Chitin　　Chitosan

Chitin　Chitosan

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Studies on the Application of Near Infrared Spectroscopy for Analysis of Chitin and Chitosan

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Abstract

Since near-infrared(NIR) spectroscopic analysis, one of the most popular method for nondestructive measurement among various equipment analyses, does not require such treatment processes as pretreatment of using a reagent, it has been actively used in various fields for the development of analysis methods.

Chitin and chitosan, which are recognized as a representative case for the use of by-product of marine process, require deproteinization process and deacetylation process, and the current methods used for the quality management of this process require expenditure of materials or

time. In this research, NIR spectroscopic analysis has been studied for the measurement of deproteinization and deacetylation to apply the merits of NIR spectroscopic analysis to the quality management in the process of chitin and chitosan production, and the results derived from this study are summarized as follows;

First, in measuring squid pen and red snow crab shell, which are raw materials of chitin and chitosan by NIR, there were the typical peaks in 1200nm, 1510nm, 2050nm and 2180nm. Squid pen had somewhat higher peak than red snow crab shell, and it shows the same result as the result of chemical protein analysis.

In producing chitin, the amount of protein was decreased. Measuring it by NIR, reduction of protein caused by deproteinization was identified in producing chitin.

Chitosan is a derivative material made from chitin by processing the deacetylation. During this processing, acetyl groups were removed and amide bends were appeared. From NIR spectra, peaks at 1530nm and 2030nm indicated amide II peak of chitosan, and these peaks were used for identifying the differences of structure between chitin and chitosan.

Currently, IR is used for the measurement of the deacetylation degree of chitosan. But, the measurement of nonidentified sample by using the standard curve which has prepared by different deacetylation degrees found out the possibility of measurement of the deacetylation degree by

NIR. The error in measurement of nonidentified sample was below 1% and the error in the standard curve was below 0.006. These errors were very low and the accuracy of NIR was considered to be superior to the existing methods.

In using NIR to examine the stuructual changes by fat absorption of chitosan, it was identified that fat absorption occurred by the combination of C-H group in fat and amide group in chitosan.

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Dull(1978)　　"

"   .

                              ,   ,

              .

                              (Near-Infrared)

              .   C-H, N-H, O-H

          0.8   -   2.5

                                                              (Lanza.

    1983).

      1960   Norris   Ben-Gera

                          spectrophotometer

                        ,   Near Infrared Reflectance Spectroscopy (NIR)

                                                                  .

                              10

                                              .

              (NIR)   ,   ,

                              .

NIR   5

                                                                                    .

              .

                                                                                              .

                                                              .

                              NIR

                        .

                        .

                                                                                        .

                            ,

              chitin   chitosan   cellulose

                                                            ,

                    . Chitin

      chitin   chitosan

                              ,

                                                                      (Table 1).

      chitin   chitosan   ,

Table 1. Industrial applications of chitosan.

Application fields	Available functions
Wastewater Treatment	Removal of metal Flocculant and coagulant - protein - dye - amino acids
Food Industry	Removal of dye and suspended solids etc Preservative Color stabilization Animal feed additive
Medical	Bandages Blood cholesterol control Controlled release of drugs Skin bum Contact lens
Biotechnology	Enzyme and cell immobilization Protein separation Cell recovery Chromatography
Agriculture	Seed coating Fertilizer Controlled agrochemical relaese
Cosmetic	Moisturizer Face, hand and body creams Bath lotion Hair care
Pulp and Paper	Surface treatment Photographic paper
Membrane	Permeability control Reverse osmosis

,

.

chitin   chitosan

chitin   chitosan   (Fig.1), chitosan
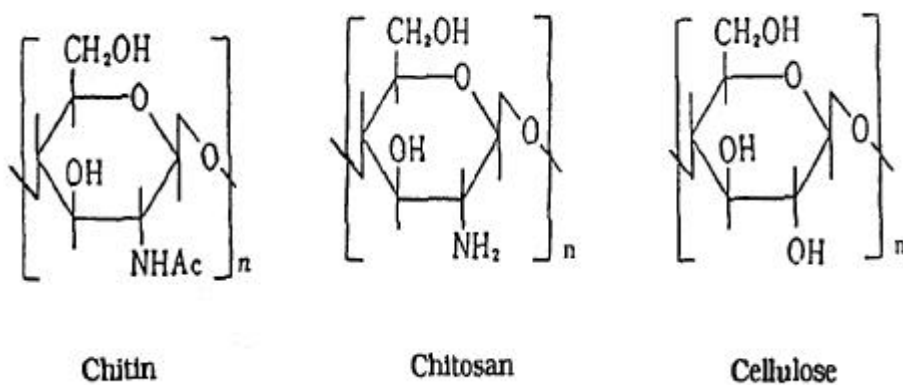
,

chitin   chitosan

.

Fig. 1. Chemical structure of chitin, chitosan and cellulose.

I.

  1-1.

                                        (*Ommastrephes*

bartrami)   40   12

    50mesh   chitin   .

  1-2.

  Chitin   chitosan

                     (*Chinonocetes japonicus*)

    50   , 20mesh

    (5)   .

  .

  1.   **chitin**

   50mesh   1N NaOH   (15)

               3

     40   chitin   (Fig.2).

  2.   **chitosan**
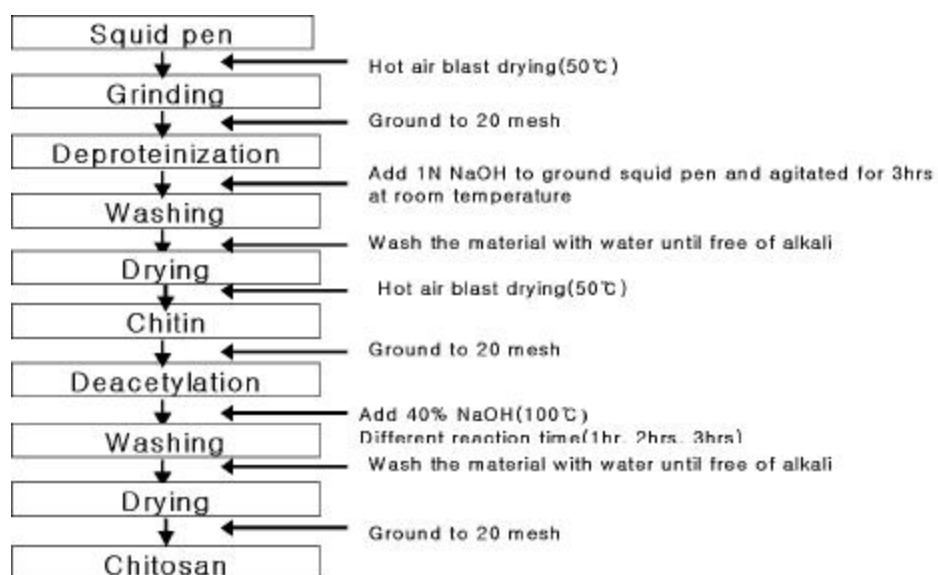
  Chitin 100g   40% NaOH   1.6   110 115   3

                 chitosan   (Fig.2).

| Squid pen |
| — Hot air blast drying(50℃) |
| Grinding |
| — Ground to 20 mesh |
| Deproteinization |
| — Add 1N NaOH to ground squid pen and agitated for 3hrs at room temperature |
| Washing |
| — Wash the material with water until free of alkali |
| Drying |
| — Hot air blast drying(50℃) |
| Chitin |
| — Ground to 20 mesh |
| Deacetylation |
| — Add 40% NaOH(100℃) Different reaction time(1hr, 2hrs, 3hrs) |
| Washing |
| — Wash the material with water until free of alkali |
| Drying |
| — Ground to 20 mesh |
| Chitosan |

Fig. 2. Preparation procedure of chitin and chitosan from squid pen.

