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APPENDICES

APPENDIX 1: EXPERIMENTAL DATA

1.1 pH Analysis

pH values of yogurts during first 5 hours of fermentation at 41˚C

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	6.35	6.26	6.31	6.35
	60	6.19	6.24	6.18	6.18
	120	6.42	5.34	5.40	5.31
	180	4.98	4.93	4.98	4.89
	240	4.72	4.75	4.67	4.59
	300	4.58	4.48	4.52	4.42

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	6.35	6.26	6.34	6.28
	60	6.27	6.26	6.26	6.27
	120	6.13	6.17	5.99	6.10
	180	5.81	5.84	5.57	5.75
	240	5.14	5.43	4.99	5.13
	300	4.50	4.58	4.48	4.47

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	6.43	6.41	6.42	6.40
	60	6.17	6.17	6.10	6.19
	120	5.54	5.50	5.49	5.69
	180	5.00	4.97	4.91	4.94
	240	4.63	4.72	4.65	4.62
	300	4.50	4.56	4.50	4.45

The pH values of yogurt during fermentation (average of 3 batches)

Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	6.37±0.026	6.31±0.050	6.35±0.032	6.34±0.034
60	6.21±0.030	6.22±0.027	6.18±0.046	6.21±0.028
120	5.69±0.219	5.67±0.254	5.62±0.183	5.70±0.228
180	5.26±0.271	5.24±0.296	5.15±0.209	5.19±0.278
240	4.83±0.157	4.96±0.231	4.77±0.110	4.78±0.175
300	4.52±0.026	4.54±0.030	4.50±0.011	4.44±0.014

pH values of yogurt during 28 days storage period at 4˚C

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	4.30	4.32	4.34	4.27
	7	4.15	4.07	4.08	4.06
	14	4.23	4.12	4.18	4.14
	21	4.15	4.13	4.15	4.11
	28	4.21	4.18	4.15	4.15

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	4.44	4.48	4.40	4.42
	7	4.28	4.28	4.22	4.14
	14	4.25	4.29	4.26	4.21
	21	4.27	4.25	4.30	4.21
	28	4.26	4.29	4.31	4.19

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	4.36	4.36	4.31	4.26
	7	4.18	4.20	4.21	4.15
	14	4.25	4.20	4.14	4.09
	21	3.96	3.99	3.90	3.82
	28	3.83	3.91	3.83	3.70

The pH values of yogurt during storage period (average of 3 batches)

Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	4.36±0.040	4.38±0.048	4.35±0.026	4.31±0.051
7	4.20±0.039	4.18±0.061	4.17±0.045	4.11±0.028
14	4.24±0.006	4.20±0.049	4.19±0.035	4.14±0.034
21	4.12±0.090	4.12±0.075	4.11±0.116	4.04±0.116
28	4.10±0.135	4.12±0.112	4.09±0.141	4.01±0.157

1.2 Titratable Acidity (TA) Analysis

Volume of NaOH (ml) used in TA analysis during 5 hours fermentation at 41˚C

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	0.36	0.36	0.36	0.36
	60	0.36	0.36	0.45	0.45
	120	0.63	0.63	0.72	0.72
	180	0.81	0.72	0.99	0.90
	240	0.90	0.90	1.08	1.08
	300	1.08	1.08	1.08	1.17

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	0.36	0.36	0.45	0.36
	60	0.45	0.45	0.54	0.54
	120	0.45	0.45	0.54	0.54
	180	0.54	0.63	0.63	0.63
	240	0.81	0.72	0.99	0.81
	300	0.99	1.08	1.08	1.08

	Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	0.18	0.09	0.09	0.09
	60	0.27	0.27	0.27	0.27
	120	0.36	0.36	0.45	0.36
	180	0.54	0.54	0.63	0.54
	240	0.63	0.63	0.72	0.63
	300	0.72	0.72	0.81	0.72

The TA values of yogurt during fermentation (average of 3 batches)

Time	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	0.30±0.060	0.27±0.090	0.30±0.108	0.27±0.090
60	0.36±0.051	0.36±0.051	0.42±0.079	0.42±0.079
120	0.48±0.079	0.48±0.079	0.57±0.079	0.54±0.103
180	0.63±0.090	0.63±0.051	0.75±0.120	0.69±0.108
240	0.78±0.079	0.75±0.079	0.93±0.108	0.84±0.130
300	0.93±0.108	0.96±0.120	0.99±0.090	0.99±0.137

