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APPENDIX

Formular and preparation of culture media, reagents, analysis of the data from the experiment and statistic analysis of Table 9 and Table 37

1. Culture media

1.1 Preparation of each of five sugars in concentration of 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 1% and 3%

1.1.1 Glucose, Sucrose, Lactose, Mannitol and Xylitol 0.01%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.005 gm.

1.1.2 Glucose, Sucrose, Lactose, Mannitol and Xylitol 0.02%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.01 gm.

1.1.3 Glucose, Sucrose, Lactose, Mannitol and Xylitol 0.03%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.015 gm.

1.1.4 Glucose, Sucrose, Lactose, Mannitol and Xylitol 0.05%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.025 gm.

1.1.5 Glucose, Sucrose, Lactose, Mannitol and Xylitol 0.1%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.05 gm.

1.1.6 Glucose, Sucrose, Lactose, Mannitol and Xylitol 1%

Sugar media 50 ml. (for each sugar)

each of five sugars 0.5 gm.

1.1.7 Glucose, Sucrose, Lactose, Mannitol and Xylitol 3%

Sugar media 50 ml. (for each sugar)

each of five sugars 1.5 gm.

1.2 Preparation of sugar media (400 ml.)

Bacto tryptone = $\frac{17 \times 400}{1,000}$ = 6.8 gm.

Bacto peptone = $\frac{3 \times 400}{1,000}$ = 1.2 gm.

Sodium chloride = $\frac{5 \times 400}{1,000}$ = 2.0 gm.

Dipotassium hydrogen phosphate (K_2HPO_4) = $\frac{2.5 \times 400}{1,000}$ = 1.0 gm.

sugar vary to each concentration of sugar.

Distilled water q.s. to 400 ml.

1.3 Preparation of tryptic soy broth

Tryptic soy broth 30 gm.

Distilled water q.s. to 1,000 ml.

2. Reagents

2.1 Preparation of p-hydroxy diphenyl

Dissolve 600 mg. of p-hydroxy diphenyl in 10 ml. of 4% sodium hydroxide solution with heating. Slowly add 70 ml. of water and mix. (Prepare freshly before use)

2.2 Preparation of sulfuric acid copper sulfate reagent

By mixing 1 ml. of 2% copper sulfate pentahydrage solution with 60 ml. of conc. sulfuric acid.

2.3 Preparation of 1N HCL

HCL 97.1 ml.

Distilled water q.s. to 1,000 ml.

2.4 Preparation of 1N NaOH

NaOH 40 gm.

Distilled water q.s. to 1,000 ml.

3. Analysis of the data from the experiments

3.1 S. mutans in 0.01% and 0.02% sugar, anaerobic condition

It was found that, after incubation with 0.01% and 0.02% of each sugar for 24 hrs in anaerobic condition, S. mutans produced more lactic acid in glucose but less in xylitol and lactose, as shown in Table 9, and more lactic acid in glucose but less in xylitol, as shown in Table 10, page 61.

(Statistic analysis of Table 9 see on page 61.

Table 9 Lactic acid production of S. mutans after incubation for 24 hrs in 0.01% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.08	0.1	0.015	0.22
Number	(1)**	(2)*	(3)*	(4)**	(5)

**(1) and (4) No significant difference

*(2) and (3) No significant difference

Table 10 Lactic acid production of S. mutans after incubation for 24 hrs in 0.02% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.161	0.102	0.04	0.19
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference.

3.2 S. mutans in 0.03% and 0.05% sugar, anaerobic condition

It was found that, after incubation with 0.03% and 0.05% of each sugar for 24 hrs in anaerobic condition, S. mutans produced more lactic acid in glucose but less in xylitol, as shown in Table 11, and more lactic acid in lactose and glucose but less in xylitol, as shown in Table 12, page 62.

