Green Tea Modulates Temporal Dynamics and Environmental Adaptation of Microbial Communities in *Daqu* Fermentation

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1. Appendix S1
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Appendix S1 Preparation and sample collection of *Daqu* blocks

1. Preparation of *Daqu* Blocks

Following the traditional protocol for Moutai-flavor *Daqu*, we prepared the *Daqu* with hard wheat and green tea as the raw materials. The process, depicted in Fig. S1A, comprised five essential steps: moistening, crushing, mixing, shaping, and fermentation in storage [1]. Four *Daqu* variants were prepared: the original *Daqu* (pure wheat with no green tea), 10% tea-added *Daqu* (10% green tea by weight of the *Daqu* block), 20% tea-added *Daqu* (20% green tea), and 30% tea-added *Daqu* (30% green tea). Each variant was produced in four blocks, each weighing 1,500 g. Each block contained 675 g water (45% of the block's total mass) and 150 g starter culture (10% of the total weight). After shaping, the *Daqu* blocks were placed in a cardboard container to simulate storage room fermentation conditions.

The preparation process, detailed in Fig. S1B, began with raw material preparation: green tea and wheat were crushed (a–c). These were then blended with starter culture and water in specified ratios, with green tea content adjusted to 0%, 10%, 20%, or 30% (d–e). Following mixing, the mixture was manually pressed into turtle-back-shaped molds, forming blocks with tight edges and a looser central core (f–i).

To replicate traditional storage conditions, the container was lined with straw at the base. Blocks were wrapped in straw and arranged in four layers (four blocks per layer), separated by flattened straw. The container was sealed, with side holes drilled for temperature monitoring; a thermometer was inserted into the blocks through these holes. Fermentation proceeded for 40 days. On days 6 and 15, the blocks were turned over to

optimize temperature and humidity distribution, ensuring uniform fermentation.

2. Sample Collection

Samples were collected from each *Daqu* variant on days 1, 6, 12, 15, 30, and 40 of fermentation (samples from days 6 and 15 were taken prior to turning). For each block, samples were taken from three locations: 1 cm beneath the top surface (inward-facing bulge), 1 cm below the bottom surface (inward-facing flat side), and the central core. Three samples from each location were pooled to form a replicate per time point. Three replicates were collected at each time point, resulting in 72 total samples (4 variants × 6 time points × 3 replicates).

Reference

1. Zuo, Q.; Huang, Y.; Guo, M. Evaluation of bacterial diversity during fermentation process: A comparison between handmade and machine-made high-temperature daqu of maotai-flavor liquor. *Ann. Microbiol.* **2020**, *13*, 14-23, doi:10.1186/s13213-020-01598-1.

