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APPENDICES

Appendix A Characterization of CM-Chitin

Table A1 Viscosity-average molecular weight of CM-chitin

conc. (g/100 ml)	Time (sec.)				η_{rel}	η_{sp}
	X1	X2	X3	Average		
0	242.25	242.28	242.28	242.27		
0.00625	252.81	252.84	252.77	252.81	1.0435	0.0435
0.01250	264.75	264.78	264.76	264.76	1.0928	0.0928
0.02500	290.13	290.15	290.11	290.13	1.1975	0.1975
0.05000	344.37	344.35	344.32	344.35	1.4213	0.4213
0.10000	477.97	477.44	476.70	477.37	1.9704	0.9704

conc. (g/100 ml)	η_{sp}/c	$\ln(\eta_{rel})/c$
0.00625	6.9586	5.1177
0.01250	7.4275	4.4708
0.02500	7.9019	3.8692
0.05000	8.4267	3.3473
0.10000	9.7040	2.9808

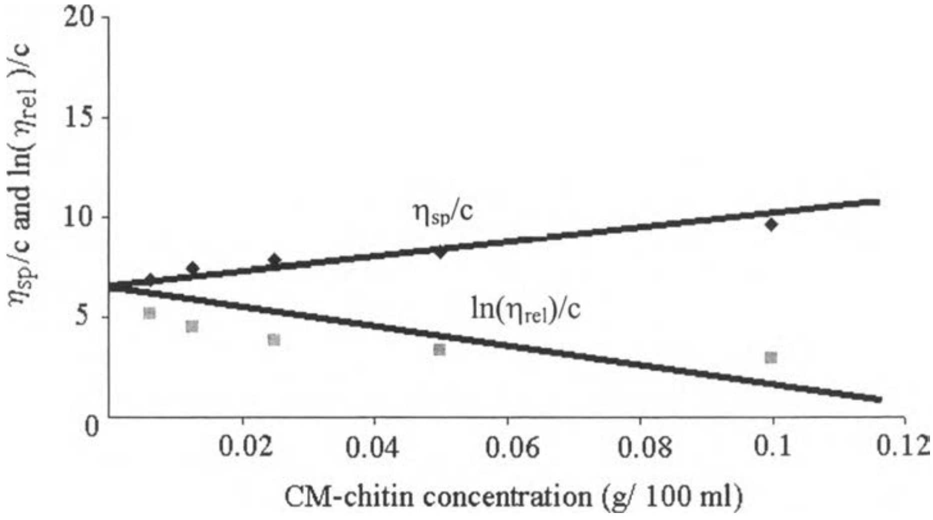


Figure A1 η_{sp}/c and ln(η_{rel})/C against concentration of CM-chitin solution.

The viscosity-average molecular weight of CM-chitin was determined based on Mark-Houwink equation. The K and a values were according to Kaneko *et al.* (1982).

$$[\eta] = 7.92 \times 10^{-5} M^{1}$$

Where [η] = intrinsic viscosity

M = Viscosity-average molecular weight

Interception: [η] = 6.4

From calculation; $M = 8.08 \times 10^{4}$

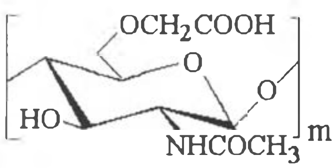
The viscosity-average molecular weight of CM-chitin obtained from calculation was 8.08×10^{4} g/mol.

Table A2 Degree of substitution of CM-chitin from elemental analysis

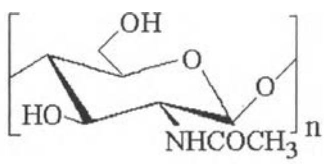
Experimental values

	%C	%N	%H
1	38.977	5.878	5.954
2	38.38	6.289	5.807
Average	38.904	5.881	6.084

Calculation of Degree of Substitution of CM-chitin



MW = $C_{10}H_{11}O_3NNa$ = 283



MW = $C_8H_{13}O_5NNa$ = 203

$$283\,m + 203\,n = 8.08 \times 10^4 \quad \text{---(1)}$$

From EA, %C = 38.904

%H = 6.084

%N = 5.881

We can find that in CM-chitin structure has N.

