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Antimicrobial Paper Based on a Soy Protein Isolate or Modified Starch Coating Including Carvacrol and Cinnamaldehyde

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Soy protein isolates (SPI) and octenyl-succinate (OSA) modified starch were used as paper coating and inclusion matrices of two antimicrobial compounds: cinnamaldehyde and carvacrol. Antimicrobial compound losses from the coated papers were evaluated after the coating and drying process, and the two matrices demonstrated retention ability that depended on the compound nature and concentration. Whereas carvacrol losses ranged between 12 and 45%, cinnamaldehyde losses varied from 43 to 76%. The losses were always higher from OSA-starch-coated papers than from SPI-coated papers. During storage in accelerated conditions, at 30 °C and 60% relative humidity, carvacrol retention from coated papers was found to be similar whatever the coating matrices and the carvacrol rate. In contrast, the retention from SPI-coated papers was particularly high for the cinnamaldehyde concentration of 30% (w/w) compared to the lowest (10% w/w) or highest concentration (60% w/w). Compared to carvacrol, faster release was observed, particurlarly when OSA-starch was used. The antimicrobial properties of the coated papers were shown against *Escherichia coli* and *Botrytis cinerea* and explained by favorable conditions of total release of the antimicrobial agents.

KEYWORDS: Cinnamaldehyde; carvacrol; coated papers; soy protein isolate; OSA-starch; antimicrobial packaging

INTRODUCTION

Microbial growth on food surfaces is a major cause of food spoilage, for example, bacterial contamination of ready to-eat or meat products and mold decay, in fruits and vegetables. Attempts have been made to improve safety and to delay spoilage by using antimicrobial sprays or dips (*1*, *2*). However, direct surface applications onto foods have limited benefits because the active substances can be neutralized on contact with food or diffuse rapidly from the surface into the food mass. An alternative is the use of antimicrobial packaging, a promising form of active packaging (*3*−*7*). It possesses attributes beyond basic barrier properties, which are achieved by adding active ingredients to the packaging systems and/or by using antimicrobial polymeric materials. Antimicrobial packaging could be more efficient than direct surface application by controlling migration of the antimicrobial agents from the packaging material to the surface of the product, thus maintaining their high concentrations where and when they are needed (*8*, *9*).

Volatile and nonvolatile antimicrobial agents can be incorporated into polymers during processing or applied onto polymer surfaces by coating or by absorption (*7*). Among potential carriers, agropolymers such as proteins and polysaccharides have drawn attention for their film-forming ability and have been used to make edible films and biodegradable active packaging (*9*−*16*). In addition, the agropolymer's film-forming properties are successfully used for the encapsulation of numerous products and have high retention ability. Encapsulation is described as a process by which the compounds may be coated or packaged in a protective wall material (*17*). Functional characteristics of an effective wall material include not only good film-forming ability but also emulsion stabilization properties, effective retention, and release ability. Proteins such as whey or soy proteins have physicochemical properties that satisfy the requirement of an encapsulating agent and have been reported as effective agents for encapsulation of fats or aroma compounds by spray-drying, the most common microencapsulation technique in the food industries (*17*, *18*). Native starches and related hydrolyzed products lack active surface properties and have to be chemically modified or used in conjunction with emulsifying agents in order to encapsulate hydrophobic products such as aroma compounds. For example, hydrophobic octenyl succinate anhydrous (OSA) groups can be grafted to native or hydrolyzed starches to impart their emulsifying abilities and decrease their viscosity (*19*). The presence of hydrophobic groups contributes

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to the absorption of oil into the matrix and increases the oil loading during the encapsulation process.

With regard to the antimicrobial agents, several compounds have already been proposed for antimicrobial food packaging, including organic acids such as sorbic acid (*12*), enzymes such as lysozyme (*5*), bacteriocins such as nisin (*20*, *21*), and natural antimicrobial compounds such as essential oils (*15*, *16*, *22*). Essential oils possess strong antimicrobial properties against foodborne pathogens, and their antimicrobial activities have often been assigned to their major components. Among them, phenolic compounds such as carvacrol ($C_{10}H_{14}O$) extracted from oregano and thyme essential oils or cinnamaldehyde (C_9H_8O), which occurs mainly in cinnamon oil, are described to be potent antimicrobial compounds (*23*−*28*).

