

# THE EFFECTS OF SULFUR CONTENT ON ANTIMICROBIAL ACTIVITY AND WATER SOLUBILITY OF SULFONATED CHITOSAN AND SULFOBENZOYL CHI

蘇俊旗、陳齊聖

E-mail: 9015666@mail.dyu.edu.tw

## ABSTRACT

SHRIMP SHELL DERIVED CHITOSAN WAS CHEMICALLY MODIFIED TO OBTAIN SULFONATED CHITOSAN ( SC ) AND SULFOBENZOYL CHITOSAN ( SBC ) IN PYRIDINE AND METHANOL SOLVENT SYSTEM RESPECTIVELY. ANTIMICROBIAL ACTIVITY AND AQUEOUS SOLUBILITY OF THESE TWO CHITOSAN DERIVATIVES AS FUNCTIONS OF SULFUR CONTENT ( RANGED FROM 1 TO 5% ) WERE STUDIED. TEST MICROORGANISM INCLUDED ESCHERICHIA COLI ( CCRC 10674 ) , SALMONELLA TYPHIMURIUM ( CCRC 10746 ) AND STAPHYLOCOCCUS AUREUS ( CCRC 12652 ) . GENERALLY SPEAKING, SOLUBILITY INCREASED WITH INCREASING SULFUR CONTENT. HOWEVER, INCREASED SULFUR CONTENT DID NOT RESULT IN HIGHER ANTIMICROBIAL ACTIVITY. IT WAS ALSO FOUND THAT THERE EXISTED OPTIMAL SULFUR CONTENT IN TERMS OF ANTIMICROBIAL ACTIVITY AND THE SENSITIVITIES TOWARD SULFUR CONTENT WERE STRAIN DEPENDENT. DEGREE OF DEACETYLATION ALSO AFFECTED THESE TWO PROPERTIES OF SC,SBC AND UNDERIVATISED CHITOSAN . SIMILAR CONCLUSION THAT THE INFLUENCE WAS STRAIN DEPENDENT WAS OBTAINED. RELATIVELY SPEAKING, SC AND SBC DEMONSTRATED SUPERIOR ANTIMICROBIAL ACTIVITY AND AQUEOUS SOLUBILITY THAN UNDERIVATISED CHITOSAN .

Keywords : DEGREE OF DEACETYLATION、SULFUR CONTENT、CHITOSAN、SULFONATED CHITOSAN、SULFOBENZOYL CHITOSAN、MINIMUM INHIBITION CONCENTRATION

## Table of Contents

1.前言	--P1
2.文獻回顧	--P4
2.1 幾丁質與幾丁聚醣的發現與分佈	--P4
2.2幾丁質與幾丁聚醣的構造	--P4
2.3幾丁質與幾丁聚醣結構間之相關性	--P5
2.4幾丁質與幾丁聚醣之理化特性	--P6
2.4.1溶解性與黏度	--P6
2.4.2與金屬離子錯合	--P6
2.4.3剪力	--P7
2.4.4熱裂解	--P7
2.4.5吸附能力	--P7
2.5幾丁質與幾丁聚醣之製備	--P8
2.5.1幾丁質之製備	--P8
2.5.2幾丁聚醣之製備	--P9
2.6幾丁聚醣去乙醯程度的測定	--P9
2.6.1膠體滴定法	--P9
2.6.2酸鹼滴定法	--P10
2.6.3中性滴定法	--P10
2.6.4紅外線光譜分析法	--P11
2.7幾丁聚醣分子量之測定	--P12
2.7.1黏度平均分子量法	--P12
2.8幾丁質與幾丁聚醣之化學修飾	--P13
2.9幾丁聚醣之抑菌機制	--P13
2.10幾丁質與幾丁聚醣之應用	--P14
2.10.1廢水處理	--P14
2.10.1.1凝聚劑	--P14
2.10.1.2吸附重金屬	--P14
2.10.1.3去除放射性元素	--P14
2.10.1.4吸附反應性染料	--P15
2.10.1.5污水處理場之污泥脫水	--P15
2.10.2醫藥方面	--P15
2.10.3食品方面	--P15
2.10.4農業方面	--P16
3.材料設備與方法	--P17
3.1實驗材料	--P17
3.1.1食品原料	--P17
3.1.2幾丁聚醣	--P17
3.1.3化學藥品	--P17
3.1.4培養基	--P17
3.1.5實驗菌株	--P17
3.2設備	--P18
3.3實驗方法	--P18
3.3.1 SULFONATED CHITOSAN之製備	--P18
3.3.2不同含硫率磺酸幾丁聚醣之製備	--P19
3.3.3 SULFOBENZOYL CHITOSAN之製備	--P19
3.3.4 不同含硫率磺酸苯幾丁聚醣之製備	--P19
3.3.5不同去乙醯度之幾丁聚醣之製備	--P19
3.3.6幾丁聚醣去乙醯程度的測定	--P19
3.3.7幾丁聚醣分子量的測定	--P20
3.3.8溶解度測定	--P20
3.3.9抑菌測試	--P20
3.3.10元素分析	--P21
4.結果於討論	--P23
4.1鹼液濃度及反應時間對幾丁質去乙醯效果之影響	--P23
4.2幾丁聚醣及其衍生物之抑菌能力	--P24
4.2.1磺酸幾丁聚醣之抑菌效果	--P24
4.2.2磺酸苯幾丁聚醣之抑菌效果	--P25
4.2.3去乙醯度對磺酸苯幾丁聚醣之抑菌效果	--P26
4.3幾丁聚醣及其衍生物之水溶性	--P27
4.3.1磺酸幾丁聚醣的水溶性	--P27
4.3.2磺酸苯幾丁聚醣的水溶性	--P27
4.3.3去乙醯度對磺酸苯幾丁聚醣之溶解度	--P28
4.4含硫率與去乙醯度之交互作用	--P29
5.結論	--P31

