6-P9

EFFICACY OF CHITOSAN-BASED ANTIBACTERIAL FILMS ON PROCESSED MEATS

Blaise Ouattara, Ronald E. Simard, Gabriel Piette, André Bégin, and Richard A. Holley

Food Research and Development Centre, Casavant 3600 Boulevard West, Saint-Hyacinthe, Québec, Canada, J2S 8E3.

INTRODUCTION A great deal has been learned over the years about microbial spoilage of meats and meat products, and its control (1, 2). Surface treatments by spraying or dipping with solutions of antimicrobial compounds such as organic acids, bacteriocins and spice extracts have been tried to inhibit microbial growth (3, 4) but their efficiency has been limited by the rapid diffusion of the antimicrobial moieties within the food. Diffusion might be slowed down by incorporating the active susbtances within the packaging material, which may help maintain high concentrations of the antimicrobial agents onto food surfaces for longer periods of time (5). In recent studies, Ouattara *et al.* (6, 7) evaluated the efficacy of various organic acids, fatty acids, and essential oils against common meat spoilage bacteria and found that acetic acid, propionic acid, lauric acid, cinnamon, and clove were the most efficient compounds. The present sudy was then undertaken to develop an antimicrobial package for meat products by incorporating acetic or propionic acids in thin chitosan films, which may also contain lauric acid or cinnamaldehyde. The objective was to evaluate the ability of the films to slowly release acetic or propionic acids. Also, the antibacterial properties of the films were determined on meat products inoculated with *Serratia liquefaciens* and *Lactobacillus sake*, and on uninoculated products.

MATERIALS AND METHODS Practical grade chitosan from crab shells (Sigma Chemical, St-Louis, MI) was used to prepare acetic acid/chitosan films (AA) and propionic acid/chitosan films (AP), according to the procedure described by Wong *et al.* (8). Acetic acid/cinnamaldehyde/chitosan films (AAC), acetic acid/lauric acid/chitrosan films (AAL), and propionic acid/cinnamaldehyde /chitosan films (APC) were prepared in the same manner, with the exception that trans-cinnamaldehyde (Aldrich Chemical, Milwaulkee, WI) or lauric acid (Sigma Chemical, St-Louis, MI) were added to the film forming solutions prior to casting and drying. Neutralized AA films (AAN) served as controls, to assess the antibacterial effect of chitosan alone.

Cooked bologna, ham, and pastrami, manufactured in Federally inspected plants, were obtained from a local grocery store. For the diffusion tests, slices (100 mm diameter x 15 mm thick) were cut from the meat products and placed into sterile petri plates. Squares of 9 cm² of each tested film were applied onto the surfaces of meat slices, and the slices were vacuum-packaged (deli #1 bags; Winpak, Montreal, QC). Packages were stored at 4°C, for 3, 6, 12, 48, and 168 h, then opened, and the chitosan films were recovered and solubilized in hydrochoric acid solution (1%, w/v). Residual acetic and propionic acids were then extracted with ethyl acetate (Burdick & Jackson Inc., Muskegon, MI) and quantified by gas chromatography using a Hewlett Packard model 5890 gas chromatograph (J & W Scientific, Folsom, CA) equiped with a DB-FFAP column (Chromatographic Specialities Inc., Brockville, ON).

The microbiological evaluation of the chitosan films was done in two separate experiments. In the first experiment, products were surface-inoculated with *L. sake* ATCC 15521 (*Ls*) or *S. liquefaciens* (*Sl*; isolated from vacuum-packaged bologna), then vacuum-packed with or without the various antimicrobial chitosan films, stored at 4°C or 10°C for 21 d, and periodically evaluated for the presence of *Ls* (MRS agar ; 25°C for 72 h) and *Sl* (BHI agar ; 35°C for 48h). In the second experiment, the same procedure was used to enumerate total lactic acid bacteria (LAB) and *Enterobacteriaceae* (Ent) on uninoculated product slices, using MRST agar (MRS containing 0.1%, w/v of thallous acetate) and VRBG agar, respectively.

RESULTS AND DISCUSSION Typical graphs illustrating the kinetics of acetic and propionic acid release from chitosan films are shown in Figure 1. Regardless of the film type, more than 75 % of the compounds were released within 3 h after film application onto the surface of bologna (Figure 1A). After 3 h, however, the patterns of desorption differed between acetic and propionic acids.

