

# Selection of a chitosan type for eggshell coating to reduce salmonella shell contamination

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Abbreviated title: Chitosan for eggshell coating

## Summary

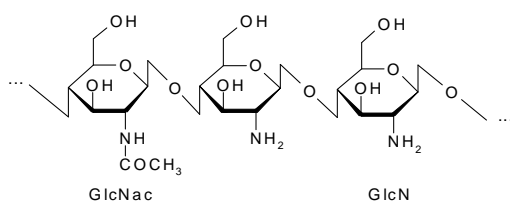
Chitosan is a natural polysaccharide derived by complete or partial (>70%) deacetylation of chitin. Because of its antimicrobial activity and film-forming properties, chitosan coating of eggshells can improve the storability of eggs and could reduce eggshell contaminations. The antimicrobial activity of eight chitosan types, with molecular weights (mw) ranging from 28 to 375 kDa, towards SE was assayed using a contact plate method. This method consisted of inoculation of SE on agar plates in the presence and absence (reference) of 0.25% chitosan, dissolved in acetic acid, at pH 5.0. After incubation for 2 h at 20°C and 60% relative humidity, the bacteria were recovered using contact plates. All chitosan types showed antimicrobial activity against SE with survival rates in the range of 24-47% compared to the reference. The chitosan type with a mw of 310-375 kDa was selected and its antimicrobial activity towards other egg-related microorganisms was evaluated. Survival rates of *Acinetobacter baumannii*, *Alcaligenes* sp., *Carnobacter* sp., *Pseudomonas* sp., *Serratia marcescens* and *Staphylococcus warneri* were 50, 31, 20, 5, 3 and 8% respectively. Thus, chitosan appears to be a promising antimicrobial component for egg coating.

Keywords: chitosan, antimicrobial activity, *Salmonella* Enteritidis, eggs

## Introduction

Chitosan is a natural polysaccharide (Figure 1) and is derived by complete or partial (>70%) deacetylation of chitin (poly- $\beta$ -1,4-D-N-acetylglucosamine).

Figure 1. The chemical structure of chitosan



Chitosan has received increased attention for its commercial applications in the biomedical, food and chemical industries. It has also been documented to possess a film-forming property for use as edible films or coatings, which can improve the storability of perishable foods. It is biocompatible, nonantigenic, non-toxic and biofunctional and its biological safety has been demonstrated by feeding trials with domestic animals.

Chitosan has antimicrobial activity towards fungi, yeast and Gram-negative and -positive bacteria. In general, the antimicrobial activity of chitosan is higher when the deacetylation degree is higher and the molecular weight (mw) and the pH are lower (below pH 6.3) (No *et al.*, 2007).

Because of its antimicrobial activity and film-forming properties, chitosan coating of eggshells can improve the storability of eggs (Bhale *et al.*, 2003) and could reduce eggshell contaminations.

In the present study, eight chitosan types are compared, based on their antimicrobial activity towards SE. One selected chitosan type will also be used to assess the antimicrobial activity towards other egg-related microorganisms.

## Materials and methods

### Bacterial strains and cultures

Seven bacterial strains were used – *Salmonella enterica* serovar Enteritidis MB1409, *Acinetobacter baumannii* (MB2793), *Alcaligenes* sp. (MB2794), *Carnobacter* sp. (MB2796), *Pseudomonas* sp. (MB2797), *Serratia marcescens* (MB2795) and *Staphylococcus warneri* (MB2792) – that were isolated from egg contents, albumen or yolk, by our institute (De Reu *et al.*, 2006). The strains, stored on Protect Beads at -80°C, were resuscitated by incubation overnight in buffered peptone water (BPW; Oxoid, Basingstoke, UK) at 30°C. These cultures were then plated on tryptone soya agar (TSA; Oxoid) and incubated overnight at 30°C. One colony was picked and grown in 10 ml BPW at 30°C for 18 h. These cultures were then diluted to obtain a final concentration of approximately 1000 cfu per 450 µl.

