# Chito-oligosaccharides (COS) functionality: Antimicrobial activity and antioxidant capacity *in vitro*

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## Introduction

Chitosan has several biological properties useful for the food industry, but the most attractive is its potential use as a food preservative. *Salmonella* Typhimurium and *Clostridium difficile* are related with diarrhoea, abdominal pain, nausea and vomiting. *C. difficile* can be responsible for the development of pseudomembranous colitis. Chitosan is known to have important functional activities but its poor solubility makes it difficult to use in food applications. However, the low viscosity and good solubility of chito-oligosaccharides (COS) make them especially attractive in an important number of useful applications such as antitumoral, antimicrobial, antifungal, lipid binder, etc[1].

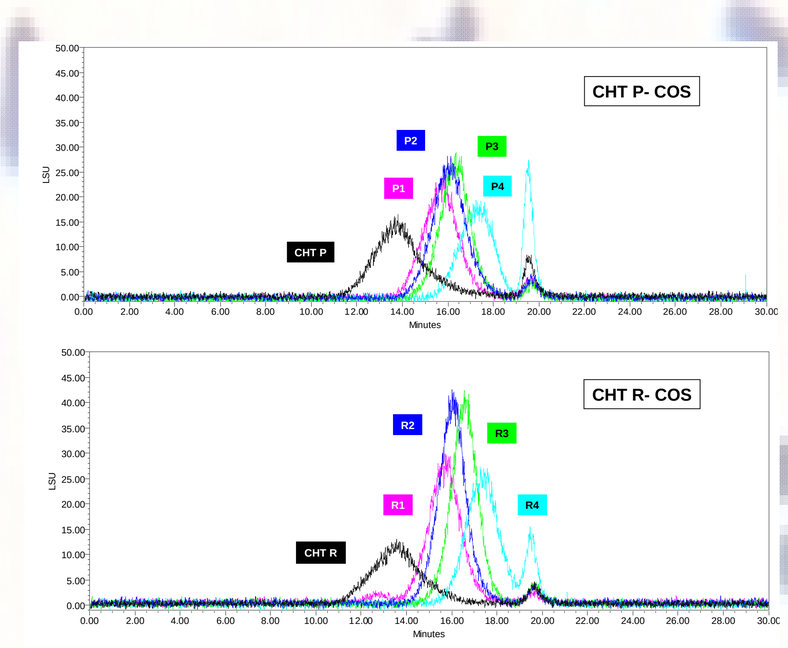


**Figure 1. Molecular weight distribution**

## Materials and Methods

In the present work, COS were obtained by enzymatic hydrolysis with chitosanase[2] from shrimp (*Pandalus borealis*) chitosans (CHT P and CHT R) with different molecular weight (MW) and deacetylation degree (DD). COS were isolated using ultrafiltration obtaining four fractions per each chitosan (P1-P4 and R1-R4). Each fraction was characterized based on its deacetylation degree by UV-VIS and molecular weight by GPC (Figure 1. Table 1). The effect of COS on *S.* Typhimurium and *C. difficile* was investigated measuring the optical density *in vitro* at 24h. The ferric reducing antioxidant power (FRAP) and scavenging effect on DPPH radical have been also measured.

| COS | *MW (Da)* | *DD (%)* |
|---|---|---|
| CHT P | 179000 | 86±0.8 |
| P1 | 30588 | 77±3.5 |
| P2 | 17247 | 82±1.6 |
| P3 | 11641 | 83±1.6 |
| P4 | 2745 | 92±0.8 |
| CHT R | 261000 | 65±2.5 |
| R1 | 29570 | 53±2.0 |
| R2 | 17754 | 54±1.5 |
| R3 | 7858 | 62±1.7 |
| R4 | 2237 | 63±0.7 |

