

Title: Metabolomic differences between non-hydrothermal treated water-soluble (WSPs) and hydrothermally treated water-insoluble (WIPs) Maitake polysaccharides fermented by *Lactobacillus acidophilus* and *L. plantarum*

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Abstract: Bacterial Metabolite through a fermentation process is a growing trend and a promising alternative for use as functional components. Non-hydrothermal water-soluble (WSPs) and hydrothermally treated water-insoluble (WIPs) Maitake polysaccharides were fermented with *Lactobacillus acidophilus* (LA) and *Lactobacillus plantarum* (LP). Chemical composition analysis indicated that Maitake polysaccharides contained 58.22 ± 1.35 % total sugar and 31.46 % β -glucan, essential for metabolites production. 6-glucanase was used to degrade the WIPs, and hydrothermally treated WIP fibers exhibited smooth microstructure. Hence, the LA and LP bacteria investigated the potential fermented metabolic activities and differences between WSPs(Sp1)and WIP(Sp3) Maitake polysaccharides using LC-MS, and 887 metabolites were identified. Using Venn, Partial least squares discriminant analysis (PLS-DA), VIP Metabolites, and other multivariate statistical analysis methods, metabolites were expressed differently in all samples. Due to hydrothermal processing, WIP induced the highest growth of LA and LP, with an abundance of isocitrate metabolites. Furthermore, 50 metabolite correlations were identified, leading to the classification of 6 distinct metabolic groups. Thus, the study offers the initial comprehensive analysis of metabolites in *Lactobacillus*-fermented Maitake polysaccharides, aiding in understanding its metabolic interactions and facilitating progress in food engineering research.

Keywords: β -glucan, Hydrothermal treatment, 6-glucanase degradation, Metabolomics, LC-MS analysis, *Lactobacillus acidophilus*, *Lactobacillus plantarum*

DOI: <https://doi.org/10.1016/j.ijbiomac.2024.132709>