Table 11 Lactic acid production of S. mutans after incubation for 24 hrs in 0.03% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.103	0.1	0.261	0.28
Number	(1)	(2)*	(3)*	(4)	(5)

*(2) and (3) No significant difference

Table 12 Lactic acid production of S. mutans after incubation for 24 hrs in 0.05% conc. of sugars

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.181	0.015	0.408	0.401
Number	(1)	(2)	(3)	(4)*	(5)*

*(4) and (5) No significant diffenence.

3.3 S. mutans in 0.1% and 1% sugar, anaerobic condition

It was found that, after incubation with 0.1% and 1% of each sugar for 24 hrs in anaerobic condition, S. mutans produced more lactic acid in lactose but less in xylitol, as shown in Table 13, and more lactic acid in glucose but less in xylitol, as shown in Table 14, page 63.

Table 13 Lactic acid production of S. mutans after incubation for 24 hrs in 0.1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.101	0.103	0.389	0.36
Number	(1)	(2)*	(3)*	(4)	(5)

*(2) and (3) No significant difference

Table 14 Lactic acid production of S. mutans after incubation for 24 hrs in 1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.101	0.19	0.35	0.704
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference.

3.4 S. mutans in 3% sugar, anaerobic condition

It was found that, after incubation with 3% of each sugar for 24 hrs in anaerobic condition, S. mutans produced more lactic acid in glucose but less in xylitol and sucrose, as shown in Table 15, page 64.

Table 15 Lactic acid production of S. mutans after incubation for 24 hrs in 3% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.16	0.206	0.36	1.37
Number	(1)*	(2)* **	(3) **	(4) **	(5)

*(1) and (2) No significant difference

**(2), (3), and (4) No significant difference

3.5 S. sanguis in 0.01% and 0.02% sugar, anaerobic condition

It was found that, after incubation with 0.01% and 0.02% of each sugar for 24 hrs in anaerobic condition, S. sanguis produced more lactic acid in lactose but less in xylitol mannitol, sucrose and glucose, as shown in Table 16, and more lactic acid in lactose but less in xylitol, mannitol and sucrose, as shown in Table 17, page 65.

Table 16 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.01% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0	0	0.204	0
Number	(1)*	(2)*	(3)*	(4)	(5)*

*(1), (2), (3) and (5) No significant difference

Table 17 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.02% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0	0	0.302	0.05
Number	(1)*	(2)*	(3)*	(4)	(5)

*(1), (2) and (3) No significant difference

3.6 S. sanguis in 0.03% and 0.05% sugar, anaerobic condition

It was found that, after incubation with 0.03% and 0.05% of each sugar for 24 hrs in anaerobic condition, S. sanguis produced more lactic acid in lactose but less in xylitol, mannitol, glucose and sucrose, as shown in Table 18, and more lactic acid in lactose but less in xylitol and mannitol, as shown in Table 19, page 66.

Table 18 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.03% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.011	0	0.203	0
Number	(1)*	(2)*	(3)*	(4)	(5)*

*(1), (2), (3) and (5) No significant difference

Table 19 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.05% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.1	0	0.25	0.05
Number	(1)*	(2)	(3)*	(4)	(5)

*(1) and (3) No significant difference

3.7 S. sanguis in 0.1% and 1% sugar, anaerobic condition

It was found that, after incubation with 0.1% and 1% of each sugar for 24 hrs in anaerobic condition, S. sanguis produced more lactic acid in lactose but less in xylitol, mannitol and glucose, as shown in Table 20, and more lactic acid in lactose but less in mannitol, as shown in Table 21, page 67.

Table 20 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.1	0	0.205	0
Number	(1)*	(2)	(3)*	(4)	(5)*

*(1), (3) and (5) No significant difference

Table 21 Lactic acid production of S. sanguis after incubation for 24 hrs in 1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.03	0.105	0	0.19	0.02
Number	(1)*	(2)	(3)	(4)	(5)*

*(1) and (5) No significant difference

3.8 S. sanguis in 3% sugar, anaerobic condition

It was found that, after incubation with 3% of each sugar for 24 hrs in anaerobic condition, S. sanguis produced more lactic acid in xylitol but less in mannitol, as shown in Table 22, page 68.

