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APPENDICES

Appendix A

Growth curve

Lactobacillus plantarum TISTR 850 from stock culture were sub-cultured and grown in anaerobic condition at 37 °C in MRS broth until it became activated culture. Afterwards, they were inoculated into a klette flask containing 20 mL MRS broth and incubated in anaerobic condition at 37 °C for 60 minutes or grown to optical density of 0.1 at 600 nm (OD₆₀₀). The cell suspension was pipetted (500 µL) from the klette flask into three klette flasks containing 50 mL MRS broth and one flask containing 50 mL MRS broth. The flasks were incubated at 37 °C in anaerobic condition. The first three klette flasks were measured by spectrophotometer (Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, USA) at 600 nm every one or two hours until constant absorbance was obtained. The average amount of bacteria in the flask was determined by spreading 100 µL of the cell suspension on to a plate containing MRS agar and incubated at 37 °C overnight. The average bacterial counts were shown in log CFU/mL. Similar experiments were conducted for *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* DMST 17303, and *Salmonella* Typhimurium ATCC 13311 but using TSB as the medium in flasks and TSA as the plate count agar.

Table A1 Average absorbance at 600 nm of *Lactobacillus plantarum* TISTR 850 growth and the average bacterial count at different times.

Time (hr)	Average absorbance (600 nm)	Average plate count (log CFU/mL)
0	0.035±0.000	ND
2	0.035±0.001	ND
4	0.036±0.004	4.83
6	0.042±0.003	5.79
8	0.060±0.009	6.85
9	0.096±0.034	ND
10	0.184±0.027	7.60
11	0.397±0.048	7.83
12	0.748±0.072	ND
13	1.104±0.060	8.71
14	1.393±0.060	ND
15	1.570±0.035	ND
16	1.712±0.021	ND
17	1.797±0.034	ND
18	1.890±0.006	ND
20	1.964±0.001	ND
22	2.052±0.008	ND

ND means "Not Detected"

The values shown in the table are average from 3 measurements.

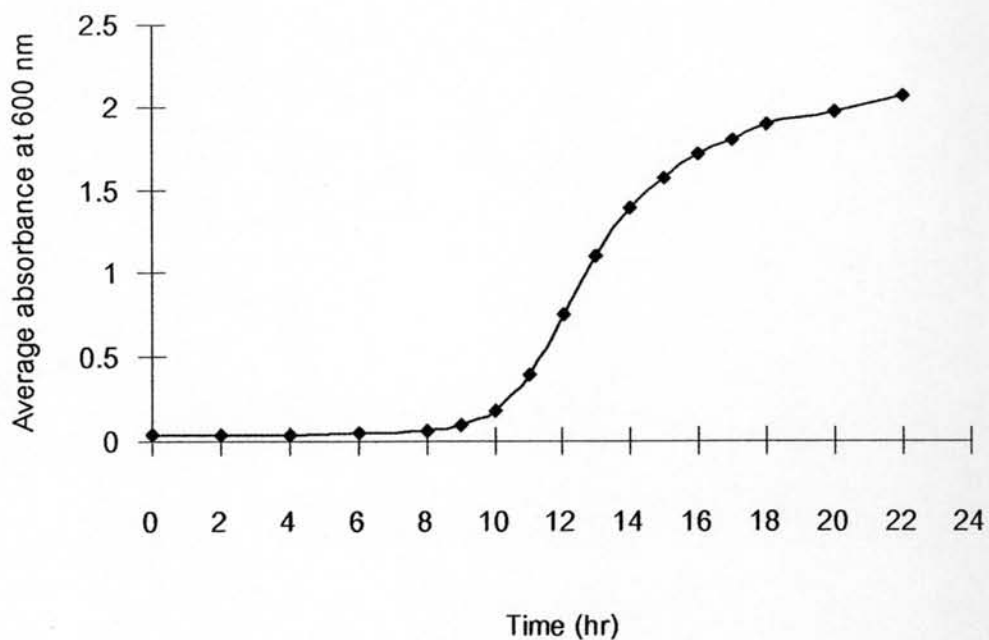


Figure A1 Growth curve of *Lactobacillus plantarum* TISTR 850.

