Antimicrobial Effectiveness of Bioactive Packaging Materials from Edible Chitosan and Casein Polymers: Assessment on Carrot, Cheese, and Salami

Maria del Rosario Moreira, Mariana Pereda, Norma E. Marcovich, and Sara I. Roura

Abstract: Antimicrobial packaging is one of the most promising active packaging systems for controlling spoilage and pathogenic microorganisms. In this work, the intrinsic antimicrobial properties of chitosan (CH) were combined with the excellent thermoplastic and film-forming properties of sodium caseinate (SC) to prepare SC/CH film-forming solutions and films. The antimicrobial effectiveness of SC, CH, and SC/CH coatings on the native microfloras of cheese, salami, and carrots was evaluated. *In vitro* assays through the test tube assay indicated that the most significant antimicrobial effect was achieved by CH and SC/CH solutions on carrot and cheese native microfloras. SC film-forming solutions did not exert antimicrobial activity on any of the native microflora studied. SC, CH, and SC/CH films stored in controlled environments showed that the retention of the antimicrobial action was observed until 5-d storage, at 65% relative humidity in both temperatures (10 °C and 20 °C). *In vivo* assays were also performed with SC, CH, and SC/CH applied as coatings or wrappers on the 3 food substrates. CH and SC/CH applied at both immersion and wrapper exerted a significant bactericidal action on mesophilic, psychrotrophic, and yeasts and molds counts, showing the 3 microbial populations analyzed a significant reduction (2.0 to 4.5 log CFU/g). An improvement of the bactericidal properties of the CH/SC blend respect to those of the neat CH film is reported. The ionic interaction between both macromolecules enhances its antimicrobial properties.

Keywords: edible coatings, food system, microbiological quality, native microflora, preservatives

Practical Application: The continuous consumer interest in high quality and food safety, combined with environmental concerns has stimulated the development and study of biodegradable coatings that avoid the use of synthetic materials. Among them, edible coatings, obtained from generally recognized as safe (GRAS) materials, have the potential to reduce weight loss, respiration rate, and improve food appearance and integrity. They can be used in combination with other food preservation techniques in order to extend the effectiveness of the food preservation chain. Moreover, antimicrobial films and coatings have innovated the concept of active packaging and have been developed to reduce, inhibit, or delay the growth of microorganisms on the surface of food in contact with the package. The use of antimicrobials packaging films to control the growth of microorganisms in food can have a significant impact on shelf-life extension and food safety. In addition, antimicrobial films can be prepared by the combination of inherent antimicrobial materials (that is, CH), with good film-forming protein-based ones (that is, SC). Therefore, the objective of this work is to study the performance of 2 biodegradable and edible biopolymers and their combination as natural packages for selected food products.

Introduction

The greatest losses in food are attributed to microbiological alterations, which decrease their shelf life and increase the risk of foodborne illness (Quintavalla and Vicini 2002). Many chemical and physical processes have been developed to preserve food (Durango and others 2006). Among them, packaging plays a prominent role in maintaining food quality (Quintavalla and Vicini 2002; Durango and others 2006).

Because of its renewable and biodegradable nature, edible film wrappers derived from proteins and polysacharides could potentially replace some conventional synthetic packaging materials used to preserve and protect foods (Audic and Chaufer 2005; Schou and others 2005). Moreover, antimicrobial packaging is a promising form of active food packaging that helps improving food safety and shelf life by controlling microbial contamination, that is, reducing the growth rate and maximum growth population and/or extending the lag-phase of the target microorganism, or by

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inactivating microorganisms by contact (Quintavalla and Vicini 2002). Antimicrobial packaging has been found highly effective in controlling or arresting spoilage and pathogenic microorganisms that contaminate foods (Dutta and others 2009). In this context, chitosan (CH) films and coatings have shown great aptitude for their application in food preservation (Dutta and others 2009) mainly due to the inherent antimicrobial properties in conjunction with the cationicity of the natural polysaccharide (Arvanitoyannis and others 1998).

CH, derived from chitin, is obtained from waste products of the shellfish industry (Xu and others 2005). It is biodegradable, nontoxic, biocompatible, and bioadhesive (Remuñán-López and Bodmeier 1997) and possesses unique functional, nutritional, and biomedical properties. Some of its applications include seed coatings, film forming and controlled release of food ingredients, nutrients, bioactive compounds, and drugs (Lazaridou and Biliaderis 2002).

Proteins also show properties that are advantageous in the preparation of packaging biomaterials, for example, their ability to form networks, their plasticity, and elasticity (Arvanitoyannis 1999). Sodium caseinate (SC) is obtained by acid precipitation of casein, the main protein in cow milk, and presents thermoplastic and film-forming properties due to its random coil nature and its ability to form weak intermolecular interactions (Arvanitoyannis 1999; Audic and Chaufer 2005; Longares and others 2005). Nevertheless, caseinate films applied by immersion on squash slices showed limited antimicrobial properties (Ponce and others 2008).

On the other hand, polysaccharide-protein mixed systems are increasingly used in various food, pharmaceutical, and biotechnology applications. The protein-polysaccharide complexes could exhibit better functional properties than that of the proteins and polysaccharides alone (Pogaku and others 2007). Thus, it is also important to look for the combinations of biopolymers that yield a better performance as a natural and edible package. CH, bearing positively charged groups, can interact and form 3-dimensional networks with molecules containing opposite charges, such as caseinate. In previous works (Pereda and others 2008, 2009), it was demonstrated that films prepared from mixtures SC/CH exhibited improved mechanical properties (tensile and impact strengths) and lower equilibrium moisture content than either CH or SC neat ones, due to the strong interactions (mainly electrostatic forces) developed during the polyelectrolytic complexation of the 2 macromolecules. However, the antimicrobial efficiency of this mixture, when used as a coating or packaging for food, was not evaluated.

Thus, the objective of this work is to evaluate the antimicrobial effectiveness, as food coatings/wrappers, of SC, CH, and mixtures (SC/CH) film-forming solutions and films. Glycerol was used as plasticizer to obtain flexible films that could be folded and manipulated without breakage. The antibacterial efficiency of the 3 films was assayed through *in vitro* tests performed on 3 different native microfloras: cheese, salami, and carrots. Furthermore, the effect of exposing the edible films to different relative humidity (RH) (40% and 65%) and temperature (10 and 20 °C) environmentals on the bacteriostatic or bactericidal retention was evaluated by storing them for 5 d at the selected conditions. *In vivo* assays were also performed on SC, CH, SC/CH applied as film-forming solutions and wrapping on the different food substrates.

