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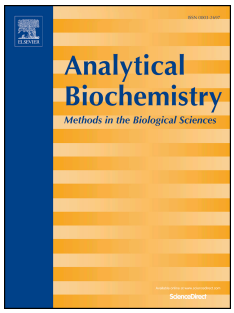

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Optimization of the quenching and extraction procedures for a metabolomic analysis of *Lactobacillus plantarum*

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Abstract

This study evaluated the quenching and extraction procedures using different solvents at different temperatures for *Lactobacillus plantarum* ZS2058. Eight quenching methods that used readily available organic solvents (methanol and ethanol) or cryoprotectants (glycerol and dimethyl sulfoxide) were evaluated quantitatively, and their effects on cell membrane integrity and metabolic inactivation were assessed by propidium iodide and energy charge assays, respectively. The combination of 20% methanol and 0.9% sodium chloride (−4 ºC), with the lowest propidium iodide labelling rate of 3.06 ± 0.16% and the highest energy charge value of 0.849 ± 0.003, outperformed most of its counterparts and was adopted for further use. To retrieve most of the cellular metabolites, four extraction solvent systems, including methyl *tert*-butyl ether/methanol/water, methanol/water, acetonitrile/water and acetonitrile/methnol/water, were evaluated. And acetonitrile/methnol/water was found to be the most efficient one as, among other solvents, it yielded the highest metabolite abundances for most of the metabolites. Furthermore, the protocol was subjected to analytical validation using a series of selected representative metabolites and yielded a linear range of 0.00128 to 100 μg mL^{-1} with coefficients of 0.9953 to 0.9999 and recovery levels of 90% to 110%. These results suggest the reliability of the proposed method for *L. plantarum* ZS2058.

Keywords: microbial metabolomics; quenching; extraction; recovery; *Lactobacillus plantarum*