To design biodegradable antimicrobial packaging, a base paper was coated with soy protein isolates (SPI) and/or with (OSA)-starch solutions containing carvacrol and cinnamaldehyde selected as antimicrobial agents. The coated papers were assigned for the retention and the controlled release of the active compounds. Thus, the effect of the coating and drying process on the cinnamaldehyde and carvacrol retention from the papers as a function of both coating matrices was investigated. The ability of the coated papers to release these compounds over time in accelerated conditions of storage was studied. Finally, the inhibitory effect of the coated papers was determined against the bacterium *Escherichia coli* and the mold *Botrytis cinerea*.

MATERIALS AND METHODS

Materials. A commercial base paper (70 g/m^2) was provided by Ahlstrom Research and Services and was used as support for coating. SPIs were purchased from Seah International (SAMPROSOY 90 NB; Wimille, France). According to the supplier, the product had an 8% moisture content and contained 91.8% proteins. OSA waxy maize starch (Cleargum, CO 01) was given by Roquette (Lestrem, France). Carvacrol, cinnamaldehyde (the antimicrobial agents), and 2-nonanol (used as internal standard) were purchased from Sigma Aldrich (St Quentin Fallavier, France). Growth media such as plate count agar medium (PCA), potato dextrose agar (PDA), and nutrient broth (NB) were purchased from Biokar Diagnostic (Beauvais, France).

Strains. *E. coli* (I.P.54127) and *B. cinerea* (MUCL30158) cultures were obtained from the Pasteur Institut (Paris, France) and from the catholic university of Louvain (Louvain, Belgium). *E. coli* was cultivated on PCA medium and kept at −80 °C in 20% (v/v) glycerol. *B. cinerea* was harvested on PDA medium and kept on PDA slants at 4 °C.

Preparation of Coating Solutions. SPI (10% w/v) or OSA-starch (20% w/v) was dissolved in distilled water heated to 50 °C. Then the solutions were continuously stirred for 30 min at 50 °C. After cooling of the solutions to 25 °C, carvacrol and cinnamaldehyde at concentration sof 10, 30, and 60% (w/w of SPI) were added. Homogenization was generally carried out with an Ultra-Turrax (T-25, IKA Labotechnik) at 8000 rpm for 10 min. For cinnamaldehyde and OSA-starch the homogenization was performed at a higher rate, that is, 20500 rpm.

For combined OSA-starch/SPI coating solutions, a 5% w/v SPI solution and a 20% (w/v) OSA-starch solution were separately prepared as described above. Then carvacrol (30% w/w of the final dry matter of the solution) was added to the 5% w/v of SPI solution and homogenized with an Ultra-Turrax (T-25, IKA Labotechnik) for 10 min at 8000 rpm. OSA-starch solution was added to the SPI−carvacrol coating solutions, and the OSA-starch/SPI/carvacrol solution was continuously stirred overnight at 25 °C.

Viscosity Measurements of Coating Solutions. The shear viscosity measurement was carried out on a Physica rheolab MC1 viscosimeter (Paar, Physica, Stuttgart, Germany) equipped with an MSZ1 DIN/double-gap measure cell. Samples were equilibrated at the analysis temperature (25 °C), gently mixed, and then poured into the instrument. The instrument was previously equilibrated at 25 °C, and the test was run immediately. The rate of rotation from the outer cylinder increased from 65 to 400 s^{-1}.

Coating Process and Drying of Papers. The coating process was performed at 25 °C: a support paper was maintained on an iron perforated plate under partial vacuum (21 cm × 30 cm), and the coating solution was applied by an adjustable micrometer thin-layer chromatography applicator (Braive Instrument, Chécy, France). Then, coated papers were dried for 3 h at 23 ± 2 °C and at 50 ± 5% relative humidity (RH).