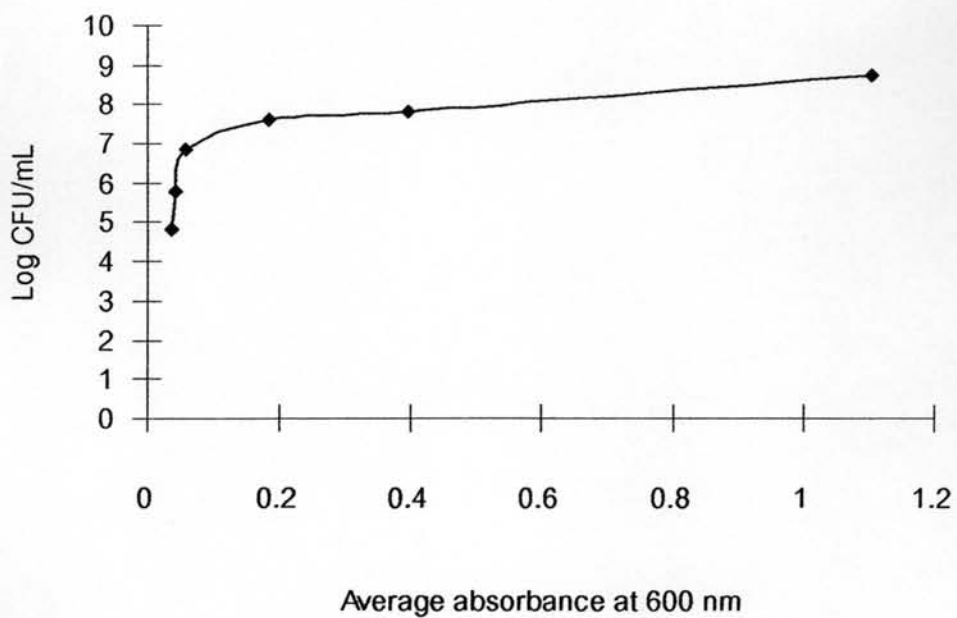


Figure A2 The relationship between average *Lactobacillus plantarum* TISTR 850 count (log CFU/mL) and average absorbance at 600 nm.

Table A2 Average absorbance at 600 nm of *Staphylococcus aureus* ATCC 25923 growth and the average bacterial count at different times.

Time (h)	Average absorbance (600 nm)	Average plate count (log CFU/mL)
0	0.005±0.001	4.58
1	0.007±0.003	5.00
2	0.012±0.006	6.05
4	0.015±0.006	6.31
5	0.039±0.006	6.39
6	0.179±0.016	7.08
7	0.594±0.011	7.54
8	1.020±0.017	8.81
9	1.249±0.018	ND
10	1.475±0.022	ND
11	1.607±0.028	ND
12	1.697±0.041	ND

ND means "Not Detected"

The values shown in the table are average from 3 measurements.

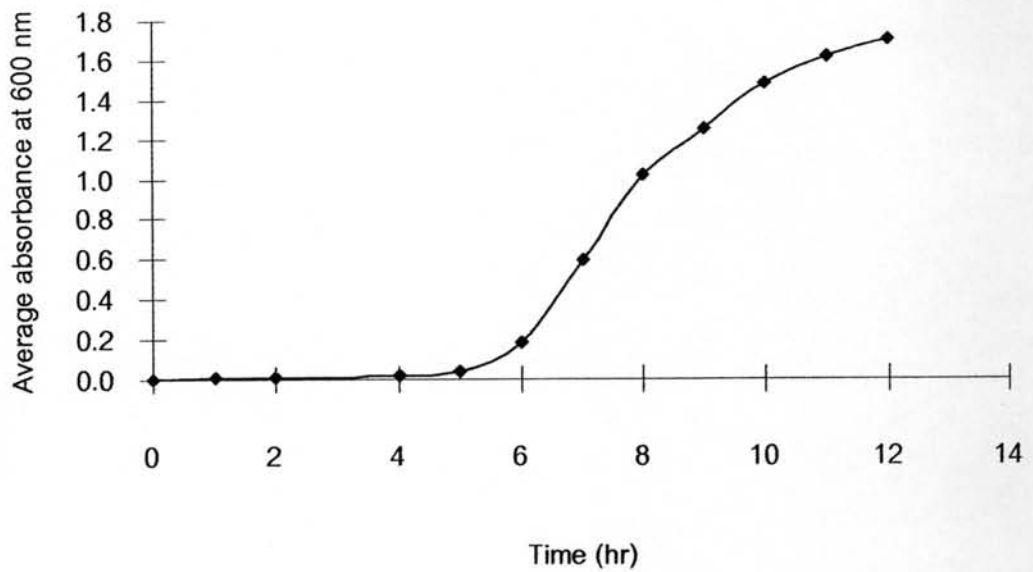


Figure A3 Growth curve of *Staphylococcus aureus* ATCC 25923.