That: $N = \frac{8.08 \times 10^4 \times 5.881}{100} = 4751.848$

Thus, $14\,m + 14\,n = 4751.848$ ---(2)

Divide (2) by 14 $m + n = 339.418$

$m = 339.418 - n$ ---(3)

Replace (3) in (1), $283(339.418) - 283\,n + 203\,n = 8.08 \times 10^4$

$80\,n = 15255.294$

$n = 190.691$

$m = 148.727$

Therefore, fraction $m = 148.727/339.418 = 0.44$

$n = 190.691/339.418 = 0.56$

The degree of carboxymethylation was estimated to be 0.44.

APPENDIX B FTIR Spectra of CM-Chitin/Silk Fibroin Blend Films

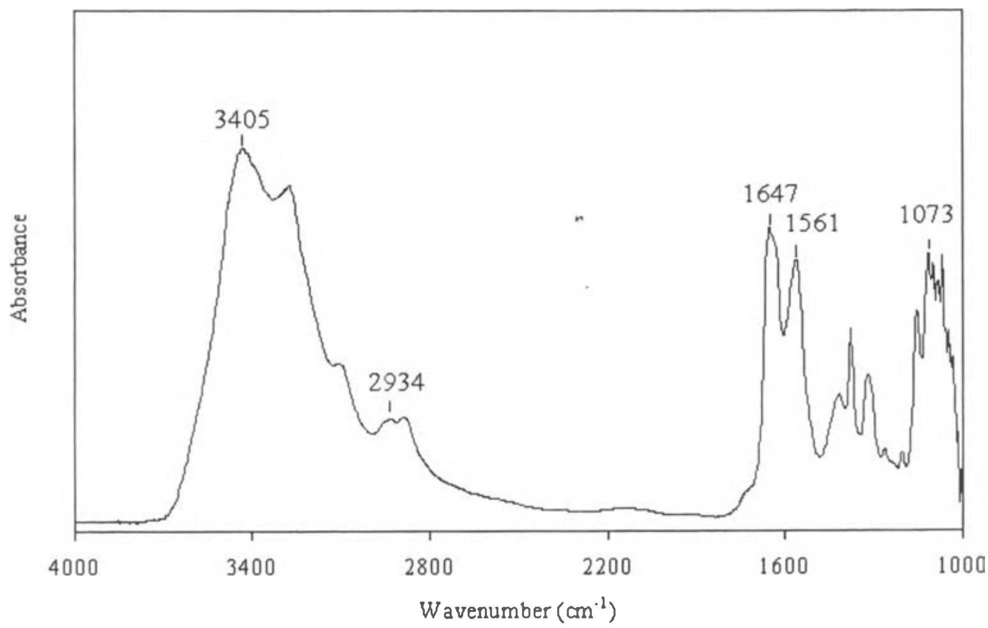


Figure B1 FTIR spectrum of CM-chitin film.

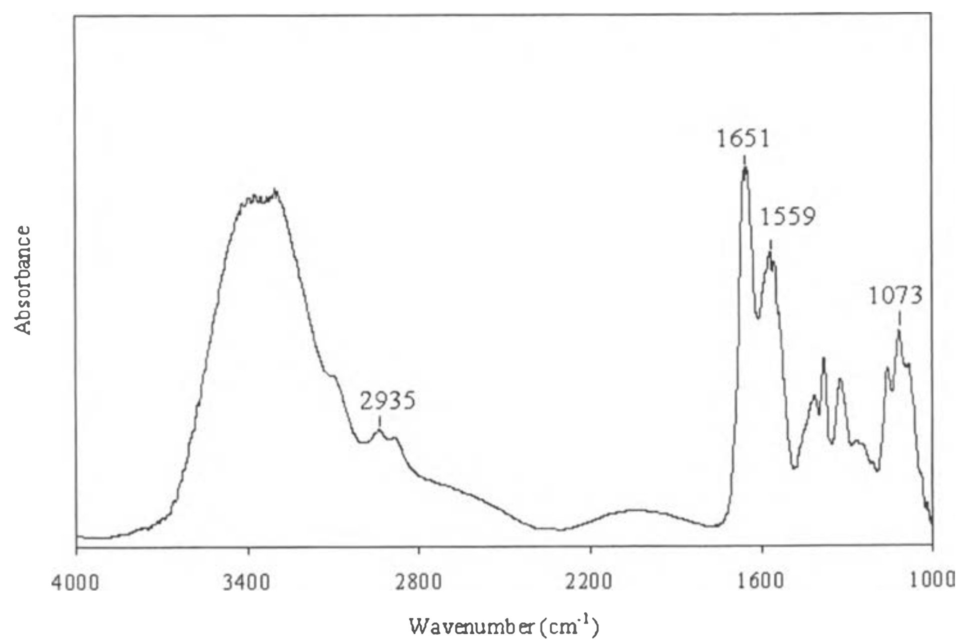


Figure B2 FTIR spectrum of the blend film containing 80% CM-chitin.

