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The effect of the combined use of high pressure treatment and antimicrobial edible film on the quality of salmon carpaccio

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ABSTRACT

Fish carpaccio is a ready-to-eat product with a very limited shelf life. In the present work, the use of high pressure treatment (HP) and/or antimicrobial edible film was studied in order to improve quality and stability of salmon carpaccio. In a preliminary part of the work, a film composed of gelatin plus chitosan incorporating clove essential oil was selected, based on its physicochemical and antimicrobial properties. Eugenol and β-caryophyllene, the main volatile components of the film, migrated to salmon muscle, the release being favored by HP and storage time. Concurrently, reducing power of the muscle increased, resulting in prevention of lipid oxidation derived from either HP or refrigerated storage. HP treatment reduced total microbial counts by 1.5 log cycles from the onset of storage, whereas the film reduced it by 2 log cycles after 3 days. The combination of HP and edible film exerted the most intense antimicrobial effect, total bacterial counts, luminescent bacteria, H_2S-producing organisms, pseudomonads, enterobacteria, and lactic acid bacteria remaining constant or under detection limit over the whole storage period (11 days). The combined use of HP treatment and gelatin–chitosan–clove essential oil film is an effective way of improving quality and stability of salmon carpaccio.

1. Introduction

Fish and seafood are much-appreciated foods worldwide owing to their nutritional quality. However, the organoleptic, hygienic, and nutritional quality of fresh fish diminishes over time, mainly as a result of microbial growth. Nowadays there is a growing trend towards consumption of minimally processed products with sensory properties similar to those of raw products. Specifically, carpaccio, sushi, and other fishery products that are consumed without thermal treatment are becoming more and more popular worldwide. In these specific cases, cutting, manipulating, and packaging processes are a source of microbial contamination that limits their shelf life under refrigerated storage, sometimes to just one day. This means that they must be produced and distributed almost daily, which is an added difficulty that hinders the availability of carpaccio and also increases its price. Consequently, the search for preservation methods in order to extend its shelf life and maintain both hygienic and sensory quality is a real challenge. Commercially, the preservation method most commonly employed is the addition of antimicrobials; however, research on new preservation treatments and technologies is necessary, owing to the growing consumer demand for a reduction of synthetic preservatives. In this connection, essential oils have demonstrated their effectiveness extending fish shelf life, and recent works can be found on this topic (Huang et al., 2018; Yuan et al., 2017). However, a current trend in food preservation is the application of hurdle treatments, as "a deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles) that any microorganisms present should not be able to overcome" (Leistner, 1992), contributing to diminish sensorial impact. Examples of the application of various hurdles to extend shelf life and improve quality of fish can be found in the literature: salting, smoking, and high pressure on dolphinfish (Montero et al., 2007); salting, modified atmosphere packaging, and oregano essential oil on sea bream (Goulas and Kontominas, 2007), or high pressure and functional edible films on cold-smoked sardine or trout fillets (Albertos et al., 2014; Gomez-Estaca et al., 2007). However, to the best of our knowledge there is scarcely any information about fish carpaccio treated by combined hurdles. Thus, the objective of the present work was to extend the shelf life of a highly perishable food product, salmon carpaccio, by the application of high pressure treatment in combination with an antimicrobial edible film. Furthermore, the physicochemical and antimicrobial properties of the edible films were evaluated in relation to the matrix employed (gelatin or gelatin plus chitosan) and the addition of clove essential oil.

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2. Materials and methods

2.1. Preparation of carpaccio

Carpaccio was prepared from salmon (*Salmo salar*) acquired in a local supermarket. Portions of approximately 10 × 8 × 5 cm were frozen at −40 °C in a blast freezer (Frigoskandia laboratory freezer, Helsingborg, Sweden) and stored frozen at −20 °C for 2 days prior to slicing. The portions were semi-thawed and slices ~1.5 mm thick were prepared with a slicing machine. The slices were vacuum packed (≈60 g/pack) into flexible bags properly separated with polyethylene film or edible film to avoid contact between slices.

2.2. Formulation of the edible films

The film-forming solutions were prepared using commercial fish gelatin, mainly from catfish (Lapi Gelatin, Florence, Italy), alone or in combination with chitosan (Guinama, Valencia; deacetylation degree 95%). Gelatin film-forming solutions were prepared with a concentration of 8 g of gelatin/100 mL of distilled water. For gelatin–chitosan film-forming solutions, 6 g of gelatin and 2 g of chitosan per 100 mL solution were used. Chitosan was previously dissolved in 30 mL of 0.15 M acetic acid. Sorbitol (0.15 g/g gelatin or gelatin plus chitosan) plus glycerol (0.15 g/g gelatin or gelatin plus chitosan) was employed as a plasticizer for all formulations. For the clove-added films, food-grade clove essential oil (Eladiet, Barcelona, Spain) was added to a concentration of 0.75 mL/g biopolymer (gelatin or gelatin plus chitosan), using soya lecithin as an emulsifying agent (160 mg/g oil), and homogenizing with an Ultra-Turrax blender (12,000 rpm, 1 min) (T25 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany). All mixtures were warmed and stirred at 45 °C to obtain a good blend, the pH was adjusted to 6, and the films were obtained by casting an amount of 40 mL on Perspex plates (144 cm^2) and drying at 45 °C in a forced-air oven for 15 h to yield a uniform thickness [200 μm ($p \leq 0.05$)] in all cases. Four different types of films were obtained: a gelatin film (G); a gelatin–chitosan film (G-Ch); a gelatin clove-added film (G-C); and a gelatin–chitosan clove-added film (G-Ch-C). Prior to analyses the films were conditioned in desiccators for 2 d at 22 °C to 58% relative humidity.

