Trimethylamine removal in salmon protein hydrolysates by a novel monooxygenase strategy

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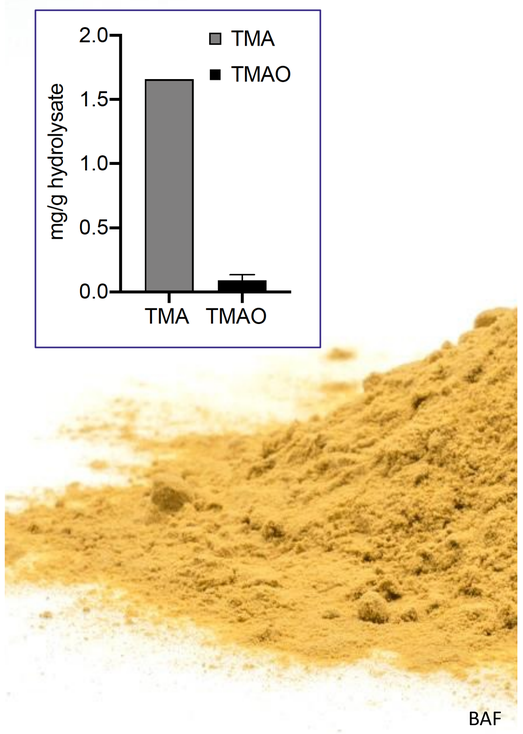
We present a novel enzyme strategy to remediate a fish-smelling molecule in salmon peptide ingredients to make these applicable in food.

Introduction

Enzyme-based conversion of marine biomass to high-quality peptide ingredients is a promising strategy in bio-based industries. Despite their good nutritional profile and food grade quality, their utility in food products is limited by the fish smell. A well-known contributor to the smell is trimethylamine (TMA). Current strategies to mask or remove the odor are not effective or give rise to undesirable side effects. We have shown that trimethylamine monooxygenases (Tmms) can oxidize TMA into the odorless TMA *N*-oxide (TMAO) in hydrolysates.



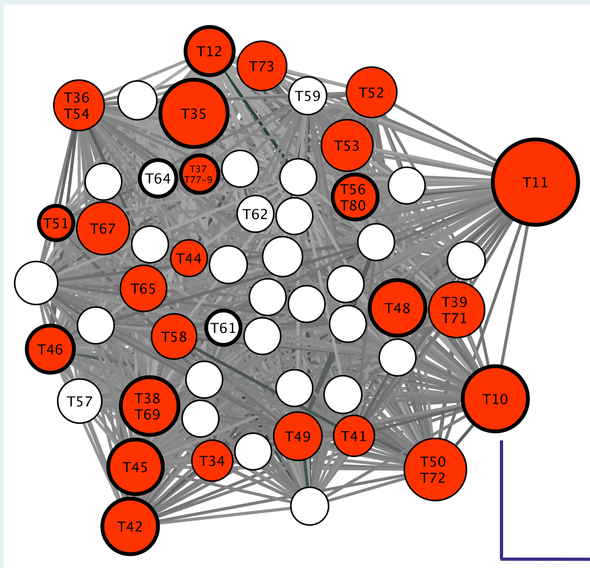
The processing of fish for human consumption gives rise to 50-70% by-products, such as heads, frames, viscera, blood, and trimmings (left). These are treated in advanced biorefineries with proteases to generate food-grade peptide ingredients (right). The image inset shows the presence of TMA (distinct smell of fish) and TMAO (odor free) in a salmon protein hydrolysate (L60).

Impact

Improving the taste and smell of fish protein hydrolysates will promote an upgrade of products to higher-value markets and promote more sustainable utilization of fish by-products from aquaculture and fisheries.