Coated Paper Characterization. *Moisture Content Evaluation*. Moisture content of carvacrol free coated paper was evaluated in an oven at 105 °C for 24 h. However, the moisture content of papers containing carvacrol or cinnamaldehyde cannot be evaluated by this method, because aroma is partially eliminated and the residual content estimated by the extraction method was not negligible.

Coated Weight Determination. Dry coated weight was determined from the weight of a defined surface of coated and uncoated paper. The dry coated weight was obtained as followed: dry coated weight (g/m^2) = coated weight (g/m^2) − carvacrol or cinnamaldehyde content (g/m^2) − moisture content (g/m^2).

Carvacrol and Cinnamaldehyde Extraction from Coated Papers. The following extraction procedure was used to quantify the residual amount of coated papers. Pieces of coated papers (3 cm × 3 cm) were immersed in a water and *n*-pentane mixture (50:50 v/v). One hundred microliters of an internal standard solution (10 g/L of 2-nonanol) was added, and the mixture was shaken for 16 h under magnetic agitation (300 min^{-1}). The organic phase containing carvacrol or cinnamaldehyde and 2-nonanol was removed, dried over anhydrous sodium sulfate, and analyzed by gas chromatography. The analysis was carried out on a Varian 3800 GC-FID (Les Ulis, France) equipped with a CP-Sil 5 column (Varian) (15 m × 0.32 mm, film thickness = 0.25 μm) and a flame ionization detector (FID; hydrogen, 30 mL/min; and air, 300 mL/min). Hydrogen was used as a carrier gas with a flow rate of 2 mL/min. The oven temperature was programmed to rise from 60 to 150 °C at 4 °C/min, then at 15 °C/min to 250 °C, and held at 250 °C for 10 min. Injector and detector temperatures were adjusted at 250 °C. Injections were done in split mode with a 1:20 ratio. Quantification of the compounds was performed using the internal standard for which the response coefficient was determined; it was about 0.99 ± 0.03 for carvacrol and 1.2 ± 0.03 for cinnamaldehyde. The extraction yields were estimated by depositing a known quantity of the compound on the coated papers and by applying the extraction procedure described above. The extraction yields were found to be about 87 ± 5% (10 replications) for SPI−carvacrol, 64 ± 3% for SPI−cinnamaldehyde, 82 ± 4% for OSA-starch−carvacrol, and 99 ± 1% for OSA-starch−cinnamaldehyde. The extraction was done in triplicate from two different coated papers.

Determination of Carvacrol and Cinnamaldehyde Losses from Coated Paper after the Coating and Drying Process. The losses of carvacrol and cinnamaldehyde after the coating and drying process were calculated by comparing the residual amount on coated paper determined by extraction procedure and the theoretical content of the agent from the same coated paper. The theoretical content of carvacrol or cinnamaldehyde (in g/m^2) was calculated in relation with the dry coated weight by multiplying the latter by the percentage of added compound in grams per 100 g of dry matter (SPI or OSA-starch). The losses were expressed in percentage for comparison purposes.

For instance, the theoretical carvacrol quantity, for a SPI-coated paper with a dry coated weight of 10.7 g/m^2 and prepared with a solution containing 10% carvacrol, was equal to 1.07 g/m^2 (**Table 1**). The residual amount of carvacrol for this paper was found to be 0.749 g/m^2, and consequently the losses were estimated to be 0.321 g/m^2 (i.e., 30%).

Kinetic Retention of Antimicrobial Agent from Coated Papers. Pieces of coated papers (3 cm × 3 cm) were put in an oven at 30 °C and at a constant relative humidity of 60 ± 5%. The relative humidity was adjusted due to a humidified air flux (25 mL/min) through the oven (volume of about 370 cm^3). Coated papers were taken from the oven at prescribed time intervals; aroma compound content was immediately determined by extraction method.