3.   chitin

,

20mesh   .   2N HCl

10   3   1N NaOH

        100   3   pH

                        50   chitin   (Fig.3).

4.   chitosan

Chitin   20mesh   chitin   47% NaOH   13

0   1   , 2   , 3

                            50

            (Fig.3).

5.   chitosan

                        chitosan   0.1M acetate - 0.1M sodium acetate

buffer(pH 4.0)   1%   lipase   chitosan   1

: 0.3   40   24   incuvation   ,

        100   15   autoclaving   . Inactivation

glass filter   chitosan   , chitosan

    0.1M NaOH   (5000rpm, 10   )

                                              (Vacuum Freezer
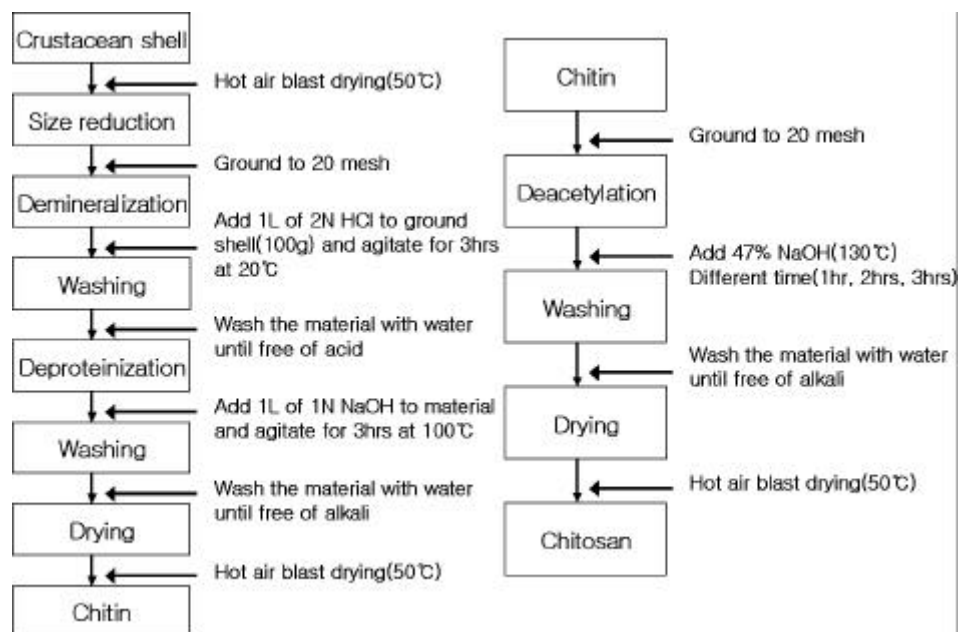
dryer, SFDSM 12, Samwon Freezing Engineering Co.)

            (Fig.4).

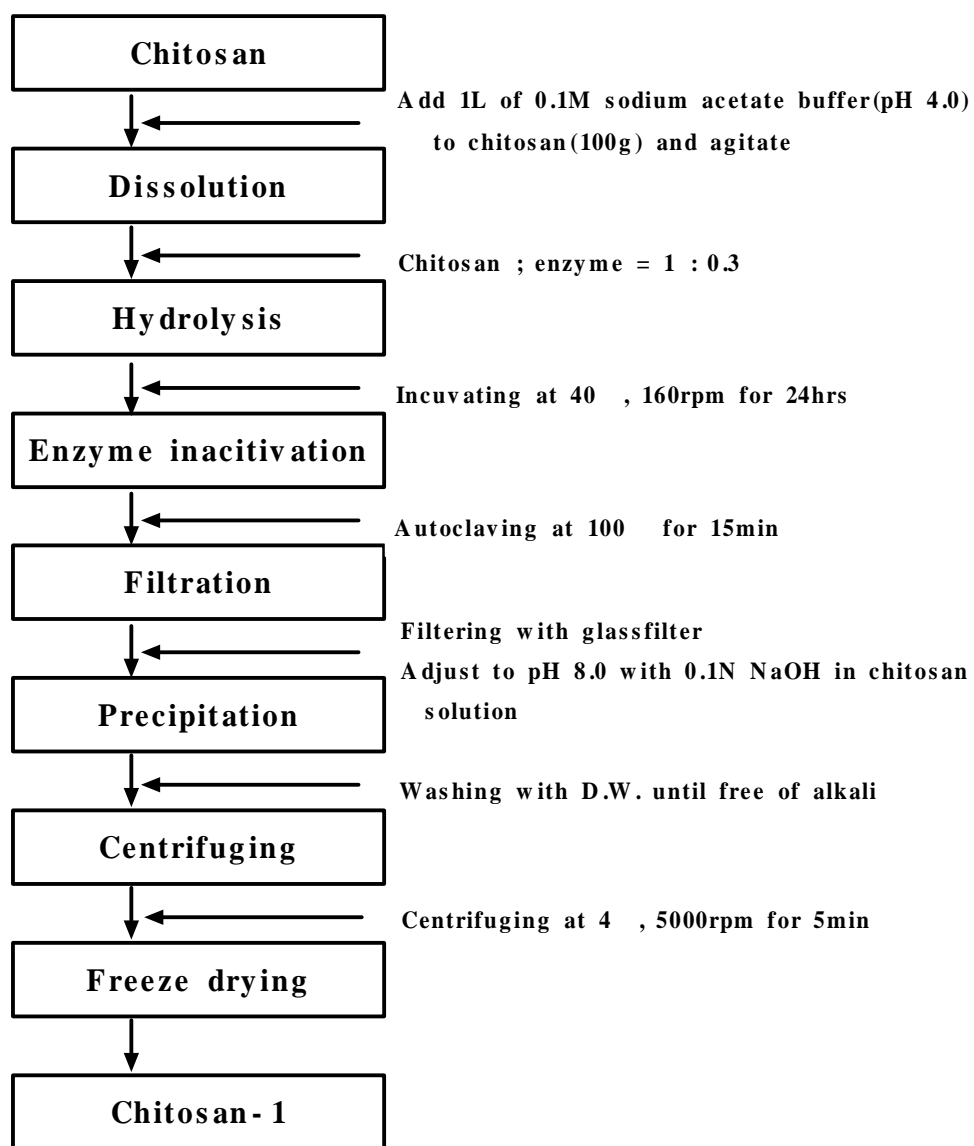**Fig. 3. Preparation procedure of chitin and chitosan from red snow crab shell.**

```
┌─────────────────────────┐
│        Chitosan         │
└─────────────────────────┘
            │         Add 1L of 0.1M sodium acetate buffer(pH 4.0)
            ↓ ←──────────    to chitosan(100g) and agitate
┌─────────────────────────┐
│       Dissolution       │
└─────────────────────────┘
            │         Chitosan ; enzyme = 1 : 0.3
            ↓ ←──────────
┌─────────────────────────┐
│       Hydrolysis        │
└─────────────────────────┘
            │         Incuvating at 40  , 160rpm for 24hrs
            ↓ ←──────────
┌─────────────────────────┐
│   Enzyme inacitivation  │
└─────────────────────────┘
            │         Autoclaving at 100    for 15min
            ↓ ←──────────
┌─────────────────────────┐
│       Filtration        │
└─────────────────────────┘
            │         Filtering with glassfilter
            ↓ ←──────────  Adjust to pH 8.0 with 0.1N NaOH in chitosan
┌─────────────────────────┐     solution
│      Precipitation      │
└─────────────────────────┘
            │         Washing with D.W. until free of alkali
            ↓ ←──────────
┌─────────────────────────┐
│      Centrifuging       │
└─────────────────────────┘
            │         Centrifuging at 4  , 5000rpm for 5min
            ↓ ←──────────
┌─────────────────────────┐
│      Freeze drying      │
└─────────────────────────┘
            │
            ↓
┌─────────────────────────┐
│       Chitosan- 1       │
└─────────────────────────┘
```

Fig. 4. Preparation procedure of low molecular weight chitosan.

