



Supplementary Materials

Enhancing the Chemical Composition of Kombucha Fermentation by Adding Indian Gooseberry as a Substrate

Tharinee Klapiyapamornkun ¹, Toungporn Uttarotai ², Sunanta Wangkarn ³, Panee Sirisa-ard ⁴,
Suwalee Kiatkarun ⁵, Yingmanee Tragoolpua ^{1,6,*} and Sakunnee Bovonsombut ^{1,*}

¹ Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

² Department of Highland Agriculture and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

³ Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

⁴ Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

⁵ Tea Gallery Group (Thailand) Co., Ltd., Chiang Mai 50000, Thailand

⁶ Research Center in Bioresources for Agriculture, Industry, and Medicine, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

* Correspondence: yingmanee.t@cmu.ac.th (Y.T.); sakunnee.b@cmu.ac.th (S.B.)

Supplementary Data

Table S1. Total phenolic content changes during fermentation.

Time (days)	Total phenolic content (mg GAE/ml)				
	TK	IGJK	IGK	DIGK	IGJ
0	0.78 ^c ± 0.01	4.18 ^a ± 0.02	3.80 ^{ab} ± 0.63	2.92 ^b ± 0.94	3.09 ^{ab} ± 0.07
3	1.20 ^c ± 0.01	4.89 ^{ab} ± 0.54	5.18 ^a ± 0.27	5.78 ^a ± 0.15	4.05 ^b ± 0.07
6	0.67 ^e ± 0.03	4.51 ^c ± 0.10	4.84 ^b ± 0.00	5.16 ^a ± 0.15	2.93 ^d ± 0.17
9	0.69 ^e ± 0.08	4.79 ^c ± 0.28	5.26 ^b ± 0.00	6.02 ^a ± 0.38	3.12 ^d ± 0.16
12	0.63 ^d ± 0.09	5.50 ^b ± 0.13	5.28 ^b ± 0.01	6.30 ^a ± 0.00	3.40 ^c ± 0.12
15	0.80 ^d ± 0.02	5.30 ^b ± 0.15	5.59 ^b ± 0.21	6.34 ^a ± 0.28	3.55 ^c ± 0.10
18	0.62 ^d ± 0.09	5.65 ^b ± 0.60	5.58 ^b ± 0.38	7.03 ^a ± 0.00	3.67 ^c ± 0.29
21	0.82 ^d ± 0.02	5.72 ^b ± 0.10	5.92 ^b ± 0.72	7.08 ^a ± 0.55	3.85 ^c ± 0.08

Values are expressed as mean ± standard deviation. Different letter in the same raw indicate significantly different values ($p < 0.05$). ND: not detected. Traditional kombucha (TK), Indian gooseberry juice kombucha (IGJK), Indian gooseberry kombucha (IGK), dried Indian gooseberry kombucha (DIGK) and Fermented Indian gooseberry juice (IGJ).

Table S2. Total flavonoid content changes during fermentation.

Time (days)	Total flavonoid content (mg QE/ml)				
	TK	IGJK	IGK	DIGK	IGJ
0	0.91 ^c ± 0.06	2.81 ^a ± 0.24	1.84 ^b ± 0.06	1.87 ^b ± 0.38	0.61 ^c ± 0.03
3	1.32 ^b ± 0.01	2.77 ^a ± 0.16	2.79 ^a ± 0.30	2.87 ^a ± 0.04	0.77 ^c ± 0.09
6	0.61 ^d ± 0.16	2.00 ^b ± 0.03	1.79 ^c ± 0.02	2.33 ^a ± 0.00	0.56 ^d ± 0.01
9	0.84 ^c ± 0.07	2.21 ^{ab} ± 0.13	1.90 ^b ± 0.29	2.53 ^a ± 0.07	0.56 ^c ± 0.01
12	0.64 ^c ± 0.00	2.04 ^b ± 0.00	2.21 ^b ± 0.21	2.68 ^a ± 0.07	0.60 ^c ± 0.03
15	0.70 ^c ± 0.03	2.53 ^b ± 0.08	2.43 ^b ± 0.15	3.04 ^a ± 0.21	0.61 ^c ± 0.02
18	0.28 ^b ± 0.07	2.29 ^a ± 0.23	2.14 ^a ± 0.36	2.70 ^a ± 0.25	0.64 ^b ± 0.09
21	0.72 ^c ± 0.05	2.59 ^b ± 0.05	2.55 ^b ± 0.09	3.16 ^a ± 0.14	0.64 ^c ± 0.03

Values are expressed as mean ± standard deviation. Different letter in the same raw indicate significantly different values ($p < 0.05$). ND: not detected. Traditional kombucha (TK), Indian

gooseberry juice kombucha (IGJK), Indian gooseberry kombucha (IGK), dried Indian gooseberry kombucha (DIGK) and Fermented Indian gooseberry juice (IGJ).

Table S3. The results of HPLC analysis of organic acids during fermentation.