2.3. High pressure treatment

A Stansted Fluid Power Iso-Lab 900 high pressure food processor (Model: FPG7100:9/2C, Stansted Fluid Power Ltd., Harlow, Essex, UK) was employed. Pressure was set at 250 MPa, temperature at 7 °C and the treatment time was 15 min. Temperature of the immersion medium (distilled water) was regulated by a thermocouple connected to a programmed temperature controller (model IA/2230 AC, INMASA, Barcelona, Spain). Pressure was increased by 2.5 MPa/s, and after high pressure treatment was completed the pressure returned to that of the atmosphere after approximately 3 s.

Four batches, all of them vacuum packed in flexible bags (type BB4L, Cryovac, Barcelona, Spain), were prepared: salmon carpaccio held at atmospheric pressure without active edible film (S batch), pressurized salmon carpaccio (S-HP), salmon carpaccio covered with G-Ch-C edible film (S-F), and salmon carpaccio covered with G-Ch-C edible film and pressurized (S-HP-F). The G-Ch-C edible film was selected from the four films tested because of its physicochemical and antimicrobial properties. After high pressure treatment, all batches were stored at 5 °C ± 1 °C and periodic analyses were performed.

2.4. Physicochemical and antimicrobial properties of the films

2.4.1. Mechanical properties

A puncture test was performed in quintuplicate to determine the breaking force and deformation of the films at the breaking point. Films were placed in a cell 5.6 cm in diameter and perforated to the breaking point using an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) with a round-ended stainless-steel plunger (ø = 3 mm) at a cross-head speed of 60 mm/min and with a 100-N load cell. Breaking force was expressed in N and breaking deformation in %, according to Gomez-Estaca et al. (2011).

2.4.2. Water solubility

It was determined as previously described by Gomez-Estaca et al. (2011). Briefly, film portions measuring 4 cm^2 were placed in aluminum capsules with 15 mL of distilled water and shaken gently at 22 °C for 15 h. The solution was then filtered through Whatman No. 1 filter paper to recover the remaining undissolved film, which was desiccated at 105 °C for 24 h. Film solubility was expressed in %.

2.4.3. Antimicrobial properties

The antimicrobial activity of the films was determined against the following microorganisms, which were obtained from the Spanish type culture collection (CECT): *Staphylococcus aureus* CECT 240, *Clostridium perfringens* CECT 486, *Listeria monocytogenes* CECT 4032, *Photobacterium phosphoreum* CECT 4192, *Pseudomonas aeruginosa* CECT 110, *Brochothrix thermosphacta* CECT 847, *Aeromonas hydrophila* CECT 839T, *Citrobacter freundii* CECT 401, and *Shewanella putrefaciens* CECT 5346T. For this purpose, spread plates of BHI Agar (plus 1% NaCl in the case of *P. phosphoreum*) were inoculated with 100 μL of these bacteria grown overnight (~10^8 cfu/mL), and circular pieces of the various films (1.5 cm diameter) were laid on the inoculated plate's surface and after incubation the observed inhibition zones – surrounding clear areas – were considered as a measurement of the antimicrobial activity. The organisms were incubated at 37 °C excepting *A. hydrophila* and *S. putrefaciens*, incubated at 30 °C, *B. thermosphacta* at 25 °C, and *P. phosphoreum* at 15 °C. In addition, *C. perfringens* was grown under anaerobic conditions (Gas-Pack, Anaerogen; Oxoid). The inhibition area was measured using specific software for digital image analysis (MIP 4 ADV, ver. 1. Consulting de Imagen Digital, S.L. & Microm, Spain). Results are expressed as the percentage of inhibition with respect to the total plate surface.

2.5. Storage trial on salmon carpaccio

2.5.1. Microbiological analyses

A total amount of 10 g of muscle was collected and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 mL of buffered 0.1% peptone water (Oxoid, Basingstoke, UK) in a vertical laminar-flow cabinet (mod. AV 30/70 Telstar, Madrid, Spain). After 1 min in a Stomacher blender (model Colworth 400, Seward, London, UK), appropriate dilutions were prepared for the following microorganism determinations: (i) total bacterial counts (TBC) on spread plates of Iron Agar 1% NaCl incubated at 15 °C for 3 days; (ii) H_2S-producing organisms, as black colonies, on pour plates of Iron Agar incubated at 15 °C for 3 days; (iii) luminescent bacteria on spread plates of Iron Agar 1% NaCl incubated at 15 °C for 5 days; (iv) pseudomonads on spread plates of Pseudomonas Agar Base (Oxoid) with added CFC (Cetrimide, Fucidine, Cephalosporine) supplement for *Pseudomonas* spp. (Oxoid) incubated at 25 °C for 48 h; (v) Enterobacteriaceae on double-layered plates of Violet Red Bile Glucose agar (VRBG, Oxoid) incubated at 30 °C for 48 h [after first adding 5 mL of Tryptone Soy Agar (Merck, Darmstadt, Germany) and incubating at room temperature for 1 h]; (vi) lactic acid bacteria (LAB) on double-layered plates of MRS Agar (Oxoid) incubated at 30 °C for 72 h. All microbiological counts are expressed as the log of the colony-forming units per gram (log cfu/g) of sample. All analyses were performed in triplicate.

2.5.2. Chemical analyses

The total volatile basic nitrogen (TVBN) was determined according to the method described by Antonacopoulos and Vyncke (1989) and the