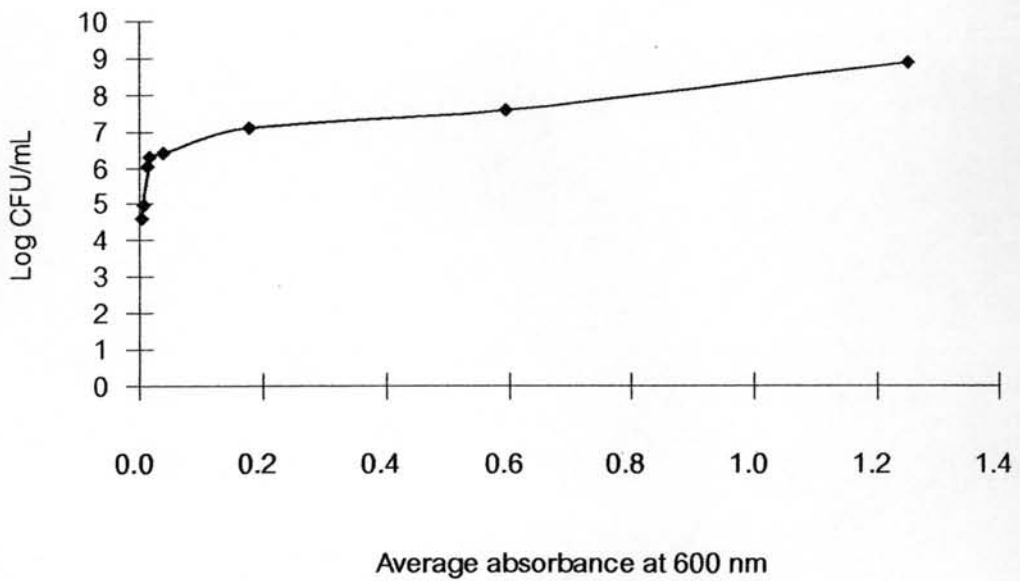


Figure A4 The relationship between average *Staphylococcus aureus* ATCC 25923 count (log CFU/mL) and average absorbance at 600 nm.

Table A3 Average absorbance at 600 nm of *Listeria monocytogenes* DMST 17303 growth and the average bacterial count at different times.

Time (h)	Average absorbance (600 nm)	Average plate count (log CFU/mL)
0	0.004±0.000	6.11
1	0.003±0.002	ND
2	0.006±0.004	ND
3	0.019±0.003	ND
4	0.057±0.005	8.10
5	0.172±0.015	8.41
5.5	0.270±0.014	ND
6	0.435±0.015	8.91
6.5	0.591±0.010	9.19
7	0.687±0.005	9.35
7.5	0.741±0.002	9.41
8	0.782±0.002	9.38
8.5	0.812±0.002	ND
9	0.843±0.003	9.44
9.5	0.871±0.006	ND
10	0.893±0.006	9.45

ND means "Not Detected"

The values shown in the table are average from 3 measurements.

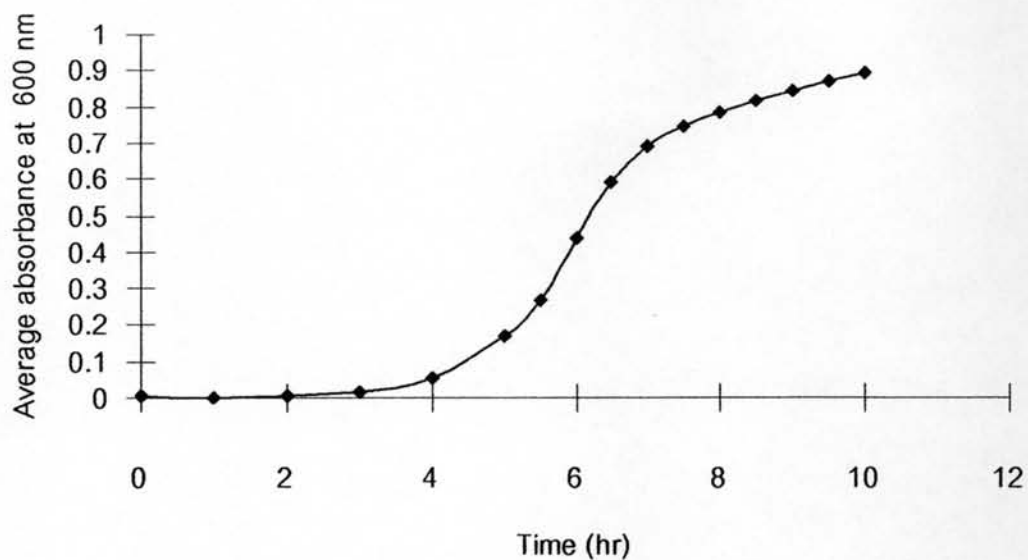


Figure A5 Growth curve of *Listeria monocytogenes* DMST 17303.