Table 1. Carvacrol and Cinnamaldehyde Losses from SPI- and OSA-Starch-Coated Papers after Coating and Drying Process as a Function of Initial Concentration Introduced in the Coating Solutions

coating matrix	antimicrobial agent	initial concn (w/w of SPI, OSA) in coating solutions	dry coated wt (g/m^2)	loss (%)
SPI	carvacrol	10	10.7 ± 2.1	30.0 ± 6.0
		30	9.7 ± 1	17.6 ± 2.7
		60	13.6 ± 2	12.0 ± 1.1
	cinnamaldehyde	10	14.5 ± 2.4	59.5 ± 7.2
		30	13.0 ± 3.1	64.1 ± 8.0
		60	11.3 ± 2.7	43.0 ± 7.3
OSA-starch	carvacrol	10	12.4 ± 1.9	45.1 ± 2.5
		30	10.2 ± 2.0	17.1 ± 5.6
		60	10.5 ± 2.2	40.1 ± 8.5
	cinnamaldehyde	10	11.7 ± 1.2	76.0 ± 8.5
		30	12.2 ± 2.4	77.5 ± 4.6

Antimicrobial Test. Nutrient broth and PCA medium (Biokar Diagnostic) were used as a basal medium for *E. coli*. PDA medium (Biokar Diagnostic) was used for the growth of *B. cinerea*.

E. coli strain was first inoculated in the appropriate broth for 24 h; subsequently, cells from this culture were then inoculated in fresh medium and incubated at 30 °C for 16 h, to obtain 10^{8-9} cells/mL. The obtained *E. coli* suspension was used to inoculate a fresh medium at 1% (v/v), which was shaken at 120 rpm and 30 °C during 4 h to obtain 10^{8-9} cells/mL. *E. coli* suspensions of 10^{5-6}, 10^{4-5}, 10^{3-4} cells/mL were prepared by serial dilution in sterile tryptone salt, and 30 μL of this inoculum was sprayed on the agar surface of a Petri dish for antimicrobial testing.

For *B. cinerea*, spores of 7-day-old cultures were first harvested with sterile distilled water with 0.1% Tween 80 (v/v). The concentration of the conidia suspensions was determined using a hemocytometer (Malassez cell) with an optical microscope at 400 magnification, and 10^4 spores were inoculated in the center of the Petri dish for antimicrobial testing.

Ten milliliters of molten agar medium was poured into sterile Petri dishes of 5.5 cm diameter. Inoculated media with *E. coli* and *B. cinerea* were placed in jars of 1 L (on the bottom). The coated papers were put in the lid of the jar. Jars were kept at 30 °C for 48 h with *E. coli* and at 22 °C for 14 days with *B. cinerea*, respectively. Controls were carried out in the same way with an uncoated paper with and without antimicrobial agent.

Growth bacterial inhibition was expressed in terms of $\log_{10}(N/N_0)$, where N is the CFU number formed on the agar surface exposed to carvacrol and cinnamaldehyde and N_0 is the number of CFU on the control Petri dish, in the absence of antimicrobial agent. For fungi, growth delay and mycelia diameter measurements were taken into account to assess the antimicrobial effect.

RESULTS AND DISCUSSION

Effect of Carvacrol and Cinnamaldehyde Addition on Viscosity of SPI and OSA-Starch Coating Solutions. SPI and OSA-starch were used at the same time as paper coating and inclusion matrices of two antimicrobial agents: cinnamaldehyde and carvacrol. Paper-coating solution rheology is an important parameter in the coating process as it influences the type of coating equipment that can be used as well as the resulting coated weight. Viscosity measurement can also provide information for understanding protein state and interactions, particularly in the presence of added components. The inclusion of any additional additives in the coating solutions could alter the rheological properties of the resulting solutions and affect the entire coating process (*28*). If the SPI coating solution (10% w/v) followed a shear thinning behavior as previously reported (*29*), the OSA-starch solution (20% w/v) displayed a Newtonian behavior (data not shown); that is, its viscosity was independent of the shear rate. In the case of a Newtonian behavior, the rate of entanglements disruption is less important than the rate of re-entanglement, and polymers are free to move independently in solution without interpenetration (*30*).