Materials and Methods

SC powder, composed of 88.9 wt% of protein (Pro) and small amounts of lactose, lipids, attached moisture, and ashes, was obtained from Lactoprot Deutschland GMBH (Kaltenkirchen, Germany). The average protein molecular weight was 22600 g/mol (Gerrard 2002). CH (deacetylation degree, DD = 98%) was supplied by ACOFAR (Mar del Plata, Argentina). Glycerol was purchased from DEM Chemicals (Mar del Plata, Argentina).

Preparation of film-forming solutions

Solutions were prepared according to our previous work (Pereda and others 2008), which is detailed below.

SC aqueous solutions with protein concentrations of Pro 2.5% (w/v) were prepared by dispersing SC powder (2.81 g) in distilled water with continuous stirring for 3 h at room temperature. Appropriate amounts of glycerol were added to achieve glycerol/protein (Gly/Pro) weight ratio of 0.28.

CH solutions (2%, w/v) (Xu and others 2005) were prepared by dispersing CH powder in acetic acid solution (1%, v/v) with magnetic stirring at 23 °C, final pH = 4.4. Glycerol content was added to achieve a glycerol/ chitosan (Gly/CH) weight ratio of 0.28. Subsequently, in order to remove insoluble impurities, filtration was performed by using a filter paper.

SC/CH composite solutions (weight ratio CH/Pro = 0.8/1) were prepared by mixing 100 mL of the 2% (w/v) CH solution with 100 mL of 2.5% (w/v) SC solution. Glycerol was added to achieve a Gly/ (Pro + CH) weight ratio of 0.28. Preparation of the polyelectrolyte complex between caseinate and CH required a careful control of the solution pH. SC is remarkably heat-stable at pH = 6.5 (Guo and others 1989) and highly insoluble at the isoelectric point, pH between 3.8 and 4.0 (Lieske and Konrad 1994). CH shows best solubility in a 1% (v/v) acetic acid solution (pH = 4.4), but remains soluble at pH lower than 6.4. In this work, the mixed solution had a pH of 5.0, and no phase separation was observed. Since CH is a cationic biopolymer, while caseinate acts as a macroanion at this pH (pH > pI), a complexation of the 2 polymers was expected as a result of their intimate mixture and conditions of film preparation (Cheng and others 2003; Pereda and others 2008). Lower CH/SC weight ratios were not compatible, probably due to the higher pH of the mixed solution, that is, in these conditions CH solubility could be affected and also the amount of $-NH_3^+$ groups available to interact with the $-COO^-$ groups of caseinate decrease.

Preparation of films

Films were prepared according to the usual casting method (Gontard 1991; Ali and others 1997; Gennadios 2002; Orliac and others 2003; Mali and others 2004; Pommet and others 2004); that is, the film-forming solutions were poured into Teflon Petri dishes (diameter = 14 cm) and were dried at 35 °C (SC and CH/SC films) and at 80 °C (CH films), for approximately 10 h in a convection oven (Srinivasa and others 2004; Mayachiew and Devahastin 2008). After the excess of water was evaporated, the obtained films were peeled off from the plates and kept in a closed reservoir at a constant RH and temperature (23 ± 2 °C) for 3 d. Films prepared from SC solutions were odorless and transparent, while those prepared from CH and SC/CH solutions were also odorless but presented a yellow hue.

In vitro assays: antimicrobial effectiveness of film-forming solution and films

Native microflora preparation from food substrates. Native microfloras from carrot, cheese, and salami were prepared from 10 g of raw material macerated in 90-mL phosphate buffer solution (0.1 mol/L), using a Stomacher 400 Circulator Homogenizer

(pH 7.2) and incubated overnight at 37 °C, in agreement with the procedure reported by Moreira and others (2007).

Agar diffusion method. The sensitivity of the native microfloras (cheese, carrot, and salami) to the film-forming solutions was determined by the agar diffusion method. An inhibition zone assay was conducted by inoculating brain heart infusion agar (BHI) (Britania, Buenos Aires, Argentina) with an overnight culture of the indicator microorganisms (0.1 mL of inoculums: microbial load approximately 10^6 CFU/mL). A 30 μL of the different solutions were poured into agar wells (5- to 6-mm dia), according to the methodology described by Ponce and others (2003) and Moreira and others (2005). The dishes were incubated at 37 °C for 1 to 2 d and the inhibition zones were measured. The sensitivity to the different antimicrobial solutions was classified by the diameter of the inhibition halos as: not sensitive, diameters less than 8 mm; sensitive, diameters 9 to 14 mm; very sensitive, diameters 15 to 19 mm; and extremely sensitive, diameters larger than 20 mm (Ponce and others 2003). Each assay was performed in duplicate in 3 independent experimental runs. For each food substrate, growth controls without adding the film-forming solutions were inoculated to ensure that viable organisms were present and to confirm the initial cell charge. Moreover, contamination controls without microbial cells were conducted with the film-forming solutions to establish their initial contamination level. Finally, control plates with 1% aqueous acetic acid, without CH and casein, at the same pH (pH 5.0), were conducted to verify the nonantimicrobial properties of the solvent used in the film-forming solution.

Diffusion-type assay. The sensitivity of the native microfloras (Cheedar cheese, carrot, and Salami di Milano) to different bioactive films was determined by the diffusion-type assay. SC, CH, and SC/CH films were aseptically cut into 1.5 cm × 1.5 cm (2.25 cm^2 area) using a sterile cutter. The squares were then aseptically placed on the surface of the inoculated BHI agar with 0.1 mL of inoculums containing indicator microorganisms in the range of 10^6 CFU/mL (CFU is colony-forming unit). After 1 to 2 d of incubation at 37 °C, the area of the inhibition zone developed around the edible film square was measured by tracing it on paper, to finally measure it using an area measurements system (Delta T-Devices Ltd., England). The results reported here are the averages of 4 measurements.

Tube-assay method. Test tubes with 5 mL of BHI broth were inoculated with 1-mL inoculums obtained from the native microfloras of carrot, cheese, and salami (approximately 10^4 to 10^5 CFU/mL). After that 4 mL of CH, SC/CH film-forming solutions, and acetic acid solvent (2%) were added. At 0 times and after 24-h incubation at 37 °C the optical density of the broths at 610 nm was measured with the UV-Visible spectrophotometer (Shimadzu Corp. UV 1601 PC UV-Visible, Kyoto, Japan).