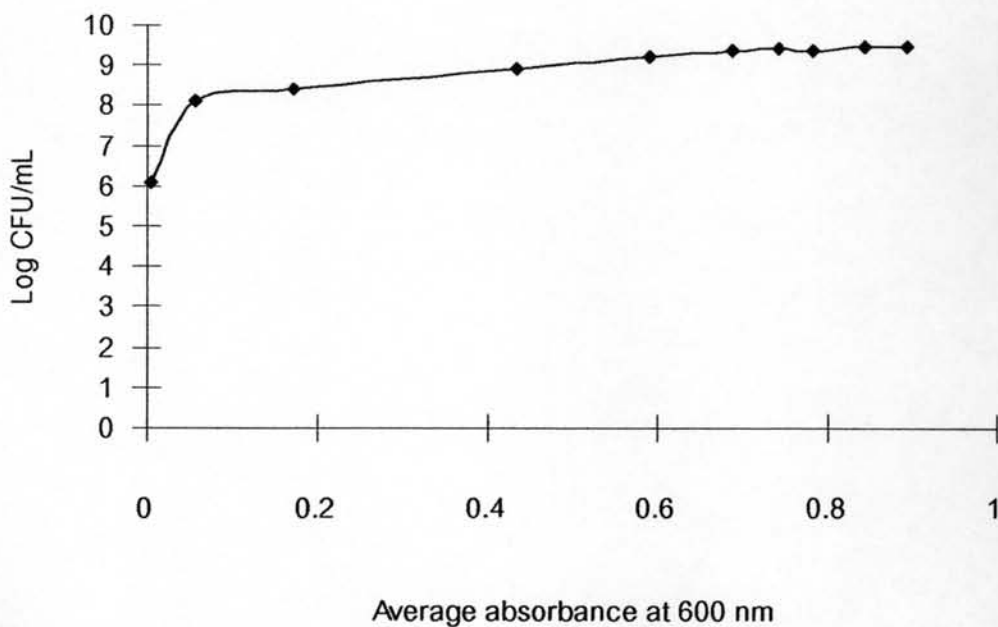


Figure A6 The relationship between average *Listeria monocytogenes* DMST 17303 count (log CFU/mL) and average absorbance at 600 nm.

Table A4 Average absorbance at 600 nm of *Salmonella* Typhimurium ATCC 13311 growth and the average bacterial count at different times.

Time (h)	Average absorbance (600 nm)	Average plate count (log CFU/mL)
0	0.000±0.000	4.37
2	0.000±0.000	5.44
4	0.014±0.005	ND
5	0.096±0.021	8.00
5.5	0.167±0.018	ND
6	0.378±0.017	7.81
6.5	0.552±0.027	ND
7	0.802±0.045	8.77
7.5	1.057±0.025	ND
8	1.205±0.081	9.57
8.5	1.279±0.016	ND
9	0.356±0.021	9.81
9.5	1.430±0.018	ND
10	1.489±0.024	10.18
10.5	1.540±0.040	ND
11	1.608±0.036	ND

ND means "Not Detected"

The values shown in the table are average from 3 measurements.

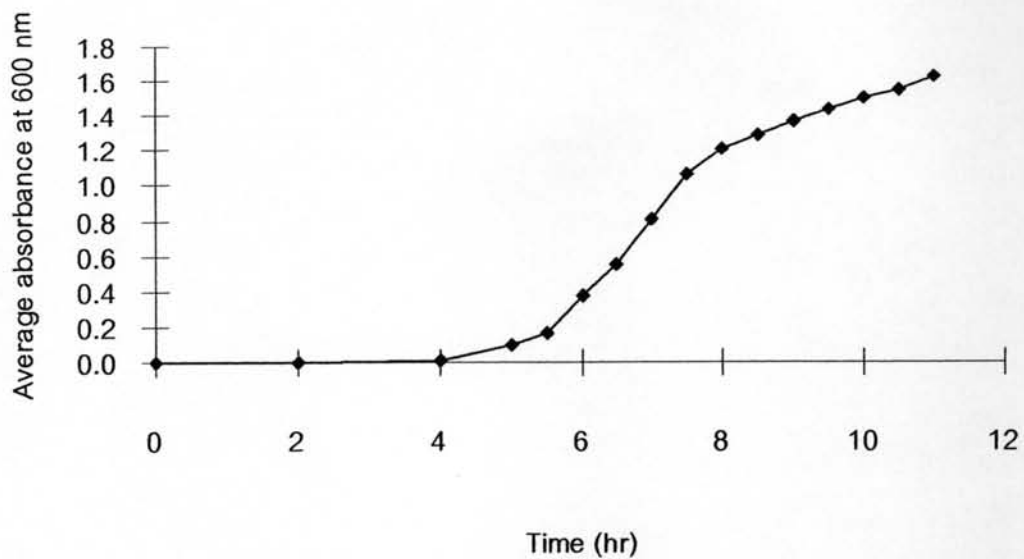


Figure A7 Growth curve of *Salmonella Typhimurium* ATCC 13311.