Table 22 Lactic acid production of S. sanguis after incubation for 24 hrs in 3% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.4	0.15	0	0.25	0.08
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference

3.9 S. mutans in 0.01% and 0.02% sugar, aerobic condition

It was found that, after incubation with 0.01% and 0.02% of each sugar for 24 hrs in aerobic condition. S. mutans produced more lactic acid in lactose but less in sucrose, as shown in Table 23, and more lactic acid in lactose but less in mannitol as shown in Table 24, page 69.

Table 23 Lactic acid production of S. mutans after incubation for 24 hrs in 0.01% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.06	0	0.102	0.22	0.04
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference

Table 24 Lactic acid production of S. mutans after incubation for 24 hrs in 0.02% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.02	0.061	0.011	0.22	0.141
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference

3.10 S. mutans in 0.03% and 0.05% sugar, aerobic condition

It was found that, after incubation with 0.03% and 0.05% of each sugar for 24 hrs in aerobic condition, S. mutans produced more lactic acid in glucose but less in xylitol and sucrose, as shown in Table 25, and more lactic acid in glucose but less in sucrose, as shown in Table 26, page 70.

Table 25 Lactic acid production of S. mutans after incubation for 24 hrs in 0.03% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0	0.102	0.22	0.35
Numaer	(1)*	(2)*	(3)	(4)	(5)

*(1) and (2) No significant difference

Table 26 Lactic acid production of S. mutans after incubation for 24 hrs in 0.05% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.02	0	0.15	0.221	0.35
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference

3.11 S. mutans in 0.1% and 1% sugar, aerobic condition

It was found that, after incubation with 0.1% and 1% of each sugar for 24 hrs in aerobic condition, S. mutans produced more lactic acid in glucose but less in sucrose, as shown in Table 27, and more lactic acid in glucose but less in xylitol, mannitol and sucrose, as shown in Table 28, page 71.

Table 27 Lactic acid production of S. mutans after incubation for 24 hrs in 0.1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.06	0	0.111	0.165	0.39
Number	(1)	(2)	(3)	(4)	(5)

Every values significant difference

Table 28 Lactic acid production of S. mutans after incubation for 24 hrs in 1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.112	0.18	0.17	0.202	0.35
Number	(1)*	(2)* **	(3)* **	(4) **	(5)

*(1), (2) and (3) No significant difference

**(2), (3) and (4) No significant difference

3.12 S. mutans in 3% sugar, aerobic condition

It was found that, after incubation with 3% of each sugar for 24 hrs in aerobic condition. S. mutans produced more lactic acid in glucose but less in xylitol, mannitol and lactose, as shown in Table 29, page 72.

Table 29 Lactic acid production of S. mutans after incubation for 24 hrs in 3% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0.17	0.25	0.17	0.221	0.35
Number	(1)*	(2) **	(3)*	(4)* **	(5)

*(1), (3) and (4) No significant difference

**(2) and (4) No significant difference

3.13 S. sanguis in 0.01% and 0.02% sugar, aerobic condition

It was found that, after incubation with 0.01% and 0.02% of each sugar for 24 hrs in aerobic condition, S. sanguis produced more lactic acid in glucose but less in xylitol, mannitol, sucrose and lactose, as shown in Table 30, and more lactic acid in glucose but less in xylitol and mannitol, as shown in Table 31, page 73.

