Antimicrobial Activity and Preservative Effects of Chitosan in Cosmetic Products

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Key Words

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Abstract

Chitin and chitosan have been almost neglected until 1960's, although they are the second largest biomass on earth. Chitosan is a partially deacetylated chitin and belongs to the class of cationic biopolymers.

We investgated the antimicrobial activity of chitosan as natural preservatives in cosmetic products. Antimicrobial activity of chitosan against some microorganisms was investigated. The results indicated that chitosan had an effectiveness against some bacteria.

We found that chitosan had minimum inhibitory concentrations (MICs) as low as 100 ppm to *S. aureus* ATCC 6538, *E. coli* ATCC 1634 and *P. aeruginosa* KCTC 2004. But there was not effective to *Asp. niger* ATCC 1374 at 1,000 ppm.

Also, formulas preserved with chitosan have been subjected to preservative efficacy tests to some microorganisms. Formula preserved with 0.5% chitosan had an effective antimicrobial activity against the Gram (+) and Gram (-) bacteria but not fungi.

It is possible to determine the formulas with chitosan, which would be effective to reduce the artificial preservatives.

I. Introduction

Chitin is a main component of crab and shrimp shell, which is abandoned from processing plants. Chitin is the second most plentiful natural polymer. Chitosan is prepared from chitin by chemical N-acetylation. Chitosan is the name used for low acetyl forms of chitin and is primarily composed of glucosamine, 2-amino-2-deoxy-D-glucose. [1](Fig.1)

Chitosan's key properties are its ability to act as a catioinc flocculent, humectant, viscosifier and selective chelator of metal ions. Chitosan is being used as a non-toxic cationic polymer in hair treatment and skin care. Clear solutions form clear films that adhere to skin or hair, primarily due to chitosan's cationic character. Chitosan is an excellent moisturizer, attributes to wound healing, make it an attractive biopolymer for cosmetics and personal care applications.[2]

Cosmetic products are subject to microbial contamination and spoilage. Preservatives are added to cosmetics to inhibit the growth of bacteria, yeast and molds while the product is in trade channels and in the hand of consumers. The type of preservative and the concentration used are determined by preservative efficacy testing.[3]

Despite the many chemicals presently in use to preserve to cosmetic, toiletry and perfumery products, newer and more complex compounds with greater and often very specific activities are continnually appearing on the market.[4]

Chitosan, a high molecular weight cationic polysaccharide, has been shown to be fungicidal against several fungi. Inhibitory of chitosan has already been reported by several reserchers for soliborne phytopathogenic fungi.[5,6]

To measure the antimicrobial activity of chitosan in cosmetic products, USP, CTFA and Linear regression method are used.[7]

In this report, we studied the antimicrobial activity and preservative effects of chitosan in cosmetic products.

2. Experiment

2-1. Microorganisms, Media and Materials

2-1-1. Test organisms

The following microorganisms were used: *S. aureus* ATCC 6538, *E. coli* ATCC 1634, *P. aeruginosa* KCTC 2004 and *Asp. niger* ATCC 1374.

2-1-2.Media

Test strains were cultured in TSALT, Nutrient Broth, Potato Dextrose Broth and Potato Dextrose Agar for bacteria and fungi. Commercially prepared dehydrated culture media used in this study were manufactured by Difco Laboratories.

2-1-3. Materials

Crab shell chitosan was purchased from Showa chemical co. (Japan) and ground to a fine powder.

2-2. Antimicrobial activity of chitosan

Culture media containing 0, 100, 200, 300 and 1,000 ppm of purified chitosan were prepared. Test organisms were grown on nutrient broth for bacteria and potato dextrose broth for fungi. Growth of bacteria was incubated at 35 for 4 days on a rotary shaker and measured by OD (optical density) at 660 nm. Fungi were incubated for 5 days at 25 with orbial shaking (120 rpm). At the end of the incubation period, the mycelial dry weght was determined. Cell growth was measured by turbidity and dry cell weight (D.C.W.) method in bacteria and fungi.

