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PREPARATION OF MULTIFUNCTIONAL LOW MOLECULAR WEIGHT CHITOSAN AND ITS APPLICATION IN COSMETICS.

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Abstract

The aim of this study is to elucidate the anti-microbial activity and anti-oxidative activity of water-soluble chitosan with a molecular weight of 5,000-200,000. Water-soluble chitosans have demonstrated a regular anti-microbial activity on the tested strians by the paper disk method. In the MIC (Minimum Inhibitory Concentration) test, CC-01(MW=5,000) with the lower MW showed the higher MIC value than the higher MW chitosan. The MW of chitosan increase, the MIC decreases. MICs of 4 chitosans(CC-02~CC-05) against *S. aureus* ATCC 65389, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231 were 7.0-39.0μM, whereas MICs of chitosans against *A. niger* were over 2.0mM. Formula containing chitosan showed higher anti-microbial activities than the formula made with the chemical preservatives(Methylparaben 0.2% and Imidazolidinyl Urea 0.3%). Among 5 water-soluble chitosans, CC-03(MW=92,163) showed the most potent anti-oxidative activity (IC_{50} : 0.2mM). In conclusion, the water-soluble low molecular weight chitosan could be served as natural preservatives and antioxidant in cosmetics.

Introduction

Chitin extracted from the rind of crabs and shrimps was secondary abundantly natural polysaccharide after cellulose[1,2]. Chitosan is made with deacetylation of chitin. Chitin and Chitosan have the repeated sequence [Chitin: Poly(β-(1→4)-2-acetamido-2-deoxy-D-glucose), Chitosan: Poly(β-(1→4)-2-amino-2-deoxy-D-glucose)][3]. They were perceived as useless materials at one time. But, recent studies of them elucidated the physical property and many biological function[4]. The Chitin and chitosan are a non-toxic material with various functions anti-microbial activity[5], anti-oxidative activity, lowering collesterol[6], anti-cancer[7], immuno activating effects[8] etc.

The anti-microbial activity of chitosan was firstly reported by Allan and Hadwiger[9] and clarified by Kendra and Hadwiger[5]. Also, Naita suggseted the anti-microbial mechanism of chitosan that free

amino group(positive charge) at C-2 of glucosamine form an ionic bond with negative charge at cell wall of microbes and inhibit the microbial growth.

Chitosan is insoluble in most solvents and chitosan is soluble in organic acid such as acetic acid, succinic acid, lactic acid and malic acid. But, the use of chitin and chitosan is limited because of its insolubility in water, coagulating protein, high pH and high viscosity.

In this experiment, we prepared water-soluble low molecular weight chitosan between 5,000-200,000 and elucidated their anti-microbial activities and anti-oxidative activities.

Methods

Chitosans

5 water-soluble chitosan with a molecular weight between 5,000 and 200,000 were used. 5 chitosan were prepared by microfiltration & ultrafiltration membrane system. The deacetylation degree of the chitosan is about 85%. The chitosan was purchased from the R&C International Ltd.(Korea).

Determination of the molecular weight

The molecular weights of 5 chitosans were determined by chromatographic method using gel permeation chromatography(Waters 2690, Waters Company, U.S.A). The pullulan (Show Denko Company, Japan) was used as standard material.

Strains and media

5 strains-gram positive coccus bacteria (*Staphylococcus aureus* ATCC 65389), gram negative rod bacteria (*Escherichia coli* ATCC 8739 , *Pseudomonas aeruginosa* ATCC 9027), yeast (*Candida albicans* ATCC 10231) and mold (*Aspergillus niger* ATCC 9642) - were purchased from KCTC (Korean Collection for Type Cultures). For the bacterial growth and the subculture, we used nutrient broth and nutrient agar (Difco). For the fungi, we used PDA (Difco) and YM agar (yeast extract 0.3%, peptone 0.5%, malt extract 0.5%, glucose 1.0%, agar 2.0%).

Anti-microbial effects of chitosan

Anti-microbial activities of chitosans were determined by the size of clear zone on the plate using paper disk method[10]. The culture temperature of bacteria and fungi were 37 C and 25, respectively.

Minimum Inhibitory Concentration (MIC) of chitosan

We determined the MICs of chitosans as the minimal concentration that microorganism could not grow at microbe grown agar plates treated with various concentration of chitosan. We observed the microbial growth at 72 hours after cultivation.

The preservative efficacy of formula contained chitosan

Each chitosan was added to evaluate the preservative efficacy of the formulated lotion with the final chitosan concentration of 0.1%(w/v) and 0.5.%.(w/v). The cultured microbes were suspended in the sterilized water. 0.1ml of the microbial suspension was inoculated in 30g of formula and incubated during 1-7 days. The bacterial number was counted at 1, 2, 4, 8 and 24 hours after inoculation. The viable cell counts of fungi were counted at 1, 3, 5, and 7 days after inoculation.