Table 30 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.01% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0	0	0	0.26
Number	(1)*	(2)*	(3)*	(4)*	(5)

*(1), (2), (3) and (4) No significant difference

Table 31 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.02% conc. of sugars.

	sugars				
	Xylitol	Sucroee	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.07	0	0.05	0.33
Number	(1)*	(2)**	(3)*	(4)**	(5)

*(1) and (3) No significant difference

**(2) and (4) No significant difference

3.14 S. sanguis in 0.03% and 0.05% sugar, aerobic condition

It was found that, after incubation with 0.03% and 0.05% of each sugar for 24 hrs in aerobic condition, S. sanguis produced more lactic acid in glucose but less in xylitol and mannitol, as shown in Table 32, and more lactic acid in glucose but less in xylitol and mannitol, as shown in Table 33, page 74.

Table 32 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.03% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.07	0	0.02	0.221
Number	(1)*	(2)	(3)*	(4)	(5)

*(1) and (3) No significant difference

Table 33 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.05% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.105	0	0.04	0.203
Number	(1)*	(2)	(3)*	(4)	(5)

*(1) and (3) No significant difference

3.15 S. sanguis in 0.1% and 1% sugar, aerobic condition

It was found that, after incubation with 0.1% and 1% of each sugar for 24 hrs in aerobic condition, S. sanguis produced more lactic acid in glucose but less in xylitol, mannitol and lactose, as shown in Tabee 34, and more lactoc acid in glucose but less in xylitol and mannitol, as shown in Table 35, page 75.

Table 34 Lactic acid production of S. sanguis after incubation for 24 hrs in 0.1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.125	0	0	0.333
Number	(1)*	(2)	(3)*	(4)*	(5)

*(1), (3) and (4) No significant difference

Table 35 Lactic acid production of S. sanguis after incubation for 24 hrs in 1% conc. of sugars.

	sugars				
	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.14	0	0.081	0.282
Number	(1)*	(2)	(3)*	(4)	(5)

*(1) and (3) No significant diffenence

3.16 S. sanguis in 3% sugar, aerobic condition

It was found that, after incubation with 3% of each sugar for 24 hrs in aerobic condition, S. sanguis produced more lactic acid in glucose but less in xylitol and mannitol, as shown in Table 36, page 76.

Table 36 Lactic acid production of S. sanguis after incubation for 24 hrs in 3% conc. of sugars.

	Xylitol	Sucrose	Mannitol	Lactose	Glucose
Absorbancy at 570 nm	0	0.14	0	0.13	0.35
Number	(1)*	(2) **	(3)*	(4) **	(5)

*(1) and (3) No significant difference

**(2) and (4) No significant difference

3.17 S. mutans in 1% mannitol and 3% glucose, anaerobic condition

It was found that, after incubation with 1% mannitol and 3% glucose of each pH for 24 hrs in anaerobic condition, S. mutans produce more lactic acid at pH 7 but less at pH 5 and pH 9, as shown in Table 37, and more lactic acid at pH 6 but less at pH 8 and pH 9, as shown in Table 38, page 77.

Table 37 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 1% mannitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.575	1.075	0.875	0
Number	(1)*	(2)	(3)	(4)	(5)*

*(1) and (5) No significant difference

Table 38 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 3% glucose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.28	0.33	0.26	0.195	0.205
Number	(1)*	(2)	(3)*	(4)**	(5)**

*(1) and (3) No significant difference

**(4) and (5) No significant difference

3.18 S. mutans in 1% lactose and 1% sucrose, anaerobic condition

It was found that, after incubation with 1% lactose and 1% sucrose of each pH for 24 hrs in anaerobic condition, S. mutans produced more lactic acid at pH 6, pH 7, pH 8 and pH 9 but less at pH 5, as shown in Table 39, and more lactic acid at pH 6 and pH 7 but less at pH 5 and pH 8, as shown in Table 40, page 78.