TA values of yogurt during 28 days storage period at 4˚C

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	1.08	1.08	1.17	1.08
	7	1.35	1.35	1.53	1.44
	14	1.08	1.35	1.35	1.35
	21	1.17	1.35	1.35	1.17
	28	1.35	1.44	1.44	1.35

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	0.99	0.99	1.08	1.17
	7	1.08	1.08	1.17	1.26
	14	1.17	1.26	1.35	1.26
	21	1.35	1.26	1.53	1.35
	28	1.44	1.26	1.44	1.44

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	0.81	0.90	0.90	0.90
	7	0.90	0.90	0.90	0.99
	14	0.90	0.90	0.99	0.99
	21	0.90	0.90	0.99	0.90
	28	0.99	0.99	1.08	0.99

TA values of yogurt during storage period (average of 3 batches)

Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	0.96±0.079	0.99±0.051	1.05±0.079	1.05±0.079
7	1.11±0.130	1.11±0.130	1.20±0.182	1.23±0.130
14	1.05±0.079	1.17±0.137	1.23±0.120	1.20±0.108
21	1.14±0.130	1.17±0.137	1.29±0.158	1.14±0.130
28	1.26±0.137	1.23±0.130	1.32±0.120	1.26±0.137

1.3 Viable Cell Count Analysis (CFU)

Viable *Streptococcus thermophilus* count (cfu/ml^{-1}) in yogurt during 28 days storage period at 4˚C

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	76 x 10^6	73 x 10^6	51 x 10^6	90 x 10^6
	7	153 x 10^6	132 x 10^6	148 x 10^6	147 x 10^6
	14	131 x 10^6	171 x 10^6	194 x 10^6	121 x 10^6
	21	72 x 10^6	96 x 10^6	101 x 10^6	67 x 10^6
	28	71 x 10^6	89 x 10^6	113 x 10^6	119 x 10^6

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	115 x 10^6	65 x 10^6	95x 10^6	120 x 10^6
	7	106 x 10^6	172 x 10^6	165 x 10^6	129 x 10^6
	14	117 x 10^6	97 x 10^6	97 x 10^6	113 x 10^6
	21	89 x 10^6	80 x 10^6	25 x 10^6	109 x 10^6
	28	104 x 10^6	27 x 10^6	39 x 10^6	58 x 10^6

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	121 x 10^6	150 x 10^6	111 x 10^6	147 x 10^6
	7	87 x 10^6	110 x 10^6	117 x 10^6	111 x 10^6
	14	124 x 10^6	102 x 10^6	89 x 10^6	86 x 10^6
	21	130 x 10^6	173 x 10^6	145 x 10^6	147 x 10^6
	28	101 x 10^6	143 x 10^6	97 x 10^6	42 x 10^6

Viable *S. thermophilus* count (10^6 cfu/ml^{-1}) during storage period (average of 3 batches)

Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	104±14.10	96±027.09	85±17.93	119±16.46
7	115±19.61	138±18.14	143±14.05	129±10.39
14	124±4.04	123±23.87	126±33.74	106±10.58
21	97±17.21	116±28.70	90±35.04	107±23.10
28	92±10.53	86±33.51	83±22.47	73±23.45

Viable *Lactobacillus* spp. count (cfu/ml[1]) in yogurt during 28 days storage period at 4˚C

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
1st Batch	0	26.3×10^6	16.3×10^6	21.4×10^6	31.5×10^6
	7	19.2×10^6	17.3×10^6	14.5×10^6	19.4×10^6
	14	6.1×10^6	7.6×10^6	19.4×10^6	32×10^6
	21	6.8×10^6	8.6×10^6	13.2×10^6	8.5×10^6
	28	5.2×10^6	8.9×10^6	7.9×10^6	6.1×10^6

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
2nd Batch	0	6.7×10^6	6.5×10^6	10.2×10^6	10.9×10^6
	7	15.4×10^6	7.2×10^6	10.4×10^6	8.3×10^6
	14	6.4×10^6	9.7×10^6	6.8×10^6	5.6×10^6
	21	1.7×10^6	8.5×10^6	4.7×10^6	2.5×10^6
	28	2.9×10^6	2.7×10^6	1.6×10^6	2.6×10^6

	Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
3rd Batch	0	5.9×10^6	5.6×10^6	5.3×10^6	6.1×10^6
	7	12.8×10^6	4.9×10^6	13.5×10^6	14×10^6
	14	13.2×10^6	2.6×10^6	3.4×10^6	14.4×10^6
	21	16.3×10^6	2.6×10^6	7.4×10^6	8.5×10^6
	28	3.9×10^6	2.9×10^6	9.7×10^6	4.3×10^6

Viable *Lactobacillus* spp. count (10^6 cfu/ml[1]) during storage period (average of 3 batches)

Day	Plain-yogurt	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt
0	12.9±6.67	9.4±3.42	12.3±4.76	16.1±7.79
7	15.8±1.85	9.8±3.80	12.8±1.23	14±3.21
14	8.5±2.31	6.6±2.10	9.8±4.86	14.4±8.78
21	8.2±4.27	6.5±1.98	8.4±2.50	8.5±3.52
28	4±0.66	4.8±2.03	6.4±2.45	4.3±1.01

1.4 DPPH Assay

Absorbance reading (517nm) of DPPH assay in yogurt during 28 days storage period at 4˚C

		0	7	14	21	28
1st Batch	Control	0.331	0.406	0.445	0.394	0.384
	Plain-yogurt	0.272	0.351	0.333	0.375	0.332
	Cinnamon-yogurt	0.265	0.254	0.375	0.344	0.318
	Licorice-yogurt	0.263	0.304	0.390	0.382	0.325
	Garlic-yogurt	0.289	0.314	0.315	0.374	0.382

		0	7	14	21	28
2nd Batch	Control	0.367	0.355	0.496	0.337	0.334
	Plain-yogurt	0.316	0.255	0.457	0.278	0.276
	Cinnamon-yogurt	0.320	0.276	0.466	0.250	0.273
	Licorice-yogurt	0.297	0.273	0.465	0.291	0.300
	Garlic-yogurt	0.334	0.307	0.470	0.294	0.322

		0	7	14	21	28
3rd Batch	Control	0.362	0.360	0.519	0.342	0.333
	Plain-yogurt	0.297	0.288	0.494	0.280	0.302
	Cinnamon-yogurt	0.280	0.250	0.442	0.287	0.293
	Licorice-yogurt	0.286	0.277	0.441	0.222	0.287
	Garlic-yogurt	0.323	0.314	0.477	0.286	0.309

Percentage inhibition (%) of DPPH in yogurts at 28 days during storage period

		0	7	14	21	28
1st Batch	Plain-yogurt	17.82	13.54	25.16	4.82	13.54
	Cinnamon-yogurt	19.93	37.43	15.73	12.69	17.18
	Licorice-yogurt	20.54	25.12	12.35	3.04	15.36
	Garlic-yogurt	12.68	22.66	29.21	5.07	0.52

		0	7	14	21	28
2nd Batch	Plain-yogurt	13.89	28.16	7.86	17.50	17.36
	Cinnamon-yogurt	12.80	22.25	6.04	25.81	18.26
	Licorice-yogurt	19.07	23.09	6.25	13.64	10.17
	Garlic-yogurt	8.99	13.52	5.24	12.75	3.59

		0	7	14	21	28
3rd Batch	Plain-yogurt	21.39	20	4.81	18.12	9.30
	Cinnamon-yogurt	34.02	30.55	14.83	16.37	12.01
	Licorice-yogurt	22.68	23.05	15.02	35.08	13.81
	Garlic-yogurt	10.56	12.77	8.09	16.08	7.20

Absorbance reading (517nm) of DPPH assay in yogurts during storage period (average of 3 batches)

	0	7	14	21	28
Control	0.362	0.373	0.486	0.357	0.350
Plain-yogurt	0.297	0.298	0.428	0.311	0.303
Cinnamon-yogurt	0.280	0.260	0.427	0.293	0.294
Licorice-yogurt	0.286	0.284	0.432	0.298	0.304
Garlic-yogurt	0.323	0.311	0.420	0.318	0.339

Percentage inhibition (%) of DPPH in yogurts during storage period (average of 3 batches)