Effects of temperature and relative humidity on the film antibacterial action. SC, CH, and SC/CH films were exposed for 5 d to 2 different temperatures (10 and 20 °C) and 2 RH conditions (40% and 65%) using an environmental chamber (SCT-Pharma, Argentina), to determine the retention of the antimicrobial activity. Each assay was performed in duplicate in 3 independent experimental runs.

In vivo assays: antimicrobial effectiveness of edible coatings on carrot, cheese, and salami

The 3 edible coatings/films were applied to carrot, cheese, and salami slices in 2 ways, by immersion and as a wrapper. Before coating or film application, carrot slices (0.5-cm thickness) were washed by immersing them in tap water (containing 200 ppm of hypochlorite sodium) for 60 s, followed by water rinsing and drained. Subsequently, carrot slices were immersed in the different film-forming solutions for 180 s at 20 °C and then were allowed to drain the remaining liquid. After that, carrot slices were dried by exposing them to flowing air at 30 °C and 50% RH for 50 min. Cheese and salami slices were directly immersed in the film-forming solutions (without prior washing) and subjected to further drying under the same conditions described for carrot slices. Control samples were food slices without coatings.

To test the films when used as wrappers, food slices were packaged using the different films. Each slice was wrapped in the film, simulating a package where all the faces of the food brought into contact with the film. The envelope was filled with light pressure made by the fingers ensuring full contact. Final samples obtained (coated and packaged foods) were stored for 5 d in a controlled environmental, maintained at 65% RH and 10 °C.

Microbiological determinations. For microbiological studies, diced treated food (10 g) was macerated in 90 mL PO_4K_3 buffer solution (pH 7.2) using a homogenizer (Stomacher 400 Circulator Homogenizer). The enumeration and differentiation of mesophilic and psychrotrophic aerobic bacteria were performed on PCA (plate count agar) (Britania) after 48 h at 36 ± 1 °C and 7 d at 6 ± 1 °C, respectively. The yeast and mold count was determined on YGC Agar (Yeast Extract Glucose Chloramphenicol) (Britania), at 20 ± 1 °C for 4 to 5 d (Ponce and others 2008). Microbial counts were conducted by duplicate on 3 independent lots.

Statistical analysis

For *in vitro* assays, differences in antimicrobial properties between film-forming solutions and films were calculated by 1-way analysis of variance (ANOVA) using a statistical package (MATLAB). Whenever differences were significant, a 95% confidence level was used. For *in vivo* studies, a Student's *t*-test was employed to calculate the differences in antimicrobial properties between edible coatings and wrappers. Whenever differences were significant, a 95% confidence level was used (Khuel 2001).

Results and Discussion

In vitro assays: antimicrobial effectiveness of SC, CH, and SC/CH film-forming solutions and films

Initially, the susceptibility of the native microfloras obtained from carrot, cheese, and salami to the film-forming solutions and films (SC, CH, and SC/CH) as determined by the agar diffusion method and by diffusion-type assay, respectively (Table 1). The initial contamination of SC, CH, and SC/CH film-forming solutions was previously evaluated and no contamination was observed. As film-forming solutions, a slightly inhibitory strength ($P < 0.05$) was exerted by CH and SC/CH on cheese and salami microfloras. Not significant antimicrobial effects were observed on the native microflora of carrot (see Table 1). It was observed that CH and SC/CH solutions were not diffused into the agar medium. The native microfloras were grown around the wells without significant inhibition zones. The highly viscous CH solutions could be the cause of the relatively small inhibition halo. Ponce and others (2008) also reported limited antimicrobial action of CH film-forming solutions on squash native microflora. These authors attributed their results to the inocula size (approximately 10^6 to 10^7 CFU per Petri dish), indicating that the high number of bacteria may exceed CH inhibition activity. Zivanovic and others (2005) also found similar results with pure CH films. Coma and

others (2002) also reported a poor inhibitory activity of the CH film-forming solution in agar medium.

Nevertheless, the sensitivity of the native microflora from different foods to CH solution was reported previously. Bautista Baños and others (2005) informed that in carrot, strawberry, and papaya the use of CH films outperformed the behavior of ipridione compounds and chemicals, such as thiabendazole, fungicides commonly used to reduce watery rot, mold gray, and anthracnose. A possible explanation of the CH antimicrobial behavior can be found in the positive nature of its amino groups, which can interact and form polyelectrolyte complexes with the acidic polymers produced in the bacterial cell surfaces (lipopolysaccharide, teichoic, or theicuronic acids, and capsular polysaccharide), leading to losses of protein components and other intracellular constituents of microorganisms (Coma and others 2002; Pranoto and others 2005). It was also reported that CH inhibits the deterioration caused by different bacteria through its capacities to bind water and inhibit various enzymes and through its ability to adsorb nutrients normally used by bacteria (Ouattara and others 2000a). Several studies have shown that the effect of CH on some fungi is mainly due to alterations induced in the cell membrane functions, by interacting with the highly electronegative cell surface, which leads to changes in permeability, metabolic disorders, and in some cases, the death of the microorganisms (Fang and others 1994).

Finally, the solution of SC did not exert antimicrobial activity on the native microflora of any food studied. Similar results were reported by Ponce and others (2008) and Seydim and Sarikus (2006) working on edible films prepared from whey protein.

Due to the low diffusivity of CH biopolymer in agar diffusion method, the inhibitory effects of CH and SC/CH film-forming solutions were determined by tube-assay method. Figure 1 shows the inhibitory effects exerted by CH and SC/CH film-forming solutions after 24 h of incubation at 37 °C. The native microflora of carrot was strongly inhibited ($P < 0.05$) by acetic acid (2%) and by CH and SC/CH solutions (Figure 1A). The native microflora of cheese was significantly ($P < 0.05$) inhibited by CH and SC/CH film-forming solutions, while the effect observed from acetic acid was lower. On the other hand, the film-forming solutions and acetic solvent exerted a slightly inhibition on the native microflora of salami (Figure 1B and C, respectively). It is well known that CH shows its antibacterial activity only in an acidic medium, which is usually ascribed to the poor solubility of this biopolymer at high pH (Liu and others 2004). This reported antimicrobial activity might be the effect of dissolved CH in acidic media, such as acetic acid (Devlieghere and others 2004). The poor effect of the acetic acid solvent on cheese and salami microfloras could be attributed

Table 1–Antimicrobial activities of SC, CH, and SC/CH films and film-forming solutions against native microfloras of carrot, cheese, and salami.

