

**Enhancement of Anti-Diabetic Potential of Probiotic-Fermented Soymilk: GC-SPME  
Metabolomic Profiling and  $\alpha$ -Amylase Kinetic Analysis**

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*Abstract*

Soybean is recognized as a valuable functional food owing to its high-quality protein, isoflavones, peptides and other bioactive constituents that contribute significantly to the prevention and management of metabolic disorders.

Soymilk fermentation with probiotic bacteria has gained significant attention due to its potential to enhance nutritional quality and generate bioactive metabolites associated with diabetes management. The present study evaluated the anti-diabetic potential of soymilk fermented using six probiotic bacterial cultures through metabolomic profiling and  $\alpha$ -amylase inhibition kinetics. Freshly prepared soymilk was fermented individually with selected probiotic strains under controlled conditions. The fermented samples were analysed for  $\alpha$ -amylase inhibitory activity to assess their ability to reduce starch hydrolysis and postprandial glucose release. Enzyme kinetics studies were performed using varying substrate concentrations and also kinetic parameters were determined through Lineweaver–Burk analysis to understand the mode of inhibition.

Metabolic profiling of fermented and unfermented soymilk samples was carried out using Gas Chromatography–Solid Phase Microextraction (GC-SPME). The analysis revealed significant variations in volatile and semi-volatile metabolites among the probiotic treatments. Fermentation resulted in the reduction of undesirable beany-flavor compounds such as hexanal and the formation of several bioactive metabolites including organic acids, alcohols, aldehydes, and phenolic derivatives that may contribute to anti-diabetic activity.

Among the six probiotic cultures, selected strains exhibited higher  $\alpha$ -amylase inhibitory activity along with favorable enzyme kinetic behavior, indicating improved potential for glycemic control. Correlation between metabolite composition and enzyme inhibition suggested that fermentation-induced biochemical transformations enhanced the functional properties of soymilk. The findings demonstrate that probiotic fermentation can be an effective strategy to improve the anti-diabetic potential and sensory quality of soymilk, supporting its development as a functional food for diabetes management.

Key words: Diabetes, Soymilk, Probiotic,  $\alpha$ -amylase inhibition, GC-SPME, Metabolites, Fermentation

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