

The ability of absorption and physicochemical properties of chitosan prepared from fungi

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Abstract

The physicochemical properties of fungal chitosan at 95°C and 40°C acid treatment was as follows respectively. The nitrogen content was 6.71%, 6.91%, the viscosity 2.23cps, 2.21cps, the acetylation 12.0%, 12.7% and the molecular weight 3.12×10^5 Dalton, 3.01×10^5 Dalton. The absorbency band of reference, FCs-40 and FCs-95 in I.R. spectra was almost in accord with one another. In solid state NMR spectra, methyl group(-CH₃) was observed lightly. That means which deacetylation was well occurred. Carbonyl group(C=O) was not observed. C₁ to C₆ in solid state NMR was well observed seperately enough.

Introduction

Commercially available chitosan is usually manufactured from crustacean shell chitin by deacetylating with a hot(over 100°C) concentrated sodium hydroxide solution(30~50%). This treatment eliminates the acetate groups from the amine of C₂ group. This process of chitosan prepared from crustacean shells requires large amounts of heat and caustic alkali. And such conversion appears to have limited potential for industrial acceptance because of seasonal and limited supply, high processing cost, processing difficulties, and inconsitent physicochemical characteristics of chitosan. Recent advances in fermentation technology suggested that many of this problems can be overcome by culturing fungi. In the functional properties, chitosan is a biopolymer used for the production of biodegradable films, viscosity-control agents, dye binding capacity, emulsion stability agents, flocculating and chelating agents in wastewater treatments with great potential for industrial use due to its high amine content and polycationic nature. The object of this study was to produce chitosan(called fungal chitosan or FCs) from the mycelium of *Absidia coerulea*, then, to experiment of physicochemical properties of fungal chitosan and of properties of function involving the ability of pigment

absorption, the ability of emulsification, and the absorption force of fat.

Materials and Methods

Strains : *Absidia coerulea* IFO5301

Culture Instrument : Continuous Bio-reactor(KF-25L, Korea Fermentor)

Materials

Chitosans : SCs(Sigma Co., USA), FCs40(acid treatment at 40°C),
FCs95(acid treatment at 95°C),
JCs(Commercial products, Japan)

Chitins : SCt(Sigma Co., USA), MCt(MIT bioeng. lab., USA)

Edible colorant : FD&C Red No. 3

Edible oil : Soybean oil, Dongbang Co.

Methods

Chemical compositions : AOAC methods

Viscosity : Capillary viscometer, Cannon Ins. Co., USA

Molecular weight : Mark-Houwink viscosity equation

Acetylation degree : Sannan et al. methods

I.R. spectrum : Bruker IFS 66 FT-IR, Germany

NMR spectrum : Bruker MSL 200, Germany

Dye binding capacity : UV spectrophotometer, Absorbance at 505nm

Fat binding capacity & Emulsification : Lin et al. methods

Results and Discussion

In flask incubation, *Absidia coerulea* IFO5301 had highest yield in FCs/DCW, 258mg/200ml at initial pH 6.5, temperature 27°C, culture time 6days. In batch bioreactor, the optimum cultivation condition was that cell suspended solution was 70ml, aeration rate 0.5 l/min, agitation rate 800rpm, culture time 36hr. In continuous bioreactor, the optimum substrate flow rate was 4 l/day. Among the cultivation hours, 24hr, 36hr, 48hr, the yields of FCs/AIM was 58.5% in 36hr, higher than the others. In the experiment according to the temperature of acid treatment, the yield of FCs was higher at 95°C than at 40°C. The colors were, in turn, cream white and brown. The chemical composition and physicochemical properties of fungal chitosan at 95°C and 40°C acid treatment was as follows respectively. The moisture was 6.58%, 6.57%, the nitrogen content 6.71%, 6.91%, the yield of FCs/DCW 16.10%, 15.64%, the solubility 99.05%, 99.13%, the viscosity 2.23cps, 2.21cps, the acetylation 12.0%, 12.7% and the molecular weight 3.12×10^5 Dalton, 3.01×10^5 Dalton. From the above facts, according