	Diameters of inhibition zone[a] (mm)			Inhibition area[b] (cm^2)		
	Film forming solution			Edible films		
Native microflora	SC	CH	SC/CH	SC	CH	SC/CH
Carrot	< 8	8 ± 1	< 8	< 2.25	3.0 ± 0.1	3.0 ± 0.2
Cheese	< 8	10 ± 1	12 ± 2	< 2.25	3.3 ± 0.3	4.2 ± 0.5
Salami	< 8	9 ± 1	10 ± 1	< 2.25	3.3 ± 0.4	4.2 ± 0.6

The sensitivity to the different antimicrobial agents was classified by the diameter of the inhibition halos as: not sensitive, diameters less than 8 mm; sensitive, diameters 9 to 14 mm; very sensitive, diameters 15 to 19 mm; and extremely sensitive, diameters larger than 20 mm.
[a]The agar wells diameter (5 mm) is included. Each assay was performed by duplicate in 3 independent experimental runs.
[b]Initial area of bioactive films = 2.25 cm^2. The results presented were the average of 4 measurements.

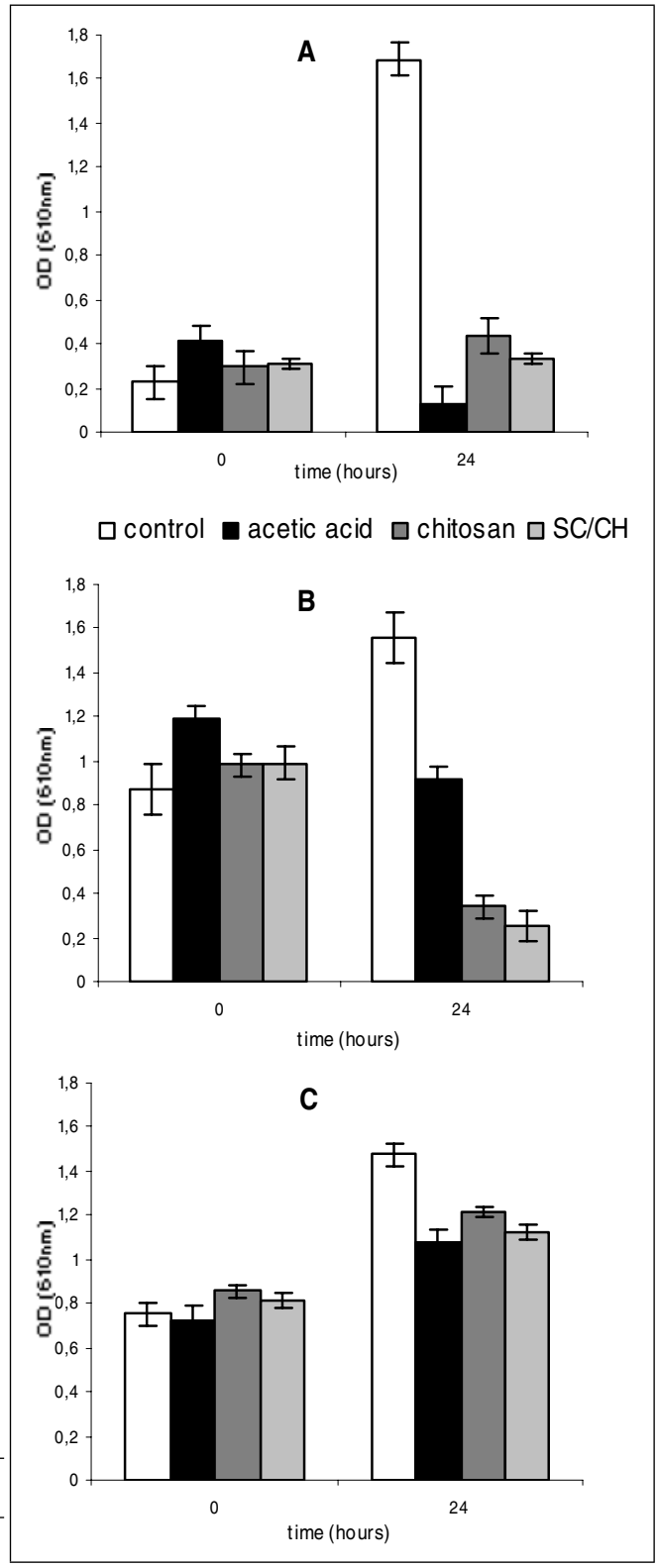

Figure 1–Antimicrobial activities of CH and SC/CH film-forming solutions and acetic acid against native microfloras of carrot (A), cheese (B), and salami (C).

to a resistance induced by the buffering capacity of these protein foods. In this sense, the lower buffer capacity of the vegetable matrix would enhance the inhibitory effect of the acid solvent on carrot native microflora.

Regarding films performance, no growth of the native microfloras occurred below the CH and SC/CH discs, indicating that only microorganisms in direct contact with the active sites of the films were inhibited. The most significant ($P < 0.05$) antimicrobial effects were observed in CH and SC/CH samples on the microflora of cheese and salami (Table 1). As in solution form, caseinate film did not show antimicrobial effects in any food tested. The low inhibition areas showed the scarce diffusion of CH and SC/CH films into the medium as was observed in well agar method. However, Cagri and others (2001) using the same technique to evaluate the antibacterial effects of films, reported that whey protein edible ones were more suited for foods that have pH values near 5.2, such as meats and cheeses.

To quantify the effects of storage in controlled environments maintained at fixed temperature and RH on the retention of the bacteriostatic or bactericidal action, the 3 edible films (SC, CH, and SC/CH) were stored for 5 d at 40% and 65% RH and 2 temperatures (10 and 20 °C). Figure 2 and 3 show the inhibition areas obtained from CH, SC, and SC/CH films on microbial growth of carrots, cheese, and salami native microfloras. The initial area of bioactive film was 2.25 cm^2. During storage, the antimicrobial action of the films decreases with time, independently of the temperature and RH selected for the tests (Figure 2 and 3), probably because the available amino groups of CH become quickly saturated by binding to surface components of bacteria (Coma and others 2002). Nevertheless, at 5 d the higher antimicrobial retention was observed at 65% RH for temperatures, 10 and 20 °C. The major effects ($P < 0.05$) were always observed with SC/CH films and with cheese and salami as food substrate microfloras. While SC films did not show any antimicrobial property (data not shown), its blend with CH improved its strength as biopreservative.

It is known that the antimicrobial action of CH is influenced by intrinsic factors, such as the deacetylation and polymerization degree, the chemical or nutrient composition of the substrate or both, and the environmental conditions (for example, substrate water activity or moisture) (Devlieghere and others 2004; Dutta and others 2009). The highly deacetylated CH has more antimicrobial character than those with a higher proportion of acetylated amino groups, because of their greater solubility and charge density (Dutta and others 2009). Besides, the excellent compatibility of CH with other substances is attributed to the presence of a high density of amino and hydroxyl groups in its structure (Park and others 2004).