Table 39 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 1% lactose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.08	0.075	0.085	0.085
Number	(1)	(2)*	(3)*	(4)*	(5)*

*(2), (3), (4) and (5) No significant difference

Table 40 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 1% sucrose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.14	0.22	0.23	0.15	0.17
Number	(1)*	(2) **	(3) **	(4)*	(5)

*(1) and (4) No significant difference

**

3.19 S. mutans in 0.01% xylitol, anaerobic condition

It was found that, after incubation with 0.01% xylitol of each pH for 24 hrs in anaerobic condition, S. mutans produced more lactic at pH 5, pH 6, pH 7 and pH 8 but less at pH 9, as shown in Table 41, page 79.

Table 41 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 0.01% xylitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.025	0.025	0.02	0.02	0.01
Number	(1)*	(2)*	(3)*	(4)*	(5)

*(1), (2), (3) and (4) No significant difference

3.20 S. sanguis in 1% mannitol and 0.1% glucose, anaerobic condition

It was found that, after incubation with 1% mannitol and 0.1% glucose of each pH for 24 hrs in anaerobic condition, S. snaguis produced mare lactic acid at pH 6 but less at pH 5, pH 8 and pH 9, as shown in Table 42, and more lactic acid at pH 6 but less at pH 5, as shown in Table 43, page 80.

Table 42 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 1% mannitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.105	0.095	0	0
Number	(1)*	(2)	(3)	(4)*	(5)*

*(1), (4) and (5) No significant difference

Table 43 Lactic acidpproduction of S. sanguis after incubation for 24 hrs in each pH of 0.1% glucose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.26	0.22	0.19	0.19
Number	(1)	(2)	(3)	(4)*	(5)*

*(4) and (5) No significant difference

3.21 S. sanguis in 3% lactose and 3% sucrose, anaerobic condition

It was found that, after incubation with 3% lactose and 3% sucrose of each pH for 24 hrs in anaerobic condition, S. sanguis produced more lactic acid at pH 9 but less at pH 5, pH 6, pH 7 and pH 8, as shown in Table 44, and more lactic acid at pH 6, pH 7, pH 8 and pH 9 but less at pH 5, as shown in Table 45, page 81.

Table 44 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 3% lactose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0	0	0	0.04
Number	(1)*	(2)*	(3)*	(4)*	(5)

*(1), (2), (3) and (4) No significant difference

Table 45 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 3% sucrose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.08	0.1	0.1	0.1	0.1
Number	(1)	(2)*	(3)*	(4)*	(5)*

*(2), (3), (4) and (5) No significant difference

3.22 S. sanguis in 0.01% xylitol, anaerobic condition

It was found that, after incubation with 0.01% xylitol of each pH for 24 hrs in anaerobic condition, S. sanguis produced none lactic acid at every pH as shown in Table 46, page 82.

Table 46 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 0.01% xylitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0	0	0	0
Number	(1)	(2)	(3)	(4)	(5)

Every values no significant difference

3.23 S. mutans in 0.05% mannitol and 3% glucose, aerobic condition

It was found that, after incubation with 0.05% mannitol and 3% glucose of each pH for 24 hrs in aerobic condition, S. mutans produced more lactic acid at pH 6 but less at pH 5, pH 7, pH 8 and pH 9, as shown in Table 47, and more lactic at pH 6 but less at pH 5, as shown in Table 48, page 83.

Table 47 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 0.05% mannitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.005	0	0	0
Number	(1)*	(2)	(3)*	(4)*	(5)*

*(1), (3), (4) and (5) No significant difference

Table 48 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 3% glucose

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.17	0.25	0.2	0.215	0.2
Number	(1)	(2)	(3)*	(4)*	(5)*

*(3), (4) and (5) No significant difference

3.24 S. mutans in 3% lactose and 3% sucrose, aerobic condition

It was found that, after incubation with 3% lactose and 3% sucrose of each pH for 24 hrs in aerobic condition, S. mutans produced more lactic acid at pH 6, pH 7, pH 8 and pH 9 but less at pH 5, as shown in Table 49, and equal lactia acid at every pH, as shown in Table 50, page 84.

