IMPACT OF CHITOSAN ON GROWTH INHIBITION OF MICROORGANISMS ISOLATED FROM FISHERY PRODUCTS

Cruz, Z.[1], Lauzon, H.L.[2], Olabarrieta I.[1], Arboleya, J.C.[1], Nuin, M.[1], Amarita, F.[1], Martínez de Marañón, I.[1]*

[1]AZTI-TECNALIA, Txatxarramendi Ugartea z/g, 48395 SUKARRIETA, SPAIN

* iolabarrieta@suk.azti.es

[2.] Icelandic Fisheries Laboratories, Skulagata 4, IS-101 Reykjavik, ICELAND

Abstract

The elaboration of antimicrobial films and coatings is a promising way of reducing the addition of preservatives into food matrix and improving its safety during storage (1). The potential of chitosan to achieve this objective has focused considerable attention worldwide. Literature reports vary somewhat. This antimicrobial effect showed to depend considerable with the type of chitosan, the target microorganism and the product where is applied. (2)

The objective of this work was to select the most suitable type and formulation of chitosan to inhibit or retard microbial growth in fishery products, especially Lightly Preserved Fish Products (LPFPs). For this purpose, two types of chitosan showing considerable differences in molecular weight but presenting a high deacetylation degree were chosen. Several formulations were prepared with different chitosan concentrations and organic acid solvents. These combinations were used to test the antimicrobial activity, against two different spoilage bacteria and two pathogens/surrogate bacteria isolated from LPFPs. The antimicrobial activity was evaluated by microplate inhibitory assay at 8°C.

The most inhibiting preparation turned out to be the formulation of higher chitosan concentration (0.02%) containing acetic acid. Chitosan formulations inhibited or retarded the growth of some bacteria isolated from LPFPs. Therefore, this biopolymer shows potential to be used as a natural preservative to increase the shelf life of fishery products.

Introduction

Chitosan edible films and coatings have been studied during last years to extend the shelf life of food, e.g. in the storage of fruits and vegetables (3,4) meat products (5) and seafood products (6). The antibacterial and antifungal activity of chitosan has been reported widely in the scientific literature mainly on the basis of *in vitro* trials against microorganisms (7). Such studies have led to suggestions that chitosan could be used as a novel food preservative.

Minimum inhibitory concentrations (MICs) vary several orders of magnitude depending on the type of chitosan used (e.g. degree of deacetylation**,** chain length and concentration), the testing conditions (e.g. pH, temperature, medium) and target microorganism (8). Therefore, the success of its application will be a result of an appropriate combination of all these parameters.

Materials and methods

Chitosan formulations

Food Grade chitosans (ChitoClear®) of two different molecular weights and deacetylation degree of more than 95 % were supplied by Primex ehf (Iceland). Combination of two selected chitosans at low (1%w/v) and high (2% w/v) initial concentration with two different organic acids (lactic acid at 1% and acetic acid at 0,5%) as solvent led to 8 chitosan formulations and 2 controls: 1-LMW LA (1% w/v low viscosity chitosan in lactic acid), 2-LMW LA (2% w/v low viscosity chitosan in lactic acid), 1-LMW AA (1% w/v low viscosity chitosan in acetic acid), 2-LMW AA (2% w/v low viscosity chitosan in acetic acid), 1-HMW LA (1% w/v high viscosity chitosan in lactic acid), 2-HMW LA (2% w/v high viscosity chitosan in lactic acid), 1-HMW AA (1% w/v high viscosity chitosan in acetic acid), 2-HMW AA (2% w/v high viscosity chitosan in acetic acid), LA (control, lactic acid) and AA (control, acetic acid).

Bacterial strains

Various spoilage (*Photobacterium phosphoreum*, *Shewanella putrefaciens*) and pathogenic/surrogate (*Listeria innocua*, *Listeria monocytogenes*) bacteria were used for the assessment of the antibacterial activity of the chitosan preparations. These strains are part of the EU collection of the Hurdletech project within the integrated project SeafoodPlus.

Microplate Inhibitory Assay

Microtiter plates (96 wells, Linbro, ICN Biomedicals Inc., Ohio, USA) were used to evaluate the antimicrobial activity of the chitosan preparations towards the 4 bacterial cocktails. Chitosan preparations were added to VIB (1% NaCl, pH 7.2) as 1% v/v, resulting in a final oncentration of 0, 0.01 and 0.02% (w/v). Bacterial growth at 8ºC was followed by absorbance measurements at 590 nm using a Titertek Multiskan Plus spectrophotometer (model MKII, Flow Laboratories) on days 3, 6 and 13. Absorbance measurements on day 0 were used as the blank values. Initial bacterial inoculation level was about log 3-4 CFU/ml. Each inhibitory assay was tested in duplicate.

Results and discussion

1-LMW LA, 2-LMW LA and 1-LMW AA formulations led to the development of turbidity when added to the microbiological medium (pH 7.2) and therefore, testing their inhibitory properties towards bacteria by using a spectrophotometric method was not possible.