**Table1. Molecular weight (MW) and deacetylation degree (DD)**

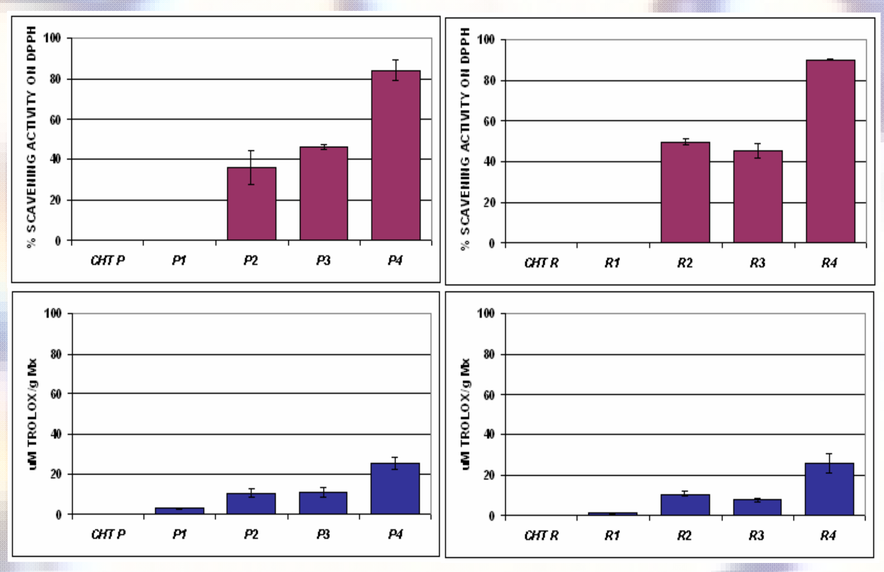


**Figure 2. % Scavenging effect on DPPH and ferric reducing antioxidant power expressed as µmol/g sample**

| COS | *S.* Typhimurium | *C. difficile* |
|---|---|---|
| CHT P | 12.4±4.9 | 7.8±2.4 |
| P1 | 0 | 100 |
| P2 | 2.8±0.0 | 100 |
| P3 | 100 | 100 |
| P4 | 69.1±8.8 | 100 |
| CHT R | 6.9±1.9 | 14.6±2.1 |
| R1 | 10.1±2.1 | 66.3±3.6 |
| R2 | 18.4±0.6 | 34.9±1.4 |
| R3 | 8.3±1.9 | 40.2±2.0 |
| R4 | 6.5±0.8 | 18.5±3.0 |

**Table 2. % Inhibition respect to the control of chitosan and COS**

## Discussion

The FRAP and scavenging effect on DPPH radical (Figure 2) seem to be more related with molecular weight (Mw) than with deacetylation degree (DD) being major in low Mws. CHT P and CHT R did not present any antioxidant activity. P1 and R1 with similar and high Mw did not scavenge DPPH radical and presented very low FRAP. P2, P3, R2 and R3 with a Mw between 8-18KDa showed similar values for the effect on DPPH radical and FRAP. The scavening effect on DPPH radical increased notably with low Mw COS (P4 and R4). Also, the FRAP values were major in these COS than high Mw-COS. However the DD (Table 1) did not influence on the measured antioxidant activity as other authors have reported[3]. The calculated inhibition respect to the positive control (Table 2) showed that the obtained COS are not effective inhibiting *S.* Typhimurium but P3 and P4 with a low Mw and high DD (Table 1). P1-P4 are very efficient inhibiting *C. difficile* (Table 2); however, COS from CHT R presented low IRC but R1. Then, the high DD of COS from CHT P (Table 1) seems to be a limiting factor for the inhibition of this bacteria and Mw does not seem effective in this case. Therefore, Mw and DD of COS appear as decisive factors to develop their biological properties, although depend on the property in different way. So, COS seem to depend on Mw for antioxidant activity and on deacetylation of the chains for the antimicrobial activity on *C. difficile* and DD and Mw on *S.* Typhimurium.

## References

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