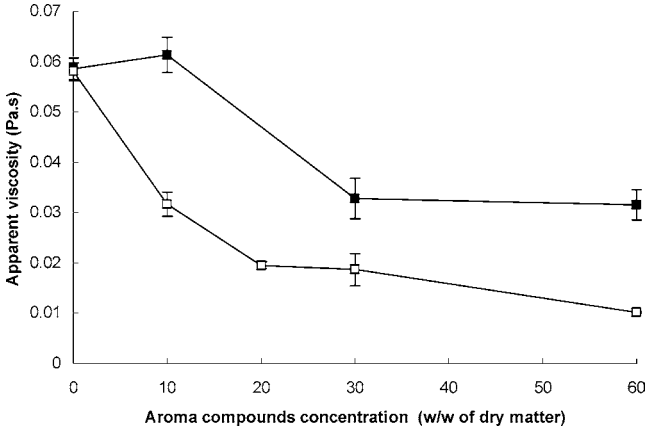

Figure 1. Apparent viscosity (shear stress = 109 s^{-1}) of SPI solutions (10% w/v) as a function of aroma compound concentration added to the solutions: (■) carvacrol–SPI solutions prepared at 50 °C; (□) cinnamaldehyde–SPI solutions prepared at 50 °C.

Whereas soy protein solution flow behavior was not significantly modified by the presence of carvacrol or cinnamaldehyde (data not shown), the apparent viscosity was strongly affected. Increasing aroma compound concentrations decreased the apparent viscosity of SPI solutions from 0.056 to 0.01 Pa·s^{-1} for cinnamaldehyde and to 0.03 Pa·s^{-1} for carvacrol (**Figure 1**). The influence of carvacrol and cinnamaldehyde addition on solution viscosity could be related to the modification of the protein conformation. Addition of organic compounds has been previously demonstrated to affect protein conformation in aqueous solution by thermodynamic measurement (*31*). In a previous study (*29*), it was found that the addition of carvacrol led to the formation of unstable soy protein aggregates observed by optical microscopy and confirmed by an increase of particle size diameter. The apparent viscosity of SPI solutions was affected in a similar way by cinnamaldehyde addition, suggesting that similar changes in soy protein conformation may occur in the presence of both carvacrol and cinnamaldehyde.

For OSA-starch solutions, neither flow behavior nor apparent viscosity was affected by carvacrol and cinnamaldehyde addition (data not shown). The viscosity remained low (about 0.015 Pa·s^{-1}) except for a slight increase of viscosity (from 0.016 to 0.026 Pa·s^{-1}) observed when carvacrol was added at high percentage (50−60% w/w of OSA-starch).

Carvacrol and Cinnamaldehyde Losses after Coating and Drying Process from SPI- and OSA-Starch-Coated Papers. Retention of the antimicrobial compound is one of the most important features of the coating process. It depends on the total compound amount retained in the coating matrices after drying (at ambient temperature) compared to the initial compound quantity introduced in the coating solutions. Thus, carvacrol and cinnamaldehyde losses were determined after the coating and drying process of papers by subtracting the extracted quantity remaining on dried papers from the calculated quantity deposited on paper (**Table 1**). Carvacrol and cinnamaldehyde losses were always higher for OSA-starch-coated papers than for SPI-coated papers. They ranged for carvacrol and OSA-starch matrices between 17 and 45% against 12 and 30% in the case of SPI

