Engineering fungal biotransformation toolbox to optimize the purity, flavor and techno-functional properties of alkaline-extracted BSG fractions

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Brewer's spent grain (BSG), the largest by-product of brewing, represents an underexploited protein resource (15–25%d.b.). Despite high recovery by traditional alkaline extraction, long time and high quantity of solvents are needed, limiting its viability at industrial scale. Alternatively, reactive extrusion achieves high recoveries at much shorter times and using 10x less solvent. However, the resulting fractions suffer from low protein purity, limited solubility, and undesirable grassy and earthy off-flavors, restricting their food applications.

In this study, BSG protein fractions obtained by reactive extrusion were subjected to submerged fermentation as single substrate using highest-developed fungal strains with distinct enzymatic profiles to evaluate their effects on protein characteristics, aroma profiles, and techno-functional properties.

Fermentation enhanced protein purity in a strain-dependent manner. *Trametes versicolor* and *Coprinellus micaceus*, characterized by strong lignocellulose-degrading enzymatic activities, showed the most pronounced effects, likely through enzyme-mediated disruption of the lignocellulosic matrix and selective degradation of non-protein components, facilitating protein enrichment. Within 3 days of fermentation, this increased 2-fold the protein purity (from 32% to 64%), accompanied by a 2–3-fold increase in protein solubility. These structural modifications further improved techno-functional properties, particularly enhancing emulsifying performance.

In parallel, fermentation with *Stropharia rugosoannulata* significantly modulated the flavor profile of BSG protein fractions. This strain, characterized by active amino acid-transforming metabolism, showed the most pronounced effect, likely through enzyme-mediated conversion of amino acid-derived precursors via microbial pathways involving alcohol formation and subsequent esterification (e.g., alcohol dehydrogenase and esterification-related enzymes), leading to the reduction of grassy and earthy off-notes and the development of distinct fruity characteristics, with key compounds including ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl 2-methylpropanoate.

Overall, this study establishes targeted fungal fermentation as a programmable biotransformation platform for precise tuning of protein purity, flavor architecture, and

techno-functional performance, unlocking scalable upcycling of BSG and other lignocellulosic side streams.

Keywords： brewer’s spent grain; fungal fermentation; protein enrichment; aroma modulation

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