Supplementary Material

Probiotic-fermented tomato with hepatic lipid metabolism modulation effects: analysis of physicochemical properties, bioactivities, and potential bioactive compounds

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Table S1 The ingredients and energy of the normal chow diet and high-fat diet.

Ingredient	Normal chow diet (g/kg)	High-fat diet (g/kg)
Casein	190	267
L-Cystine	3	4
Maltodextrin	33	157
Sucrose	332	89
Soybean Oil	23	33
Lard	19	301
Corn Starch	299	0
Cellulose	62	67
Mineral Mix, M1020	27	66
Vitamin Mix, V1010	10	13
Choline Bitartrate	2	3
t-Butylhydroquinone	0.014	0.067
Calorie sources (kcal %)	Normal chow diet (kcal %)	High-fat diet (kcal %)
Proteins	20	20
Carbohydrates	70	20
Carbohydrates	10	60
Total calorie (kcal/g)	3.85	5

Table S2 Metabolite elution procedures and instrument parameters for non-targeted metabolomics analysis based on UPLC-QTOF/MS.

Item	Information and parameters
Chromatographic column	ZORBAX Eclipse Plus C18 column (2.1×100 mm, 1.8 μm-Micron)
Mobile phase	(A) water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid for positive and negative ion modes
UHPLC	Injection volume, 5 μL; column temperature, 35℃; flow rate, 0.3 mL/min
MS	Drying gas (N_2) flow rate, 10 L/min; gas temperature, 325°C; fragmentor 175 V; capillary voltage 3500 V; nebulizer, 25 psi; acquisition range 50-1200 m/z
Elution procedures	Positive and negative ion mode: 0-5 min, 0-15% B; 5-6 min, 15-25% B; 6-11.5 min, 25-42% B; 11.5-12.5 min, 42-44% B; 12.5-14 min, 44-50% B; 14-15 min, 50% B; 15-17 min, 50-60% B; 17-19 min, 60-70% B; 19-26 min, 70% B; 26-28 min, 70-98% B; 28-29 min, 98-10%; 29-32 min, 10% B

Table S3 The elution procedure and instrument parameters for quantification analysis based on UPLC-QQQ-MS.

Item	Information and parameters
Chromatographic column	Zorbax Eclipse XDB-C18 column (4.6×250 mm, 5 μm-Micron)
Mobile phase	(A) water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid for positive and negative ion modes
HPLC	Injection volume, 5 μL; column temperature, 30℃; flow rate, 0.3 mL/min
MS	Drying gas (N_2) flow rate, 12 L/min; gas temperature, 350°C; fragmentor 175 V; capillary voltage 4000 V; nebulizer, 35 psi
Elution procedures	Positive and negative ion mode: 0-2 min, 10-50% B; 2-5 min, 50-80%; 5-22 min, 80%; 22-26 min, 80-98%; 26-28 min, 98%

Table S4 The optimized mass spectrometry parameters and standard curve information for quantification analysis based on UPLC-QQQ-MS.

Componds	Retention time (min)	Calibration curve equation	R^2	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (eV)	LOD (ng/mL)	LOQ (ng/mL)
Chlorogenic acid	11.340	Y=27.618X-580.43	0.9995	353.3	191.0	100	10	7.663	30.966
Capsiate	12.231	Y=4440X-1800.2	0.9933	307.4	156.9	130	30	7.369	28.746
Tiliroside	11.316	Y=2646X-1042.7	0.9966	593.5	284.8	105	40	8.660	34.259
Irisflorentin	15.570	Y=2376.3X+177.3	0.9986	387.4	270.9	125	45	13.56	56.023
Homoeriodictyol	13.337	Y=676.35X+24.345	0.9964	301.3	133.9	130	35	10.264	37.659

Note: X indicats concentration, Y indicats peak area.

Table S5 The gene primers used for real-time quantitative PCR.