Table 49 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 3% lactose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.036	0.04	0.045	0.045
Number	(1)	(2)*	(3)*	(4)*	(5)*

*(2), (3), (4) and (5) No significant difference

Table 50 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 3% sucrose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.09	0.1	0.1	0.09	0.095
Number	(1)	(2)	(3)	(4)	(5)

Every values no significant difference

3.25 S. mutans in 0.01% xylitol, aerobic condition

It was found that, after incubation with 0.01% xylitol of each pH for 24 hrs in aerobic condition, S. mutans produced none lactic acid at every pH, as shown in Table 51, page 85.

Table 51 Lactic acid production of S. mutans after incubation for 24 hrs in each pH of 0.01% xylitol

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0	0	0	0
Number	(1)	(2)	(3)	(4)	(5)

Every values no significant difference

3.26 S. sanguis in 1% mannitol and 0.1% glucose, aerobic condition.

It was found that, after incubation with 1% mannitol and 0.1% glucose of each pH for 24 hrs in aerobic condition, S. sanguis produced more lactic acid at pH 7 but less at pH 5 and pH 8, as shown in Table 52, and more lactic acid at pH 7 but less at pH 5 and pH 9, as shown in Table 53, page 86.

Table 52 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 1% mannitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.015	0.164	0	0.025
Number	(1)*	(2)	(3)	(4)*	(5)

*(1) and (4) No significant difference

Table 53 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 0.1% glucose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0.08	0.09	0.07	0
Number	(1)*	(2)	(3)	(4)	(5)*

*(1) and (5) No significant difference

3.27 S. sanguis in 3% lactose and 3% sucrose, aerobic condition

It was found that, after incubation with 3% lactose and 3% sucrose of each pH for 24 hrs in aerobic condition, S. sanguis produced more lactic acid at pH 9 but less at pH 5 and pH 6, as shown in Table 54, and equal lactic acid at every pH, as shown in Table 55, page 87.

Table 54 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 3% lactose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0	0.018	0.01	0.04
Number	(1)*	(2)*	(3)**	(4)**	(5)

*(1) and (2) No significant difference

**(3) and (4) No significant difference

Table 55 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 3% sucrose.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0.08	0.1	0.09	0.095	0.095
Number	(1)	(2)	(3)	(4)	(5)

Every values no significant difference

3.28 S. sanguis in 0.01% xylitol, aerobic condition

It was found that, after incubation with 0.01% xylitol of each pH for 24 hrs in aerobic condition, S. sanguis produced non lactic acid at every pH, as shown in Table 56, page 88.

Table 56 Lactic acid production of S. sanguis after incubation for 24 hrs in each pH of 0.01% xylitol.

	pH				
	5	6	7	8	9
Absorbancy at 570 nm	0	0	0	0	0
Number	(1)	(2)	(3)	(4)	(5)

Every values no significant difference

4. Statistic analysis of Table 9

S. mutans 0.01% sugar, anaerobic condition.

Sugar (i) / Culture No. (j)	Mannitol (Xi1)	$(Xi1)^2$	Glucose (Xi2)	$(Xi2)^2$	Lactose (Xi3)	$(Xi3)^2$	Sucrose (Xi4)	$(Xi4)^2$	Xylitol (Xi5)	$(Xi5)^2$	Total
1	0.12	(0.014)	0.225	(0.051)	0.014	(0)	0.081	(0.007)	0	(0)	
2	0.11	(0.012)	0.23	(0.053)	0.018	(0)	0.08	(0.006)	0	(0)	
3	0.09	(0.008)	0.2	(0.04)	0.014	(0)	0.075	(0.006)	0	(0)	
4	0.08	(0.006)	0.24	(0.058)	0.015	(0)	0.085	(0.007)	0	(0)	
5	0.1	(0.01)	0.22	(0.048)	0.015	(0)	0.08	(0.006)	0	(0)	
$\Sigma_j X_{ij} = Xi$	0.5		1.1		0.075		0.4		0		$2.075\ (\Sigma_{ij} X_{ij})$
$\Sigma_j X_{ij}^2$		(0.05)		(0.25)		(0)		(0.032)		(0)	$0.332\ \{\Sigma_{ij} (X_{ij})^2\}$
$\overline{Xi}$ (Absorbancy at 570 nm)	0.1		0.22		0.015		0.08		0		