Supplementary table

Table S1 Measurements of endogenous factors in distinct type of *Daqu*

Endogenous factors	Time points	Original *Daqu*	10% tea-added *Daqu*	20% tea-added *Daqu*	30% tea-added *Daqu*
Saccharifying power /U	Day 1	598.33±6.01a	568.33±1.67b	540.00±2.89c	528.33±1.67d
	Day 6	520.00±8.66a	426.67±4.41b	381.67±4.41c	345.00±2.89d
	Day 12	370.00±2.89a	360.00±2.89b	335.00±2.89c	305.00±2.89d
	Day 15	391.67±6.67c	343.33±1.67d	438.33±7.26a	415.00±0.01b
	Day 30	425.00±2.89b	418.33±4.41b	485.00±2.89a	431.67±6.01b
	Day 40	430.00±0.01d	461.67±1.67c	513.33±6.01a	498.33±4.41b
Temperature / ℃	Day 1	19.67±0.67a	21.33±0.33a	19.33±0.33a	20.67±0.33a
	Day 6	55.33±2.67a	55.00±1.15a	51.67±0.33a	53.67±0.67a
	Day 12	54.33±1.20a	53.00±0.01a	47.67±0.67b	51.67±0.88a
	Day 15	42.67±3.38a	51.00±4.16a	43.00±2.52a	49.67±0.33a
	Day 30	18.67±0.88b	18.33±0.67b	31.00±3.06a	26.33±3.33ab
	Day 40	13.67±0.67a	11.33±0.88a	11.67±1.20a	12.33±0.67a
Starch content / (g/100g)	Day 1	58.94±0.93a	55.12±0.54b	48.23±1.04c	41.84±0.19d
	Day 6	55.28±0.82a	49.40±1.56b	46.58±0.54b	40.62±0.26c
	Day 12	50.95±0.83a	47.78±0.26b	43.03±0.56c	36.33±0.07d
	Day 15	44.08±0.73a	42.97±0.14a	38.11±0.52b	33.42±0.11c
	Day 30	40.79±0.30a	36.75±0.37b	34.53±0.49c	30.69±0.53d
	Day 40	39.16±0.34a	36.28±0.27b	33.19±0.38c	28.10±0.33d
Acidity / (mmol/10 g)	Day 1	0.22±0.01d	0.33±0.01c	0.47±0.02b	0.58±0.01a
	Day 6	0.58±0.01d	0.83±0.01c	0.98±0.01a	0.89±0.01b
	Day 12	1.04±0.01d	1.15±0.01c	1.44±0.01a	1.29±0.02b
	Day 15	0.44±0.01d	0.57±0.01c	0.83±0.01b	1.06±0.02a
	Day 30	0.24±0.01d	0.39±0.01c	0.80±0.02a	0.74±0.03b
	Day 40	0.33±0.02d	0.54±0.02c	0.64±0.03b	0.82±0.01a
Moisture / (%)	Day 1	37.60±2.71a	37.64±0.12a	38.96±3.76a	39.00±1.79a
	Day 6	24.50±3.02a	33.26±2.87a	35.63±1.97a	28.54±1.32a
	Day 12	17.55±1.06c	22.28±0.02ab	26.64±2.68a	19.33±0.31bc
	Day 15	16.68±0.07b	20.50±0.11ab	23.21±2.40a	17.94±0.28b
	Day 30	15.78±0.03a	17.18±0.70a	19.59±3.15a	16.67±0.75a
	Day 40	14.03±0.11a	16.33±0.53a	18.10±3.25a	14.51±0.10a

Note: The difference is significant ($P < 0.05$) when the values (means ± standard deviation) of the same row are different. The mean differences were assessed via Analysis of Variance (ANOVA), with pairwise comparisons conducted using the Least Significant Difference (LSD) test.

Table S2 The relative importances (%) of the five assembly processes in communities of different *Daqus* generated from the null model

Kingdom	*Daqu* types	HeS	HoS	DL	HD	Drift	Stochastic process	Deterministic process
Bacteria	Original *Daqu*	20	0	23	3	55	80	20
	10% tea-added *Daqu*	5	1	37	3	55	94	6
	20% tea-added *Daqu*	14	0	60	1	25	86	14
	30% tea-added *Daqu*	22	1	36	1	39	77	23
Fungi	Original *Daqu*	1	0	40	1	58	99	1
	10% tea-added *Daqu*	3	0	85	1	11	97	3
	20% tea-added *Daqu*	4	0	79	0	17	96	4
	30% tea-added *Daqu*	10	2	46	1	42	88	12

Note: HeS=Heterogeneous Selection, HoS=Homogeneous Selection, DL=Dispersal Limitation, HD=Homogenizing Dispersal

Table S3 Fit of the Sloan's neutral model in microbial communities across different *Daqus*

Kingdom	Daqu types	R^2	*m*	medium (%)	high (%)	low (%)
Bacteria	Original *Daqu*	0.03	0.30	75.10	11.30	13.60
	10% tea-added *Daqu*	0.15	0.22	79.20	8.70	12.20
	20% tea-added *Daqu*	-0.071	0.19	76.00	10.00	14.00
	30% tea-added *Daqu*	0.30	0.32	80.20	7.60	12.10
Fungi	Original *Daqu*	0.58	0.30	80.30	9.00	10.60
	10% tea-added *Daqu*	0.34	0.08	70.50	15.60	14.00
	20% tea-added *Daqu*	0.31	0.06	72.90	15.40	11.70
	30% tea-added *Daqu*	-0.383	0.11	63.00	19.30	17.70

Supplementary figure

Fig. S1 Flow chart of *Daqu* preparation process (A) and the production procedure (B). In panel B: (a) raw material: green tea; (b) 50% of the green tea crushed to a particle size of 2-5 mm; (c) crushed wheat; (d) starter culture; (e) mixing of ingredients; (f)-(g) compression and molding; (h)-(i) molded *Daqu* blocks; (j)-(k) wrapped in straw and placed in box containers; (l) fermentation.