Results and discussion

Measurement of the molecular weights

The molecular weight of five kind of chitosan were determined by G.P.C. The average MW of the chitosans was 5,000-200,000 daltons(Table 1).

Table 1. Average molecular weights of chitosan.

Chitosan	Molecular Weight(Da)
CC-01	5,000
CC-02	75,470
CC-03	92,163
CC-04	127,600
CC-05	155,770

Anti-microbial activities of the chitosans

To study the effect of MW of chitosans on anti-microbial activities, we measured the size of clear zone as anti-microbial activity by paper disk method. Table 2 represents the result of the anti-microbial activities of the chitosan. Four kinds of chitosan(CC-02, CC-03, CC-04, and CC-05) showed the similar anti-microbial activities, whereas CC-01 didn t inhibit the growth of fungi.

Table 2. Anti-microbial activities of various chitosans. (Unit : mm)

Strains	CC-01	CC-02	CC-03	CC-04	CC-05
Staphyrococcus aureus ATCC 65389	11.63 0.48	11.75 0.50	11.63 0.25	12.00 0.00	11.75 0.96
Escherichia coli ATCC 8739	11.00 0.00	11.28 0.59	11.63 0.48	11.13 1.03	11.25 0.50
Pseudomonas aeruginosa ATCC9027	10.25 0.29	11.50 0.58	10.75 0.29	12.38 0.75	11.25 0.96
Candida albicans ATCC 10231	-	11.00 0.00	11.13 0.25	11.75 0.50	10.75 0.50
Aspergillus niger ATCC.9642	-	11.00 0.00	11.25 0.29	11.25 0.29	10.63 0.48

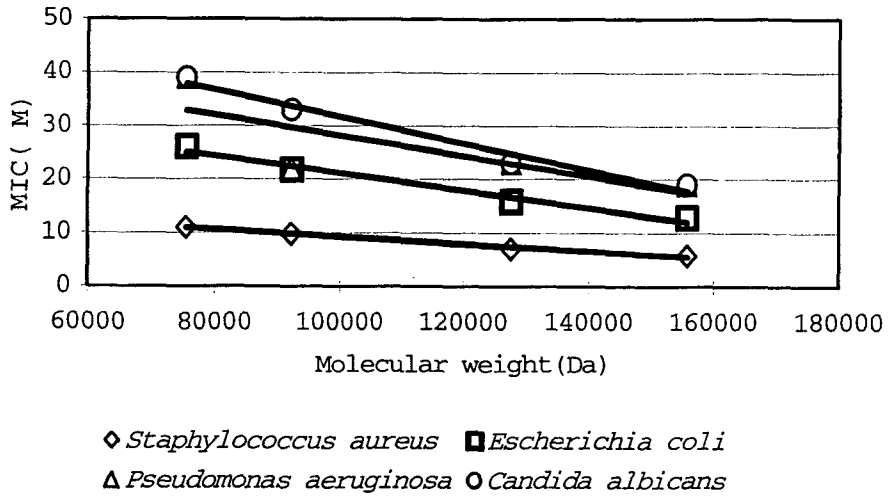
Minimum Inhibitory Concentration (MIC) of chitosans

Table 3 represents the MICs of 5 chitosans. From the results, the molecular weight of chitosan increased, and the MICs of chitosan decreased. Figure 1 represents the molecular weight effect on inhibition of microbial growth. Conclusively, high MW of chitosan has a potent anti-microbial activity.

Table 3. Minimum inhibitory concentration(MIC) of chitosans. (Unit : μM)

Strains	CC-01	CC-02	CC-03	CC-04	CC-05
Staphyrococcus aureus ATCC 65389	600	11.0	9.8	7.0	5.8
Escherichia coli ATCC 8739	>600	26.0	21.7	15.7	12.8
Pseudomonas aeruginosa ATCC9027	>600	39.0	22.0	23.0	19.0
Candida albicans ATCC 10231	>2000	39.0	33.0	23.0	19.0
Aspergillus niger ATCC 9642	>2000	>2000	>2000	>2000	>2000

Fig.1. Relationship between molecular weight of chitosan and minimum inhibitory concentration(MICs) against 4 microorganisms



The preservative efficacy of formulas containing chitosan

The preservative efficacy of formulas containing chitosan was studied. The final concentration of chitosan in the formula were 0.1%(w/v) and 0.5%(w/v) respectively. Figure 2 shows the preservative efficacy of chitosan. The five chitosans showed a good preservative efficacy. Chitosans showed stronger preservative efficacy against *C. albicans* than that of chemical preservatives(0.2% Methylparaben and 0.3% Imidazolidinyl Urea). From these results, It was clear that chitosan could serve as substitutes for chemical preservatives.

