagenesis) are interesting. These led to the creation of the MuTanPL1 mutant, which can handle heat better and works best at 60°C. Researchers have achieved impressive results by using this enzyme to process green tea infusion: they have significantly increased the content of free catechins and gallic acid, improved the infusion's sensory properties and increased its antioxidant activity.

These findings underscore the immense potential of MuTanPL1 in the beverage sector, particularly with respect to enhancing the quality and health-promoting properties of tea products. Furthermore, the paper emphasises the significance of protein engineering methodologies in enhancing the functionality of enzymes.

However, the article does not provide a detailed analysis. It does not analyse the molecular mechanism. This mechanism is responsible for improving the thermal stability of TanPL1 after mutagenesis. The authors have announced that this aspect will be discussed in a subsequent article, which has left some readers dissatisfied but also generated interest in further research.

The article is a valuable addition to the development of enzymatic biotechnology and its applications in the food industry. The results presented are well documented. The research approach is innovative and well-founded.

I have a question about this article. What is the reason for the absence of the results of the antioxidant activity of tea samples tested using three methods against DPPH? Were these results provided, or not? If they are done, would it be worth having them added?

Authors' response:

Thank you very much for taking the time to review this manuscript. We would like to appreciate your kind help and efforts to revise the paper, thanks for all the recognition and recommendation.

Thank you for highlighting the need to investigate molecular mechanisms; for that, our next paper will address the changes in molecular interactions and solvent accessibility of the mutated amino acid residues.

We appreciate your inquiry regarding the DPPH antioxidant activity results obtained

using the three methods. All the data were indeed available, and as suggested, we have incorporated this information into the manuscript (line 670–683, pp. 17), where the detailed results and discussion are presented in red.