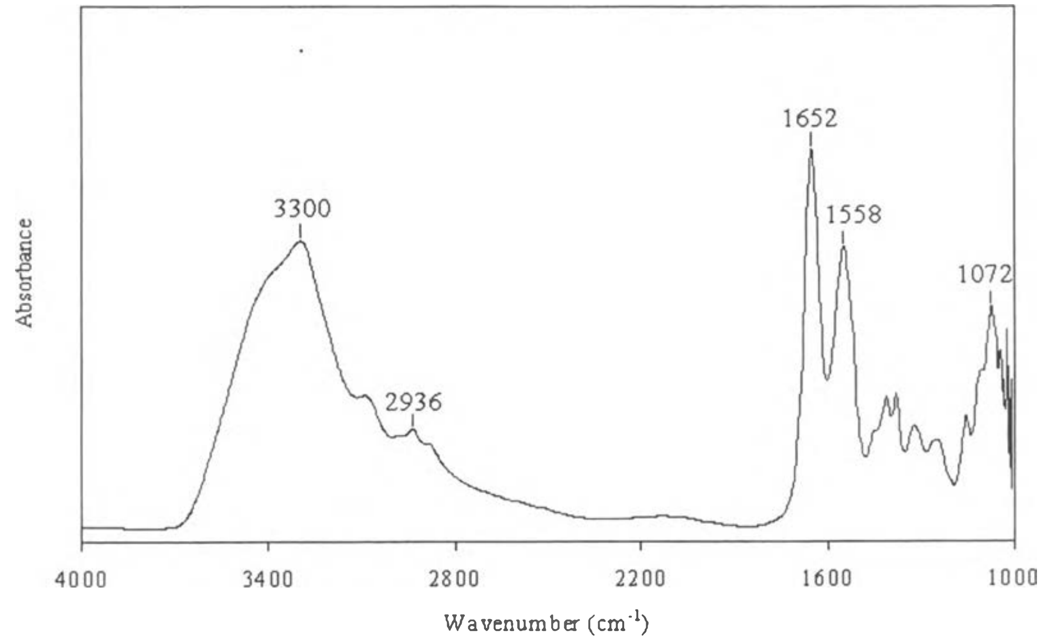


Figure B3 FTIR spectrum of the blend film containing 60% CM-chitin.