2-3 Preparation of formula

One cosmetic formula was employed to verify the antimicrobial effectiveness. Table 1 shows the formulas to apply the preserved with chitosan in preservative free cosmetic, compared with general formula containing preservative. All cosmetic materials used were satisfying the regulation of CTFA.

2-4. Preservative challenge testing

S. aureus and *P. aeruginosa* were cultured in TSA media at 37 for 18hrs, while *Asp. niger* were cultured in potato dextrose agar at 25 for 4 days respectively and suspended in sterilized saline. They were inoculated into each product by 10^6 cfu/g bacteria and 10^5 cfu/g fungi. To study the preservative effects, 1g each of the culture was sampled by the time of 0, 2, 4, 24hrs, 2, 7 and 14days and D-value was obtained by APC. The acceptance criteria of above-mentioned method using D-value are shown in Fig.3.

3. Results and Discussion

3-1. Antimicrobial activity of chitosan

Chitosan was added to the nutrient broth medium to obtain chitosan concentrations of 0, 100, 200, 300 and 1,000 ppm. Test results are shown in Fig.4, Fig.5 and Fig.6. Chitosan was effective in inhibiting growth of *S. aureus*, *P. aeruginosa* and *E. coli*. At the concentration of 100 ppm, chitosan completely inhibited the growth of bacteria. Minimun concentration values against some microbial spectrum were determined and values of 100 ppm or less were obtained in bacteria. However there was not effective to *Asp. niger* at 1,000 ppm of chitosan. (Fig.7) At all the treatment levels growth occured, it was found that the final mold mats were similar to the controls and various treatments. The results show that chitosan has considerable activity against the various organisms used in this study. Bacteria were found to be much more susceptible to chitosan activity than fungi. It is worth noting however that the MIC values obtained against bacteria are quite low and it suggests that chitosan has an appreciably high bacteriostatic activity.

3-2. Preservative effects of chitosan

The change of preservative effects of chitosan was tested by USP, CTFA and Linear regression method. In this experiment the formula contained chitosan was 0.5%. Table 1 shows the formula for lotion samples to apply the chitosan in preservative free cosmetic, compared with general formula containing preservatives. Methylparaben and Diazolidinylurea are widely used in cosmetics as preservatives.

3-2-1. The effect of chitosan on *S. aureus*

The result is shown in Fig. 8. Product preseved with 0.5% chitosan was very effective on *S. aureus*. The D-value of formula with 0.5% chitosan and formula with preservative were 4hrs respectively. It shows that there was a preservative effect of chitosan on *S. aureus*.

3-2-2. The effect of chitosan on *P. aeruginosa*

The result for *P. aeruginosa* is shown in Fig.9. It is recognized that parabens are not effective against all Gram-negative organisms — especially *Pseudomonas* spp. When compared with formula with preservatives it is a little effective on formula with 0.5% chitosan.

3-2-3. The effect of chitosan on *Asp. niger*

The result for *Asp. niger* is shown in Fig.10. After inoculation, *Asp. niger* was decreased by APC but 2 days later it was rebounded. Formula with 0.5% chitosan was not effective on *Asp. niger*. Some fungi have chitinase and chitosanase, so they can degrade the glycoside bond of chitin.

4. Conclusion

Minimum inhibitory concentration (MICs) of chitosan were less than 100 ppm on *S. aureus*, *P. aeruginosa*, *E. coli*, but not effective at 1,000 ppm on *Asp. niger*.

The preservative system based on chitosan was shown to possess a wide antibacterial activity.

It is worth noting that the MIC values obtained against bacteria are quite low and it suggests that chitosan has an appreciably high bacteriostatic activity. If chitosan-microbicide combination systems are used, the concentration of the microbicide could be reduced to a lower level on cosmetic products.