	0	7	14	21	28
Plain-yogurt	17.95±2.16	20.10±4.22	11.93±6.33	12.88±4.33	13.42±4.00
Cinnamon-yogurt	22.65±6.23	30.29±4.38	12.13±3.09	17.92±3.93	16±1.92
Licorice-yogurt	20.99±1.04	23.86±0.68	11.11±2.59	16.52±9.42	13.14±1.53
Garlic-yogurt	10.77±1.06	16.62±3.17	13.58±7.55	10.92±3.33	3.14±1.93

1.5 Bacterial Growth Inhibition Assay

Disk diffusion inhibition zone value (mm) for herbal-yogurts during 14 days storage period at 4˚C for 3 different strains

First Batch

	Days	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt	Plain-yogurt
UM-1	0	10	11	7.1	11
	7	14.1	13	8	13
	14	12	12	10	12
UM-2	0	10	12	7.1	8
	7	13	11	7.1	10
	14	12	11	10	11
UM-3	0	10	10	12	11
	7	11	13	14	12
	14	12	14	14	13

Second Batch

	Days	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt	Plain-yogurt
UM-1	0	9	11	9	10
	7	10	12	10	9
	14	9	11	11	10
UM-2	0	9	8	10	11
	7	11	9	8	12
	14	9	11	12	9
UM-3	0	11	11	10	10
	7	13	10	12	11
	14	10	10	10	12

Third Batch

	Days	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt	Plain-yogurt
UM-1	0	9	12	9	10
	7	11	13	9	13
	14	9	10	11	10
UM-2	0	10	10	9	9
	7	11	11	10	12
	14	13	10	9	10
UM-3	0	11	10	11	10
	7	11	13	11	10
	14	13	12	12	12

Disk diffusion inhibition zone value (mm) in herbs and herbal-yogurts during 14 days storage period for 3 different strains (average of 3 batches)

	Days	Cinnamon-yogurt	Licorice-yogurt	Garlic-yogurt	Plain-yogurt
UM-1	0	9.3±0.33	11.3±0.33	8.3±0.63	10.3±0.33
	7	11.6±1.23	12.6±0.33	9±0.57	11.6±1.33
	14	10±1.00	11±0.57	10.6±0.33	10.6±0.66
UM-2	0	9.6±0.33	10±1.15	8.6±0.85	9.3±0.88
	7	11.6±0.66	10.3±0.66	8.3±0.85	11.3±0.66
	14	11.3±1.20	10.6±0.33	10.3±0.88	10±0.57
UM-3	0	10.6±0.33	10.3±0.33	11±0.57	10.3±0.33
	7	11.6±0.66	12±1.00	12.3±0.91	11±0.57
	14	11.6±0.88	12±1.18	12±1.18	12.3±0.33

Disk diffusion inhibition zone value (mm) in herbs for 3 different strains

First Batch

Strain number	Cinnamon	Licorice	Garlic
UM-1	22	11	11
UM-2	15	11	10
UM-3	19	14	12

Second batch

Strain number	Cinnamon	Licorice	Garlic
UM-1	22	11	11
UM-2	13	11	12
UM-3	21	14	14

Third Batch

Strain number	Cinnamon	Licorice	Garlic
UM-1	22	11	10
UM-2	14	11	10
UM-3	19	14	11

Disk diffusion inhibition zone value in herbs for 3 different strains (average of 3 batches)

Strain number	Cinnamon	Licorice	Garlic
UM-1	22±0.00	11±0.00	10.6±0.33
UM-2	14±0.57	11±0.00	10.6±0.66
UM-3	19.6±0.66	14±0.00	12.3±0.88

APPENDIX 2: MEDIA PREPARATION AND REAGENTS

MEDIA

2.1 Buffered Peptone Water

20g of Buffered peptone water was suspended in 1L distilled water, mixed well and distributed into final containers. The mixture was then autoclaved at 121˚C for 20 min. The pH of media at 25˚C was 7.2 ± 0.2.

2.2 M17 Agar

The media was used to cultivate *Streptococcus thermophilus*. M17 agar (48.25g) was dissolved in 950ml distilled water by bring gently to boil. The mixture was sterilized by autoclaving at 121˚C for 20 min. After cooling down to 50˚C, 50ml of sterile Lactose solution was added (10% w/v) and mixed well with the M17 solution. The melted agar (20 ml) was poured carefully into disposable petri dishes and was left for 30 min to solidify. The pH of media at 25˚C was 6.9 ± 0.2.