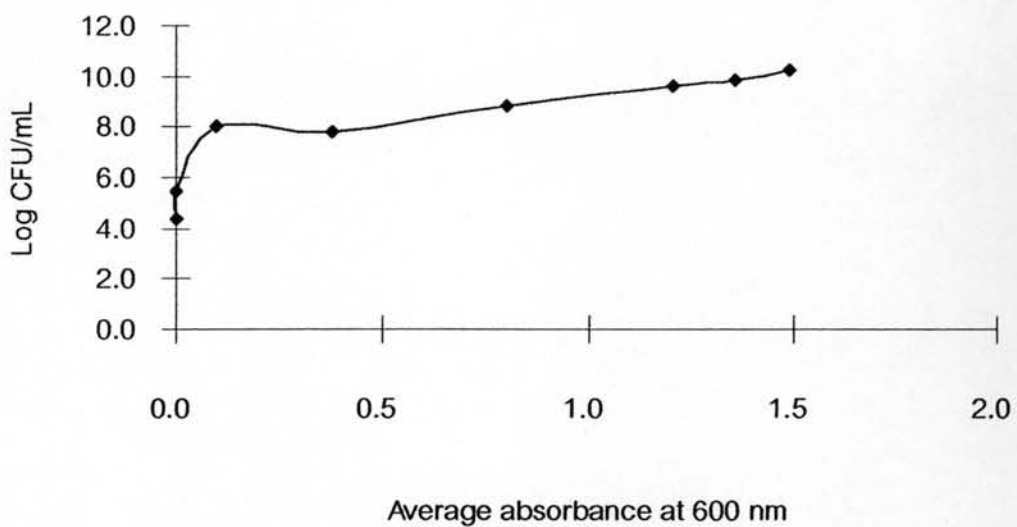


Figure A8 The relationship between average *Salmonella Typhimurium* ATCC 13311 count (log CFU/mL) and average absorbance at 600 nm.

Appendix B

Sample and Reagent Preparations

1. Preparation of 10 mL gelatin-nisin solution (3% w/w nisin concentration and 20% - 24% w/v gelatin concentration)

Material and chemicals

- 20% w/v gelatin solution

Nisin	0.066 g
Gelatin	2.0 g
Glacial acetic acid	5.96 mL
Distilled water	2.56 mL

- 22% w/v gelatin solution

Nisin	0.066 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 24% w/v gelatin solution

Nisin	0.066 g
Gelatin	2.4 g
Glacial acetic acid	5.75 mL
Distilled water	2.47 mL

Procedure

1. Mix amino acid and distilled water to make 70:30 v/v acetic acid: water solvent.

2. Add 2.0, 2.2, and 2.4 g gelatin and 0.066 g nisin in 8.52, 8.37, and 8.22 mL of the solvent respectively, and stir at room temperature ($\sim 25^{\circ}\text{C}$) in order to obtain homogeneous gelatin-nisin solution.

2. Preparation of 10 mL gelatin-nisin solution (22% w/v gelatin concentration and 0% – 3% w/w nisin concentration)

Material and chemicals

- 0% w/w nisin concentration

Nisin	0 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 0.6% w/w nisin concentration

Nisin	0.0132 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 1.2% w/w nisin concentration

Nisin	0.0264 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 1.8% w/w nisin concentration

Nisin	0.0396 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 2.4% w/w nisin concentration

Nisin	0.0528 g
Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

- 3.0% w/w nisin concentration

Nisin	0.066 g
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Gelatin	2.2 g
Glacial acetic acid	5.86 mL
Distilled water	2.51 mL

Procedure

1. Mix amino acid and distilled water to make 70:30 v/v amino acid: water solvent.

2. Add 2.2g gelatin and 0, 0.0132, 0.0264, 0.0396, 0.0528, and 0.066 g nisin in 8.37mL of the solvent respectively, and stir at room temperature ($\sim 25^{\circ}\text{C}$) in order to obtain homogeneous gelatin-nisin solution.

3. Preparation of 1,000 mL 0.85% w/v sodium chloride

Material and chemicals

NaCl	8.5 g
Distilled water	

Procedure

Sodium chloride was dissolved in distilled water. The distilled water was added to reach 1,000 mL final volume.

4. Preparation of 100 mL 1 N hydrochloric acid

Material and chemicals

HCl (conc)	8.29 mL
Distilled water	91.71 mL

Procedure

Mix hydrochloric acid and distilled water to make 1 N HCl.

5. Preparation of 250 mL 0.02 N hydrochloric acid

Material and chemicals

HCl (conc)	0.41 mL
Distilled water	249.59 mL

Procedure

Mix hydrochloric acid and distilled water to make 0.02 N HCl.

Appendix C

Nisin Standard Curve

Table C1 The width of inhibition zone of *Lactobacillus plantarum* determined by agar diffusion technique.

Nisin concentration (mg/mL)	The width of the inhibition zone (cm)		
	Replication 1	Replication2	Average
0.02	1.440	1.460	1.450
0.03	1.540	1.570	1.555
0.04	1.730	1.720	1.725
0.05	1.775	1.750	1.763
0.10	2.050	2.050	2.050
0.15	2.140	2.150	2.145
0.20	2.220	2.245	2.233
0.25	2.400	2.360	2.380
0.30	2.420	2.435	2.428

The nisin standard curve plotted between the width of inhibition zone (Y-axis) and log of nisin concentration in $\times 10^{-2}$ mg/mL (X-axis) was made for each experiment. It is shown in Figure C1.