Gene	Forward primer	Reverse primer
AMPK	GACAAGCCCACCTGATTC	TTCCTTCGTACACGCAAA
ACC	CAACTTTGTGCCCACGGTTA	TTTGTCAGGAAGAGGCGGAT
CPT-1	GGACTCCGCTCGCTCATT	GAGATCGATGCCATCAGGGG
SREBP-1c	CAGACTCACTGCTGCTGACA	GATGGTCCCTCCACTCACCA
FAS	TGCCCAAGTGACTGACATCA	CATCCCCATTGACTGTGCAG
PPARα	TGCCTTCCCTGTGAACTGAC	TGGGGAGAGAGGACAGATG G
GAPDH	TCAAGAAGGTGGTGAAGCAGG	TCAAAGGTGGAGGAGTGGGT

Table S6 Contents of organic acids and sugars in UFT, LFT, and SFT.

Organic acid	UFT	LFT	SFT
Citric acid	6.694 ± 0.147[a]	6.634 ± 0.352[a]	6.694 ± 0.718[a]
Isovaleric acid	2.102 ± 0.008[a]	2.164 ± 0.058[a]	2.138 ± 0.152[a]
Butyric acid	0[c]	0.502 ± 0.043[a]	0.377 ± 0.033[b]
Acetic acid	1.154 ± 0.027[a]	1.364 ± 0.114[a]	1.284 ± 0.095[a]
Lactic acid	1.838 ± 0.042[b]	11.182 ± 0.278[a]	11.080 ± 0.437[a]
Malic acid	0.679 ± 0.014[a]	0.139 ± 0.018[b]	0.012 ± 0.006[c]
Total	12.467 ± 0.238[b]	21.984 ± 0.686[a]	21.585 ± 1.413[a]
Sugar	UFT	LFT	SFT
Fructose	15.918 ± 0.801[a]	0[b]	0[b]
Glucose	16.074 ± 0.773[a]	1.249 ± 0.035[b]	1.244 ± 0.235[b]
Sucrose	0.538 ± 0.004[a]	0.566 ± 0.026[a]	0.555 ± 0.069[a]
Total	32.531 ± 0.24[a]	1.815 ± 0.058[b]	1.799 ± 0.276[b]

Note: unit: mg/mL; different lowercase letters represented statistically significant differences between groups ($P < 0.05$).

Table S7 Contents of free amino acids in UFT, LFT, and SFT.

Taste	Free amino acid	UFT	LFT	SFT
Umami amino acid	Asp	1.801 ± 0.092[a]	1.822 ± 0.019[a]	1.834 ± 0.022[a]
	Glu	9.216 ± 0.353[b]	9.888 ± 0.074[a]	9.184 ± 0.090[b]
	Total	11.017 ± 0.445[b]	11.710 ± 0.057[a]	11.018 ± 0.073[b]
Sweet amino acid	Thr	0.450 ± 0.023[b]	0.556 ± 0.005[a]	0.558 ± 0.006[a]
	Ser	2.843 ± 0.260[a]	2.114 ± 0.025[b]	1.317 ± 0.053[c]
	Gly	0.036 ± 0.001[c]	0.129 ± 0.010[a]	0.087 ± 0.007[b]
	Ala	0.222 ± 0.021[a]	0.059 ± 0.016[b]	0.058 ± 0.015[b]
	Total	3.552 ± 0.282[a]	2.857 ± 0.007[b]	2.021 ± 0.064[c]
Bitter amino acid	His	4.120 ± 0.281[b]	5.227 ± 0.075[a]	5.182 ± 0.062[a]
	Val	0.139 ± 0.009[a]	0[b]	0[b]
	Ile	0.148 ± 0.011[a]	0[b]	0[b]
	Leu	0.118 ± 0.014[a]	0[b]	0[b]
	Arg	0.179 ± 0.012[a]	0.075 ± 0.001[a]	0.163 ± 0.117[a]
	Phe	0.444 ± 0.072[a]	0.073 ± 0.003[b]	0.042 ± 0.037[b]
	Total	5.148 ± 0.298[a]	5.375 ± 0.077[a]	5.387 ± 0.203[a]
Tasteless amino acid	Cys	0[b]	0.060 ± 0.008[a]	0.004 ± 0.008[b]
	Tyr	0.212 ± 0.010[a]	0[b]	0[b]
	Lys	0.270 ± 0.010[a]	0.210 ± 0.002[b]	0.207 ± 0.027[b]
	Total	0.482 ± 0.017[a]	0.270 ± 0.010[b]	0.211 ± 0.031[c]
Total		20.199 ± 0.899[a]	20.213 ± 0.039[a]	18.170 ± 1.026[b]

Note: unit: mg/100 mL; different lowercase letters represented statistically significant differences between groups ($P < 0.05$).