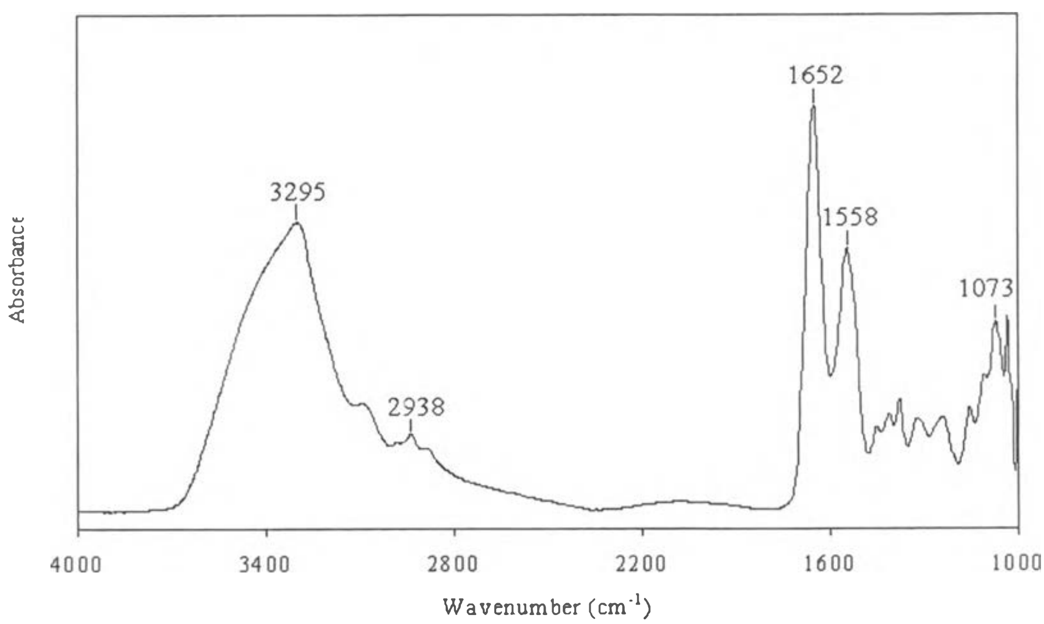


Figure B4 FTIR spectrum of the blend film containing 50% CM-chitin.

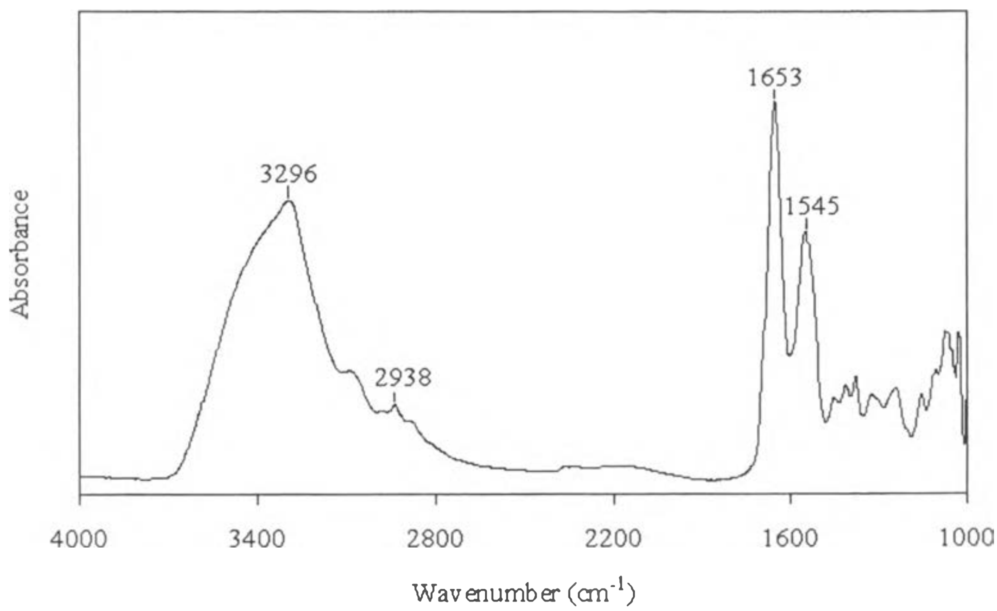


Figure B5 FTIR spectrum of the blend film containing 40% CM-chitin.