Time (days)	Samples	Organic acid (mg/ml)			
		Gluconic acid	DSL	Ascorbic acid	Acetic acid
0	TK	1.44 ^c ± 0.06	0.13 ^b ± 0.02	0.05 ^e ± 0.00	0.49 ^d ± 0.01
	IGJK	3.62 ^a ± 0.14	0.93 ^a ± 0.33	9.12 ^a ± 0.24	35.15 ^a ± 2.12
	IGK	2.47 ^b ± 0.16	0.50 ^b ± 0.07	6.70 ^c ± 0.72	14.66 ^c ± 1.09
	DIGK	2.13 ^b ± 0.31	0.73 ^a ± 0.34	5.58 ^d ± 0.02	23.71 ^b ± 4.38
	IGJ	3.43 ^a ± 0.40	0.75 ^a ± 0.25	7.87 ^b ± 0.06	26.70 ^b ± 0.59
3	TK	1.86 ^d ± 0.49	0.64 ^b ± 0.16	0.10 ^d ± 0.00	1.12 ^c ± 0.08
	IGJK	4.21 ^a ± 0.92	0.38 ^b ± 0.08	5.99 ^b ± 0.10	33.67 ^b ± 0.80
	IGK	2.58 ^c ± 0.73	0.48 ^b ± 0.06	6.61 ^a ± 0.06	35.51 ^b ± 0.67
	DIGK	2.77 ^c ± 0.81	1.90 ^a ± 0.23	5.22 ^c ± 0.15	58.43 ^a ± 3.96
	IGJ	3.71 ^b ± 0.77	1.66 ^a ± 0.18	5.79 ^b ± 0.00	31.98 ^b ± 2.95
6	TK	2.02 ^d ± 0.00	0.22 ^c ± 0.00	0.02 ^d ± 0.00	4.33 ^e ± 0.00
	IGJK	5.12 ^b ± 0.06	0.94 ^b ± 0.14	5.61 ^a ± 0.04	39.77 ^b ± 0.74
	IGK	3.16 ^c ± 0.00	0.31 ^c ± 0.00	5.56 ^a ± 0.00	26.93 ^d ± 0.00
	DIGK	3.28 ^c ± 0.00	1.62 ^a ± 0.00	5.33 ^b ± 0.00	63.05 ^a ± 0.00
	IGJ	5.54 ^a ± 0.00	1.56 ^a ± 0.00	5.18 ^c ± 0.00	36.06 ^c ± 0.00
9	TK	3.85 ^e ± 0.00	1.52 ^d ± 0.00	0.04 ^e ± 0.00	4.87 ^e ± 0.00
	IGJK	8.85 ^b ± 0.00	2.08 ^{bc} ± 0.00	9.61 ^b ± 0.00	35.68 ^b ± 0.00
	IGK	5.79 ^d ± 0.00	1.83 ^{cd} ± 0.00	10.35 ^a ± 0.00	24.99 ^d ± 0.00
	DIGK	6.72 ^c ± 0.22	4.04 ^a ± 0.24	9.52 ^c ± 0.04	52.28 ^a ± 0.05
	IGJ	10.20 ^a ± 0.00	2.24 ^b ± 0.00	9.21 ^d ± 0.00	31.25 ^c ± 0.00
12	TK	5.03 ^c ± 0.52	1.20 ^c ± 0.04	0.04 ^c ± 0.00	6.71 ^d ± 0.56
	IGJK	10.10 ^a ± 0.30	1.60 ^{bc} ± 0.09	9.13 ^b ± 0.11	43.33 ^b ± 1.06
	IGK	5.79 ^c ± 0.52	1.83 ^{ab} ± 0.22	10.47 ^a ± 0.23	33.69 ^c ± 2.87
	DIGK	8.24 ^b ± 0.00	2.14 ^a ± 0.00	10.01 ^a ± 0.00	56.92 ^a ± 0.00
	IGJ	11.24 ^a ± 0.07	1.28 ^c ± 0.15	8.84 ^b ± 0.16	34.50 ^c ± 2.22
15	TK	5.73 ^c ± 0.92	0.72 ^d ± 0.14	0.07 ^c ± 0.01	10.26 ^c ± 1.31
	IGJK	10.22 ^a ± 0.41	0.39 ^e ± 0.03	6.44 ^b ± 0.22	34.81 ^b ± 1.08
	IGK	5.33 ^c ± 0.84	1.52 ^a ± 0.01	7.41 ^a ± 0.31	32.64 ^b ± 2.97
	DIGK	7.94 ^b ± 1.36	1.15 ^b ± 0.09	6.18 ^b ± 0.16	73.39 ^a ± 3.66
	IGJ	11.48 ^a ± 0.55	0.95 ^c ± 0.07	6.31 ^b ± 0.14	31.03 ^b ± 0.98
18	TK	3.86 ^b ± 0.26	0.44 ^c ± 0.21	0.05 ^d ± 0.00	16.37 ^d ± 1.95
	IGJK	11.76 ^a ± 1.81	0.90 ^{bc} ± 0.11	8.82 ^c ± 0.13	44.50 ^b ± 5.10
	IGK	4.25 ^b ± 1.13	0.98 ^{bc} ± 0.36	9.57 ^b ± 0.32	33.13 ^c ± 4.29
	DIGK	11.11 ^a ± 0.73	1.68 ^a ± 0.27	10.20 ^a ± 0.57	73.42 ^a ± 1.80
	IGJ	12.05 ^a ± 0.76	1.03 ^b ± 0.23	8.52 ^c ± 0.22	35.95 ^c ± 1.47
21	TK	10.46 ^b ± 0.82	0.75 ^{bc} ± 0.11	0.14 ^e ± 0.07	16.71 ^d ± 1.06
	IGJK	13.34 ^a ± 0.97	0.45 ^c ± 0.17	6.28 ^c ± 0.02	36.21 ^b ± 0.92
	IGK	6.93 ^c ± 1.68	1.04 ^{ab} ± 0.31	6.69 ^b ± 0.10	36.24 ^b ± 1.59
	DIGK	15.35 ^a ± 1.39	1.26 ^a ± 0.01	6.95 ^a ± 0.21	78.28 ^a ± 0.67
	IGJ	15.03 ^a ± 0.39	0.44 ^c ± 0.13	5.91 ^d ± 0.06	31.76 ^c ± 0.82