Growth of the bacterial cocktails in VIB with no added chitosan and organic acid was used as the reference growth behaviour, “none” sample and its maximum absorbance value at day 13 is represented by the dotted line. The minimal absorbance at day 13 is shown by a straight line, which represents therefore, the highest microbial growth inhibition of the tested formulations.

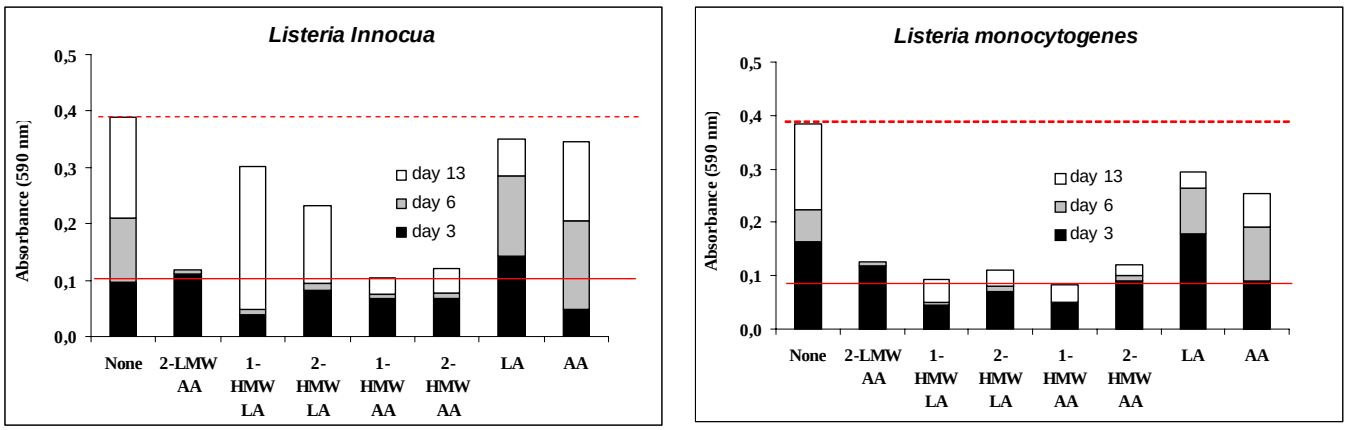

Figure 1 : Growth of pathogenic/surrogate bacteria at 8ºC as influenced by the chitosan preparations.

Figure 1 shows that *L. innocua* was more resistant to chitosan than *L. monocytogenes*. Acetic acid was a more effective solvent for chitosan to inhibit *L. innocua* growth.

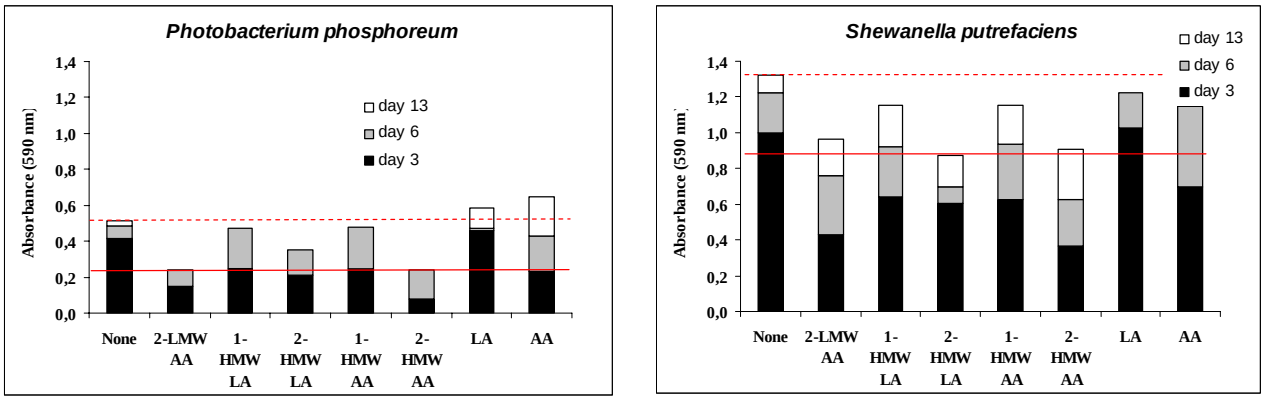

Figure 2 : Growth of spoilage bacteria at 8ºC as influenced by the chitosan preparations.

As for *L. innocua*, Figure 2 demonstrates the better efficiency of acetic acid as a solvent for chitosan to inhibit the growth of spoilage bacteria. The inhibition increased with the chitosan concentration. Among tested microorganisms, *Shewanella putrefaciens* showed the higest resistivity against chitosan.

Conclusions

Chitosan retards the growth of bacteria isolated from fishery products and its activity depends on concentration and the organic acid used as solvent. Additional studies will be carried out on lightly preserved fish products in order to point out the suitability of chitosan to reduce their surface bacterial contamination.

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