Figure 1 : Percentages of acetic and propionic acids remaining in chitosan films over time, after application onto product surfaces. Influence of film type, measured on bologna (A) and of product type, measured with AAC film (B).

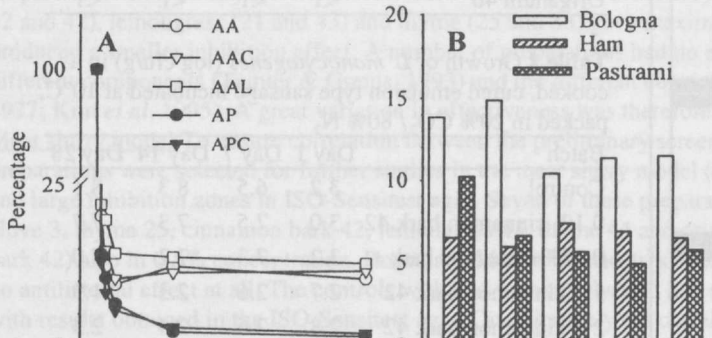

Table 1 : Inhibitory effect of chitosan films against *Sl*, Ent, *Ls*, and LAB after 21 d storage at 4°C[1].

	Sl	Ent	*Ls*	LAB
Control	8.38_a	5.70_a	6.10_a	6.52_a
AA	5.19_c	0.90_c	5.78_c	5.89_b
AAC	4.25_d	CI[2]	5.31_d	6.26_a
AAL	6.70_b	3.01_b	5.62_c	5.86_b
AAN	8.43_a	-[3]	6.82_a	-
AP	6.65_b	-	5.21_d	-
APC	4.61_{cd}	-	5.01_d	-

[1]. $\log_{10}$ CFU/cm²; numbers bearing similar letters in the same columns are not significantly different ($P > 0.05$; LSD); measured on uninoculated pastrami (Ent, LAB) or on cooked ham surface-inoculated with *Sl* and *Ls*. [2]. Complete inhibition, no growth detected. [3]. Not determined.

For acetic acid, diffusion essentially stopped after 3 h and residual amounts found in chitosan films were higher than 10 % of the initial concentration over the whole experimental period. In contrast, diffusion of propionic acid continued after 3 h, and less than 2 % of the initial concentration remained at the end of the experimental period. Also, residual amounts of acetic acid were higher in AAL films than in the other two films (AA and AAC; Figure 1A) and the acetic acid remaining in AAC films after 3 h was higher on bologna than on ham or pastrami (Figure 1B).

Theoretically, the release of acetic and propionic acids from chitosan films can be compared to the swelling-controlled release of drugs (9). As such, the desorption of hydrophilic compound from the film results from the diffusing aqueous phase entering the polymer matrix. Thus, the high rate of desorption observed in the first 3 h is a consequence of the hydrophilic nature of chitosan and should, theoretically, decrease with the incorporation of hydrophobic compound such as lauric acid and cinnamaldehyde within the chitosan matrix. Indeed, a decrease in diffusion rates of both acetic and propionic acids from chitosan films immersed in water was observed when the film contained cinnamaldehyde or lauric acid, at concentrations of 0.5% w/w and 1.0% w/w, respectively (results not shown). Also, the hypothesis of a swelling-controlled mechanism for acid release is consistent with the fact that diffusion of acid from chitosan is fastest on the product with the wettest surface (pastrami).

The inhibitory effect of acid-loaded chitosan films against *Ls* and *Sl* after 21 days storage at 4°C is presented in Table 1. In general, bacterial growth was inhibited in the presence of the chitosan-based antibacterial films, with the exception of neutralized acetic acid/chitosan films. The strongest effect was observed on *Sl*, with films in which cinnamaldehyde was co-incorporated with acetic acid (4.13 $\log_{10}$ CFU/cm^2 unit reduction, compared to the control) or propionic acid (3.77 $\log_{10}$ CFU/cm^2 unit reduction), while *Ls* was found to be more resistant to the release of acids, with only 0.79 and 1.09 $\log_{10}$ CFU/cm^2 unit reduction produced by AAC and APC films, respectively. On the other hand, co-incorporation of lauric acid did not enhance the effectiveness of the films. Similar findings were observed with the Ent and LAB flora indigenous to cooked ham (Table 1) and with the Ent flora indigenous to bologna and pastrami (Table 2). Furthermore, acid-loaded chitosan films were very effective on products with a dry surface (bologna) and completely eliminated *enterobacteriaceae* from these products stored at either 4°C or 10°C for 21 days.