to the temperature of acid treatment, the components and physicochemical properties of two kinds of fungal chitosan had little difference. From the I.R. spectra, the absorption bands at 1655 cm^{-1} (amide I), 1550 cm^{-1} (amide II) of fungal and reference chitosans became very weak, similarly. In solid state NMR spectra, reference and two FCs were also in accord with one another. In 2.1ppm of solid state NMR spectra, resolution that was appeared methyl group(-CH₃) was observed lightly. That means which deacetylation was well occurred. Carbonyl group(C=O) part was not observed. C₁ to C₆ in solid state NMR was well observed separately enough. Chemical shift of ¹³C-NMR spectra of fungal and reference chitosans were in good agreement with slight experimental deviation. In the experiment of functional properties of FCs-40, the ability of pigment absorption was 44.68mg dye/g sample, the ability of emulsification 65%, the absorption force of fat 416%.

Conclusion

The physicochemical properties of fungal chitosans(FCs95 and FCs40) were as follows respectively. The moisture was 6.58%, 6.57%, the nitrogen content 6.71%, 6.91%, the yield of FCs/DCW 16.10%, 15.64%, the solubility 99.05%, 99.13%, the viscosity 2.23cps, 2.21cps, the acetylation 12.0%, 12.7% and the molecular weight 3.12×10^5 Dalton, 3.01×10^5 Dalton. The absorbency band of reference, FCs40 and FCs95 in I.R. spectra was almost in accord with one another. In solid state NMR spectra, reference and two FCs were also in accord with one another. Carbonyl group(C=O) part was not observed. C₁ to C₆ in solid state NMR was well observed separately enough. In the experiment of functional properties of FCs40, the ability of pigment absorption was 44.68mg dye/g sample, the ability of emulsification 65%, the absorption force of fat 416%.

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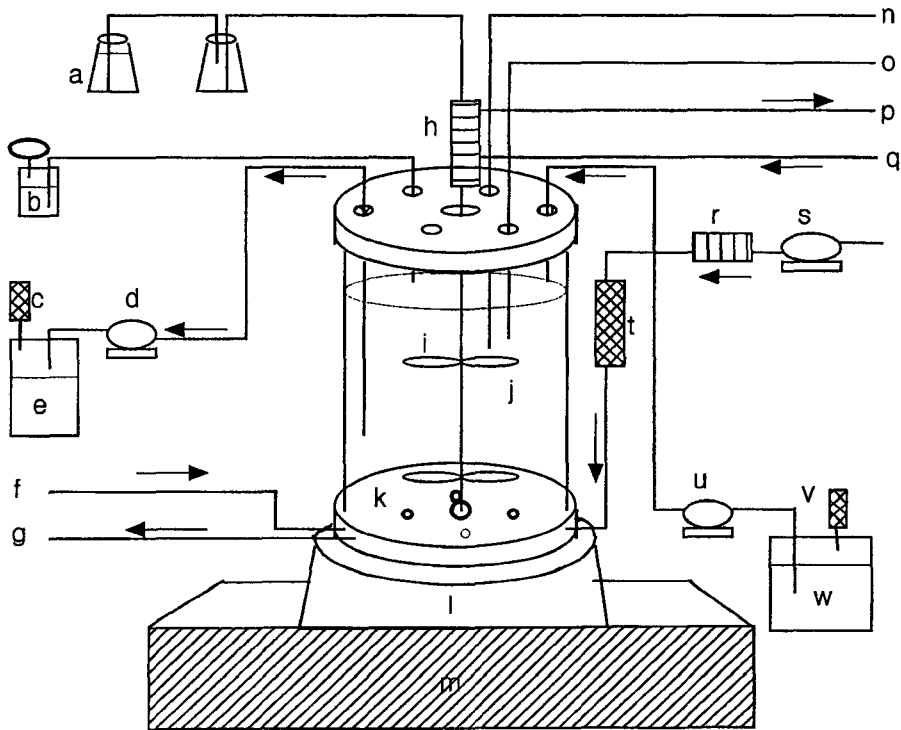


Fig. 1. Apparatus of continuous-type bioreactor.

