Biological properties of chitosan

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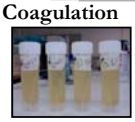
International Functional Foods Conference
6-8th May 2008 Porto
Portugal

INTRODUCTION

Biopolymers are receiving more and more attention as alternatives to synthetic polymers in several technological processes ranging from environmental, food and health applications. Among them, chitosan is one of the most promising, being produced from heterogenous alkaline de-N-acetylation of chitin. Chitosan exhibits several attractive and promising biological activities such as hemostatic, antimicrobial and immunostimulant. Non-chemically modified chitosan is only soluble at acid pH. Therefore, pH and acid solvents, affect its antibacterial activity. In this work, several biological properties of chitosans such as, anti-bacterial, coagulant, flocculant and biofilm formation capability upon *Staphylococcus aureus*, *S. epidermidis* and *Escherichia coli* have been explored .

MATERIALS & METHODS

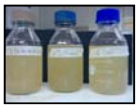
Coagulation



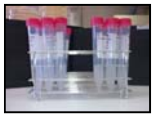
S. aureus grown for 24h at 37ºC with and without chitosan

Addition of rabbit serum to supernatant

Flocculation



Resuspend cells (pellet) in phosphate buffer, 10 mM, pH



Rest undisturbed for 24 h

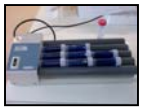
O.D. at 620 nm,

% flocculation: 1— (OD sample/ OD blank)×100.

Biofilm formation

S.aureus

Discard supernatant and add 1ml of tryptan blue



Rotate the tubes for uniform staining

Decant the tubes and allow to dry

RESULTS & DISCUSSION

Coagulating ability

☛ The results obtained were positive for all cases. Impossible to reach a conclusion of its action upon the microorganisms or upon the coagulase system. Chitosan is, itself, a coagulating agent (Okamoto et al., 2003).

Flocculation

		HMW	LMW
2 h	*S. aureus*	53.1	51.9
	S. epidermidis	65.1	76.3
	E. coli	81.7	81.8
24 h	*S. aureus*	93.7	38.9
	S. epidermidis	91.1	95.3
	E. coli	91.6	94.6

☛ Cell flocculation could be attributed to both chitosans, however, significantly higher for high MW chitosan towards *S. aureus* (94%). Low MW chitosan showed higher affinity towards flocculation of *E. coli* and *S. epidermidis*, contradicting previous studies (Strand et al. 2001), where *E. coli* flocculated better in the presence of higher MW chitosans. However, the fact that low MW chitosans penetrate easily the membrane of Gram negative bacteria promoting their flocculation has also been reported by other authors (Zheng and Zhu, 2003; Liu et al. 2006).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

	High weight	Low weight
	MIC/MBC	MIC/MBC
S. aureus	0.25 /0.50-1 %	0.25/0.50 %
S. epidermidis	0.25/1 %	0.15/0.50 %
E. coli	0.50/0.50 %	0.15/0.25 %

☛ MIC values for the low MW chitosan ranged from 0.15-0.25% without any difference between Gram-positive bacteria and *E. coli*; however the MBC for the Gram-positive bacteria was twice (0.50%) of that found for *E. coli*. The MIC value for the high MW chitosan was 0.25% for staphylococci and 0.50% for *E. coli* while the MBC was similar for all three isolates (0.50-1%).

Antibacterial activity

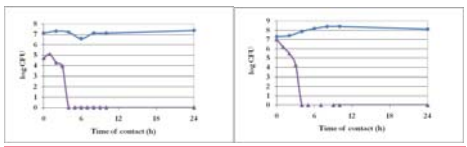


Figure 1. Antibacterial activity of *S. aureus* (left) and *S. epidermidis* (right) in the presence of chitosan (-▲-) and in the absence of chitosan (-●-)

Biofilm formation

High MW chitosan (no biofilm formation)

Low MW chitosan (biofilm formation)

☛ Sub-mic concentrations of the high molecular weight chitosan (0.10 and 0.15%) were able to effectively inhibit biofilm formation by *S. aureus*, while those of low molecular weight chitosan (0.05 and 0.025%) were not.

CONCLUSIONS

☛ Besides being described as non-toxic, immunostimulatory and biocompatible, chitosan is a powerful antimicrobial

☛ It flocculates microbial cells and prevents biofilm formation further reinforcing its antimicrobial potential

ACKNOWLEDGEMENTS

The Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT, Portugal) through program PRAXIS XXI is hereby gratefully acknowledged for funding of a post-doc fellowship (SFRH/BPD/24222/2005). Additional financial support was obtained via project BIOTEX — Bioactive Textiles using functional biopolymers (POCI/CTM/58312/2004), also funded by FCT, Portugal.

LITERATURE

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