The antibacterial properties reported here for various acid-loaded chitosan films can be related to the inhibitory effect of the incorporated compounds and the relative sensitivity of the micro-organisms. AAC and APC films exhibited the strongest antibacterial effects because cinnamaldehyde is active against gram negative and gram positive bacteria. On the contrary, lauric acid, like many long chain fatty acids, is known to be inactive against gram negative bacteria, explaining the weak efficacy of the AAL films.

CONCLUSION. This study has evaluated the feasibility of developing an antimicrobial packaging system for meat and meat products, based on active compounds incorporated into a chitosan matrix. Results obtained showed significant inhibitory effects against *S. liquefaciens* and *Enterobacteriaceae*, suggesting that such systems will be inhibitory against many pathogenic meatborne *Enterobacteriaceae*, such as *Salmonella and Escherichia coli*. However, further investigators will be faced with the resistance of spoilage lactic acid bacteria to acids, particularly in vacuum and modified atmosphere packaged meat products. Additional studies are therefore needed to find convenient carrier polymers for better control of chemical release from films and to select antimicrobial agents which are effective against lactic acid bacteria.

Table 2 : Effect of various acid-loaded chitosan films on the growth of *Enterobacteriaceae* indigenous to bologna and pastrami[1].

	Bologna				Pastrami			
	0d	11d		21d	0d	11d		21d	
		4°C	10°C	4°C	10°C		4°C	10°C	4°C	10°C
Control	1.55	CI[2]	3.20_A	0.81	4.55	2.31	3.83	4.95_A	5.70_A	6.35_A
AA		CI	0.22_B	CI	CI		CI	1.76_B	0.90_C	3.85_C
AAC		CI	0.15_B	CI	CI		CI	1.50_B	CI	3.06_D
AAL		CI	CI	CI	CI		CI	1.74_B	3.01_B	4.42_B

[1]. Numbers reported are bacterial populations, expressed as $\log_{10}$ CFU/cm², after 0, 11, and 21 day storage at 4°C or 10°C; Within the same column, means with different letters are significantly different ($P \leq 0.05$); [2]. CI: complete inhibition, no bacterial growth.

REFERENCES

(1). Greer, G.G. and Dilts, B.D. 1992. Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids. Food Res. Int. 25:355-364; (2). Holley, R.A. 1997. Asymmetric distribution and growth of bacteria in sliced vacuum-packaged ham and bologna. J. Food Prot. 60 :510-519; (3). Abugroun H.A., Cousin, N.A., and Judge, M.D. 1993. Extended shelf life of unrefrigerated prerigor cooked meat. Meat Sci. 33:207-229; (4). Hotchkiss, J.H. 1995. Safety consideration in active packaging. In *Active food packaging*, M.L. Rooney (ed.), p. 238-253. Blackie academic & professional, Bishopbriggs, Glasgow; (5). Gennadios, A., Hanna, M.A., and Kurth, L.B. 1997. Application of edible coatings on meats, poultry and seafoods : a review. Leben. Wissen. Technol. 30 :337-350; (6). Ouattara, B., Simard, R.E., Holley, R..A., Piette, G.J.-P., and Bégin, A. 1997_a. Inhibitory effect of organic acids upon meat spoilage bacteria. J. Food Prot. 60 :246-253; (7). Ouattara, B., Simard, R.E., Holley, R..A., Piette, G.J.-P., and Bégin, A. 1997_b. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37 :155-162; (8). Wong, D.W.S., Gastineau, F.A., Gregorski, , K.S., Tillin, S.J., and Pavlath, A.E. 1992. Chitosan-lipid films : Microstructure and surface energy. J. Agric. Food Chem. 40 :540-544; (9). Malley, I., Bardon J., Rollet M., Taverdet, J.L., and Vergnaud, J.M. 1987. Modelling of controlled drug-release in case of carbopol-sodium salicylate matrix in gastric liquid. Drug Dev. Ind. Pharm. 13 : 67-79.