### Chitosan

Table 1 shows the molecular weights of the four non-commercial chitosan types, derived from *Agaricus bisporus* (KitoZyme, Herstal, Belgium), and the four commercial chitosan types, derived from crab shells (Sigma-Aldrich, Bornem, Belgium).

Table 1. Molecular weights of the chitosan types tested.

Number	Type	Molecular weight (kDa)
C1	Non-commercial	42
C2	Non-commercial	50
C3	Non-commercial	28
C4	Non-commercial	65
C5	Commercial	50-190
C6	Commercial	190-310
C7	Commercial	310-375
C8	Commercial	190-375

### Contact plate method

A contact plate method was used to assess the antimicrobial effect of chitosan towards SE and other egg-related microorganisms. The contact plate method consists of the inoculation of the bacterial strains on agar plates

(bacteriological agar, Oxoid) in the presence and absence (reference) of 0.25% (w/v) chitosan, dissolved in 1% acetic acid (v/v), at pH 5.0. After incubation for 2 h at 20°C and 60% relative humidity in a climate chamber (Termaks KBP 6395 F, Solheimsviken, Norway), the bacteria were recovered using contact plates of tryptone soya agar (TSA, Oxoid). These contact plates were then incubated overnight at 30°C.

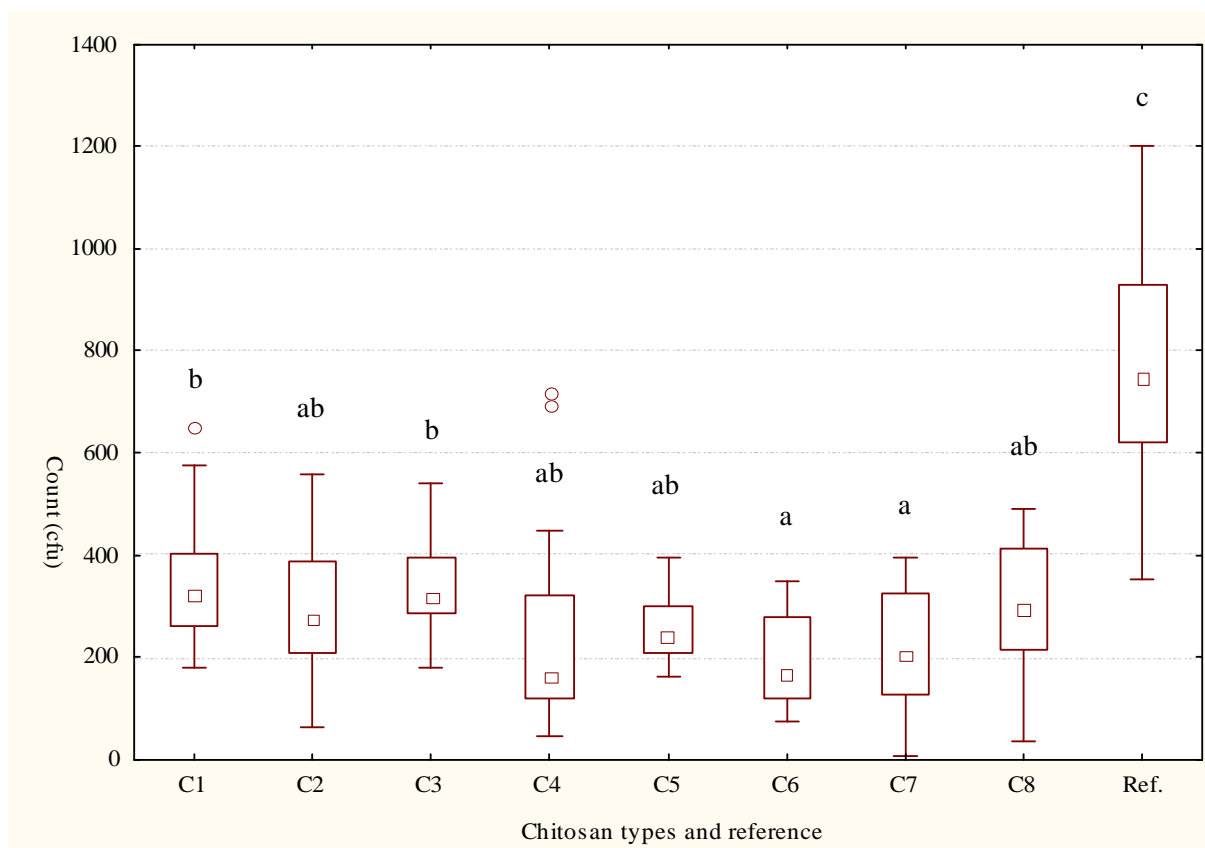
## Statistical analysis

Counts were transformed by square root transformation before statistical analysis. A one-way analysis of variance (ANOVA) was used to assess whether the mean transformed counts were influenced by the chitosan type. The Tukey's Honest Significant Difference test was used to identify pairs with significant differences. A two-sample t-test was performed on these transformed values to assess whether the mean counts recovered from the chitosan plates *versus* the reference plates were significantly different. The significance level  $\alpha$  was set at 0.05. All analysis were done in Statistica 8.0 (Statsoft Inc., Tulsa, Okla.).

## Results

All chitosan types showed antimicrobial activity against SE, as shown in Figure 1, with mean survival rates ranging from 24 to 47% compared to the reference. Both the chitosan types C6 and C7 had the strongest antimicrobial activity, but C7 displayed a more flexible film compared to C6. As a consequence, C7 was selected to evaluate its effect on the other egg-related strains.