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Table 1. Formulas of lotion samples

Raw material	*Formula* A	B	C
Ethyl cellulose	45.00	45.00	45.00
1,3-Butylene glycol	6.00	6.00	6.00
Methylparaben	—	0.20	—
Diazolidinylurea	—	0.10	—
Purified water	45.00	44.70	44.50
Tomato ext.	4.00	4.00	4.00
Chitosan	—	—	0.50
Total (%)	100.00	100.00	100.00

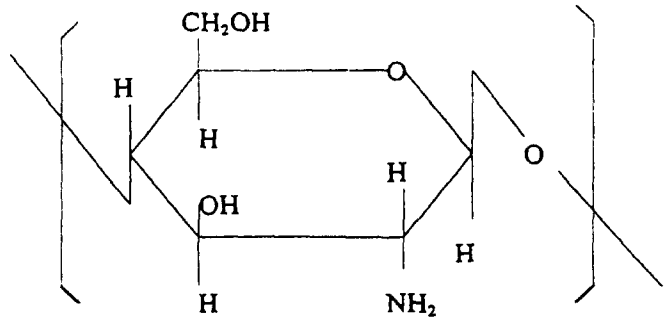

Fig. 1. The structure of chitosan

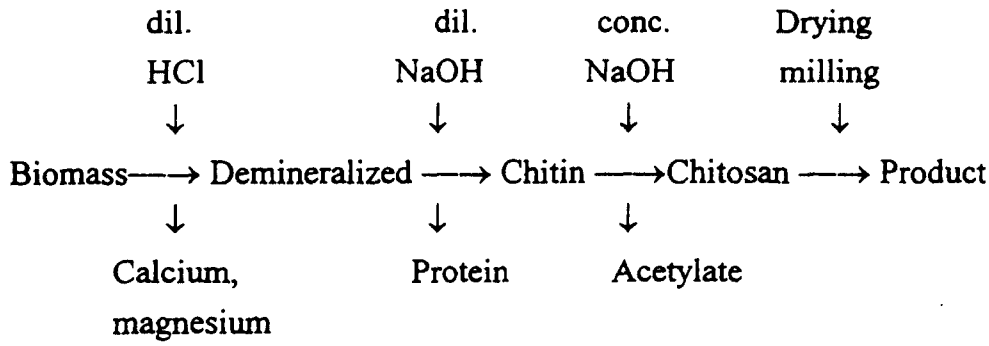

Fig. 2. Chitin and Chitosan extraction procedures from biomass.

Fig.3. Acceptance criteria by survival curves using the USP method, the CTFA method and the Linear regression method.

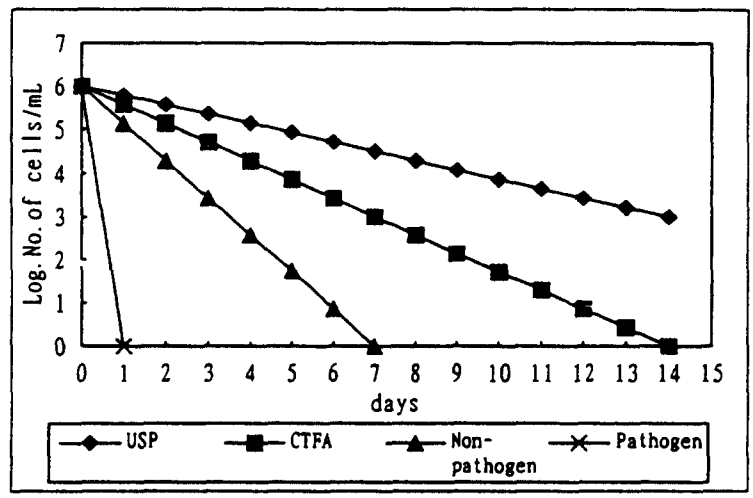

Fig. 4. Effect of chitosan concentration to the growth of *S. aureus* (35°C, 120 cycles/min).