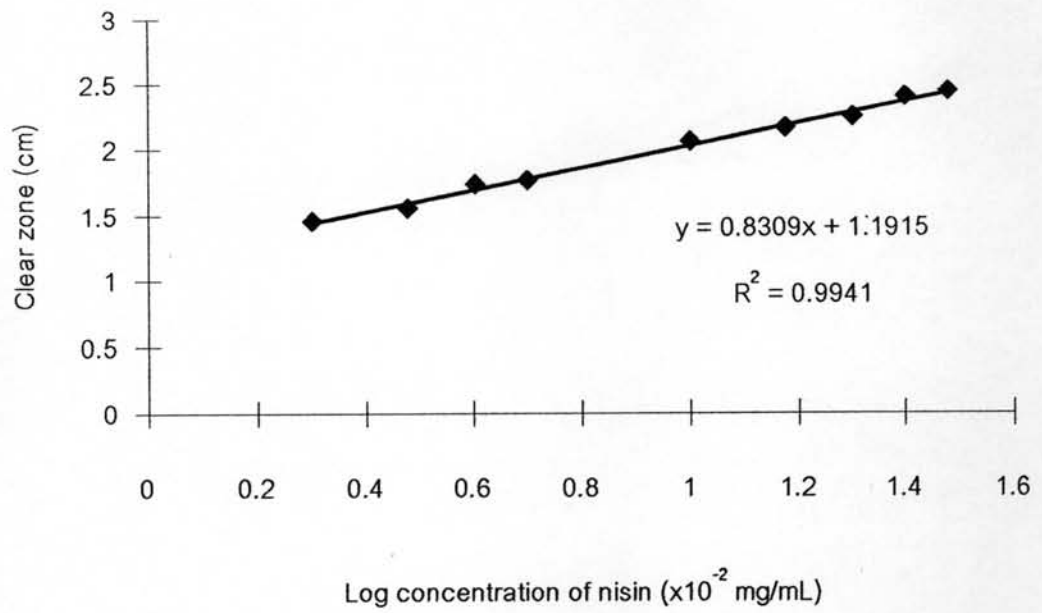


Figure C1 Nisin standard curve – relation between nisin concentrations and clear zones.

Appendix D

Results

Table D1 The width of inhibition zone of non-heated and heated nisin (0.28 mg/mL) of *Lactobacillus plantarum* TISTR 850.

Sample	The width of the inhibition zone (cm)				
	1	2	3	4	average
Nisin [Non-heated]	1.98	2.0	1.92	1.98	1.97±0.035
Nisin [Heated at 120 °C for 30 minutes]	1.90	1.90	1.95	1.92	1.92±0.024

Table D2 Viscosity and conductivity of gelatin-nisin solution (22% w/v gelatin concentration) at various initial nisin concentrations.

Initial nisin concentration (% w/w)	Properties of solution	
	Average viscosity (cP)	Average conductivity(ms/cm)
0.0	473.33±3.06	1.204±0.004
0.6	476.00±2.00	1.384±0.003
1.2	478.33±0.58	1.470±0.002
1.8	481.00±2.00	1.605±0.005
2.4	486.33±3.06	1.702±0.005
3.0	497.00±6.24	2.247±0.064

Table D3 Viscosity and conductivity of gelatin-nisin solution (3% w/w nisin concentration) at various gelatin concentrations.

Gelatin concentration (% w/v)	Properties of solution	
	Average viscosity (cP)	Average conductivity(ms/cm)
20	355.33±13.05	1.754±0.006
22	497.00±6.24	2.247±0.064
24	636.67±4.16	2.560±0.044

Table D4 Average diameter of the gelatin nanofiber and antimicrobial gelatin nanofibers (22% w/v gelatin concentration) at various initial nisin concentrations.

Initial nisin concentration (% w/w)	Average diameter (nm)	Morphological nanofiber
0.0	220.0±62.3	Smooth fiber
0.6	230.0±65.3	Smooth fiber
1.2	232.2±58.3	Smooth fiber
1.8	232.4±58.4	Smooth fiber
2.4	239.8±49.3	Smooth fiber
3.0	246.3±58.6	Smooth fiber

Table D5 Average diameter of the antimicrobial gelatin nanofibers (3% w/w nisin concentration) at various gelatin concentrations.

Gelatin concentration (% w/v)	Average diameter (nm)	Morphological nanofiber
20	232.5±45.4	Bead-on-string fiber
22	246.3±58.6	Smooth fiber
24	324.2±72.0	Smooth fiber

Table D6 Average thickness, tensile strength, Young's modulus, and elongation of crosslinked gelatin nanofiber mat (22% w/v gelatin concentration and 0% - 3% w/w initial nisin concentration).