The results presented in this section indicate that the strong interactions (mainly electrostatic forces) developed between CH and caseinate did not alter the antibacterial capacity of the carbohydrate. It means that even after complexation between SC and CH took place, a high proportion of available amino groups (DD = 98%) remains in the structure of the complex material, which can interact with the negatively charged surface of the bacteria, altering the bacterial wall permeability and inducing the loss of intracellular electrolytes and proteins, as mentioned Dutta and others (2009). In contrast, other authors observed a reduction of CH antimicrobial activity when it is mixed with starch or

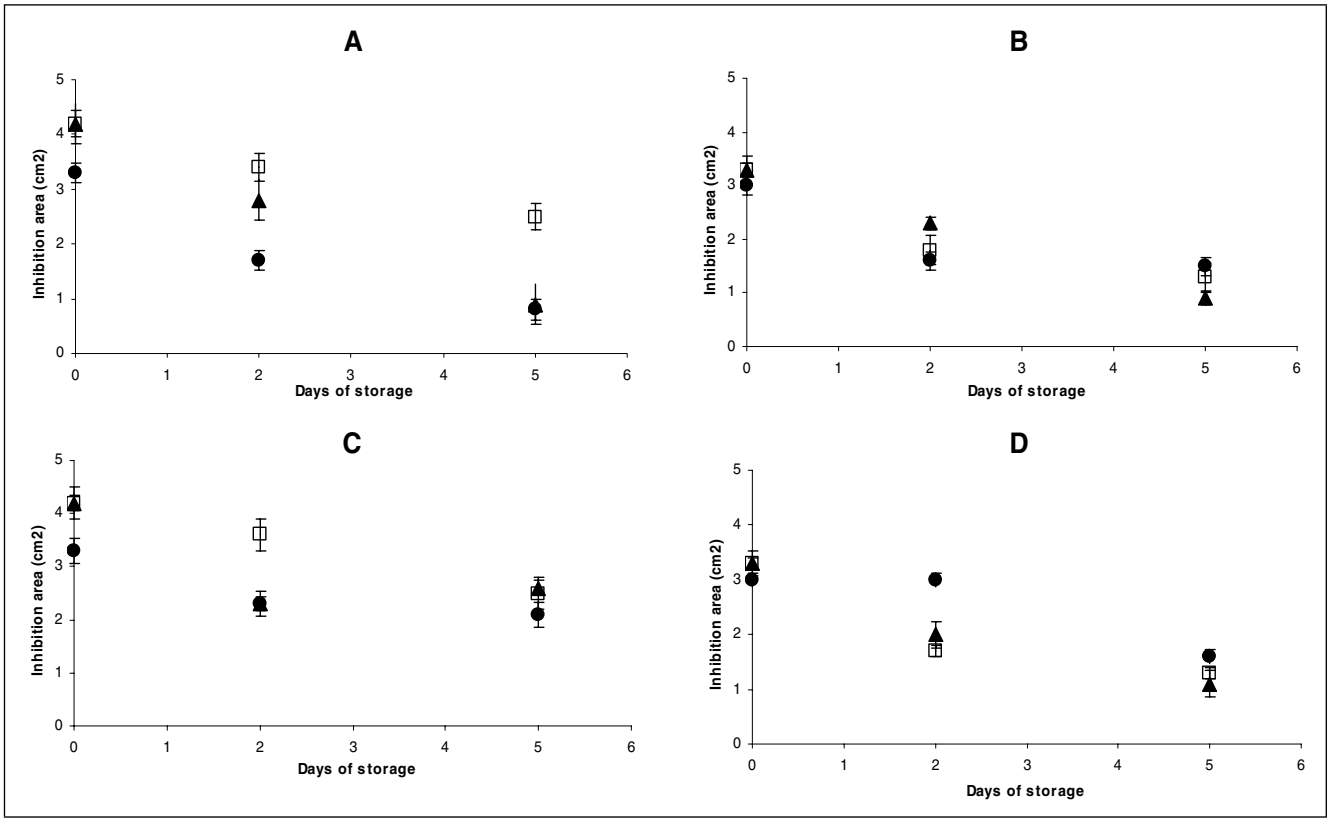

Figure 2–(A) chitosan plus casein at 40% HR and 10 °C; (B) chitosan at 40% HR and 10 °C; (C) chitosan plus casein at 40% HR and 20 °C; (D) chitosan at 40% HR and 20 °C. (●) carrot; (□) cheese; and (▲) salami native microflora. Initial area of the films = 2.25 cm^2. Each assay was performed by duplicate in 3 independent experimental runs. Bars represent mean standard error.

potassium sorbate to prepare films, which was attributed to the reduced availability of NH_3^+ groups to interact with the cell membrane (Vásconez and others 2009).

In vivo assays: antimicrobial effectiveness of edible coatings on carrot, cheese, and salami

The 3 edible films were applied to carrot, cheese, and salami slices in 2 ways, by immersion and as a wrapper. Then samples were stored for 5 d at 65% and 10 °C (selected as the better storage condition for the retention of antimicrobial effects, according to the results previously presented in Figure 2 and 3). Such conditions are not the best for food storing (ASHRAE 1994), however the challenge in applying a sanitary preservation technology, is to check its usefulness when foods are subjected to abusive storage situations. Throughout the storage period, a detailed microbial analysis of mesophilic and psychrotrophic bacteria, yeasts and molds was conducted on the treated samples and the results are shown in Figure 4 to 6.

Regarding carrot samples, SC just exerts antimicrobial effect against fungi and yeast when the film is applied by immersion. An increase in the numbers of mesophilic and psychrotrophic bacteria was observed along the storage time when SC was used (Figure 4). Because of this, the SC film was considered not suitable as a wrapping for fresh carrot slices. However, using cheese as food substrate, a significant antimicrobial effect ($P < 0.05$) at 5 d with reductions of about 3 log cycles for fungi and yeasts as well as for mesophilic and psychrotrophic bacteria was recorded. During the storage, only a slight antimicrobial action was observed in meat samples immersed or wrapped with SC, with reductions of about 1 to 1.5 log cycles, compared to control samples (Figure 4).

On the other hand, CH and SC/CH films applied as both, coatings (immersion) and wrappers, exerted a strong bactericidal action ($P < 0.05$) on 3 microbial populations analyzed, with reductions of 2 to 4.5 order log (Figure 5 and 6). Once again, the largest reductions ($P < 0.05$) were recorded for cheese and salami slices treated with CH and SC/CH either as coatings or wrappers (reductions of 3.3 to 4.8 log, with respect to the control samples).