6 .

    . (Moisture)

 AOAC(1990)   dry oven   105

                                      .

    . (Crude protein)

 AOAC(1990)   semi-micro Kjeldahl

chitin   chitin   .

    . (Crude fat)

 AOAC(1990)   Soxhlet

               .

    . (Crude ash)

 AOAC(1990)   .

 7. Chitin   chitosan   IR spectrum

           chitin   chitosan   chitin   chitosan

    200mesh   KBr   (200mesh) 100   standard pellet

              KBr   100   2   vibration mill

     10   pellet   FT-IR(Spectrum GX, Perkin

 Elmer, USA)   4,000 $^{-1}$ 500 $^{-1}$ (Fig.5).

           Sannan   (1978)   IR spectrum   2,878 $^{-1}$
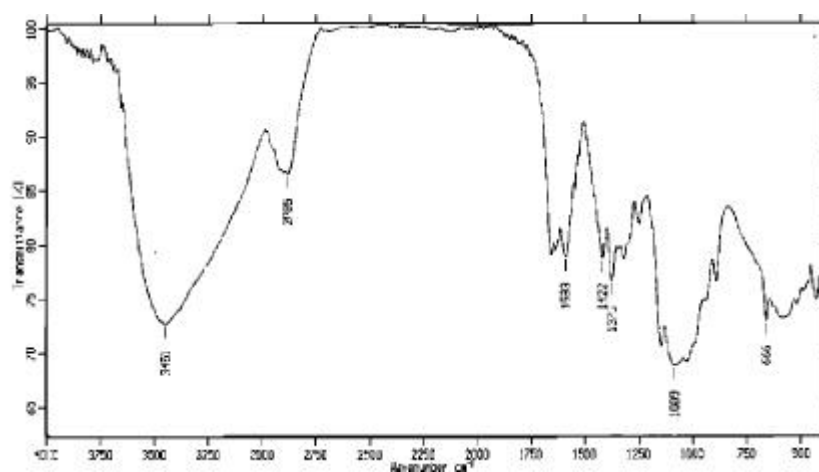
Fig. 5. Typical infrared spectra of chitosan.

1,550 $^{-1}$ (A$_{1550}$/A$_{2878}$)

(Fig.6).

8. Chitin   chitosan

Chitin   chitosan   chitosan

powder

Standard   sample   cup   (Spectrum   GX,

Perkin Elmer, USA)   .   1100 - 2400

absorbance mode   2nm   64   scan

.

9.   chitosan

Lin   (1974)   0.1M acetate- 0.1M sodium acetate buffer(pH   4.0)

500   chitosan 5g   50g   (   )

1N NaOH   pH   7.0   .

(3000rpm, 25min)   45°   30

(SFDSM 12, Samwon Freezing Engineering Co.)

pellet   .

10.

NIR   (estimated value)   (actual value)

PCR PLUS(Perkin Elmer, USA)

.
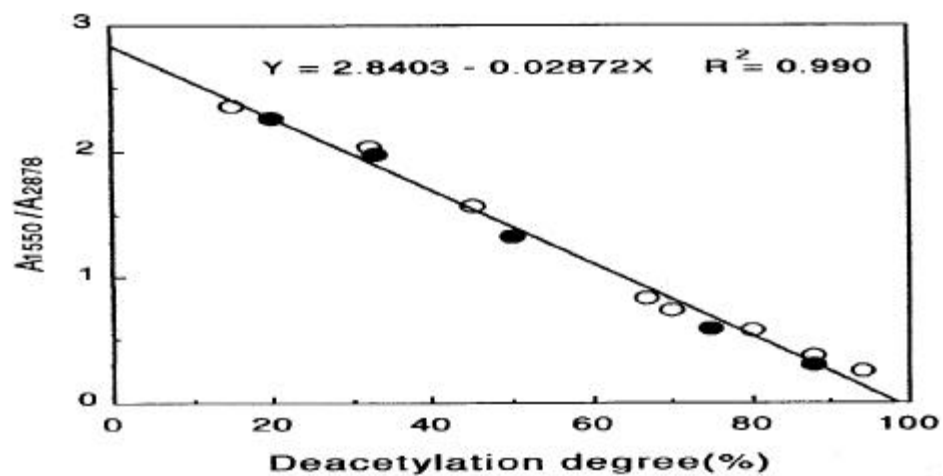
Fig. 6. Calibration line obtained by plotting the ratio of the absorbance of the bend at 1550cm^{-1} to that at 2878cm^{-1} against the degree of deacetylation (from Sannan et al., 1978).

1.

Chitin   chitin

          Table 2   Table 3   .

                    46.2%   0.8%

                                                                                                              .