## REFERENCES

1. 山口壽, 1986, 幾丁質與幾丁聚醣在食品與醫藥上之應用, 食品開發, 21 ( 8 ) :20-23. ( JAPANESE )
2. 方紹威, 1989, 幾丁聚醣的抑制微生物作用及其在低糖金橘蜜餞之應用, 國立台灣大學食品 科技研究所碩士論文.
3. 王綺芬, 1989, 蟹殼幾丁質產品理化性質測定及製備方法之研究, 國立台灣大學食品科技研究所碩士論文.
4. 王三郎, 1996, 水產資源利用學, 高立圖書有限公司, 台北.
5. 李勳宜, 1988, 草蝦幾丁聚醣之製備及其應用研究, 國立台灣大學食品科技研究所碩士論文.
6. 林孫基、劉仁煥, 1994, 以生物性高分子-幾丁質 ( CHITIN ) 衍生物處理危害性有機污染廢液 之研究, 樹德學報, 15: 147-193.
7. 林瑞洵、蔣蘇洪、張慕珊, 1992, 脫乙醯度測定方法, 化學通報, 3: 39-42.
8. 陳懿慧、李錦楓, 1996, 幾丁聚醣應用於葡萄柚汁澄清之探討, 食品科學, 23 ( 5 ) :617-628 9.