Comparing the antibacterial action exerted by CH and SC/CH applied by immersion or as wrapping, it appears that there is no marked difference in the degree of microbial inhibition, thus, it is concluded that the 2 application forms of the films are suitable to reduce the growth of yeasts and molds, mesophilic, and psychrotrophic bacteria (Figure 5 and 6). Moreover, the comparison of our results with other methods applied to reduce the microbial load in foods reveals that the reduction of microbial growth attained by using CH and SC/CH films or coatings is considerable, for example, the application of modified atmosphere in carrot slices results in a slight reduction in the number of aerobic mesophilic bacteria of only 0.4 log cycle (Amanatidou and others 2000). A great deal of studies indicated the advantage of using CH edible coatings to extend the shelf life of foods. González-Aguilar and others (2009) reported the effect of CH coating in preventing deterioration of fresh-cut papaya, suppressing mesophilic plate counts, and the growth of molds and yeast. Durango and others (2006) mentioned the use of edible antimicrobial yam starch and CH coating as a viable alternative for controlling microbiological growth in minimally processed carrots. Park and others (2005) reported the success of CH-based coatings applied on fresh strawberries, which antifungal properties contributed to extending fruit shelf life. Jiang and others (2005) informed the effects of CH coating in extending the shelf life of cold-stored litchi fruit. Ouattara

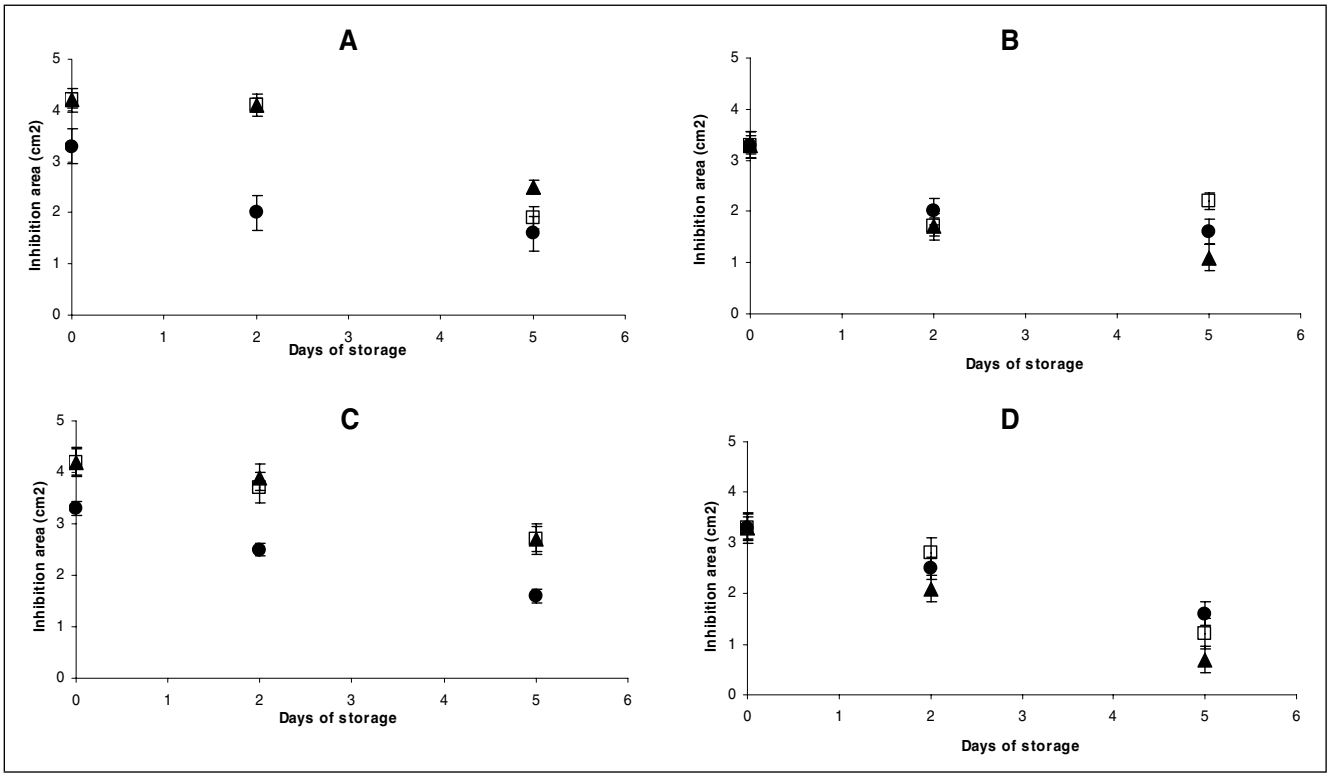

Figure 3–(A) chitosan plus casein at 65% HR and 10 °C; (B) chitosan at 65% HR and 10 °C; (C) chitosan plus casein at 65% HR and 20 °C; (D) chitosan at 65% HR and 20 °C. (●) carrot; (□) cheese, and (▲) salami native microflora. Initial area of the films = 2.25 cm^2. Each assay was performed by duplicate in 3 independent experimental runs. Bars represent mean standard error.