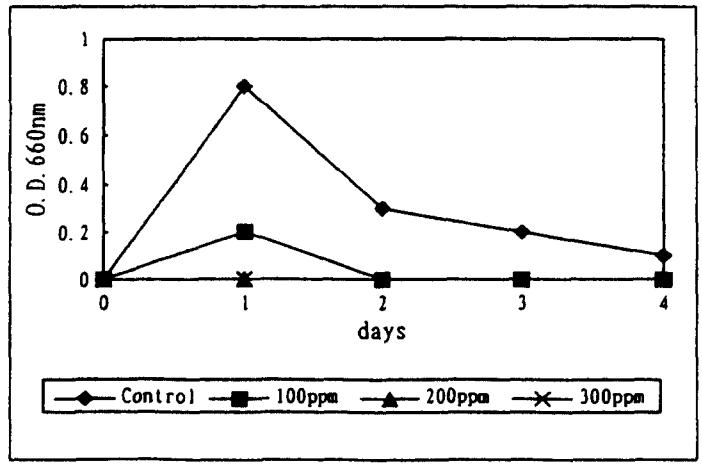

Fig. 5. Effect of chitosan concentration to the growth of *P. aeroginosa* (35°C, 120 cycles/min).

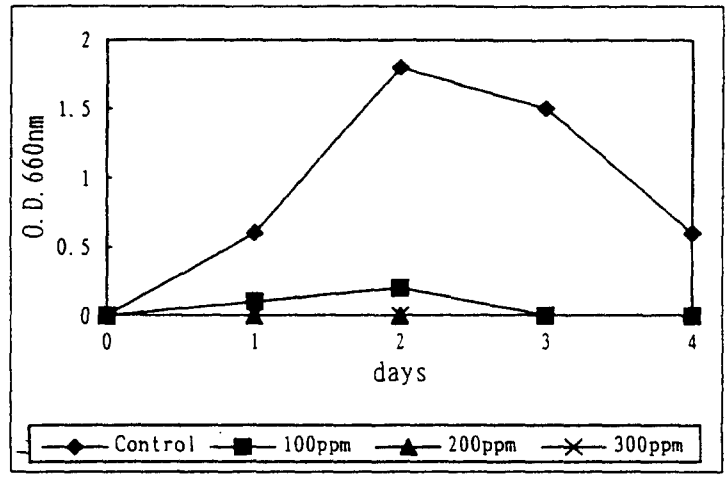

Fig. 6. Effect of chitosan concentration to the growth of *E. coli* (35°C, 120 cycles/min).

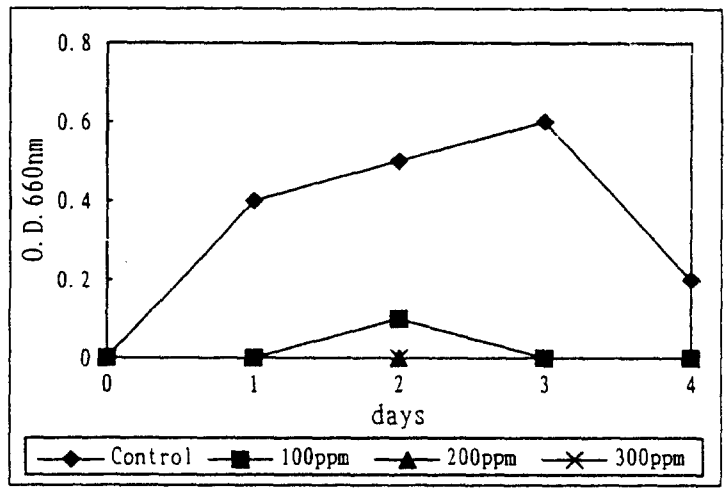

Fig.7. Effect of chitosan concentration to the growth of *Asp. niger* (25°C, 120 cycles/min).

