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Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films

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ABSTRACT

Chitosan/cyclodextrin films (CS:CD) incorporating carvacrol were obtained by casting, and conditioned at 23 °C and 75% relative humidity prior to being immersed in liquid carvacrol until they reached sorption equilibrium. In a previous work, the in vitro antimicrobial activity of these films was studied. In this work, active films were used to inhibit microbial growth in packaged chicken breast fillets. Samples of CS:CD films loaded with carvacrol, of different sizes and thus with different quantities of antimicrobial agent, were stuck to the aluminium lid used to seal PP/EVOH/PP cups containing 25 g of chicken fillets. These samples were stored for 9 days at 4 °C. The packages were hermetically sealed and it was confirmed that they provided an infinite barrier to carvacrol. The partition of the antimicrobial agent within the food/packaging system was analysed. The antimicrobial devices rapidly released a large percentage of the agent load, amounts that were gained by the adhesive coating of the lid and especially by the chicken fillets. The latter were the main sorbent phase, with average concentrations ranging between 200 and 5000 mg/Kg during the period of storage. The microbiota of the packaged fresh chicken fillets – mesophiles, psychrophiles, *Pseudomonas* spp., enterobacteria, lactic acid bacteria and yeasts and fungi – were analysed and monitored during storage. A general microbial inhibition was observed, increasing with the size of the active device. Inhibition with a 24 cm^2 device ranged from 0.3 log reductions against lactic acid bacteria to 1.8 logs against yeasts and fungi. However, the large amount of antimicrobial that was sorbed or that reacted with the fillet caused an unacceptable sensory deterioration. These high sorption values are probably due to a great chemical compatibility between chicken proteins and carvacrol.

1. Introduction

Antimicrobial packaging systems have received special attention in recent years because they can provide an important obstacle in the so-called "hurdle" technologies that are being implemented to commercialize fresher food products. In these systems, interactions between the food, the surrounding headspace and the package walls or independent devices are responsible for growth inhibition or death of pathogenic and/or spoilage microorganisms (Appendini and Hotchkiss, 2002). Although, exceptionally, this beneficial activity can be carried out by direct contact of the microorganisms with the package surface (Conte et al., 2008; Muriel-Galet et al., 2013; Zi-Xuan et al., 2012), the mechanism of activity in most packaging systems is based on mass transport processes through the packaging/food interface, and especially on the release of antimicrobial substances. The active agent can be incorporated within a suitable polymeric matrix from which it is released following diffusion mechanisms and accumulated into the food package system following thermodynamic principles. Therefore, compatibility between the agent and the various system components and diffusion from the polymeric vehicles are key parameters, and the efficiency and validity of an active packaging has to be tested for the specific product.

Recently, a biopolymeric device consisting of a combination of two biodegradable, renewable materials, chitosan and HP-β-cyclodextrins, and a known natural volatile antimicrobial compound, carvacrol, was developed and characterized. In that report, the film demonstrated a great capacity for sorption of carvacrol. The kinetics of release in ideal conditions were also measured and the in vitro antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was tested (Higueras et al., 2013; Higueras et al., 2014).

In this work, a package containing this device was designed and used as an active package containing fresh chicken fillets. The partition of carvacrol among the different components of the food/active package/environment system was studied and the effect of the active packaging on the chicken microbiota was analysed.

2. Materials and methods

2.1. Materials

Carvacrol (kosher > 98%), glycerol, acetic acid and low molecular weight chitosan were supplied by Sigma (Barcelona, Spain).

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Hydroxypropyl-β-cyclodextrins (CAVASOL® W7-HP) were provided by Wacker Fine Chemicals, S.L. (Barcelona, Spain).

2.2. Film preparation

A 1.5% chitosan (w/w) solution in an aqueous 0.5% (w/w) acetic acid solution was prepared and filtered to eliminate impurities. Glycerol plasticized chitosan/hydroxypropyl-β-cyclodextrin films were obtained by adding HP-βCD to the chitosan solution in a 1:1 proportion (w/w) with respect to CS and glycerol at 35% (g glycerol/100 g dry matter) and stirring at 1500 rpm and 37 °C until complete dissolution. Films were obtained by casting, i.e. pouring a suitable amount of the solution into a flat polystyrene tray and allowing it to dry under controlled environmental conditions (36 h, 40.0 ± 1.5 °C and 20 ± 9% relative humidity). After peeling the films from the tray, samples measuring 550 mm in diameter and 55 ± 5 μm in thickness (using a digital micrometer (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan)) were stored in glass desiccators at 75.0 ± 0.5% relative humidity (ASTM, 2007) in a temperature-controlled room at 23 ± 1 °C for at least two weeks, the time required by the samples to reach moisture equilibrium. Some of these films were used as control samples (CS:CD-Control). Others (CS:CD-Active) were immersed in carvacrol for at least 3 weeks, the time required to reach equilibrium. The analysis of the carvacrol sorbed by the samples was performed by thermal desorption and subsequent gas chromatographic analysis as described below. A more detailed description of the manufacturing procedure can be found elsewhere.