	Initial nisin concentration (% w/w)					
	0%	0.60%	1.20%	1.80%	2.40%	3.00%
Specimen thickness (μm)	47.8± 8.5	60.2± 4.5	59.6± 4.3	52.8± 2.7	99.0± 8.0	127.8± 7.0
Tensile Strength (MPa)	20.3± 3.7	13.3± 1.8	13.4± 3.5	9.2± 2.6	5.6± 2.0	2.6± 0.4
Young's Modulus (MPa)	965.9±91.9	776.5±117.0	814.0±109.5	609.4±127.2	540.7±94.2	162.8±12.9
Elongation (%)	5.9± 0.9	2.8± 0.4	3.0± 1.1	3.0± 0.4	1.7± 0.5	2.2± 0.4

Table D7 Average thickness, tensile strength, Young's modulus, and elongation of crosslinked antimicrobial gelatin nanofiber mat (3% w/w nisin concentration and 20% - 24% w/v gelatin concentration).

	Gelatin concentration (% w/v)		
	20%	22%	24%
Specimen thickness (μm)	69.6 \pm 5.1	127.8 \pm 7.0	132.8 \pm 28.6
Tensile Strength (MPa)	4.5 \pm 0.5	2.6 \pm 0.4	2.9 \pm 0.6
Young's Modulus (MPa)	272.3 \pm 27.9	162.8 \pm 12.9	215.5 \pm 15.7
Elongation (%)	2.4 \pm 0.5	2.2 \pm 0.4	1.8 \pm 0.3

Table D8 Average width of inhibition zone of nisin from antimicrobial gelatin nanofiber mat (22% w/v gelatin concentration) at various initial nisin concentrations against *Lactobacillus plantarum* for both before and after crosslinking for 5 minutes.

Initial nisin concentration (% w/w)	The average width of the inhibition zone (cm)	
	Before crosslinking	After crosslinking
0.0	0	0
0.6	1.576 \pm 0.011	1.343 \pm 0.010
1.2	1.735 \pm 0.094	1.586 \pm 0.026
1.8	1.834 \pm 0.052	1.744 \pm 0.083
2.4	1.853 \pm 0.017	1.831 \pm 0.037
3.0	1.984 \pm 0.070	1.916 \pm 0.033

Table D9 Average width of inhibition zone of nisin from antimicrobial gelatin nanofiber mat (3% w/w nisin concentration) at various gelatin concentrations against *Lactobacillus plantarum* for both before and after crosslinking for 5 minutes.

Gelatin concentration (% w/v)	The average width of the inhibition zone (cm)	
	Before crosslinking	After crosslinking
20	2.000±0.032	1.900±0.020
22	1.984±0.070	1.916±0.033
24	2.056±0.037	1.943±0.009

Table D10 Average width of inhibition zone of nisin released from crosslinked antimicrobial gelatin nanofiber mat in distilled water at 5 °C against *Lactobacillus plantarum*.

Time (hr)	The average width of inhibition zone (cm)
4	1.324±0.207
8	1.613±0.054
12	1.456±0.090
24	1.619±0.091
36	1.573±0.235
48	1.683±0.300
60	1.731±0.050
72	1.706±0.114
84	1.746±0.124
96	1.639±0.076

Table D11 Average width of inhibition zone of nisin released from crosslinked antimicrobial gelatin nanofiber mat in distilled water at 25 °C and 35 °C against *Lactobacillus plantarum*.

Time (hr)	The average width of inhibition zone (cm)	
	25 °C	35 °C
5/60	1.338±0.119	1.503±0.044
10/60	1.611±0.095	1.545±0.031
15/60	1.579±0.075	1.513±0.036
20/60	1.713±0.064	1.645±0.012
25/60	1.749±0.072	1.596±0.031
30/60	1.789±0.048	1.595±0.056
40/60	1.719±0.042	1.625±0.049
50/60	1.811±0.030	1.723±0.033
1	1.678±0.076	1.595±0.037
2	1.785±0.030	1.653±0.032
3	1.894±0.013	1.698±0.162
4	1.731±0.065	1.749±0.051
5	1.941±0.009	1.685±0.064
6	1.836±0.120	1.771±0.077
8	1.886±0.042	1.789±0.104
10	1.915±0.019	1.824±0.127
12	1.864±0.074	1.870±0.014
24	1.906±0.103	1.934±0.006
36	1.915±0.083	1.954±0.047
48	1.939±0.036	1.968±0.018

Table D12 Average width of inhibition zone (cm) of nisin released from crosslinked antimicrobial gelatin nanofiber mat in distilled water at 45 °C against *Lactobacillus plantarum*.