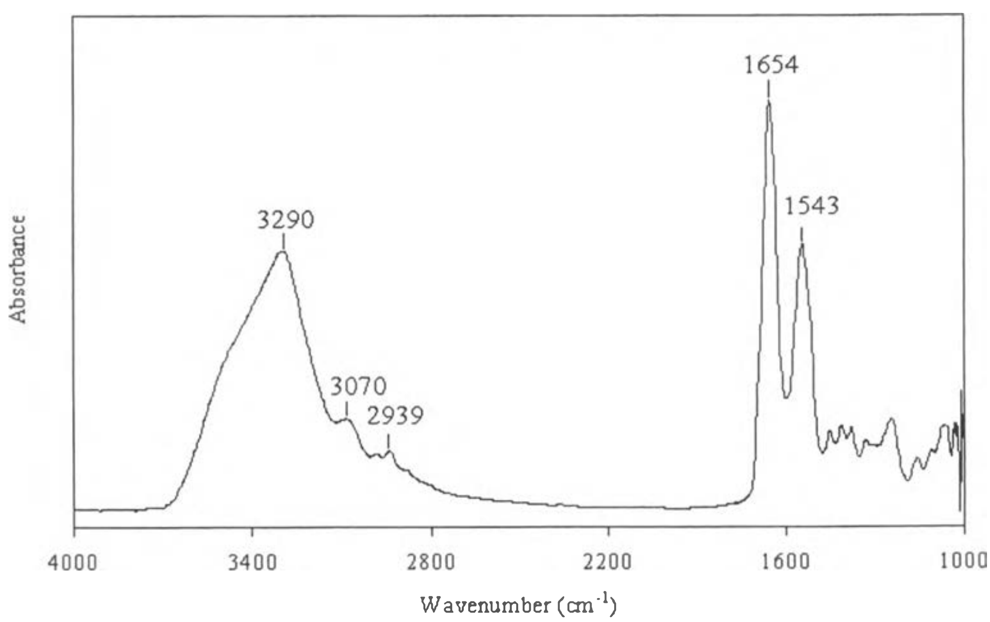


Figure B6 FTIR spectrum of the blend film containing 20% CM-chitin.

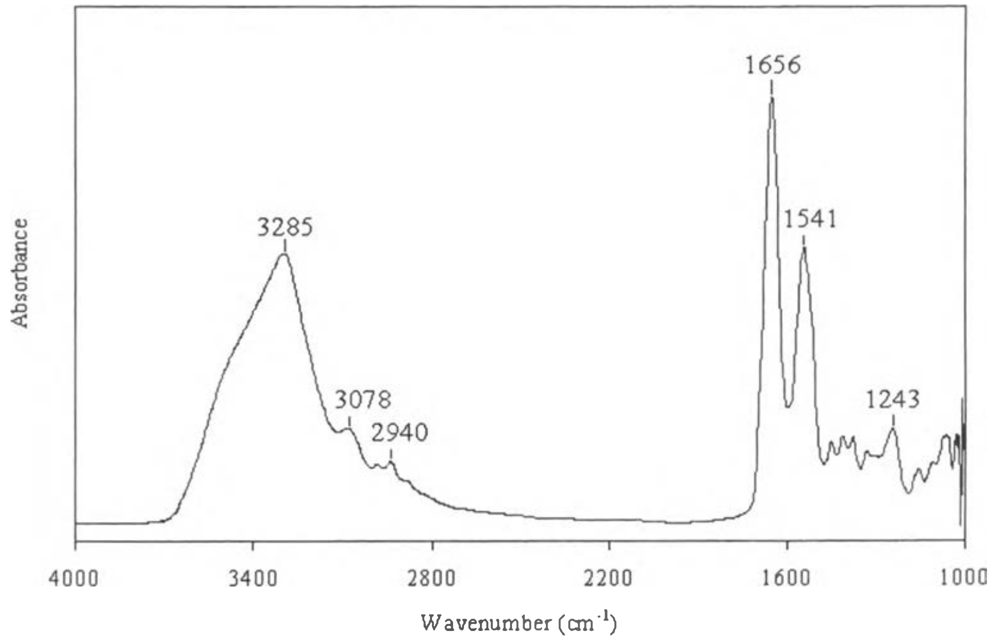


Figure B7 FTIR spectrum of silk fibroin film.

APPENDIX C Effect of Time on Equilibrium Water Content of Blend Films

Table C1 Effect of time on equilibrium water content (EWC) of blend films at 15 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	11.11	11.11	15.79	12.67	2.70
40	33.33	37.50	33.33	34.72	2.41
50	119.05	120.00	120.00	119.68	0.55
60	113.33	112.50	127.78	117.87	8.59
80	129.73	134.78	138.10	134.20	4.21
100	467.65	465.71	464.87	466.08	1.43

Table C2 Effect of time on equilibrium water content (EWC) of blend films at 30 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	14.29	22.22	26.32	20.94	6.12
40	54.17	58.33	58.33	56.94	2.41
50	130.00	133.33	128.57	130.63	2.44
60	156.25	150.00	162.50	156.25	6.25
80	151.43	169.57	154.76	158.59	9.65
100	536.84	535.29	532.35	534.83	2.28

Table C3 Effect of time on equilibrium water content (EWC) of blend films at 60 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	33.33	33.33	33.33	33.33	0.00
40	66.67	70.83	70.83	69.44	2.41
50	132.50	130.95	137.50	133.65	3.42
60	168.75	177.78	168.75	171.76	5.21
80	195.65	192.86	170.27	186.26	13.92
100	526.32	517.65	518.92	520.96	4.68