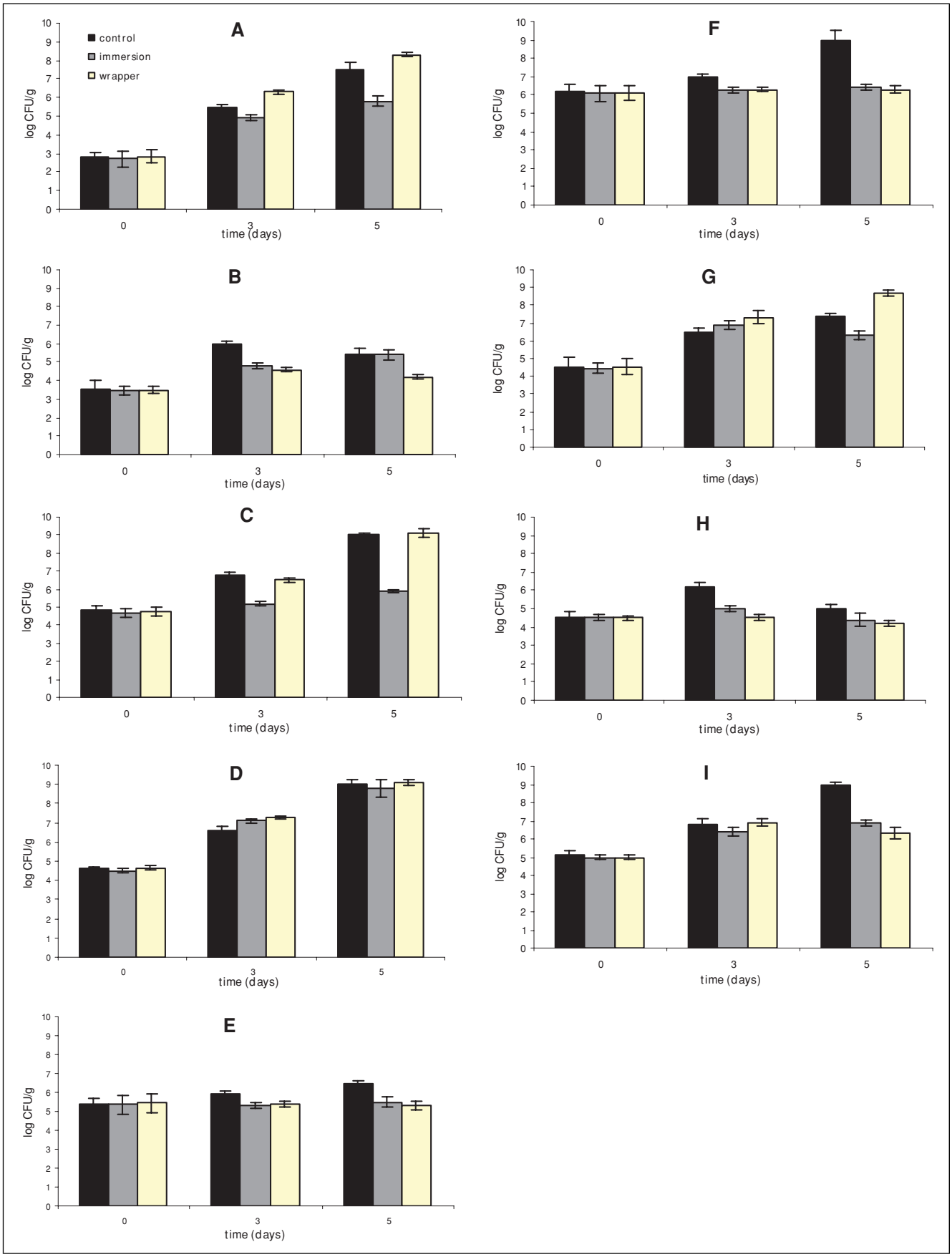

Figure 4–Antimicrobial effectiveness of sodium caseinate (SC) edible coatings on yeast and molds (A, B, C), mesophilic (D, E, F), and psychrotrophic microorganisms (G, H, I) in carrot (A, D, G), salami (B, E, H), and cheese (C, F, I). Microbial counts were conducted by duplicate on 3 independent lots. Bars represent mean standard error.

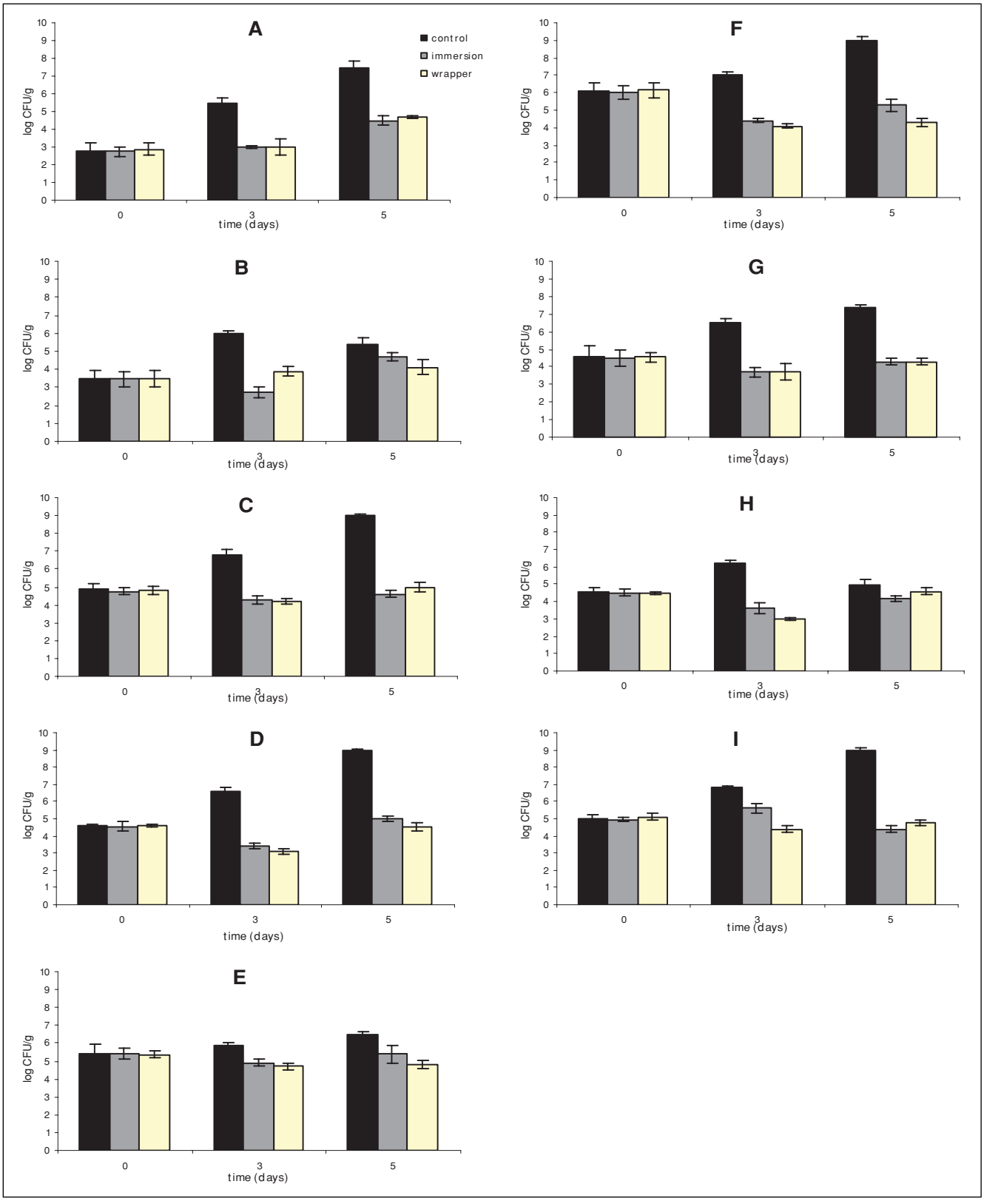

Figure 5–Antimicrobial effectiveness of chitosan (CH) edible coatings on yeast and molds (A, B, C), mesophilic (D, E, F), and psychrotrophic microorganisms (G, H, I) in carrot (A, D, G), salami (B, E, H), and cheese (C, F, I). Microbial counts were conducted by duplicate on 3 independent lots. Bars represent mean standard error.

and others (2000a, 2000b) investigated the ability of CH films to inhibit the growth of indigenous or inoculated bacteria onto the surfaces of vacuum-packed cured meat products.