Lactose Solution (10% w/v)

Lactose (10g) was dissolved in 100ml distilled water followed by autoclaving for 20 min at 121˚C. This solution was mixed thoroughly with melted M17 agar.

2.3 MRS Agar

MRS agar was used for enumeration of *Lactobacillus* ssp. MRS agar powder (62g) was mixed with 1L distilled water and was then autoclaved for 20 min at 121˚C. Upon cooling to tolerable temperature (50˚C) the agar was poured into the disposable petri dishes. The pH of media at 25˚C was 6.2 ± 0.2.

2.4 Mueller Hinton (MH) Blood Agar

This media was used to cultivate *Helicobacter pylori* using MIC method. MH agar (38g) was mixed with 950ml distilled water and the mixture was then autoclaved at 121˚C for 20 min. After cooling down (45˚C), 5% (w/v) of defibrinated sheep blood (>2 weeks old) was added to melted (50˚C) agar; and 20ml of the mixture was transferred to 9mm disposable petri dishes. The pH of media at 25˚C was 7.3 ± 0.1.

2.5 Brain Heart Infusion Broth (BHIB)

This broth was used to measure the turbidity of *H. pylori* using MacFarland machine. BHIB powder (18.5g) was suspended in 500ml distilled water; mixed thoroughly and distributed to the screw caped containers. The mix sterilized by autoclaving at 121˚C for 20 min. After cooling down, it was refrigerated at 4°C until required. The pH of media at 25˚C was 7.4 ± 0.2.

REAGENTS

2.6 NaOH Solution (1%)

NaOH pellet (4g) was dissolved in 1L purified water.

2.7 Phenolphetalein (1%)

Phenolphetalein (0.1g) was suspended in 100ml ethyl alcohol.

2.8 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) Stock Solution

DPPH (0.0236g) was dissolved in 10ml ethanol 95% and mixed thoroughly. One ml of this mixture was transferred to 9ml ethanol 95% and the mixture was thoroughly mixed. Finally, 1ml from latter mixture was added to 9ml ethanol 95% and

this mixture was used as stock solution. All preparation was carried out in the absence of light, since DPPH is sensitive to light.

2.9 Gram-staining

1. Fresh smear of bacteria (*Lactobacillus* ssp., *S. thermophilus* or *H. pylori*) prepared on a glass slide and fixed by heat.
2. The smear was flooded by Crystal violet for one minute. Excess of dye poured off and washed gently by distilled water.
3. The smear of bacteria exposed to Iodine for one minute following by washing off the excess Iodine with distilled water.
4. The bacterial smear was then washed with 95% alcohol for 30 seconds and washed with distilled water immediately after 30 seconds to stop decolorization.
5. The smear was exposed to Safranin for 30 seconds and washed away with distilled water.
6. Finally the glass slide with bacterial smear air drained, and examine by oil under microscope.

LIST OF PUBLICATIONS

International Publications

S. Behrad, M.Y. Yusof, K. L. Goh, A.S. Baba. (2009). Manipulation of Probiotics Fermentation of Yogurt by *Cinnamon* and *Licorice*: Effects on Yogurt Formation and Inhibition of *Helicobacter Pylori* Growth *in vitro. International Journal of Medicine and Medical Sciences.* 1(3), 135-139.

Conference Proceedings

1. Title: Manipulation of Probiotics Fermentation of Yogurt by *Cinnamon* and *Licorice*: Effects on Yogurt Formation and Inhibition of *Helicobacter Pylori* Growth *in vitro.*

Authors: S. Behrad, M.Y. Yusof, K. L. Goh, A.S. Baba.

Date: 25-27 December 2009

Activity: International conference of Biological and Medical Sciences (ICBMS) 2009

Venue: Thailand, Bangkok

Role: Oral presentation, Volume 60, Abstract pg. 1124.

2. Title: Manipulation of probiotic fermentation of milk by *cinnamon zeylanicum, Glycrrhiza glabra* or *Allium sativum* and their effects on inhibition of *Helicobacter pylori* growth *in vitro*.

Authors: Sara Behrad & Baba, A. S.

Date: 1-3 November 2010

Activity: Bio-Malaysia 2010

Venue: Kuala Lumpur, Malaysia

Role: Poster presentation of research work