張鈺驩, 1987, 草頭蝦中幾丁質類產品的製備方法、理化性質與應用, 國立台灣大學農業化學研究所碩士論文。 10. 蔡敏郎, 1993, 不同分子量、不同去乙酰程度的幾丁聚醣溶液的流變性質與膠囊物性的關係, 國立台灣海洋大學水產食品科學研究所碩士論文。 11. 蔡正芳, 1992, 不同去乙酰的幾丁聚醣對微生物的作用及澀味的影響, 國立台灣大學食品科技研究所碩士論文。 12. 廖萬裕, 1997, 幾丁聚醣及其衍生物之抑菌研究, 私立大葉工學院食品工程研究所碩士論文。 13. 劉瓊淑, 1994, 幾丁質, 幾丁聚醣及相關酵素之特性與應用, 食品工業, 26(11):26-36。 14. 錢明賽, 1977, 水產廢棄物蝦、蟹外殼利用之研究, 食品工業發展研究所報告 118 號。 15. 錢明賽、賴淑琪, 1979, CHITOSAN在廢水處理上之應用, 食品工業發展研究所報告 151 號。 16. AUSTIN, P. R., BRINE, C. J., CASTLE, J. E., AND ZIKAKIS, J. P. 1981. CHITIN: NEW FACETS OF RESEARCH. SCIENCE. 212: 749-753. 17. BIHARI-VARGA, M., SEPULCHRE, C., AND MOCZAR, E. 1975. THERMOANALYTICAL STUDIES ON PROTEIN-POLY-SACCHARIDE COMPLEX OF CONNECTIVE TISSUE. J. THERMAL ANAL. 7: 675. 18. BAXTER, A., DILLON, M., TAYLOR, K. D. A., AND ROBERTS, G. A. F. 1992. IMPROVED METHOD FOR I.R. DETERMINATION OF THE DEGREE OF N-ACETYLATION OF CHITOSAN. INT. J. BIOL. MACROMOL., 14: 166-169. 19. BOUGH, W. A. AND SALTER, W. L. 1978. INFLUENCE OF MANUFACTURING VARIABLES ON THE CHARACTERISTICS AND EFFECTIVENESS OF CHITOSAN PRODUCTS. I. CHEMICAL COMPOSITION, VISCOSITY, AND MOLECULAR-WEIGHT DISTRIBUTION OF CHITOSAN PRODUCTS, BIOTECH. BIO-ENG. 20: 1931. 20. CHEN C. S. AND LIAU W. Y. AND TSAI G. J. 1998. ANTIBACTERIAL EFFECTS OF N-SULFONATED AND N-SULFOBENZOYL CHITOSAN AND APPLICATION TO OYSTER PRESERVATION. FOOD PROTECTION, JOURNAL, 61(9):1124-1128. 21. DOMSZY, J. G. AND ROBERTS, G. A. F. 1985. EVALUATION OF INFRARED SPECTROSCOPIC TECHNIQUES FOR ANALYSING CHITOSAN. MAKROMOL. CHEM. 186: 1671-1677. 22. GHOSH, A. E., ARUL, J., GRENIER, J., AND ASSELIN, A. 1992. ANTIFUNGAL ACTIVITY OF CHITOSAN ON TWO POSTHARVEST PATHOGENS OF STRAWBERRY FRUIT. PHYTOPATHOLOGY. 82:398-402. 23. HADWIGER, L. A. AND BECKMAN, J. M. 1980. CHITOSAN AS A COMPONENT OF PEA-FUSARIUM ALOBI INTERACTION. PLANT PHYSIOL. 66:205-211. 24. HADWIGER, L. A. AND BECKMAN AND ADAMS, M. J. 1981. LOCALIZATION OF FUNGAL COMPONENTS IN THE PEA-FUSARIUM INTERACTION DETECTED IMMUNOCHEMICALLY WITH ANTI-CHITOSAN AND ANTI-FUNGAL CELL ANTISERA. PLANT PHYSIOL. 67:170-175. 25. HADWIGER, L. A. AND LIN, R. F. 1982. HEXAMINE ACCUMULATIONS ARE ASSOCIATED WITH THE TERMINATED GROWTH OF PUCCINIA STRIIFORMIS ON WHEAT ISOLINES. PHYSIOL. PLANT. PATHOL. 19:249-255. 26. HADWIGER, L. A., KENDRA, D. F., FRISTENSKY, B. W. AND WAGONER, W. 1986. CHITOSAN BOTH ACTIVATES GENES IN PLANTS AND INHIBITS RNA SYNTHESIS IN FUNGI. IN "CHITIN IN NATURE AND TECHNOLOGY", PP.209-214, MUZZARELLI, R., JEUNIER, C. AND GOODAY, G. (ED.), PLENUM PRESS, N.Y. 27. HIRANO, S., OHYE, Y., AND ONE, H. 1976. SELECTIVE N-ACYLATION OF CHITOSAN. CARBOHYDR. RES. 47: 315-320. 