Table S8 Detailed information of differential metabolites between UFT and LFT groups.

Retention time (min)	Metabolites	Mass	m/z	Adducts	MS/MS	Formula	VIP	FC	Trend	*P*	CAS
16.213	Capsiate	306.1839	307.191	[M+H]+	289.1802, 261.1841	$C_{18}H_{26}O_4$	1.36	2.05	↑	***	205687-01-0
21.205	[7]-Paradol	292.2033	293.2106	[M+H]+	135.0798, 69.0684	$C_{18}H_{28}O_3$	1.28	77109.17	↓	***	53172-04-6
5.381	trans-p-Coumaric acid 4-glucoside	326.1003	349.0892	[M+Na]+	291.0496, 185.0435	$C_{15}H_{18}O_8$	1.47	12.04	↓	***	14364-05-7
2.724	p-Hydroxycinnamaldehyde	148.0522	149.0593	[M+H]+	121.0267, 103.0539	$C_9H_8O_2$	1.81	2.06	↓	***	2538-87-6
5.092	Chlorogenic acid	354.0963	353.0889	[M-H]-	191.0564, 59.0137	$C_{16}H_{18}O_9$	2.00	14.28	↑	***	327-97-9
4.181	Irisflorentin	386.0997	385.0922	[M-H]-	191.9471, 126.5486	$C_{20}H_{18}O_8$	1.67	20.80	↑	***	41743-73-1
4.776	Tiliroside	594.1358	593.1285	[M-H]-	346.9605, 280.7779	$C_{30}H_{26}O_{13}$	1.47	12.56	↑	***	20316-62-5
7.858	Homoeriodictyol	302.0796	301.0723	[M-H]-	214.8608, 189.0197	$C_{16}H_{14}O_6$	1.69	13.11	↑	***	446-71-9
2.792	Cryptomeridiol 11-rhamnoside	386.265	387.2723	[M+H]+	339.1546, 158.9257	$C_{21}H_{38}O_6$	3.86	34.32	↓	***	349112-30-7
8.577	Tuliposide B	294.096	295.103	[M+H]+	207.0267, 149.0208	$C_{11}H_{18}O_9$	1.71	5.25	↓	***	19870-33-8
14.854	Isopetasoside	396.2133	395.207	[M-H]-	327.2203, 192.9570	$C_{21}H_{32}O_7$	1.62	356471.33	↓	***	69809-29-6
24.392	Dibutyl phthalate	278.1526	279.1598	[M+H]+	149.0238, 121.0296	$C_{16}H_{22}O_4$	1.93	687300.50	↑	***	84-74-2
11.1	Tetrahydrofurfuryl butyrate	172.1101	171.1028	[M-H]-	86.9752, 68.9965	$C_9H_{16}O_3$	1.58	12.29	↓	***	92345-48-7
24.385	12-Hydroxy-8,10-octadecadienoic acid	296.2357	297.2431	[M+H]+	95.0500, 81.0700	$C_{18}H_{32}O_3$	2.37	27.34	↑	***	170171-31-0
22.675	4,8,12,15-Octadecatetraenoic acid	276.2081	277.2161	[M+H]+	133.0991, 93.0699	$C_{18}H_{28}O_2$	1.66	3.22	↓	***	67329-10-6
15.49	3,4-Dimethyl-5-pentyl-2-furanheptanoic acid	294.2188	295.226	[M+H]+	165.0365, 135.0801	$C_{18}H_{30}O_3$	3.01	1681709.50	↑	***	92745-17-0
18.95	γ-Linolenic acid	278.2239	279.2311	[M+H]+	135.0794, 69.0341	$C_{18}H_{30}O_2$	1.84	10.85	↑	***	506-26-3
15.621	8R-HpODE	312.2301	313.2374	[M+H]+	141.1264, 99.0798	$C_{18}H_{32}O_4$	5.63	20.18	↓	***	-
15.627	9S,12S,13S-trihydroxy-10E-octadecenoic acid	330.2415	329.2342	[M-H]-	197.1178, 113.0959	$C_{18}H_{34}O_5$	7.24	27.70	↑	***	-
21.824	18-Oxooleate	296.2357	295.2285	[M-H]-	277.2172, 195.1372	$C_{18}H_{32}O_3$	2.13	3.72	↑	***	-
14.705	Traumatic acid	228.1362	227.1293	[M-H]-	209.1178, 88.9883	$C_{12}H_{20}O_4$	1.19	194055.50	↓	***	6402-36-4
1.489	Malic acid	134.0217	133.0144	[M-H]-	71.0140, 59.0134	$C_4H_6O_5$	4.17	74.24	↓	***	6915-15-7
1.957	L-Aspartic acid	133.0371	132.0298	[M-H]-	115.0017, 71.0123	$C_4H_7NO_4$	1.21	4.02	↑	***	56-84-8
5.757	Leucyl-Isoleucine	244.1797	243.1723	[M-H]-	83.0115, 68.9959	$C_{12}H_{24}N_2O_3$	1.64	96925.83	↓	***	-
1.594	N-Acryloylglycine	129.0426	130.0499	[M+H]+	84.0439, 56.0490	$C_5H_7NO_3$	11.06	3.87	↑	***	24599-25-5