Table C4 Effect of time on equilibrium water content (EWC) of blend films at 90 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	50.00	42.11	44.44	45.52	4.06
40	111.11	100.00	112.50	107.87	6.85
50	142.50	142.86	145.83	143.73	1.83
60	225.00	210.00	206.25	213.75	9.92
80	232.61	230.95	231.78	231.78	0.83
100	544.74	542.86	548.65	545.41	2.95

Table C5 Effect of time on equilibrium water content (EWC) of blend films at 120 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	55.56	55.56	52.63	54.58	1.69
40	125.93	137.50	129.17	130.86	5.97
50	143.75	142.50	145.24	143.83	1.37
60	200.00	200.00	193.75	197.92	3.61
80	252.17	242.86	246.77	247.27	4.68
100	565.79	561.77	559.46	562.34	3.20

Table C6 Effect of time on equilibrium water content (EWC) of blend films at 180 min

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	61.11	61.91	57.90	60.30	2.12
40	159.26	156.67	146.15	154.03	6.94
50	154.17	154.50	154.76	154.48	0.30
60	262.50	268.75	240.00	257.08	15.12
80	243.48	242.86	243.08	243.14	0.31
100	565.79	564.71	562.16	564.22	1.86

Table C7 Effect of time on equilibrium water content (EWC) of blend films at 24 h

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	77.78	77.78	66.67	74.07	6.41
40	150.00	155.00	145.83	150.28	4.59
50	180.00	181.25	166.67	175.97	8.08
60	183.33	187.50	196.15	189.00	6.54
80	254.35	252.38	253.10	253.28	1.00
100	538.24	555.88	543.24	545.79	9.09

Table C8 Effect of time on equilibrium water content (EWC) of blend films at 48 h

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	77.78	77.78	57.14	70.90	11.91
40	166.67	165.00	169.05	166.91	2.03
50	170.83	169.23	187.50	175.85	10.12
60	231.25	211.11	221.04	221.13	10.07
80	258.70	240.48	253.10	250.76	9.33
100	565.79	550.00	545.95	553.91	10.48

APPENDIX D Effect of pH on Swelling Behavior of the Blend Films

Table D1 Degree of swelling of blend films in pH buffer solution pH = 3

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	109.09	117.39	100.00	108.83	8.70
40	138.46	148.72	120.59	135.92	14.24
50	364.71	371.43	380.00	372.05	7.67
60	440.74	448.00	452.00	446.91	5.71
80	450.00	451.52	437.93	446.48	7.44
100	935.90	943.48	933.33	937.57	5.28

Table D2 Degree of swelling of blend films in pH buffer solution pH = 4

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	80.76	82.61	88.00	83.79	3.76
40	115.39	117.95	126.14	119.83	5.62
50	296.88	303.13	300.00	300.00	3.13
60	369.23	355.56	352.00	358.93	9.10
80	437.50	423.40	430.00	430.30	7.05
100	817.50	803.03	807.69	809.41	7.39

Table D3 Degree of swelling of blend films in pH buffer solution pH = 5

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	62.00	42.86	50.00	51.62	9.67
40	115.00	114.71	112.00	113.90	1.65
50	287.88	278.13	278.95	281.65	5.41
60	363.64	365.39	370.00	366.34	3.29
80	319.05	317.24	323.53	319.94	3.24
100	741.67	741.18	744.83	742.56	1.98

Table D4 Degree of swelling of blend films in pH buffer solution pH = 6

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	28.57	30.44	28.57	29.19	1.08
40	94.23	100.00	111.77	102.00	8.94
50	274.19	274.29	274.23	274.24	0.05
60	313.04	312.50	313.79	313.11	0.65
80	327.10	334.04	331.71	330.95	3.53
100	712.50	720.00	705.00	712.50	7.50

Table D5 Degree of swelling of blend films in pH buffer solution pH = 7

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	10.53	16.00	12.00	12.84	2.83
40	107.41	95.24	90.24	97.63	8.83
50	210.35	214.29	217.24	213.96	3.46
60	313.04	312.50	313.79	313.11	0.65
80	372.22	389.29	388.89	383.47	9.74
100	687.50	674.42	675.56	679.16	7.25