Values are expressed as mean ± standard deviation. Different letter in the same raw indicate significantly different values ($p < 0.05$). ND: not detected. Traditional kombucha (TK), Indian gooseberry juice kombucha (IGJK), Indian gooseberry kombucha (IGK), dried Indian gooseberry kombucha (DIGK) and Fermented Indian gooseberry juice (IGJ).

Table S4. Relative increase of phenolic content.

Time (days)	Relative change of phenolic content (%)				
	TK	IGJK	IGK	DIGK	IGJ
3	53.72	17.01	36.27	98.32	31.12
6	-14.52	7.93	27.34	76.84	-5.30
9	-11.68	14.65	38.54	106.35	0.89
12	-18.82	31.52	39.03	115.98	10.17
15	3.28	26.82	47.16	117.48	14.91
18	-20.57	35.20	46.88	140.94	18.75
21	4.85	36.77	55.97	142.87	24.50

Table S5. Relative increase of flavonoid content.

Time (days)	Relative change of flavonoid content (%)				
	TK	IGJK	IGK	DIGK	IGJ
3	44.26	-1.43	51.59	53.64	25.28
6	-32.96	-28.96	-2.65	24.59	-8.43
9	-8.47	-21.47	3.43	35.43	-8.43
12	-30.13	-27.43	20.26	43.73	-1.40
15	-23.54	-10.20	32.26	62.71	-0.47
18	-69.68	-18.76	16.29	44.42	5.15
21	-21.34	-8.06	38.81	69.09	4.68

Table S6. Relative increase of organic acids.

Time (days)	Relative change of organic acids (%)				
	Gluconic acid				
TK	IGJK	IGK	DIGK	IGJ	
3	29.17	16.38	4.29	29.98	8.30
6	39.89	41.41	27.74	53.80	61.58
9	166.79	144.47	134.44	215.61	197.58
12	248.78	179.10	134.33	287.08	227.68
15	297.08	182.47	115.87	272.82	234.73
18	167.27	224.84	72.13	421.66	251.46
21	625.22	268.60	180.40	620.96	338.35

D-saccharic acid-1,4-lactone (DSL)					
3	183.71	-59.31	-3.70	159.09	53.51
6	-82.74	1.14	-37.78	120.69	107.32
9	1066.94	124.25	265.79	452.05	196.93
12	817.44	72.44	365.45	192.77	69.37
15	454.92	-58.42	204.65	57.57	25.87
18	238.50	-3.25	154.04	128.88	15.74
21	479.24	-51.79	107.58	38.66	-42.17

Ascorbic acid					
3	95.72	-35.48	-7.96	-8.91	-27.17
6	-57.12	-39.54	-23.09	-4.15	-34.79
9	-13.97	3.47	43.12	71.26	15.84

12	-13.02	-1.70	44.89	80.19	11.20
15	46.60	-33.24	8.30	8.88	-22.33
18	5.56	-5.72	28.50	83.52	9.44
21	345.98	-32.40	-8.93	25.07	-26.03

Acetic acid					
3	124.90	-10.90	125.80	80.81	16.17
6	769.50	5.25	72.44	110.94	30.99
9	877.94	-5.58	60.02	74.91	13.52
12	1247.42	14.67	115.73	90.43	25.33
15	2329.45	-11.05	95.01	150.75	16.97
18	2682.90	29.50	138.37	145.64	34.72
21	3090.45	-2.46	124.56	161.90	11.71

(TK), Indian gooseberry juice kombucha (IGJK), Indian gooseberry kombucha (IGK), dried Indian gooseberry kombucha (DIGK) and Fermented Indian gooseberry juice (IGJ).