THE INFLUENCE OF SOME ABIOTIC FACTORS ON THE ANTIMICROBIAL POTENTIAL OF CHITOSAN

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The stratum corneum of the skin of patients with atopic dermatitis is highly susceptible to colonization by *Staphylococcus aureus* (Arikawa et al., 2002). Biopolymers have received more and more attention as alternatives to synthetic polymers in several technological processes, ranging from environmental, to food and health applications. Among them, chitosan is one of the most promising; it is produced from heterogenous alkaline de-N-acetylation of chitin. Non-chemically modified chitosan is only soluble at acid pH, but pH and acid solvents affect its antibacterial activity. The effects of such factors upon chitosan activity have been explored by some authors (Vishu Kumar et al., 2007), but it was always found to be strain-dependent. In addition, several external (abiotic factors) also influence its activity, thus making it quite difficult to draw clear-cut conclusions. To assess the potential use of chitosan to prevent and control atopic dermatitis, its antibacterial activity against skin-associated bacteria was investigated, by varying such abiotic factors as pH, ionic strength, organic acids and free fatty acids.

MATERIALS & METHODS

Chitosans: Chitosan of high molecular weight (ca. 624 kDa, >75% deacetylated), medium molecular weight (ca. 591 kDa, 75-85% deacetylated) and low molecular weight (ca. 107 kDa, 75-85% deacetylated) were obtained from Sigma (St. Louis, MO, USA).

Microorganisms: Three major skin-related bacteria were used: *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Staphylococcus* sp. ATCC 155 and *Escherichia coli* ATCC 25922.

Antimicrobial activity: A 2^5 full factorial design was followed: ionic strength, pH level, organic acids, fatty acids and presence/absence of chitosan, selected according to skin conditions, were used as parameters.
Ionic strength: 0.2 %(w/v) and 0.4 %(w/v); pH: 4 and 7; Organic acids: lactic and propionic acids (1 % (v/v)); Free fatty acids: oleic and palmitic acids (0.75 % (v/v)). Only high molecular weight chitosan was used at 0.5 % (v/v) as this MW was found as that which affected Gram positive skin microorganisms the most (Tavaria et al., 2008). The inoculants were added to each mixture (5% v/v)) and left to incubate for 24h at 37º C. Samples were collected at time zero, and then every 2 h up to 24h, for viable cell enumeration. The reduction rate was then determined as the variation of the colony forming units (CFU) between time zero and a given time.

Statistical analysis: Repeated measures oneway ANOVA was applied, using the SPSS package (v. 16.0 for windows, SPSS, IL, USA) at a 5% level of significance.

RESULTS & DISCUSSION

Effect of pH

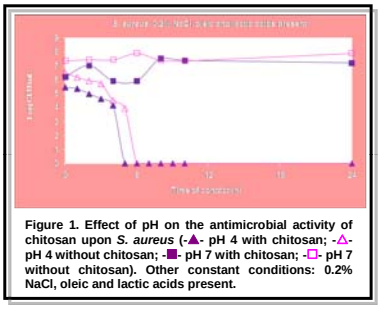

Figure 1. Effect of pH on the antimicrobial activity of chitosan upon *S. aureus* (-▲- pH 4 with chitosan; -△- pH 4 without chitosan; -■- pH 7 with chitosan; -□- pH 7 without chitosan). Other constant conditions: 0.2% NaCl, oleic and lactic acids present.

✓ **pH and the fatty acid (FFA) were the most important factors influencing anti-microbial activity.**

✓ **pH affected significantly the antimicrobial activity of the three isolates; for *S. aureus* (Figure 1), this effect was less apparent than for the other microorganisms, as also reported by Yang et al. (2005).**

✓ **At 0.2% NaCl (Figure 2), oleic acid (A) protected the cells from the antimicrobial action (even in the presence of chitosan).**

Effect of FFAs

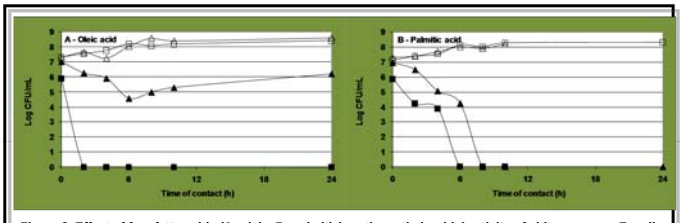

Figure 2. Effect of free fatty acids (A- oleic, B- palmitic) on the antimicrobial activity of chitosan upon *E. coli* at pH 7 [-▲- 0.2%(v/v) NaCl with chitosan; -△- 0.2%(v/v) NaCl without chitosan; -■- 0.4%(v/v) NaCl with chitosan; -□- 0.4%(v/v) NaCl without chitosan]. Other constant conditions: presence of lactic acid.

✓ **Presence of NaCl and of the organic acid was only significant ($p<0.05$) for *S. epidermidis*, in the presence of chitosan.**

✓ **At the lower NaCl concentration (0.2%), propionic acid seemed to protect bacteria from the antimicrobial action of chitosan at pH 7, while lactic acid seemed to potentiate its antimicrobial activity (Figure 3). Jo et al. (2007) described a strong inhibitory effect of lactic acid upon *E. coli*, while Cheng et al. (2003) demonstrated that, although some strains of *E. coli* exhibit an increased tolerance to lactic acid, they do not show such an adaptative/tolerant capacity towards propionic acid.**

Effect of organic acids

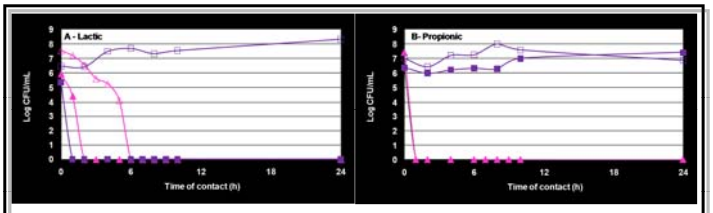

Figure 3. Effect of organic acids (A- lactic, B- propionic) on the antimicrobial activity of chitosan upon *S. epidermidis* (-▲- pH 4 with chitosan; -△- pH 4 without chitosan; -■- pH 7 with chitosan; -□- pH 7 without chitosan). Other constant conditions: 0.2% NaCl, presence of oleic acid.

Conclusions

➔ **By controlling the presence of selected abiotic factors, the action of chitosan can be targetted at inhibiting growth of unwanted microorganisms, such as *S. aureus* and *E. coli*.**

➔ **Chitosan was able to selectively inhibit growth of skin microorganisms, a requirement for use as effective antimicrobial compound.**

➔ **Chitosan applied to textiles can, in this way, change the ecology of the skin native flora, without leading to the outgrowth of pathogenic bacteria.**

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Acklowledgements

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

FCT Fundação para a Ciência e a Tecnologia
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Ciência Inovação 2010 — Programa Operacional Ciência e Inovação 2010