Table D6 Degree of swelling of blend films in pH buffer solution pH = 8

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	45.00	44.44	57.14	48.86	7.18
40	109.68	104.76	110.35	108.26	3.05
50	302.78	316.67	298.57	306.01	9.47
60	366.67	346.67	376.67	363.33	15.28
80	417.50	433.33	425.64	425.49	7.92
100	810.53	804.35	812.50	809.12	4.25

Table D7 Degree of swelling of blend films in pH buffer solution pH = 9

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	77.27	83.33	77.27	79.29	3.50
40	129.27	126.19	125.00	126.82	2.20
50	314.29	310.64	309.00	311.31	2.71
60	496.77	525.00	500.00	507.26	15.45
80	660.00	672.50	662.50	665.00	6.61
100	1132.50	1128.95	1147.37	1136.27	9.77

Table D8 Degree of swelling of blend films in pH buffer solution pH = 10

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	107.69	105.56	111.11	108.12	2.80
40	130.44	131.58	131.25	131.09	0.59
50	362.86	361.86	364.71	363.14	1.45
60	700.00	718.18	704.17	707.45	9.53
80	773.33	782.35	759.09	771.59	11.73
100	1278.65	1285.71	1283.72	1282.69	3.64

Table D9 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 3

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	814.29	837.50	825.00	825.60	11.62
0.01	364.71	371.43	380.00	372.05	7.67
0.05	134.21	136.11	143.24	137.86	4.76

Table D10 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 4

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	532.65	540.00	533.96	535.54	3.92
0.01	296.88	303.13	300.00	300.00	3.13
0.05	178.57	174.07	165.46	172.70	6.67

Table D11 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 5

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	536.67	537.50	542.11	538.76	2.93
0.01	287.88	303.13	300.00	297.00	8.05
0.05	152.17	138.78	145.83	145.59	6.70

Table D12 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 6

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	515.79	535.29	520.00	523.69	10.26
0.01	274.19	274.29	274.23	274.24	0.05
0.05	120.00	111.43	117.95	116.46	4.48

Table D13 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 7

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	520.00	524.00	536.36	526.79	8.53
0.01	210.35	214.29	217.24	213.96	3.46
0.05	123.53	131.03	130.92	128.50	4.30

Table D14 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 8

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	652.63	647.83	644.44	648.30	4.11
0.01	264.10	261.11	265.71	263.64	2.34
0.05	164.52	175.76	162.50	167.59	7.14

Table D15 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 9

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	885.71	890.91	887.50	888.04	2.64
0.01	314.29	310.64	312.90	312.61	1.84
0.05	180.56	182.14	187.18	183.29	3.46

Table D16 Degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 10

Glutaraldehyde content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
0.005	894.12	900.00	918.75	904.29	12.86
0.01	362.86	361.77	364.71	363.11	1.49
0.05	220.00	211.11	223.08	218.06	6.21

Table D17 Effect of time on degree of swelling of CM-chitin/silk fibroin: 50/50 in pH buffer solution pH = 6 and pH = 10

pH	Time (min)	Degree of swelling (%)			Average	Standard deviation
		X1	X2	X3		
6	30	285.71	274.19	276.47	278.79	6.10
	60	274.29	277.42	290.91	280.87	8.83
	90	274.23	267.74	296.77	279.58	15.24
10	120	362.86	351.61	351.61	355.36	6.49
	150	362.86	351.61	361.77	358.75	6.20
	180	362.86	358.07	361.77	360.90	2.51
6	210	268.57	261.29	276.47	268.78	7.59
	240	268.57	261.29	279.41	269.76	9.12
	270	268.57	261.29	280.65	270.17	9.78
10	300	368.57	367.74	358.07	364.79	5.84
	330	368.57	374.19	364.71	369.16	4.77
	360	365.71	367.74	364.71	366.05	1.55
6	390	267.74	277.42	278.79	274.65	6.02
	420	274.19	290.32	284.85	283.12	8.20

APPENDIX E Effect of Salt Type on Swelling Behavior of the Blend Films

Table E1 Degree of swelling of blend films in 0.25 M LiCl

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	55.56	57.16	53.45	55.39	1.86
40	162.50	162.86	176.67	167.34	8.08
50	348.57	348.65	347.50	348.24	0.64
60	878.85	860.42	881.54	873.60	11.50
80	440.00	450.00	442.86	444.29	5.15
100	716.67	708.33	693.10	706.03	11.95