28. HIRANO, S. 1988. PRODUCTION AND APPLICATION OF CHITIN AND CHITOSAN IN JAPAN. IN PROCEEDING OF THE FOURTH INTERNATIONAL CONFERENCE ON CHITIN AND CHITOSAN, PP.37-43, SKJAK-BRAEK, G. ANTHONSEN, T. AND SANDFORD, P. (ED.), ELSEVIER APPLIED SCI. PUBLISHERS, LONDON. 29. HIRANO, S., KINUGAWA, J., AND NISHIOKA, A. 1989. SULFATED DERIVATIVES OF CHITOSAN AND THEIR CHARACTERIZATION WITH RESPECT TO BIOLOGICAL ACTIVITY. IN CHITIN IN NATURE AND TECHNOLOGY, PP. 461-467, MUZZARELLI, R., JEUNIER, C. AND GOODAY, G. (ED.), PLENUM PRESS, N.Y. 30. KENDRA D. F. AND HADWIGER, L. A. 1984. CHARACTERIZATION OF THE SMALLEST CHITOSAN OLIGOMER THAT IS MAXIMALLY ANTIFUNGAL TO FUSARIUM SOLANI AND ELICITS PISATIN FORMATION IN PISUM SATIVUM. EXP. MYCOL. 8:276-281. 31. KNORR, D. 1984. USE OF CHITINOUS POLYMERS IN FOOD-A CHALLENGE FOR FOOD RESEARCH AND DEVELOPMENT. FOOD TECHNOL., JOURNAL: 85-97. 32. KNORR, D. 1991 RECOVERY AND UTILIZATION OF CHITIN AND CHITOSAN IN FOOD PROCESSING WASTE MANAGEMENT. FOOD TECHNOL., JOURNAL: 114-122. 33. KNORR, D. 1986. NUTRITIONAL QUALITY, FOOD PROCESSING, AND BIOTECHNOLOGY ASPECTS OF CHITIN AND CHITOSAN: A REVIEW. PROCESS BIOCHEMISTRY., 21(3): 90-92. 34. KURITA, K. 1986. CHEMICAL MODIFICATIONS OF CHITIN AND CHITOSAN. IN "CHITIN IN NATURE AND TECHNOLOGY", PP.287-293, MUZZARELLI, R., JEUNIER, C. AND GOODAY, G. W. (ED.), PLENUM PRESS, N.Y. 35. LANG, G. AND WELLA, T. C. 1989. THE USE OF CHITOSAN IN COSMETICS. IN "CHITIN AND CHITOSAN", PP.139-147, SKJAK-BRAEK, G., ANTHONSEN, T. AND SANDFORD, P. (ED.) ELSEVIER APPLIED SCIENCE, N.Y. 36. LEUBA, J. L., AND P. STOSSEL. 1986. CHITOSAN AND OTHER POLYAMINES: ANTIFUNGAL ACTIVITY AND INTERACTION WITH BIOLOGICAL MEMBRANES, P.215-221. IN R. MUZZARELLI, C. JEUNIER, AND G. GOODAY (ED.), CHITIN IN NATURE AND TECHNOLOGY. PLENUM PRESS. NEW YORK. 37. MIMA, S., MIYA, M., IWAMOTO, R., AND YOSHIKAWA, S. 1982. HIGHLY DEACETYLATED CHITOSAN AND ITS PROPERTIES. PROC. SECOND INTERNATIONAL CONFERENCE ON CHITIN/CHITOSAN, P.21. 38. MIMA, S., MIYA, M., IWAMOTO, R., AND YOSHIKAWA, S. 1983. HIGHLY DEACETYLATED CHITOSAN AND ITS PROPERTIES. J. APPL. POLYMER SCI., 28: 1909-1917. 39. MOORE, G. K. AND ROBERTS, G. A. F. 1980. DETERMINATION OF THE DEGREE OF N-ACETYLATION OF CHITOSAN. INT. J. BIOL. MACROMOL., 2, APRIL: 115-116. 40. MUZZARELLI, R. A. A. 1997 OXFORD. O'BRIEN, M. AND COLWELL, R. R. CHITIN. PREGAMON PRESS. A RAPID TEST FOR CHITINASE ACTIVITY THAT USES 4-METHYLBELLIFERYL-N-ACETYLD-GLUCOSAMINE. APPL. AND ENVIR. MICROB., 53: 1718-1724. 41. MUZZARELLI, R. A. A., TANFANI, F., EMANUELL, M., CHIURAZZI, E. AND PIANI, M. 1986. SULFATED N-CARBOXYMETHYL CHITOSANS AS BLOOD ANTICOAGULANTS. CHITIN IN NATURE AND TECHNOLOGY,