                              chitin

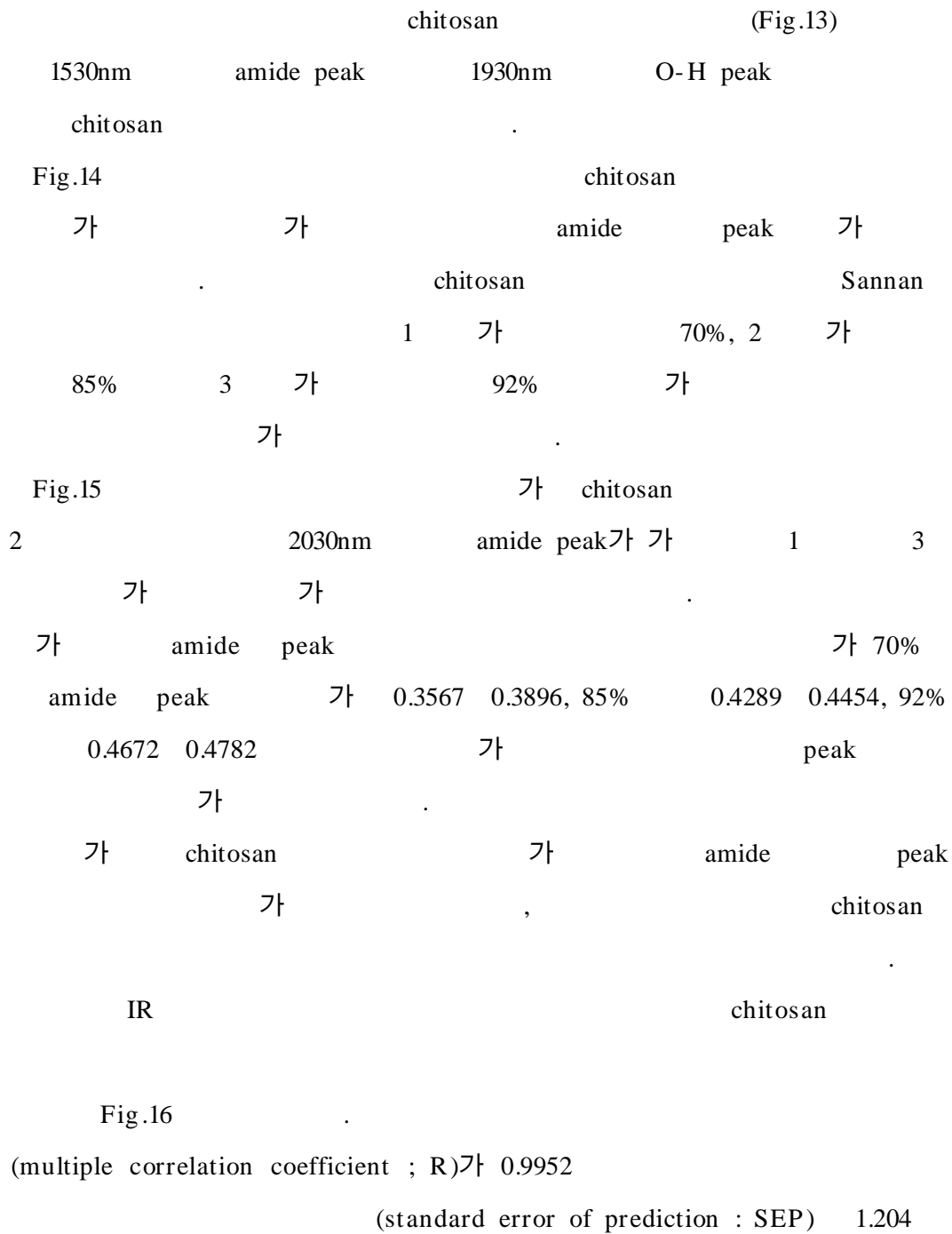   .   12.1%,

          8.3%

                    .

1100nm   2500nm   2nm   64   scan

                                        Fig.7   .   1500nm, 2180nm

                              peak   .   peak

                    N-H

                                                  peak

     . Robertson   (1989)   1200nm, 1510nm, 2050nm, 2180nm

                              peak   .

2. Chitin   chitosan

2-1   chitin

                                        chitin

Fig.8   .   chitin

Table 2. Ingredients and yield of dried shell and chitin from red snow crab (dry basis)

Composition	Shell	Chitin
Yield(%)	100	22.3
Moisture(%)	9.4	8.1
Nitrogen(%)	8.3	6.76
Crude ash(%)	46.2	0.3

Table 3. Ingredients and yield of squid pen and chitin from squid pen (dry basis)

Composition	Squid pen	Chitin
Yield(%)	100	36.1
Moisture(%)	5.6	3.7
Nitrogen(%)	12.1	8.1
Crude ash(%)	0.8	0.4
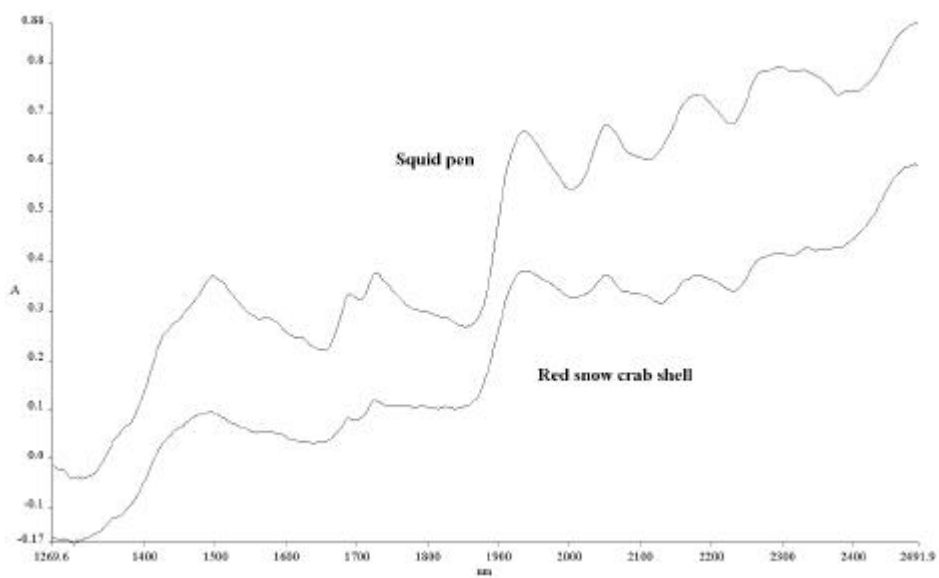
Fig. 7. Near-infrared spectra of red snow crab shell and squid pen.
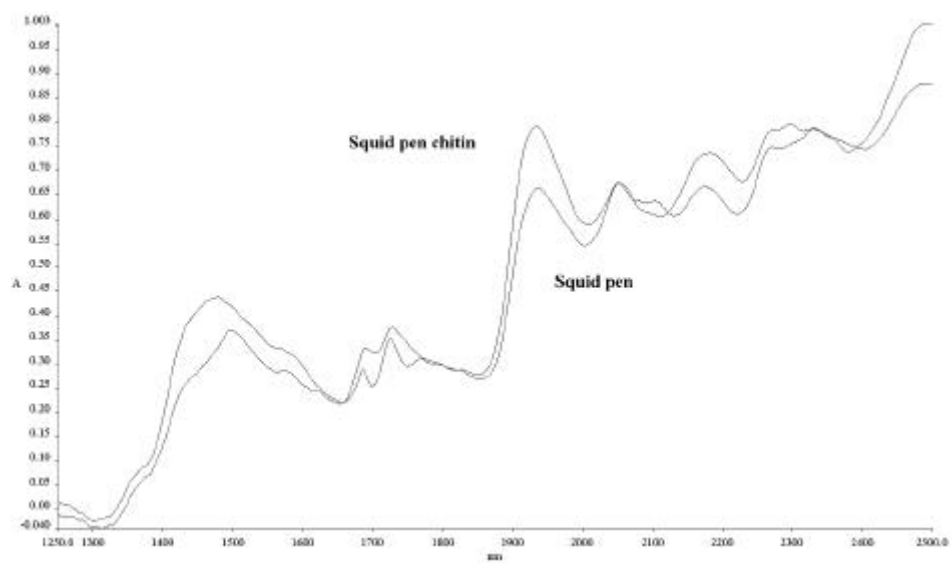
Fig. 8. Near-infrared spectra of squid pen and chitin.

1500nm 2180nm   .

N-H   peak   peak

chitin   . chitin

chitin

chitin

chitin   N-H   peak

.

1930nm   chitin   peak

O-H

chitin   O-H

chitin   .

chitin

.

2-2 chitin chitosan

chitin chitosan spectrum

Fig.9   . chitosan   1530nm 2030nm

amide   peak   .