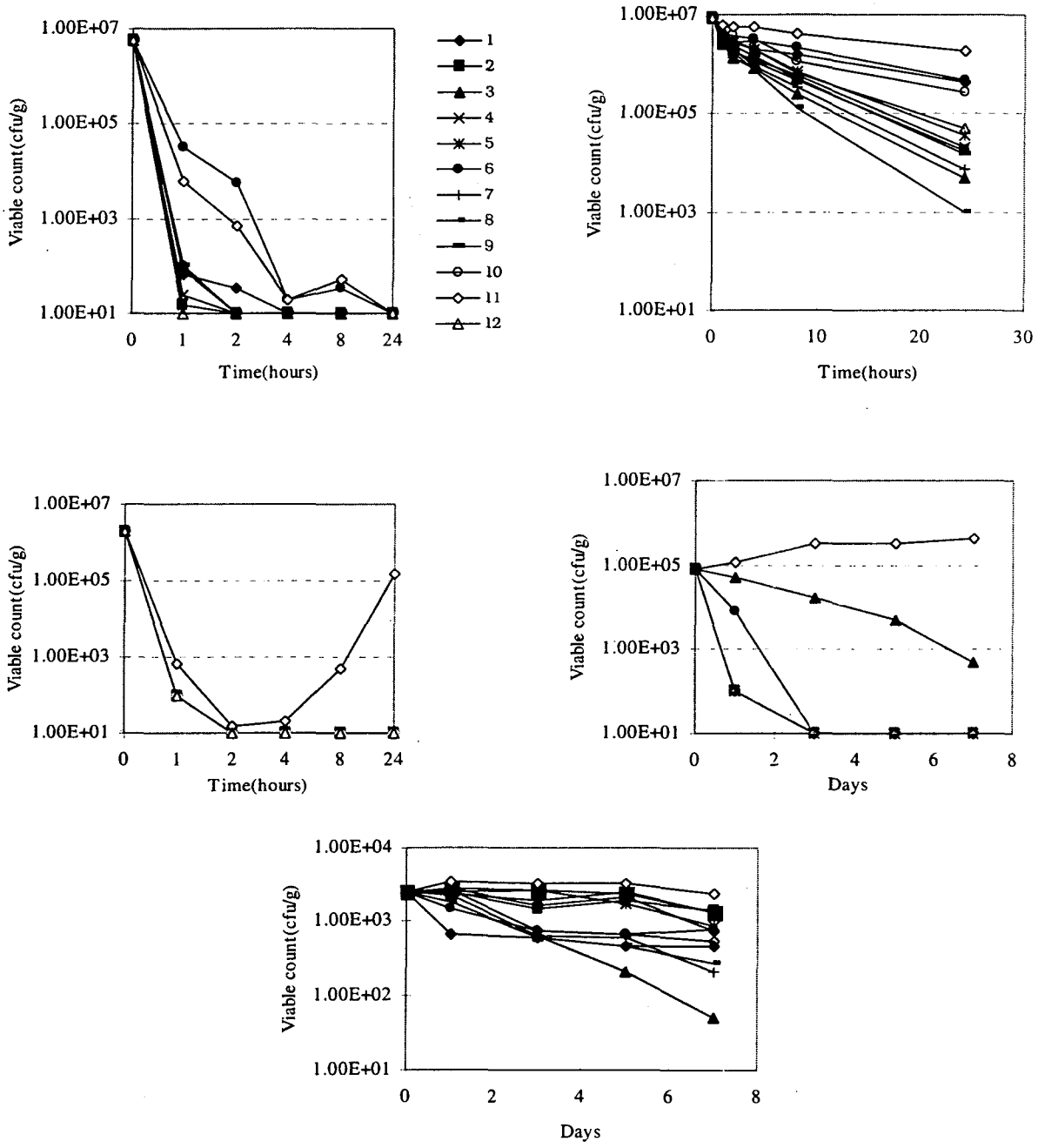


Fig 2. Survival curve of various microorganisms in emulsion containing chitosans. Final chitosan concentrations were 0.1%(w/v) and 0.5%(w/v). Molecular weights of chitosans were 1, 6 ; 5,000, 2, 7 ; 75,470, 3, 8 ; 92,163, 4, 9 ; 127,600 and 5, 10 ; 155,700. Emulsion number 11 was a preservative free formula and 12 was a formula preservered by chemical presevatives (Methyl paraben 0.2%, Imidazolidinyl urea 0.3%). Tested microorganisms are A ; Staphylococcus aureus ATCC 65389, B ; Escherichia coli ATCC 8739, C ; Pseudomonas aeruginosa ATCC 9027, D ; Candida albicansATCC 10213, and E ; Aspergillus niger ATCC9642.

Anti-oxidative activity: Among 5 water-soluble chitosans, CC-03(MW=92,163) showed the most potent anti-oxidative activity(IC_{50} : 0.2mM), but it showed lower effect than BHT. CC-03 showed a good anti-oxidative effect at low concentration (0.2mM-0.5mM) (data not shown).

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