Calculation X_{ij} is an observed value at j in sugar i

X_i is total value of sugar i

t = number of sugar = 5

r = number of cultures in each of five sugars = 5

4.1 Calculate $CT = \frac{X^2}{rt} = \frac{(\Sigma X_{ij})^2}{rt} = \frac{(2.075)^2}{5 \times 5} = 0.172$

4.2 Total SS $= \Sigma_{ij} X_{ij}^2 - CT$

$= 0.332 - 0.172 = 0.16$

4.3 Sugar SS $= \frac{X_1^2 + X_2^2 + \ldots\ldots X_n^2}{r} - CT$

$= 0.325 - 0.172 = 0.153$

4.4 Error SS = Total SS - Sugar SS

$= 0.16 - 0.153 = 0.007$

source of variation	df	sum of square	Mean square	$T = S/_E$
Sugar	4	0.16	0.04 $(SS/_{df})$ (S)	
Error	20	0.007	0.00035 $(SS/_{df})$ (E)	114.29
Total	24			

Null hypothesis none difference between sugars.

When Null hypothesis $_{\gamma}S^2 = 0$ is true

$$F = \frac{\text{sugar mean square}}{\text{error mean square}}$$

$$F_{cal} = 114.29$$

F_{tab} at df 4 and 20 = 2.87 and 4.43 at $P_{.05}$ and $P_{.01}$

$F_{cal} > F_{tab}$ at 1% by Null hypothesis

Shown $\overline{X}$ of each of five sugars significant difference

4.5 Calculate $S_{\overline{x}} = \sqrt{\frac{\text{(error mean square)}}{r}}$

$$= \sqrt{\frac{0.00035}{5}}$$

$$= 0.008$$

SSR for 5% at df 20 ตาราง ก.7 at P 2 to 5

Significant studentized Ranges for 5% level New Multiple-Range Test

P	2	3	4	5
SSR	2.95	3.10	3.18	3.25
LSR = SSR ($S_{\overline{x}}$)	0.0236	0.0248	0.0254	0.026

	sugars				
	Xylitol	Lactose	Sucrose	Mannitol	Glucose
Absorbancy at 570 nm	0	0.015	0.08	0.1	0.22
Number	(1)	(4)	(2)	(3)	(5)

Glucose-Xylitol (5) (1) = 0.22>0.026

Glucose-Lactose (5) (4) = 0.205>0.025

Glucose-Sucrose (5) (2) = 0.14>0.0248

Glucose-Mannitol (5) (3) = 0.12>0.0236

Mannitol-Xylitol (3) (1) = 0.1>0.0254

Mannitol-Lactose (3) (4) = 0.085>0.0248

Mannitol-Sucrose (3) (2) = 0.02<0.0236.

Sucrose-Xylitol (2) (1) = 0.08>0.0248

Sucrose-Lactose (2) (4) = 0.065>0.0236

Lactose-Xylitol (4) (1) = 0.015<0.0236.