Table S9 Concentrations of different phenolic compounds in different samples measured by UPLC-QQQ-MS.

Phenolic compound	UFT	LFT	SFT
Chlorogenic acid (CA)	62.451 ± 3.741	570.394 ± 21.246	151.511 ± 1.722
Capsiate (CS)	0.497 ±0.010	0.891 ± 0.024	0.738 ± 0.049
Tiliroside (TR)	0.480 ± 0.008	2.230 ± 0.034	1.691 ± 0.065
Irisflorentin (IF)	0.161 ± 0.024	4.104 ± 0.255	0.165 ± 0.010
Homoeriodictyol (HE)	1.888 ± 0.301	12.606 ± 0.533	9.607 ± 1.821

Note: unit: μg/g; different lowercase letters represented statistically significant differences between groups ($P < 0.05$).

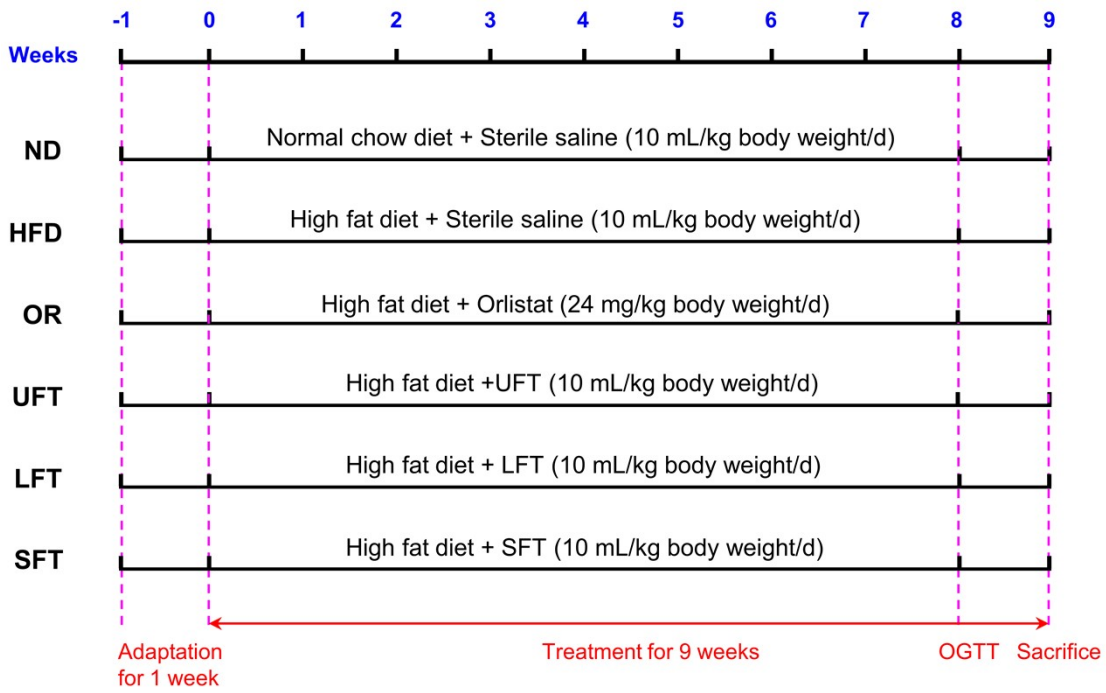

Fig. S1 The treatment flow chart of animal experiment design.

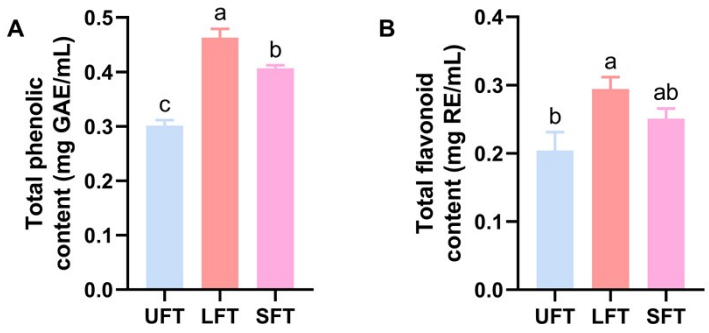

Fig. S2 Total phenolic content and total flavonoid content of tomato before and after fermentation.

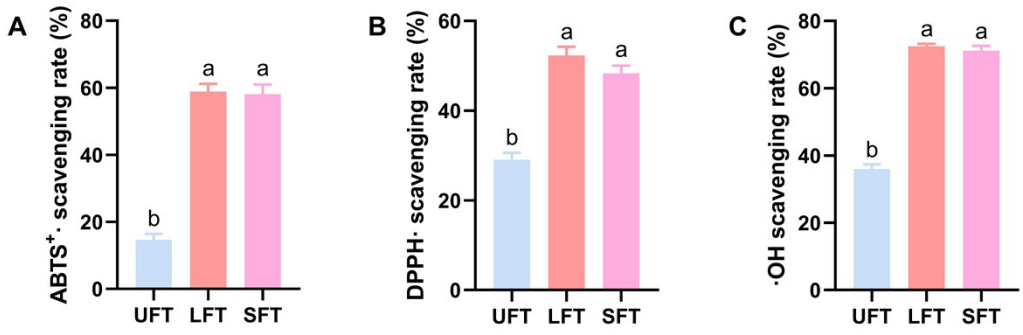

Fig. S3 Changes in antioxidant activities of tomato before and after fermentation.