- | | | | |
|--------|------------------------|----|--------------------|
| a. | Ethanol | j. | Bio-reactor vessel |
| b. | Antiform | k. | Air inlet port |
| c,t,v. | Air filter | l. | Heater |
| d,u. | Peristaltic pump | m. | Motor |
| e. | Effluent sample vessel | n. | Thermometer |
| f,q. | Water in | o. | pH meter |
| g,p. | Water out | r. | Air flowmeter |
| h. | Condensor | s. | Air compressor |
| i. | Propeller | w. | Substrate vessel |

Fungal mycelia

Ethanol treatment

Washing

Lyophilization

Dry cell

Alkali treatment
(1N NaOH, 1:40(w/v), for 15min at 121)

Washing and lyophilization

Alkali-insoluble materials

Acid treatment
(2% acetic acid, 1:50(w/v), for 12hr at 95 or 40)

Centrifuge

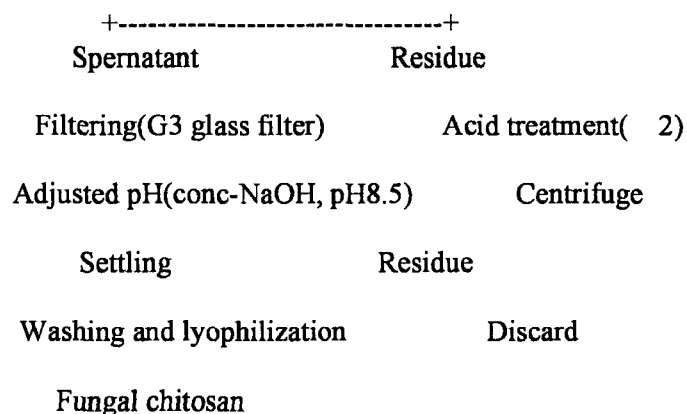


Fig. 2. Extraction procedure for fungal chitosan

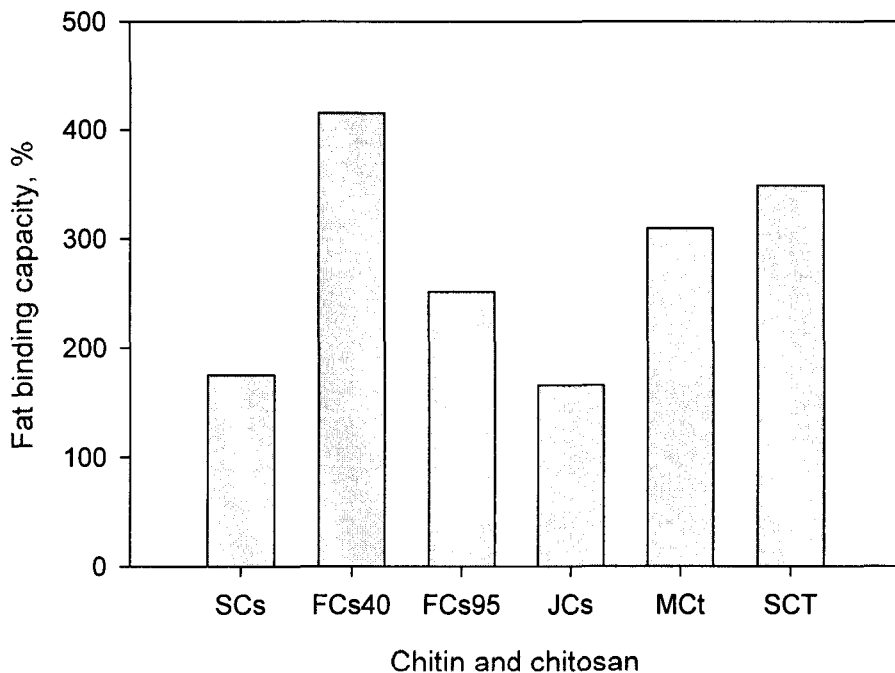


Fig. 3. Fat binding capacity of chitin and chitosan

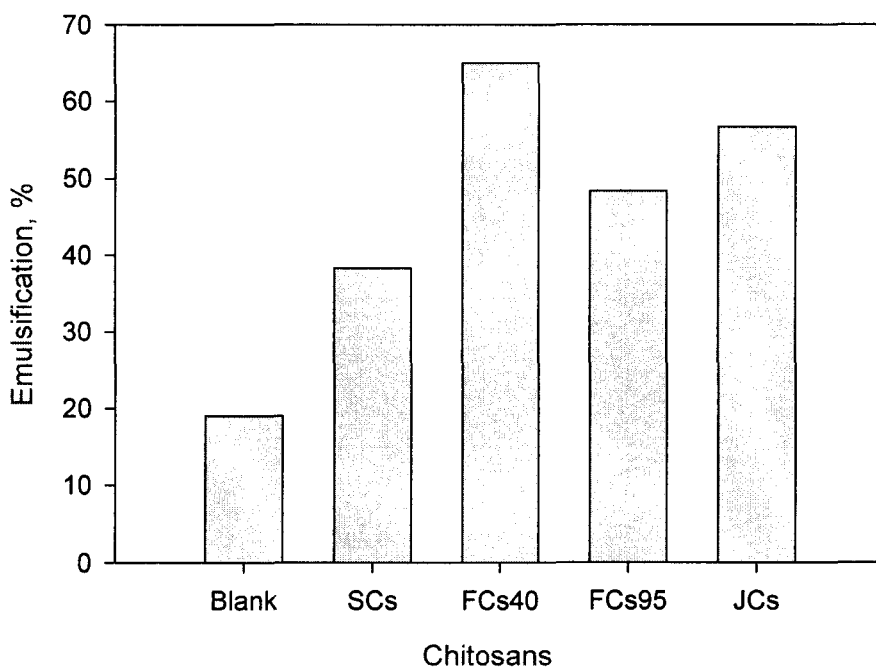


Fig. 4. Emulsification of chitosan.

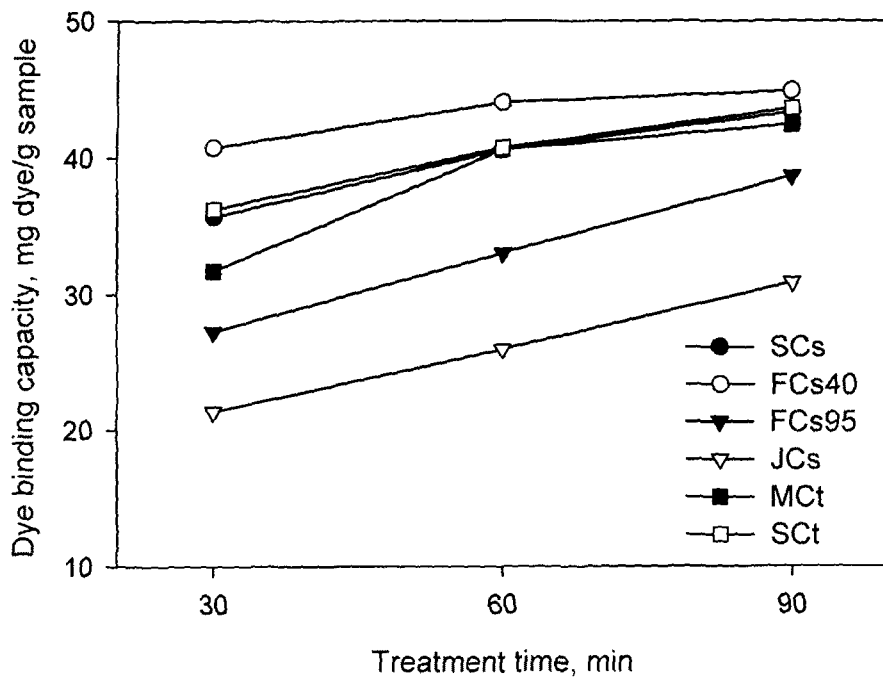


Fig. 5. Dye vinding capacity of chitin and chitosan by treatment time with dye soluton.