PP.469-467, MUZZARELLI, R., JEUNIAUX, C. AND GOODY, G. W. ( ED ), PLENUM PRESS, N.Y. 42. NIL. 1996. MARKET OF CHITIN AND CHITOSAN. BIO INDUSTRY, 13 ( 3 ) :52-61. ( JAPANESE ) 43. NISHIMURA, K., NISHIMURA, S., NISHI, N., SAIKI, I., TOKURA, S. AND AZUMA, I. 1984. IMMUNOLOGICAL ACTIVITY OF CHITIN AND ITS DERIVATIVES. VACCINE 2 : 93-99. 44. NISHI, N., NOGUCHI, J., TOCURA, S., AND SHIOTA, H. 1979. STUDIES ON CHITIN I.ACETY -LATION OF CHITIN. POLYMER JOURNAL.11 ( 1 ) : 27-32. 45. OKIEI, W., NISHIMURA, S., SOMORIN, O., NISHI, N. AND TOKURA, S. 1986. INHIBITORY ACTION OF SULFATED CHITIN DERIVATIVES ON THE HYDROLYTIC ACTIVITY OF THROMBIN. IN" CHITIN IN NATURE AND TECHNOLOGY ", PP.433-460, MUZZARELLI,R.JEUNIAUX,C.AND GOODY, G. W. ( ED ), PLENUM PRESS, N.Y. 46. PEARCE, R. B. AND RIDE, J. P. 1982. CHITIN AND RELATED COMPOUNDS AS ELICTORS OF THE LIGNIFICATION RESPONSE IN WOUNDED WHEAT LEAVES. PHYSIOL.PLANT PATHOL.20:119-129. 47. POULICEK, M., VOSS-FOUCART, M. F., AND JEUNIAUX, C. 1986. CHITINPROTEIC COMPLEXES AND MINERALIZATION IN MOLLUSK SKELETAL STRUCTURES. IN PROCEEDINGS OF THE THIRD INT -ERNATIONAL CONFERENCE ON CHITIN AND CHITOSAN, PP.7-12. PLENUM PRESS, N.Y. 48. RICO-MUNOZ, E., AND P. M. DAVIDSON. 1983. EFFECT OF CORN OIL AND CASEIN ON THE AN -TIMICROBIAL ACTIVITY OF PHENOLIC ANTIOXIDANTS. J. FOOD SCI. 48:1284-1288. 49. ROBERTS, G. A. F. 1992. CHITIN CHEMISTRY. THE MACMILLAN PRESS LTD, LONDON. 50. ROBERTS, G. A. F. AND DOMSZY, J. G. 1982. DETERMINATION OF THE VISCOMETRIC CONSTAN -TS FOR CHITOSAN. INT. BIOL. MACROMOL., 4: 374-377. 51. STANLEY, W.L., WATTERS, G.G., CHAN, B.G., CHAN, B.G., AND MERCER, J.M. 1975. LACTO- -SE AND OTHER ENZYMES BOUND TO CHITIN WITH GLUTARALDEHYDE. BIOTECH. AND BIOENG., XVII: 315-326. 52. SUDARSHAN, N. R., D. G. HOOVER, AND D. KNORR. 1992. ANTIBACTERIAL ACTION OF CHITOS -AN. FOOD BIOTECHNOL. 6(3):257-272. 53. TOEI, K. AND KOHORA, T. 1976. A CONDUCTOMETRIC METHOD FOR COLLOID TITRATIONS. ANAL -YTICA CHIMICA ACTA. 83: 59-65. 54. URAGAMI, T. 1992. SEPARATION OF ORGANIC LIQUID MIXTURES THROUGH CHITOSAN AND CHITO -SAN DERIVATIVE MEMBRANES BY PERVAPORATION AND EVAPOMEATION METHODS. IN "ADVANCES IN CHITIN AND CHITOSAN".PP.594-603, BRINE, C. J., SANDFORD, P. A. AND ZIKAKIS, J. P. ( ED ), ELSEVIER APPLIED SCIENCE. N.Y. 55. WOLFROM, M. L. JOHNSON, AND H. T. M. SHEN. 1959. THE SULFONATION OF CHITOSAN. J. AM. CHEM. SOC. 81:1764-1766.