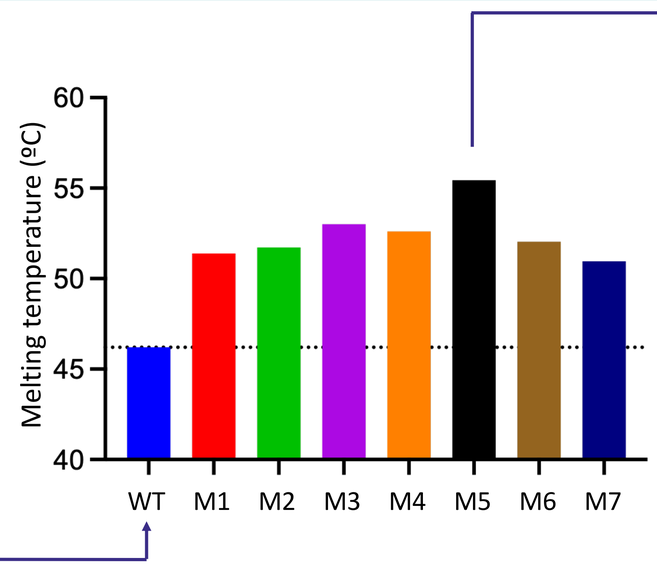
Results

Data mining for enzyme candidates



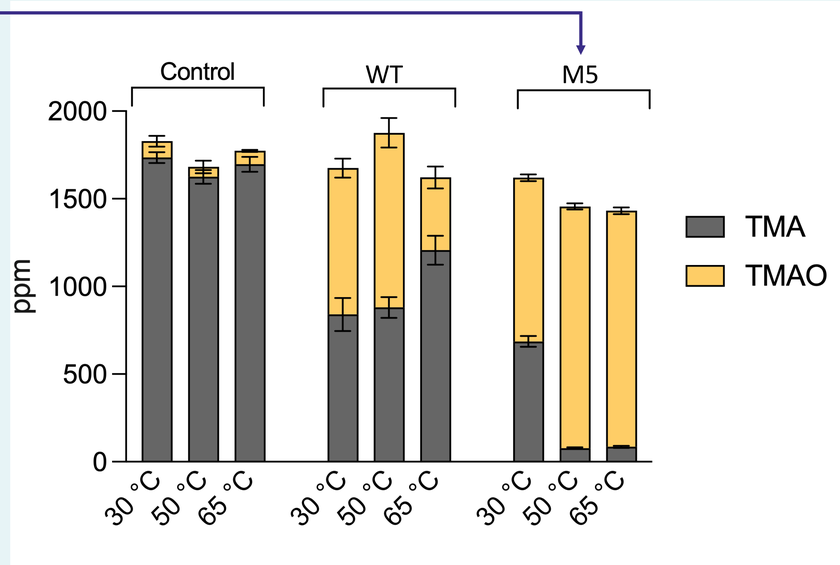
Sequence similarity network analysis was employed to identify the cluster of UniRef90 Tmms in the flavin monooxygenase family, guided by nodes with annotated Tmm sequences (red). Bacterial Tmm candidates indicated by arbitrary numbers (e.g., T10), were selected. Nodes containing at least one sequence with Tmm activity are highlighted by bold circles. Node sizes reflect the numbers of sequences.

Enzyme engineering for more heat stability



An enzyme should withstand at least 50 °C in industrial application, but none of the native enzymes did. The T10 enzyme, (here, WT), was selected for computational enzyme engineering to increase its stability. Seven mutant variants (here, M1-M7), containing up to 28 mutations, were made. Protein melting studies were conducted using circular dichroism. All mutants displayed increases in melting temperature. The most thermostable variant was M5, with 9.0 °C increase.

Mutant performance in salmon protein hydrolysate



We compared the M5 mutant's ability to convert TMA to TMAO in a salmon protein hydrolysate to that of the WT. Salmon protein hydrolysates were incubated at 30, 50 and 65 °C with no enzyme (control), WT, or M5. As there are insufficient amounts of the cofactor NADPH to drive the reaction, it was supplemented. TMA (grey) and TMAO (yellow) levels were determined using mass spectrometry. The M5 mutant outperformed the WT enzyme at all temperature with a striking 95% reduction of TMA at both 50°C and 65°C.

Conclusion

The heat stable mutant may be applied for industrial use as it can remediate TMA in fish protein hydrolysates. It may be useful for other engineering efforts to further improve its properties.

Reference

Goris M, et al. Use of Flavin-Containing Monooxygenases for Conversion of Trimethylamine in Salmon Protein Hydrolysates. Appl Environ Microbiol. 2020 Nov 24;86(24):e02105-20.

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