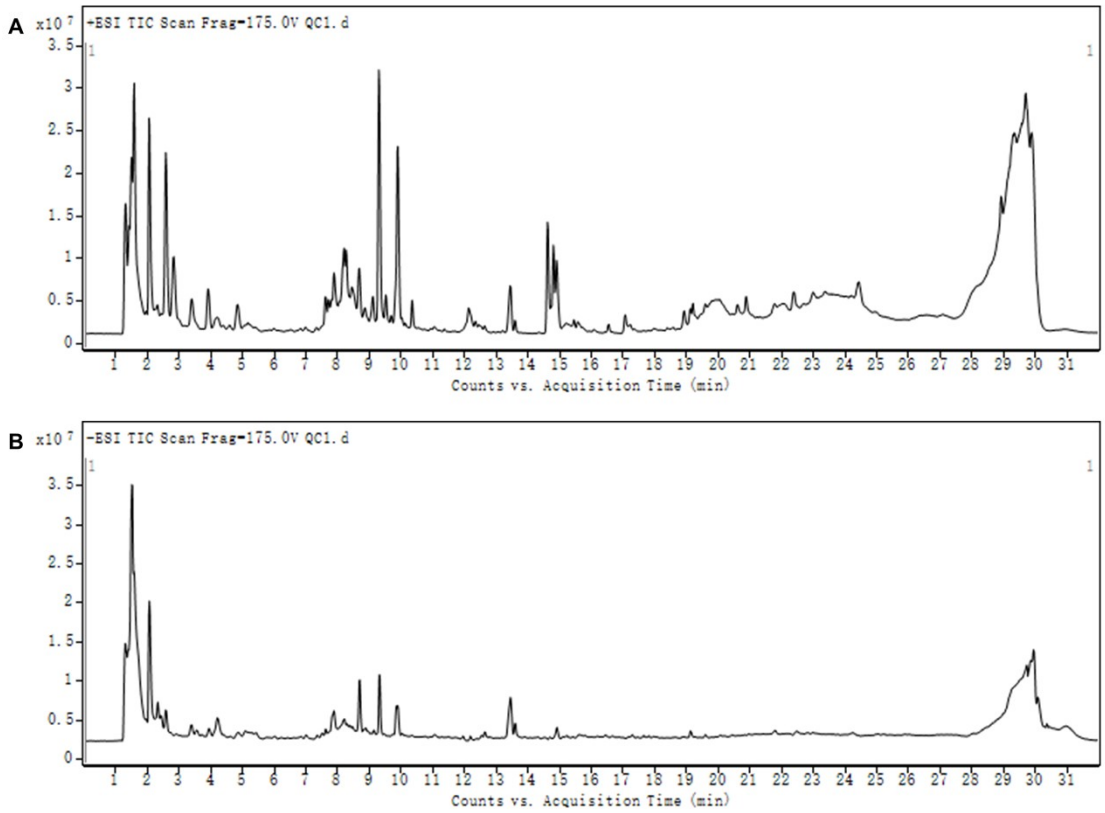

Fig. S4 Total ion chromatogram (TIC) of the QC sample in (A) positive ion mode and (B) negative ion mode.

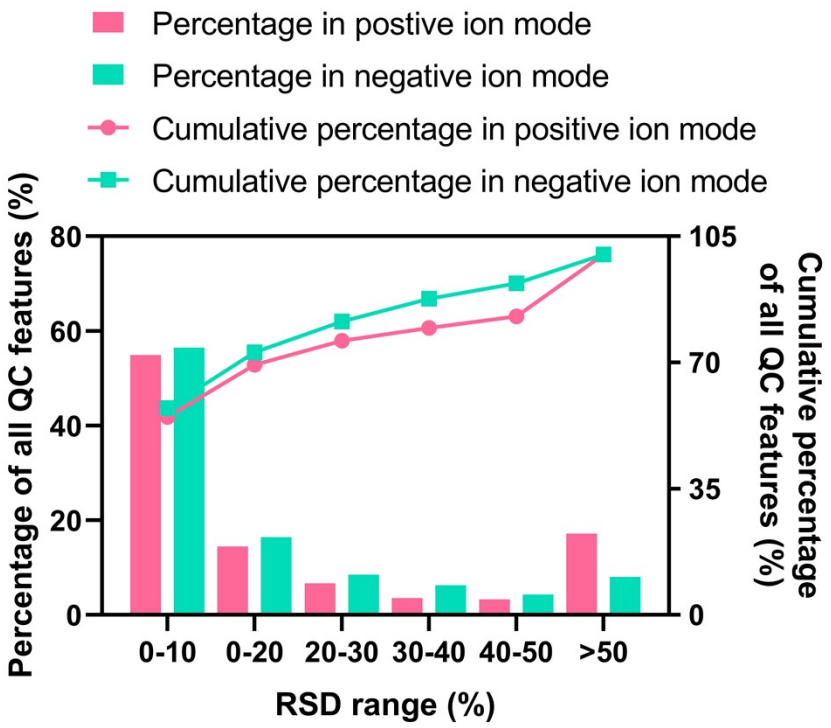

Fig. S5 Relative standard deviation (RSD) distribution of ion features in QC sample.