Chitin chitosan   2   Fig.10

.   peak

. 2

chitin chitosan peak   1530nm 2030nm

amide   peak   .
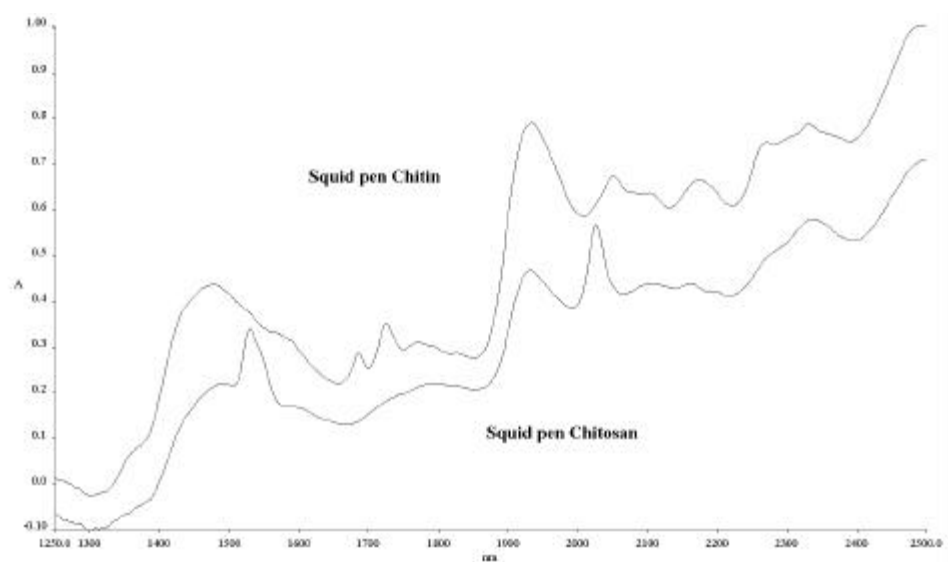
chitosan

Fig. 9. Near-infrared spectra of squid pen chitin and squid pen chitosan.
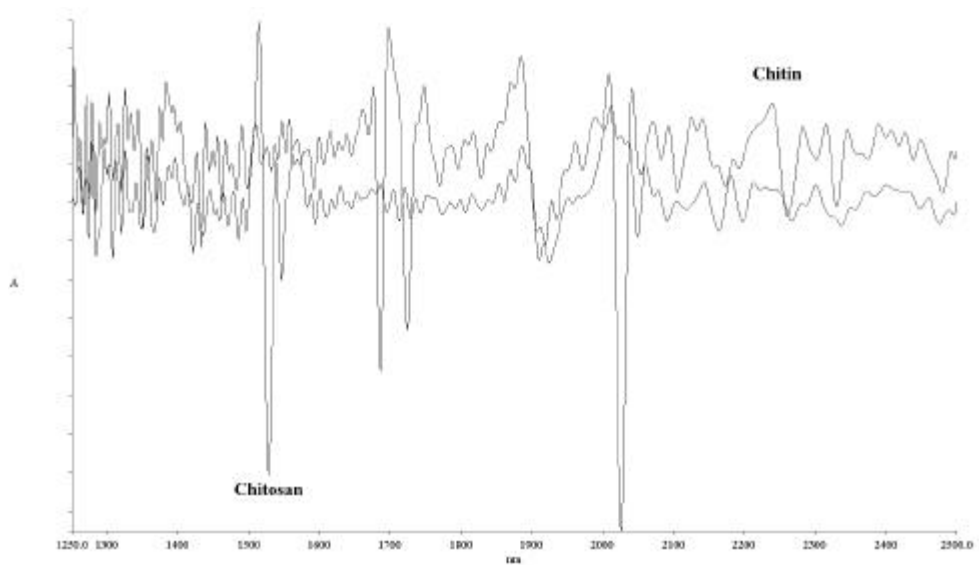
Fig. 10. Second derivative near-infrared spectra of squid pen chitin and chitosan.

chitosan   chitosan

spectrum   (Fig.11),   chitosan

                                    1530nm   2030nm   amide

        peak   .

            chitosan   ,

                    chitosan   1530nm   2030nm

amide   peak   .

2-3   chitin   chitosan

Chitosan   chitin

        chitin   amide   .

        chitin   chitosan   .

        chitin   chitosan

                                            chitin   chitosan   Fig.12

                            , chitosan   chitin

                        peak   1530nm

    2030nm   peak   .   amide II

chitosan NH_2 peak   . Chitin   amide

peak   chitosan

                chitin   chitosan

                                    .

2-4   chitosan

Chitosan
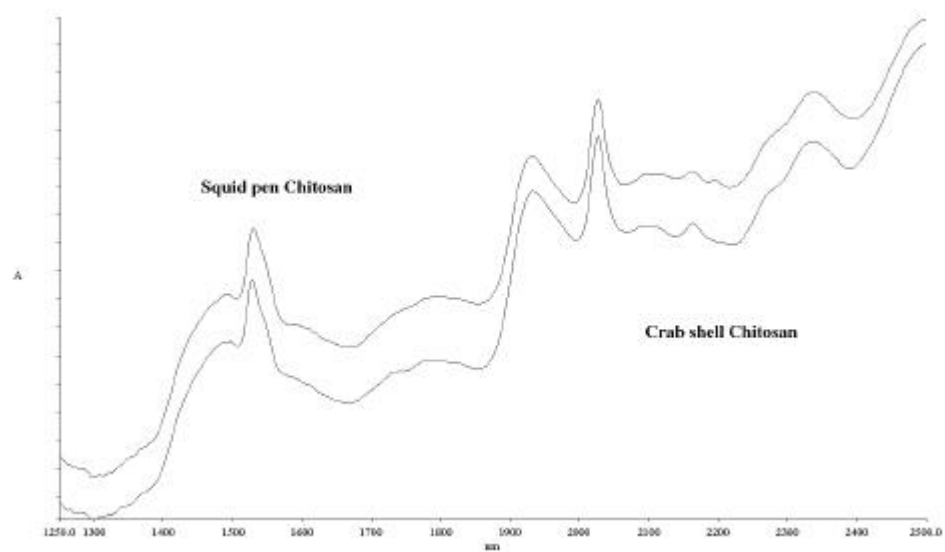
Fig. 11. Near-infrared spectra of crab shell chitosan and squid pen chitosan.
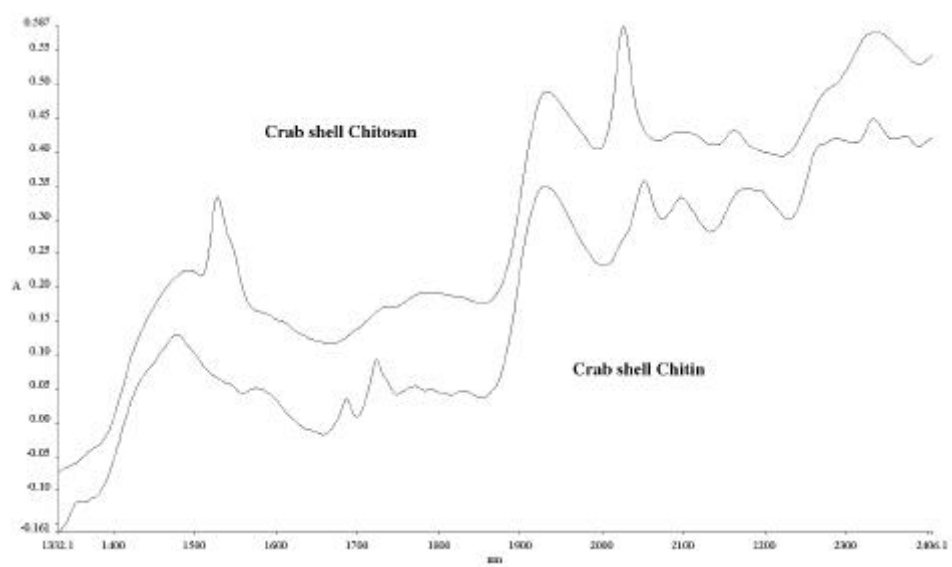
Fig. 12. Near-infrared spectra of crab shell chitin and chitosan.

chitosan　　　　　　　　　　　　(Fig.13)

1530nm　　　　amide peak　　　　1930nm　　　　O-H peak

chitosan　　　　　　　　　　　　　.

