

Effect of Okara and Biovalorized Okara Biscuits Consumption on Postprandial Circulating Amino Acid Concentrations: A Crossover Randomized Controlled Trial

Delia Pei Shan Lee, Genevieve Xin Yin Leong, Xiangning Liu, and Jung Eun Kim

National University of Singapore

Objectives: Soymilk or tofu production results in considerable okara (soybean pulp) generation. Although okara has a high protein content and contains all essential amino acids, its low water solubility and anti-nutrient presence reduces its protein digestibility. Okara fermented with *Rhizopus oligosporus* can increase its digestibility by lowering anti-nutrient content and liberating free amino acids. However, neither okara nor biovalorized okara has been evaluated for its effect on postprandial blood amino acids. This study hence explored this gap in knowledge with okara- and biovalorized okara- containing biscuits consumption.

Methods: The randomized controlled crossover trial involved 15 middle-aged and older Singaporean adults. Each participant undertook 3 separate 2-hour meal tolerance test with 100 g of control biscuits (C), biovalorized okara biscuits (RO), and autoclaved okara biscuits (AOK). RO and AOK were developed with the same recipe as C, utilizing a 20% wheat flour substitution with *R. oligosporus* fermented

okara or autoclaved okara respectively. Biscuit total and free amino acids contents were analyzed by cation-exchange chromatography. Circulating free amino acids concentrations were assessed at timepoints 0, 15, 30, 45, 60, 90, and 120 min. Incremental area under curve (iAUC) and peak concentration were assessed, with results presented as mean (95% confidence interval).

Results: Biscuit total amino acid content (mean \pm standard deviation, g/100 g) was significantly greater for RO (7.51 ± 0.22) and AOK (6.78 ± 0.39) compared to C (5.93 ± 0.33), with RO having notably greater alanine, cysteine, and proline content over AOK. Free amino acid content in biscuit (nmol/g) in ascending order was C (485 ± 11), AOK (698 ± 13), and RO (3414 ± 97). Amino acid iAUC had no significant difference, as demonstrated with total amino acids iAUC ($\mu\text{mol/L}\cdot\text{min}$) ($P = 0.568$, C: 23 649 (13 738, 33 560), RO: 23 284 (13 374, 33 195) & AOK: 17 257 (7 346, 27 168)). Peak iAUC concentration also showed no significant difference across all amino acids, with total amino acids peak iAUC concentration ($\mu\text{mol/L}$) as $P = 0.560$, C: 387 (233,540), RO: 456 (303,610) & AOK: 343 (189,497).

Conclusions: Consumption of okara- or biovalorized okara- containing biscuits does not alter postprandial amino acid responses in middle-aged and older Singaporeans.

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