Table E2 Degree of swelling of blend films in 0.25 M NaCl

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	46.67	41.67	55.56	47.96	7.03
40	147.22	151.43	145.16	147.94	3.19
50	425.00	421.43	429.63	425.35	4.11
60	901.45	924.07	911.05	912.19	11.36
80	427.78	429.03	420.83	425.88	4.42
100	831.25	838.46	847.06	838.92	7.91

Table E3 Degree of swelling of blend films in 0.25 M $CaCl_2$

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	35.00	30.00	40.00	35.00	5.00
40	166.67	160.53	162.27	163.16	3.17
50	362.86	369.23	375.93	369.34	6.54
60	846.24	887.23	865.55	866.34	20.51
80	375.00	384.44	378.95	379.46	4.74
100	593.33	612.00	611.77	605.70	10.71

Table E4 Degree of swelling of blend films in 0.25 M $FeCl_3$

CM-chitin content (%)	Degree of swelling (%)			Average	Standard deviation
	X1	X2	X3		
20	30.453	31.818	43.478	35.250	7.159
40	133.333	128.824	131.047	131.068	2.255
50	268.966	278.947	278.125	275.346	5.541
60	473.469	477.183	473.077	474.576	2.266
80	346.087	344.565	349.206	346.619	2.366
100	608.333	613.158	582.353	601.281	16.569

Table E5 Degree of swelling of blend films in water

CM-chitin content (%)	EWC (%)			Average	Standard deviation
	X1	X2	X3		
20	77.78	77.78	66.67	74.07	6.41
40	150.00	155.00	145.83	150.28	4.59
50	180.00	181.25	166.67	175.97	8.08
60	183.33	187.50	196.15	189.00	6.54
80	254.35	252.38	253.10	253.28	1.00
100	538.24	555.88	543.24	545.79	9.09

APPENDIX F Mechanical Properties of the Blend Films

Table F1 Tensile strength of the blend films

CM-chitin content (%)	Tensile strength (MPa)			Average	Standard deviation
	X1	X2	X3		
20	2.84	2.45	5.56	3.62	1.69
40	15.43	14.21	15.54	15.06	0.74
50	15.57	14.99	15.71	15.42	0.38
60	18.13	18.80	21.13	19.35	1.57
80	21.57	18.61	21.97	20.71	1.83
100	28.00	22.92	26.95	25.96	2.68

Table F2 Tensile strength of the blend film with containing 0.01% glutaraldehyde

CM-chitin content (%)	Tensile strength (MPa)			Average	Standard deviation
	X1	X2	X3		
20	6.45	9.96	5.96	7.46	2.18
40	18.97	11.91	15.81	15.56	3.54
50	20.71	17.14	16.25	18.03	2.36
60	23.80	25.70	24.88	24.79	0.95
80	26.05	27.77	26.98	26.94	0.86
100	29.86	25.98	31.67	29.17	2.91

Table F3 Elongation at break of the blend film

CM-chitin content (%)	Elongation (%)			Average	Standard deviation
	X1	X2	X3		
20	1.59	1.34	1.24	1.39	0.18
40	2.05	1.81	1.95	1.94	0.12
50	1.91	2.16	2.33	2.13	0.21
60	2.65	2.54	1.95	2.38	0.38
80	3.03	2.51	2.71	2.75	0.26
100	2.95	3.14	3.29	3.13	0.17

Table F4 Elongation at break of the blend film with containing 0.01% glutaraldehyde

CM-chitin content (%)	Elongation (%)			Average	Standard deviation
	X1	X2	X3		
20	0.71	1.26	1.24	1.07	0.31
40	2.18	1.56	1.94	1.89	0.31
50	2.41	1.86	1.88	2.05	0.31
60	1.69	2.13	1.87	1.90	0.22
80	2.15	2.04	2.09	2.09	0.06
100	3.06	3.04	3.13	3.08	0.05

APPENDIX G

Table G1 Decomposition temperature of pure and blend films

CM-chitin content (%)	Decomposition temperature (^{o}C)		Average	Standard deviation
	X1	X2		
0	299.34	299.33	299.34	0.01
20	292.93	292.62	292.78	0.22
40	289.54	286.34	287.94	2.26
50	284.49	284.48	284.49	0.01
60	279.45	279.42	279.44	0.02
80	269.09	269.30	269.20	0.15
100	265.70	265.90	265.80	0.14

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