2.3. Packaging of chicken breast fillets

Samples of chicken breast (25 g) were placed at the bottom of polypropylene (PP)/ethylene-vinyl alcohol copolymer (EVOH)/PP trays measuring 156 cm^3. The trays were sealed with adhesive aluminium foil (Miarco, Valencia, Spain) and stored at 4 °C for 0, 3, 6 and 9 days. CS:CD-Active films measuring 0.24, 4.8 and 24 cm^2 were stuck to the centre of the tray lid and constituted the small, medium and large samples, respectively. A negative control sample for each time was also prepared with CS-Control. Packages without chicken containing a medium size CS:CD-Active films were prepared too.

2.4. Quantification of carvacrol concentration

The analysis of the initial concentration of carvacrol retained in the materials was performed by thermal desorption coupled to gas chromatography using a Dynatherm Thermal Desorber Model 890/891 (Supelco, Teknokroma, Barcelona, Spain) connected in series to the column of an HP5890 gas chromatograph Series II Plus (Agilent Technologies, Barcelona, Spain) via a heated transfer line. A sample of the CS:CD-Active film was cleaned with a paper tissue to remove any excess of volatile compound on the film surface and then immediately inserted into an empty desorption tube (11.5 × 0.39 cm I.D.). The tube was placed in the desorber chamber, which was then closed. Conditions for desorption were as follows: desorption temperature, 210 °C; transfer line, 230 °C; desorption time, 7 min; He desorption flow, 8.15 mL/min. The GC was equipped with a TRB5 (30 m, 0.32 mm, 0.25 μm) column (Teknokroma, Barcelona, Spain) and a flame ionization detector. The chromatographic conditions were: 260 °C detector temperature, 7 min at 45 °C, heating ramp to 220 °C at 18 °C/min, and 1 min more at 220 °C. After the analysis, the film sample was recovered from the desorption tube and weighed on an analytical balance (Voyager model V11140, Ohaus Europe, Greifensee, Switzerland). The desorber-GC was calibrated by measuring other polymeric (polyethylene and polypropylene) film samples with known amounts of carvacrol (measured independently by gravimetry).

The same procedure was used to determine the carvacrol concentration during chicken storage within each phase of the food package/system. Briefly, samples of tray, lid, film or chicken were cut and desorbed following the previous procedure. Every package was tested three times and three packages were analysed per sample.

The carvacrol concentration in the package headspace was analysed by sampling 500 μL of gas with a gas-tight Hamilton syringe through an adhesive septum stuck to the package wall. The gas sample was immediately analysed by using a Model 6850 GC (Agilent Technologies) equipped with a semi-capillary RTX-1301 (30 m, 0.53 mm, 0.5 μm) column (Restek, Teknokroma, Barcelona, Spain). The chromatographic conditions were: 3.8 mL/min He, oven at 150 °C for 11 min, injector and detector temperatures 220 °C and 250 °C, respectively. The GC was previously calibrated by injections of a known amount of carvacrol in hexane.

2.5. Antimicrobial activity of carvacrol-CS films on chicken breast fillets

At selected times, chicken samples were transferred aseptically and weighed in a sterile Stomacher bag, diluted with 25 mL of 0.1% peptonated water (Scharlab, Barcelona, Spain) and blended in a Stomacher (IUL S.L., Barcelona) for 6 min. Ten-fold dilution series of the suspensions obtained were made in peptonated water and plated using the following selective media (Scharlab, Barcelona, Spain) and culture conditions: tryptone soy agar for total aerobic bacteria, incubated at 30 °C for 48 h, and also for total aerobic psychrophiles, incubated at 10 °C for 10 days, King B agar for *Pseudomonas* spp., incubated at 25 °C for 48 h, violet red bile glucose agar for enterobacteria, incubated at 37 °C for 24 h, MRS agar for lactic acid bacteria, incubated at 25 °C for 5 days, and malt extract agar for yeasts and fungi, incubated at 28 °C for 5 days. Tests were performed in triplicate.

2.6. Data analysis

Statistical analysis of the results was performed with SPSS commercial software (SPSS Inc., Chicago, Illinois, USA). Differences between means were assessed on the basis of confidence intervals, using the Tukey-b test at a level of significance of $P \leq 0.05$. The data are represented as average ± standard deviation.

3. Results and discussion

CS:CD-Control samples were prepared first. They were transparent, practically colourless and without discontinuities. After the immersion in carvacrol for 3 weeks, the samples were transparent and had an intense yellow colour. The initial carvacrol content of the samples was 2.3 ± 0.4 g/g of dried film (cyclodextrins and chitosan). These high uptakes were in agreement with previous studies (Higueras et al., 2013). Nevertheless, the carvacrol content in each sample was determined individually prior to use.

3.1. Quantification of carvacrol concentration in the packaging/headspace/chicken system

Fresh chicken breast fillets are commonly packaged in trays closed with a thermosealable lid, and distributed and commercialized under refrigeration. The materials used for the design of these trays are variable (polyethylene terephthalate (PET), expanded polystyrene (PS) or PP laminated or coextruded to a polyamide/polyethylene (PA/PE or EVOH/PP …)), but, since the product is normally preserved in a modified atmosphere, materials that provide a medium to high barrier to oxygen and carbon dioxide are commonly employed. These materials present this barrier because of a combination of properties that might include high crystallinity, low free volume, adequate chain rigidity and high interchain cohesive energy. With these properties, the materials also provide a high to very high barrier to organic compounds such as food aroma components, vitamins, fats or organic acids (Gavara and Catala, 2002).