Fig.14　　　　　　　　　　　　　　chitosan

amide　　　peak

.　　　　　　chitosan　　　　　　　　　　Sannan

1　　　　　　　　　70%, 2

85%　　　3　　　　　　　92%

.

Fig.15　　　　　　　　　　chitosan

2　　　　　　　2030nm　　　amide peak　　　　　1　　　3

.

amide　　peak　　　　　　　　　　　　　70%

amide　　peak　　　　　　0.3567　0.3896, 85%　　　0.4289　0.4454, 92%

0.4672　0.4782　　　　　　　　　　peak

.

chitosan　　　　　　　　　　amide　　　peak

,　　　　　　　chitosan

.

IR　　　　　　　　　　chitosan

Fig.16　　　　　.

(multiple correlation coefficient ; R)　0.9952

(standard error of prediction : SEP)　1.204
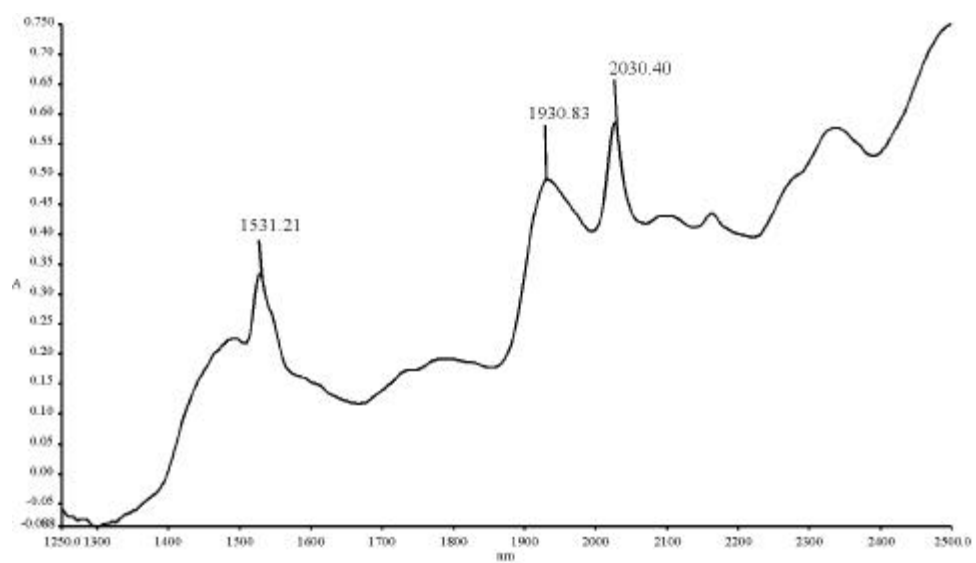
Fig. 13. Near-infrared spectra of typical chitosan.
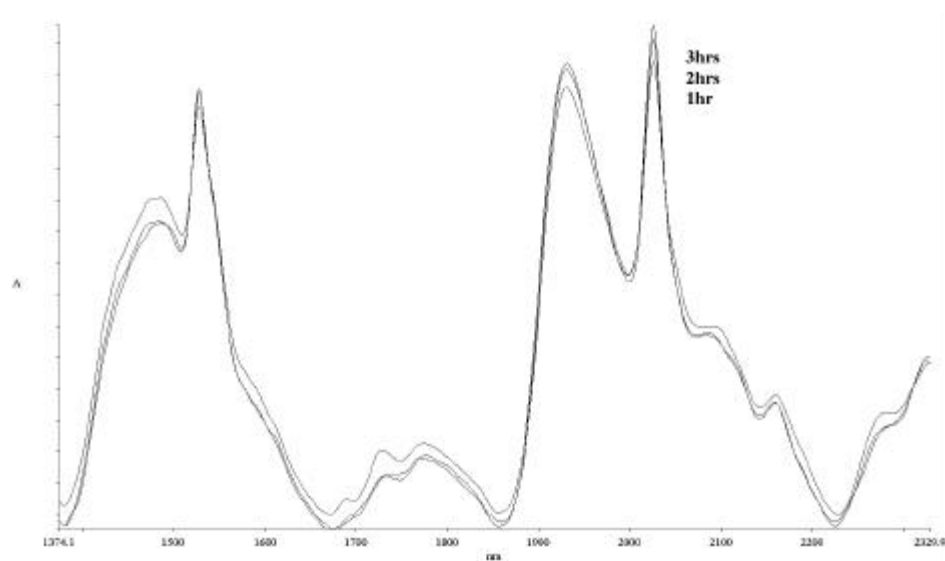
Fig. 14. Near-infrared spectra of different deacetylated chitosan.

Fig. 15. Second derivative near-infrared spectra of different
deacetylated chitosan.
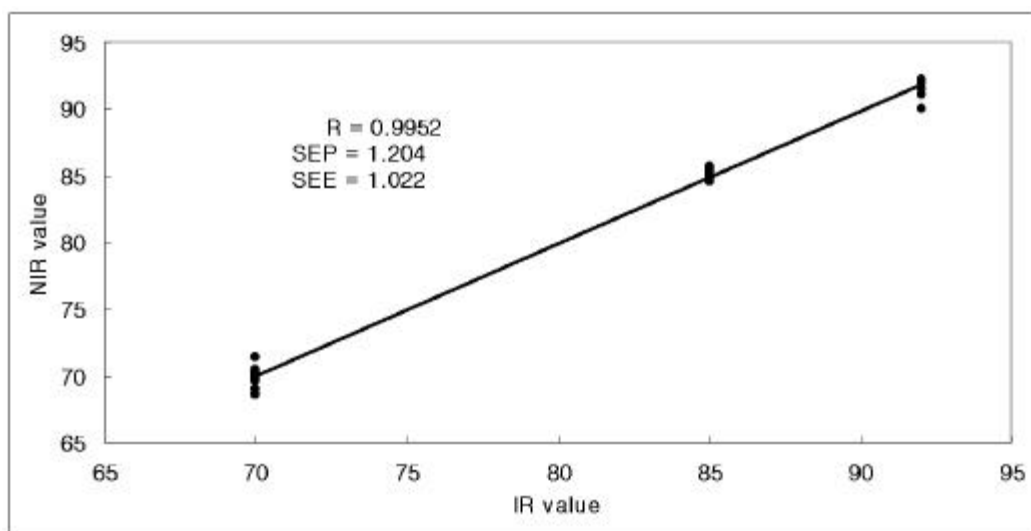
Fig. 16. Relationship between NIR method and IR method for determining the deacetylation degree of chitosan.

