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Procedia Food Science 1 (2011) 399 – 403

Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Characterization of salmon gelatin based film on antimicrobial properties of chitosan against *E. coli*

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Abstract

Salmon gelatin and chitosan are high potential biopolymer to obtain edible films with antimicrobial effect for fresh meat. Therefore, it is important to characterize the structural (glassy or rubbery) state of the gelatin film on antimicrobial properties of chitosan. Extracted salmon (*Salmo Salar*) gelatin (acid-basic extraction) and low molecular weight chitosan solutions (0; 0.25; 0.5 and 1% w/w) at pH 5.5 were prepared to obtain a final concentration to 7% w/w (gelatin + chitosan). Films were obtained by casting at 5ºC and equilibrated at 33% and 85% of relative humidity (RH) in order to obtain a glassy and rubbery state, respectively. Water content, specific volume and glass transition temperature (Tg) were measured and antimicrobial properties against *Escherichia coli* (E.c.) (105 ufc/ml) were obtained by agar plate diffusion and kinetic measurements at 37ºC. Chitosan diffusion in agar plate was determined using liquid solutions and films over agar plate. The results showed diminution of Tg (maintaining structural state) as increase chitosan concentration, increasing also specific volume and water content due their high capacity to adsorb water. Although inhibition by chitosan of bacteria growth kinetic was 100%, solutions and films samples could not diffuse in the agar. In conclusion, the chitosan could not diffuse on the films matrix and also in agar plate and therefore in food when is combined with salmon gelatin. The implication of this work have been investigate the use of edible films from marine sources with antimicrobial effects in an effort to growing demand from consumers for safer and better quality foods.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: salmon; chitosan; E. coli; gelatin, structure

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Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.
doi:10.1016/j.profoo.2011.09.061

1. Introduction

Taking advantage of natural resources that are intended as waste becomes an excellent option in the development of new biodegradable products. Gelatin is an animal protein obtained by controlled hydrolysis of collagen in bone and skin [1].

Salmon gelatin is a possible alternative to bovine gelatin. The main advantage is not associated with the risk of outbreaks of bovine spongiform encephalopathy and is accepted by Islam, can be used with minimal restrictions in Judaism and Hinduism [2]. In addition, the salmon skin is a waste major subproduct of the fish industry (about 5% of whole fish), making waste a valuable source of gelatin [3].

Meat by high water activity and its wealth of user-friendly nutrients is an excellent substrate for the growth of microorganisms. The control method used is cooling, but when the meat is not stored at an appropriate temperature can generate the growth of *Escherichia coli* that has a minimum growth temperature of 8-10 º C, which is considered the main threat poorly refrigerated meat [4].

Chitosan is a natural biopolymer derived from the deacetylation of chitin, an important component of the shells of crustaceans such as crabs, shrimps and prawns [5]. Chitosan is a cationic polysaccharide composed of glucosamine units with bonds β (1-4) [6]. This biopolymer has the advantage of being biodegradable, biofunctional, biocompatible, nontoxic and also has antimicrobial property [7]. The antimicrobial effect of chitosan is effective against a wide variety of pathogenic microorganisms and spore, including fungi, bacteria, gram-positive and gram-negative. However, its functionality and activity depends of its characteristics such as molecular weight, degree of acetylation, concentration in solution, the microorganism and the environmental conditions, especially pH, strength ion and the presence of solutes susceptible to reaction with chitosan via electrostatic interaction and / or covalent bonds that can cover or completely block the reactivity of the active amino groups [8].

The exact mechanism of the antimicrobial action of chitosan is not well known. It is assumed that the reason of antimicrobial activity is its positively charged amino group that interacts with the negatively charged bacterial cell membrane, leading to the release of proteins and other constituents of intracellular microorganisms [9]. Chitosan also acts as a chelating agent that selectively binds trace metals inhibiting the production of toxins and microbial growth. In addition, chitosan can enter into the cell as a result of the permeability of the membrane, penetrating the cell nucleus by interfering with the synthesis of messenger ribonucleic acid (RNA) and protein [7].

The aim of this work was to characterize the structural (glassy or rubbery) state of the salmon gelatin film on antimicrobial properties of chitosan against *Escherichia coli*.

2. Materials & Methods

Atlantic salmon skin (*Salmo salar*) was provided gently by Salmonoil S.A. Gelatin was extracted from skin by alkaline and acid hydrolysis [10]. The chitosan used was low molecular weight with deacetylation degree of 92% (Sigma Aldrich).

2.1 Preparation of gelatin films.

Salmon gelatine-chitosan based films were prepared at final concentration of 7% w/w. The chitosan was added at concentrations of 0, 0.25, 0.5 and 1% w/w dissolved in acetic acid solution. Stirred moderately at 50 °C until completely dissolve. Then, the solutions were adjusted to pH 5.5 with NaOH 2N. The formation of the films was performed by the method of cold casting at 5°C.

Salmon gelatine-chitosan based films were equilibrated at 20°C in salt-saturated $MgCl_2$ (33% RH) and KCl (85% RH) inside sealed containers [11], in order to obtain samples in the glassy and rubbery state, respectively. Moisture content was determined gravimetrically (24 h at 105ºC).