In this work, the improvement of the bactericidal properties of the SC/CH blend is also reported. Its antimicrobial action was higher than that exerted by SC films and also, with some food

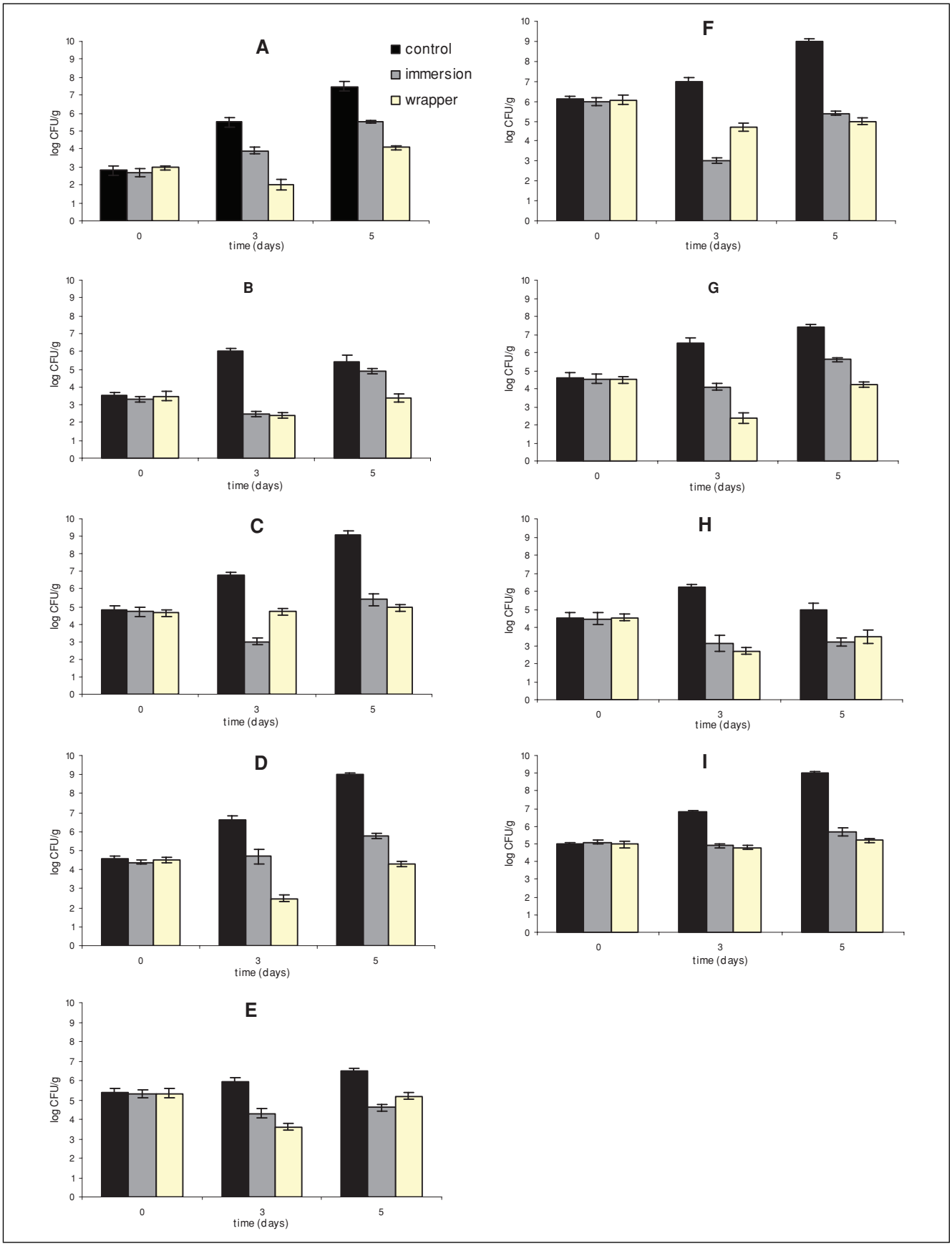

Figure 6–Antimicrobial effectiveness of SC/CH edible coatings on yeast and molds (A, B, C), mesophilic (D, E, F), and psychrotrophic microorganisms (G, H, I) in carrot (A, D, G), salami (B, E, H), and cheese (C, F, I). Microbial counts were conducted by duplicate on 3 independent lots. Bars represent mean standard error.

substrates, was better than the effect exerted by CH applied alone. The significant antibacterial activity of SC/CH film suggested that the ionic interaction between both macromolecules did not limit the antimicrobial activity of CH, on the contrary, enhance its antimicrobial properties.

These results are extremely promising, since in our previous works (Pereda and others 2008, 2009), it was also observed a synergistic effect on the mechanical (tensile and impact) and physical (contact angle, moisture absorption) properties due to the formation of the polymeric complex between SC and CH. Han and others (2009) also established that the ionic interaction between calcium caseinate and cationic carboxymethylcellulose can stabilize the protein network. Other authors obtained polyelectrolyte complexes between cationic CH and anionic polymers, including sodium alginate (Remuñán-López and Bodmeier 1997; Remuñán-López and others 1998), trifoliphosphate (TPP) (Shu and Zhu 2000), xanthan (Dumitriu and others 1994), and collagen (Sionkowska and others 2004).

Conclusions

The use of antimicrobial coating consisting of CH and SC/CH mixtures, applied as either coatings produced by food immersion in the film-forming solutions or just as packages (by wrapping the food using a film) is a good alternative for controlling the microbiota present mainly in cheese and salami. Both CH and SC/SC significantly inhibited the growth of mesophilic bacteria, psychrotrophic, yeasts, and molds.

Based on the concept of hurdle technologies, the use of such coatings/wrappers in combination with other barriers, such as hygienic processing conditions and adequate storage temperatures may contribute to improve the safety in minimally processed foods.

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