Biovalorized Okara Consumption Improves Gut Metabolites by Modulating Gut Microbiome: A Randomized Controlled Crossover Trial

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Objectives: Okara is a major food by-product of soymilk or tofu production, particularly rich in dietary fiber. Biovalorization with *Rhizopus oligosporus* can improve its nutritional quality with greater soluble fiber, bioavailable isoflavones, and antioxidants. These nutrients are fermented by the gut microbiota to generate gut metabolites involved in gut health maintenance. This study evaluates the impact of okara and biovalorized okara- containing biscuits consumption on gut health in middle-aged and older Singaporeans.

Methods: Healthy participants (n = 15) of the crossover trial were randomly allocated to 3-week interventions of 100 g of control (C), 20% flour substituted autoclaved okara- (AOK), and 20% four substituted *R. oligosporus* biovalorized okara- (RO) containing biscuits, with 3-week washout periods. Pre- and post- intervention serum short-chain fatty acids (SCFA), fecal SCFA, fecal bile acids, serum zonulin, gastrointestinal symptoms, and dietary intakes were assessed. Pre- and post- intervention fecal 16S ribosomal RNA V3-V4 sequencing was also investigated for gut microbiome analysis. Results are presented as change value mean (95% confidence interval).

Results: Amongst fecal SCFA, only valeric acid (μmol/g) observed a significant difference, higher with RO compared to AOK (0.16 (0.01, 0.30) vs −0.20 (−0.34, −0.05), $\boldsymbol{P} = 0.005$). RO and AOK had significantly higher total serum SCFA ($\boldsymbol{P} = 0.002$ & 0.018 respectively) and serum acetic acid ($\boldsymbol{P} = 0.007$ & 0.030 respectively) compared to C. RO had significant improvements with higher serum propionic acid (μmol/L) (RO: 3.04 (0.80, 5.27) vs C: −2.65 (−4.97, −0.33), $\boldsymbol{P} = 0.004$) and lower fecal lithocholic acid (mg/g) (a secondary bile acid) (RO: −0.11 (−0.20, −0.02) vs C: 0.10 (0.01, 0.19), $\boldsymbol{P} = 0.009$) contrasted to C. Although serum zonulin showed no significant difference, AOK induced a reduction in Clostridiales while RO increased *Bifidobacterium*. Okara consumption increased dietary fiber intake, with greater soluble fiber intake from RO compared to AOK and C ($\boldsymbol{P} = 0.018$ & 0.019 respectively), aligning with the greater flatulence felt by participants on RO.

Conclusions: Okara consumption improved serum SCFA rather than fecal SCFA, regardless of fermentation. Biovalorized okara consumption further improved SCFA and bile acid profiles by modulating gut microbiome.

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