(standard error of

estimate : SEE) 1.022 .

.

. (multivariate)

. Principal Component Regression(PCR)

. PCR

scaling factor

. Principal Component(PC)

PC

PC

, PC . PCR

, PC

PC

.

chitosan amide peak

R 0.9917

(SEP) 0.0069,

(SEE) 0.0061 (Fig.17).

chitosan

,
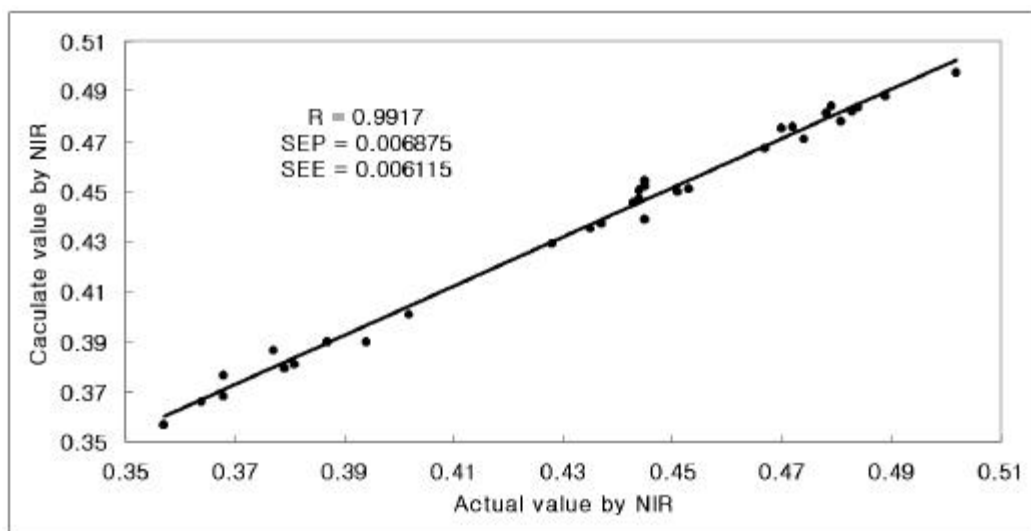
Fig. 17. Relationship between calculated deacetylation degree and actual deacetylation degree of chitosan by NIR.

．　　　　　　　　　　　　　　　　　　　　　　　chitosan

　　　　　　　　　　　Table 4　　　　　　　　　．

2%　　　　　　　　　　　　　　　　　　　　　　．

　　　　　　　　　　　　　　　　　　　　　　　　　　　　chitosan

　　　　　　　　　　　　　　　　　　　　　　　　　，

　　　　．

2-5　　　　　　　chitosan

　Chitosan　　　　　　　　　　　　chitosan　　amide　(NH^{3+})

C-H

　　　　　　　　　　　　　　　　　　　　　　　　　　．

　　　　．

　Chitosan

Fig.18　　　　　　　　　　　　　　　　　chitosan

　　chitosan　　　　　　　　　chitosan　　　　　　　　　　　　　　．

　　　　　　　chitosan　　　　　　　　　　　　chitosan

peak　　1750　　　　2150nm　　　　　　．　　　C-H

　　　　　　　　　　chitosan　　　　　　　　　　peak　　　　　．

　　　chitosan　amide

　　　　　　　　　　　　　　　　　　　　　．

　Fig.19　　　　　　　　　　2　　　　　　　　　　2030nm

　　　amide　　　peak　　　　　　　　　　　　　　peak

- 37 -

Table 4. Deacetylation degree of chitosan by IR and NIR spectra

IR	NIR	Differences	NIR spectrum area
	69.29	0.71	0.366
	69.96	0.04	0.3753
	70.17	−0.17	0.3794
70	67.97	2.03	0.3567
	69.36	0.64	0.3607
	69.91	0.09	0.3896
	70.78	−0.78	0.3896
	84.35	0.65	0.4289
	87.2	−2.2	0.4454
85	85.04	−0.4	0.4369
	85.29	−0.29	0.4294
	86.09	−1.09	0.4387
	91.68	0.32	0.476
	90.93	1.17	0.4754
92	90.86	1.14	0.4782
	91.37	0.63	0.4709
	91.06	0.94	0.4672
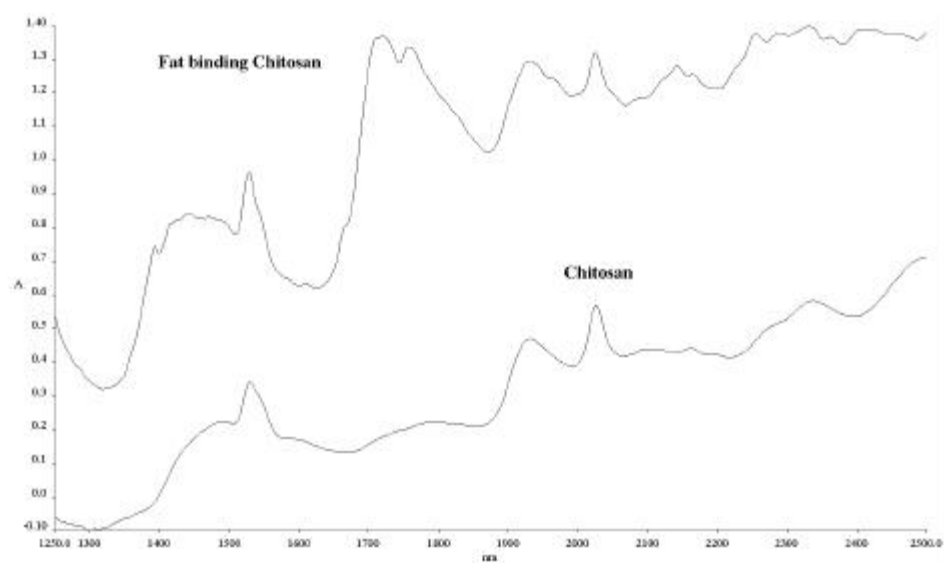
Fig. 18. Near-infrared spectra of chitosan and fat binding
chitosan.
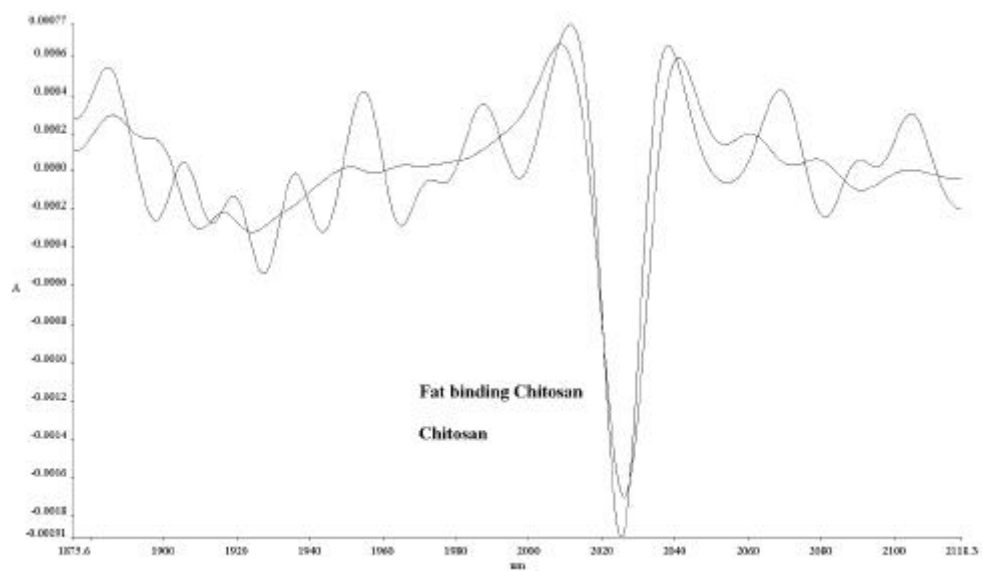
Fig. 19. Second derivative near-infrared spectra of chitosan
and fat binding chitosan.