Figure 1. Boxplot showing the antimicrobial effect of chitosan towards SE. The small square within the box marks the median. The boundaries of the box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles. Whiskers above and below the box indicate the 10<sup>th</sup> and 90<sup>th</sup> percentiles. The outliers are shown. Chitosan types and reference without common letter (a, b, c) are significantly different.



The contact plate method showed that the chitosan type C7 had also an antimicrobial effect on the other egg-related strains. Survival rates of *Acinetobacter baumannii*, *Alcaligenes* sp., *Carnobacter* sp., *Pseudomonas* sp., *Serratia marcescens* and *Staphylococcus warneri* were 50, 31, 20, 5, 3 and 8% respectively.

## Conclusion

As the survival rates of egg-related microorganisms such as *Salmonella* Enteritidis, *Acinetobacter baumannii*, *Alcaligenes* sp., *Carnobacter* sp., *Pseudomonas* sp., *Serratia marcescens* and *Staphylococcus warneri*, are significantly reduced by 0.25% (w/v) chitosan, more specifically chitosan type C7 (mw of 310-375 kDa), appears to be a promising antimicrobial component for egg coating.

## Acknowledgements

We acknowledge the financial support from the European Community, project FOOD-CT-2006-036018, also referred to as RESCAPE project (Reducing Egg Susceptibility to Contaminations in Avian Production in Europe).

## References

*BHALE, S., NO, H.K., PRINYAWIWATKUL, W., FARR, A.J., NADARAJAH, K. and MEYERS, S.P. (2003) Chitosan coating improves shelf life of eggs. Journal of Food Science 68: 2378-2383*

*DE REU, K., GRIJSPEERDT, K., MESSENS, W., HEYNDRICKX, M., UYTENDAELE, M., DEBEVERE, J. and HERMAN, L. (2006) International Journal of Food Microbiology 112: 253-260*

*NO, H.K., MEYERS, S.P., PRINYAWIWATKUL, W. and XU, Z. (2007) Applications of chitosans for improvement of quality and shelf life of foods : a review. Journal of Food Science 72: R87-R100*