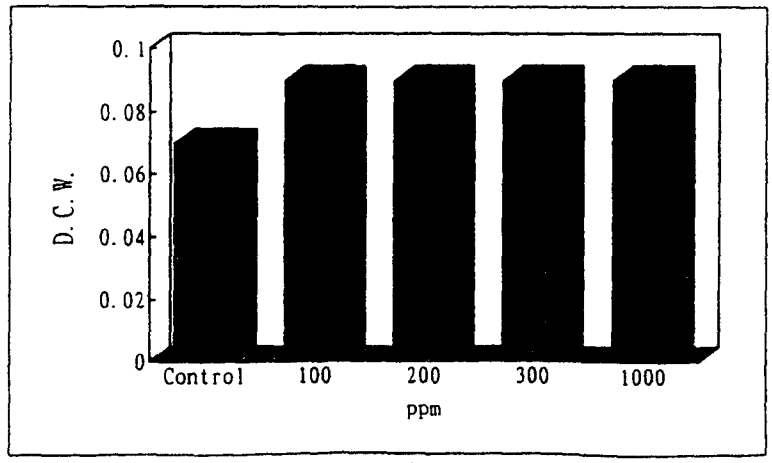

Fig. 8. Survivor curves for *S. aureus* ATCC 6538 in formula.
◆—◆: A: formula with control
■—■: B: formula with 0.2% methylparaben and 0.1% diazolidinylurea
▲—▲: C: formula with 0.5% chitosan

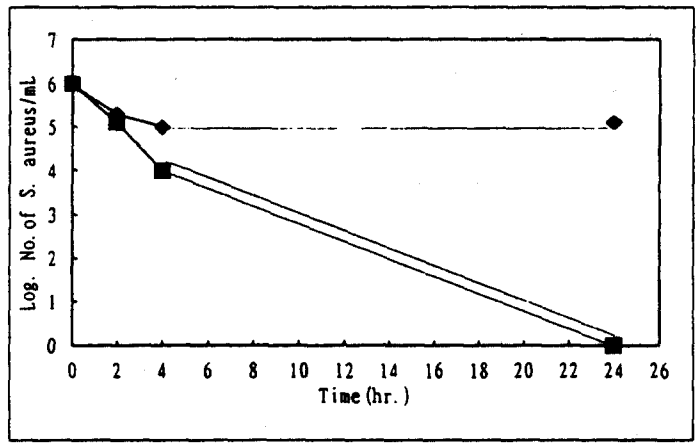

Fig. 9. Survivor curves for *P. aeroginosa* KCTC 2004 in formula.
◆—◆: A: formula with control
■—■: B: formula with 0.2% methylparaben and 0.1% diazolidinylurea
▲—▲: C: formula with 0.5% chitosan

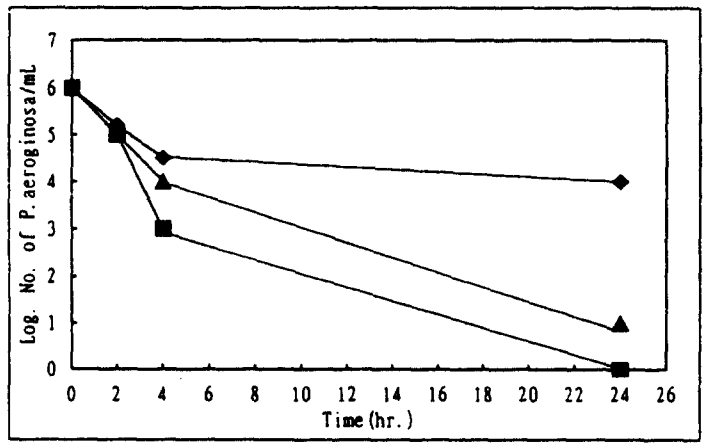

Fig. 10. Survivor curves for *Asp. niger* ATCC 1374 in formula.
◆—◆: A: formula with control
■—■: B: formula with 0.2% methylparaben and 0.1% diazolidinylurea
▲—▲: C: formula with 0.5% chitosan