5. Statistic analysis of Table 37

S. mutans 1% Mannitol, anaerobic conditon.

pH(i) Culture No.(j)	(X_{i1}^5)	$(X_{i1})^2$	(X_{i2}^6)	$(X_{i2})^2$	(X_{i3}^7)	$(X_{i3})^2$	(X_{i4}^8)	$(X_{i4})^2$	(X_{i5}^9)	$(X_{i5})^2$	Total
1	0	(0)	0.59	(0.348)	1.05	(1.103)	0.85	(0.723)	0	(0)	
2	0	(0)	0.561	(0.315)	1.1	(1.21)	0.895	(0.801)	0	(0)	
3	0	(0)	0.57	(0.325)	1.15	(1.323)	0.91	(0.828)	0	(0)	
4	0	(0)	0.58	(0.336)	1	(1)	0.845	(0.714)	0	(0)	
5	0	(0)	0.575	(0.331)	1.075	(1.155)	0.875	(0.766)	0	(0)	
$\Sigma_j X_{ij}$ = Xi	0		2.876		5.375		4.375		0		
$\Sigma_j X_{ij}^2$		(0)		(1.655)		(5.792)		(3.832)		(0)	
$\bar{X}_i$ (Absorbancy at 570 nm)	0		0.575		1.075		0.875		0		

Calculation X_{ij} is an observed value at j in pHi

X_i is total value of pHi

t = number of pH

r = number of cultures in each of five pH = 5

5.1 Calculation $CT = \frac{X^2}{rt} = \frac{(\Sigma_{ij} X_{ij})^2}{rt} = \frac{(12.626)^2}{5 \times 5} = 6.377$

5.2 Total SS $= \Sigma_{ij} X^2_{ij} - CT$

$= 11.279 - 6.377 = 4.902$

5.3 pH SS $= \frac{X_1^2 + X_2^2 + \ldots\ldots X_n^2}{5} - CT$

$= 11.261 - 6.377 = 4.884$

5.4 Error SS = Total SS - pH SS

= 4.902 - 4.884 = 0.018

Source of variation	df	Sum of sugars	Mean square	$F = \frac{S_{pH}}{E}$
pH	4	4.884	1.221 SS/df(S_{pH})	1,356.67
Error	20	0.18	0.0009SS/df(E)	
	24			

Null hypothesis none difference between pH

When Null hypothesis $\gamma^2_{pH} = 0$ is true

$$F = \frac{\text{pH mean square}}{\text{error mean square}}$$

$$F_{cal} = 1,356.67$$

F_{tab} at df 4 and 20 = 2.87 and 4.43 at P.05 and P.01

$F_{cal} > F_{tab}$ at 1% by Null hypothesis

Shown $\bar{X}$ of each of five pH significant difference.

5.5 Calculate $S_{\bar{x}} = \sqrt{\frac{\text{(error mean square)}}{r}}$

$$= \sqrt{\frac{0.0009}{5}} = 0.013$$

SSR for 5% at df 20 ตาราง ก.7 at P 2 to 5

Significant Studentized Ranges for 5% Level New Multiple-Range Test

P	2	3	4	5
SSR	2.95	3.10	3.18	3.25
LSR = SSR ($S_{\bar{x}}$)	0.038	0.04	0.041	0.042

	pH				
	5	9	6	8	7
(Absorbancy at 570 nm)	0	0	0.575	0.875	1.075
Number	(1)	(5)	(2)	(4)	(3)

$\frac{7}{(3)} - \frac{5}{(1)} = 1.075 > 0.042$ $\quad$ $\frac{8}{(4)} - \frac{9}{(5)} = 0.875 > 0.04$

$\frac{7}{(3)} - \frac{9}{(5)} = 1.075 > 0.041$ $\quad$ $\frac{8}{(4)} - \frac{6}{(2)} = 0.3 > 0.038$

$\frac{7}{(3)} - \frac{6}{(2)} = 0.5 > 0.04$ $\quad$ $\frac{6}{(2)} - \frac{5}{(1)} = 0.575 > 0.04$

$\frac{7}{(3)} - \frac{8}{(4)} = 0.2 > 0.038$ $\quad$ $\frac{6}{(2)} - \frac{9}{(5)} = 0.575 > 0.038$

$\frac{8}{(4)} - \frac{5}{(1)} = 0.875 > 0.041$ $\quad$ $\frac{9}{(5)} - \frac{5}{(1)} = 0 < 0.038$

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