Time (hr)	The average width of inhibition zone (cm)
5/60	1.753±0.071
10/60	1.725±0.021
15/60	1.834±0.008
20/60	1.800±0.048
25/60	1.818±0.028
30/60	1.820±0.027
40/60	1.915±0.025
50/60	1.731±0.005
1	1.733±0.028
2	1.918±0.021
3	1.794±0.083
4	1.843±0.012
5	1.795±0.110
6	1.941±0.037
7	1.825±0.033
8	1.840±0.042
9	1.898±0.110
10	1.824±0.073
12	1.864±0.078
24	2.030±0.022

Table D13 Average width of inhibition zone (cm) of nisin released from crosslinked antimicrobial gelatin nanofiber mat in distilled water-glycerol ($a_w = 0.955$, 0.975 , and 0.992) at $25\text{ }^\circ\text{C}$.

Time (hr)	The average width of inhibition zone (cm)		
	$a_w 0.955$	$a_w 0.975$	$a_w 0.992$
5/60	1.158±0.022	1.138±0.043	1.338±0.119
10/60	1.315±0.079	1.320±0.185	1.611±0.095
15/60	1.521±0.015	1.613±0.145	1.579±0.075
20/60	1.446±0.119	1.601±0.131	1.713±0.064
25/60	1.371±0.141	1.743±0.031	1.749±0.072
30/60	1.706±0.062	1.695±0.033	1.789±0.048
40/60	1.584±0.033	1.643±0.130	1.719±0.042
50/60	1.685±0.033	1.719±0.050	1.811±0.030
1	1.741±0.066	1.794±0.028	1.678±0.076
2	1.733±0.053	1.808±0.116	1.785±0.030
3	1.805±0.010	1.879±0.030	1.894±0.013
4	1.771±0.018	1.853±0.055	1.731±0.065
5	1.875±0.026	1.875±0.084	1.941±0.009
6	1.886±0.111	1.860±0.024	1.836±0.120
8	1.806±0.034	1.950±0.032	1.886±0.042
10	1.820±0.052	1.886±0.035	1.915±0.019
12	1.850±0.041	1.888±0.064	1.864±0.074
24	1.830±0.023	1.995±0.010	1.906±0.103
36	1.970±0.039	2.093±0.030	1.915±0.083
48	1.868±0.028	2.090±0.026	1.939±0.036

Table D14 Antimicrobial activity of crosslinked antimicrobial gelatin nanofiber mat against *Staphylococcus aureus* ATCC 25923 (10^6 CFU/mL).

Time (hr)	Average log CFU/mL	
	Crosslinked gelatin nanofiber	Crosslinked antimicrobial gelatin nanofiber
0	5.68±0.51	5.68±0.51
2	5.32±0.97	3.14±0.55
4	5.83±0.73	2.83±0.53
8	6.28±0.56	2.66±0.52
12	6.46±0.36	2.47±0.51
24	6.61±0.78	2.10±0.58
48	6.93±0.74	0.41±0.70

Table D15 Antimicrobial activity of crosslinked antimicrobial gelatin nanofiber mat against *Listeria monocytogenes* DMST 17303 (10^6 CFU/mL).

Time (hr)	Average log CFU/mL	
	Crosslinked gelatin nanofiber	Crosslinked antimicrobial gelatin nanofiber
0	6.27±0.02	6.27±0.02
2	6.93±0.03	0.65±0.58
4	7.07±0.02	0.17±0.30
8	7.13±0.08	0
12	6.97±0.06	0
24	6.92±0.18	0
48	7.05±0.14	0

Table D16 Antimicrobial activity of crosslinked antimicrobial gelatin nanofiber mat against *Salmonella* Typhimurium ATCC 13311 (10^6 CFU/mL).

Time (hr)	Average log CFU/mL	
	Crosslinked gelatin nanofiber	Crosslinked antimicrobial gelatin nanofiber
0	6.03±0.14	6.03±0.14
2	6.27±0.27	4.84±0.47
4	6.60±0.01	4.54±0.50
8	7.03±0.03	4.71±0.38
12	7.12±0.03	4.85±0.17
24	7.17±0.06	5.85±0.73
48	7.21±0.07	7.95±0.03

Table D17 Inhibition zone of crosslinked antimicrobial gelatin nanofiber mat against *Lactobacillus plantarum* TISTR 850 at different times.

Time (month)	The width of the inhibition zone (cm)								
	1	2	3	4	5	6	7	8	average
0	1.830	1.845	1.885	1.850	1.775	1.795	1.735	1.795	1.814±0.048
1	1.840	1.870	1.850	1.850	1.835	1.880	1.800	1.855	1.848±0.024
3	1.920	1.925	1.835	1.915	1.875	1.880	1.885	1.930	1.896±0.033
5	1.880	1.885	1.835	1.850	1.850	1.855	1.870	1.850	1.859±0.017

CURRICULUM VITAE

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