.   chitosan   C-H

amide

chitosan

.

2-6   **chitosan**

Chitosan   chitosan

Table 5   *in vitro*   chitoasn

chitosan

Fig.20   Fig.21   .

in vitro   chitosan   24kDa

chitosan   4kDa   chitosan

.   24kDa

chitosan   4kDa   chitosan

.

in vitro

peak

Table 6   .   24kDa

chitosan   4kDa   chitosan   659.3%,

338.8%   chitosan   2   ,

peak   17.66, 12.82   chitosan

chitosan   .

Table 5. Degree of deacetylation and molecular weight of chitosan

		Degree of deacetylation(%)	Molecular weight
Chitosan[a]	70H	70	28kDa
	70L		4kDa
Chitosan[b]	85H	85	26kDa
	85L		4kDa
Chitosan[c]	92H	92	24kDa
	92L		4kDa

[a] Deacetylation ; added 47% NaOH to chitin for 1hr at 130

[b] Deacetylation ; added 47% NaOH to chitin for 2hrs at 130

[c] Deacetylation ; added 47% NaOH to chitin for 3hrs at 130
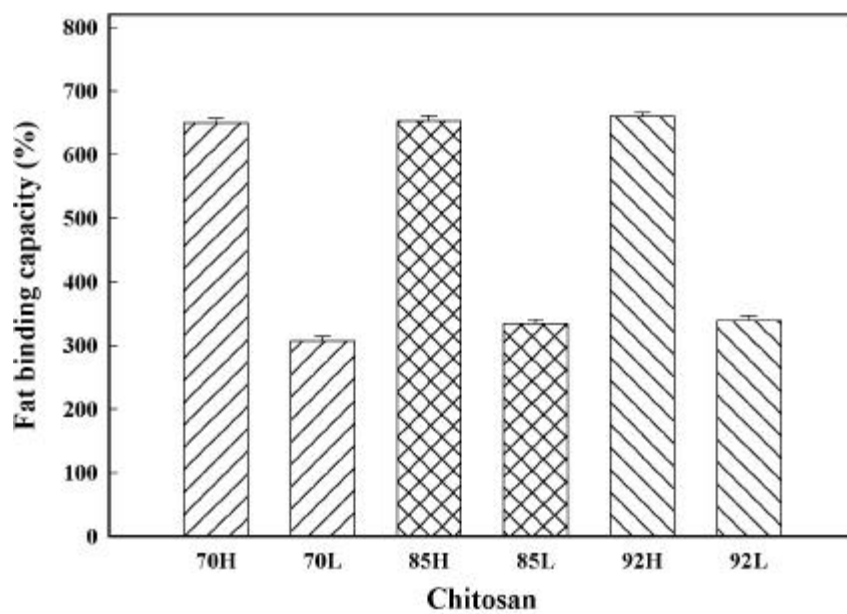
Fig. 20. Fat binding capacity of chitosan.

70H；DD70%, Mw28kDa chitosan, 70L；DD70%, Mw4kDa chitosan
85H；DD85%, Mw26kDa chitosan, 85L；DD85%, Mw4kDa chitosan
92H；DD92%, Mw24kDa chitosan, 92L；DD92%, Mw4kDa chitosan
DD；Degree of deacetylation(%), Mw；Molecualr weight.
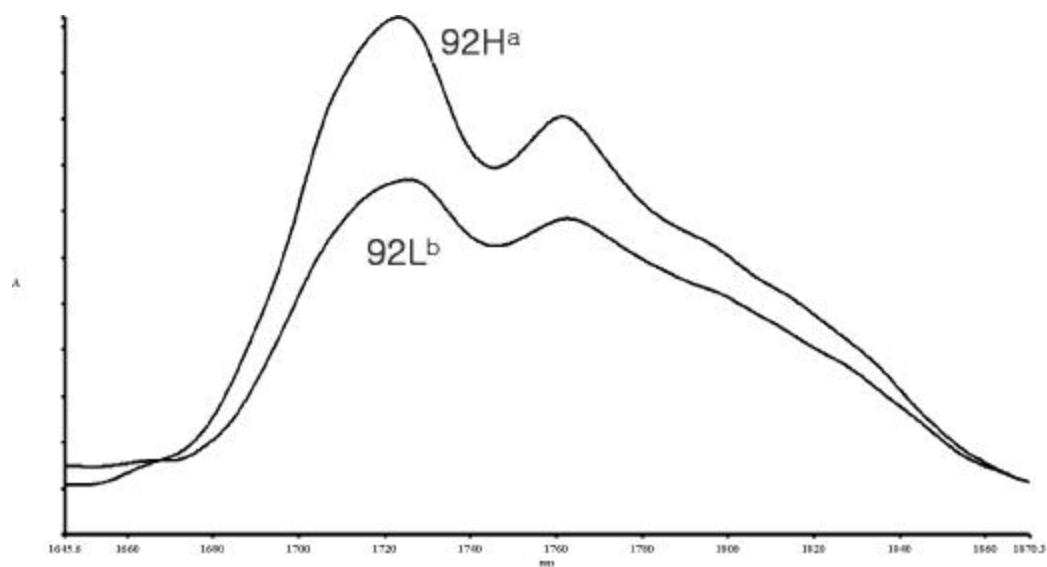
Fig. 21. Near-infrared spectra of fat binding chitosan.

[a]92H; deacetylation degree(%) ; 92(%) Mw 24kDa
[b]92L; deacetylation degree(%) ; 92(%) Mw 4kDa

Table 6. Fat binding capacity and corrected area by NIR analysis as molecular weight of chitosan

Chitosan	Fat binding capacity (%)	Corrected area by NIR analysis
92H[a]	659.3± 6.8	17.66
92L[b]	338.8± 8.5	12.82

[a]92H ; deacetylation degree (%) ; 92 (%) Mw 24kDa

[b]92L ; deacetylation degree (%) ; 92 (%) Mw 4kDa

.

chitin   chitosan

.   chitin   chitosan

chitin   chitosan

.

chitin   chitosan   (squid pen)   (red

snow crab shell)   NIR   1200nm, 1510nm, 2050nm

2180nm   peak

peak

.

Chitin   NIR

chitin

.

Chitosan   chitin

amide   NIR

chitosan   1530nm, 2030nm NH_2 amide   peak

chitin   chitosan   .

chitosan   IR

NIR

1%

0.006

.

Chitosan   NIR

C-H   chitosan   amide

,   chitosan

.

Chitosan   Mw 24kDa

chitosan   Mw 4kDa   chitosan

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·   ,

·

,

,   ,   ,

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,   ,

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,   ,   ,   ,

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Perkin Elmer   ,   ,   ,

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