

Supplementary Materials

Fermented soybean foods (natto) ameliorate age-related cognitive decline by hippocampal TAAR1-mediated activation of CaMKII/CREB/BDNF signaling pathway in senescence-accelerated mouse prone 8 (SAMP8)

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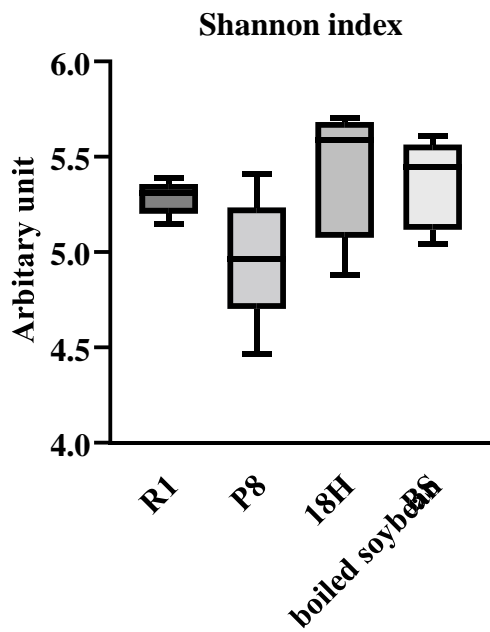
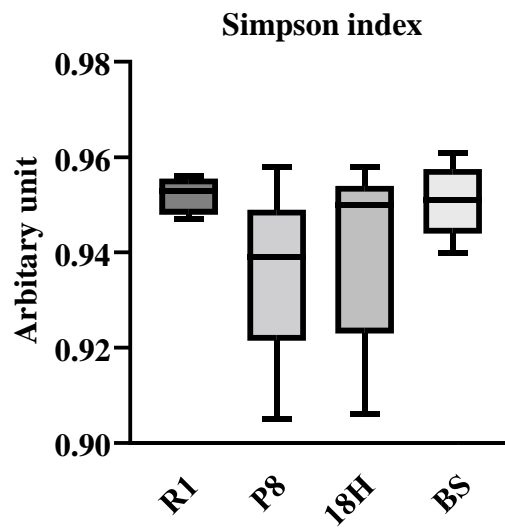
Supplementary Methods

Microbiological analysis

Five mice were randomly chosen from each group, and genomic DNA (gDNA) was extracted from the cecal contents using the NucleoSpin DNA Stool Extraction Kit (Takara Bio, Shiga, Japan). The purified gDNA samples were sent to GeneWiz (South Plainfield, NJ, USA) for 16S rRNA gene sequencing analysis for microbiota profiling with barcoded amplicons of the V3-V4 regions (n=3). Sequencing results were analyzed using QIIME 1.9.1, and taxonomic assignment of operational taxonomic units (OTUs) was performed using the Silva v132 database. Analysis of alpha diversity was performed to determine the characteristics of the gut microflora of different groups.

Metabolome analysis

The powders of 14-h, 18-h, 22-h, and 42-h fermented natto (14N, 18N, 22N, and 42N) were obtained from Takano Foods Co. Ltd. (Ibaraki, Japan). Natto samples (n=1) were transferred to Human Metabolome Technologies, Inc. (HMT, Tsuruoka, Japan) for metabolite analysis, using capillary electrophoresis (CE) coupled with Fourier transform mass spectrometry (CE-FTMS) based on the method described in HMT's ω Scan package.

A**B**

Supplementary Figure 1. Effects of supplementation with 18N and boiled soybean (BS) on the α diversity of flora in cecal contents. (A) Shannon index. (B) Simpson index. R1, SAMR1 control; P8, SAMP8 control; 18N, natto fermented for 18 h.

Supplementary Table 1. Representative difference in natto prepared by different fermentation times.

Name	Relative area				Ratio (42N/18N)
	14N	18N	22N	42N	
Dimethylaminoethanol	0.012	0.012	0.0067	0.00054	0.05
Ethylacetimidate	0.11	0.096	0.069	0.011	0.11
Arg-Asp	0.0052	0.0071	0.0039	0.00084	0.12
Succinic acid	0.033	0.050	0.033	0.0061	0.12
Ribulose 5-phosphate	0.013	0.0059	0.0043	0.00087	0.15
Citric acid	0.12	0.060	0.033	0.015	0.25
2' or 5'-Deoxyadenosine	0.0036	0.0022	0.0019	0.00062	0.28
Leu-Ile or Leu-Leu	0.14	0.17	0.096	0.048	0.28
Mucic acid	0.0025	0.0011	0.0005	0.00032	0.29
6-Aminohexanoic acid	0.0059	0.0031	0.0026	0.0010	0.32
Adenosine	0.046	0.036	0.027	0.012	0.33
Ile-Gln or Leu-Gln	0.058	0.073	0.043	0.025	0.34
Fructose 1,6-diphosphate	0.0065	0.0028	0.0035	0.0010	0.36
Val-Asp	0.0072	0.010	0.0057	0.0040	0.40
Glucose 6-phosphate	0.00096	0.0023	0.0013	0.00092	0.40
Ala-Arg or Arg-Ala	0.0052	0.0050	0.0039	0.0020	0.40
Met-Ala	0.0011	0.0016	0.00089	0.00064	0.40
Met-Thr	0.00072	0.00068	0.00032	0.00028	0.41
Gly-Gly	0.011	0.018	0.0074	0.0073	0.41
Mannosamine	0.011	0.021	0.014	0.0093	0.44

The top 20 compounds are shown in descending order of ratio (42N/18N).