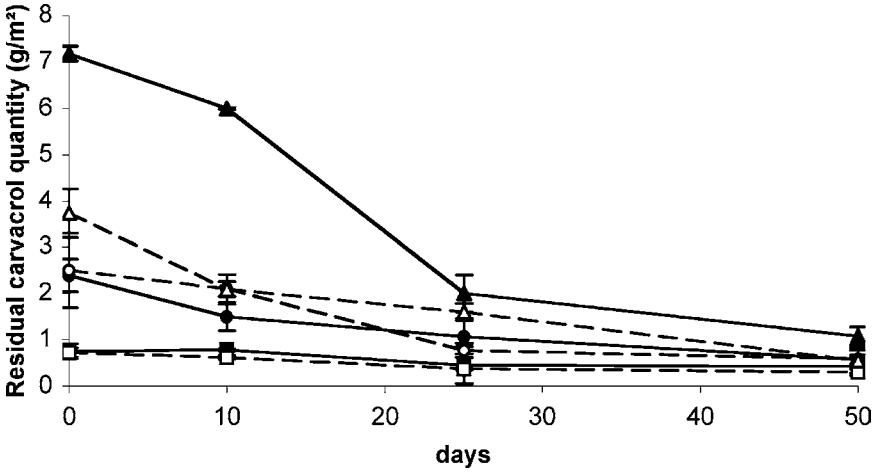

Figure 2. Kinetic carvacrol retention during storage at 30 °C and 60% relative humidity from papers coated with SPI and carvacrol (■) 10% (w/w), (●) 30% (w/w), and (▲) 60% (w/w) and with OSA-starch (□) 10% (w/w), (○) 30% (w/w), and (△) 60% (w/w).

matrices. For cinnamaldehyde, the losses were particularly high with OSA-starch and could be explained by a more difficult distribution of this compound in the matrix compared to carvacrol. Coating with a solution containing 60% of cinnamaldehyde (w/w of OSA-starch) could not be done because of the solution instability. The stability of the coating solutions was assessed with optical microscopy observation and by storage at 25 °C during 7 days to enhance flocculation and coalescence.

When cinnamaldehyde was used, no effect of concentration on rate losses was observed, whereas with carvacrol, highest losses were obtained with a concentration rate of 10%.

Generally accepted principles are that stronger interactions between aroma compounds and matrices are favored by the addition of lower aroma concentration: the size of emulsion droplets decreases with oil concentration, and smaller emulsion droplets are more physically stable than larger emulsions droplets (*33*, *34*). Unexpectedly, for SPI-coated papers, carvacrol loss percentages were significantly higher for the lowest 10% initial carvacrol concentration than for the higher 30 and 60% (w/w) concentrations in the SPI coating solution. The sole hypothesis could rely on Kim et al.'s study (*18*) demonstrating specifically that the SPI emulsion containing 30% of essential oil (w/w) exhibited a lower concentration of large particle size than the solution containing 10% (w/w). The efficiency of a matrix to completely coat the oil droplet during the homogenization is related to the ability to provide uniformly sized globules as found for a content of 30% (w/w). A more "complete" interaction of SPI with emulsified oil during homogenization at higher oil concentrations was shown to aid in dispersing larger size aggregates into smaller particles (*18*).

Moreover, SPIs are known for their excellent foaming properties due to their ability to unfold and to locate at the interface between air and water (*35*, *36*). During the coating and drying step, an important air/solution interface may be developed on the surface of the thin coating layer. Unfolding of the SPI molecule on this surface combined with the presence of large size carvacrol droplets, particularly at low concentrations prone to form a cream layer, could favor the migration toward the surface of a carvacrol quantity easily eliminated during the drying step.

The difference in cinnamaldehyde and carvacrol retentions from coating matrices may be explained by their own physicochemical properties and particularly their hydrophobic nature: according to their log *P*, carvacrol (with a log *P* of 3.52) is more hydrophobic than cinnamaldehyde (log *P* of 1.82). The better carvacrol retention by soy proteins suggested that hydrophobic interactions are preferentially involved as previously described with other aroma compounds (*31*, *37*). In most cases, the interactions between proteins and flavor compounds are reversible, involving hydrophobic and hydrogen bonding (*38*). Soy proteins and particularly 11S globulin exhibit high capacity of retention for lipophilic molecules due to their specific quaternary structures characterized by hydrophobic cavity (*37*).

OSA-starch is known to interact with aroma compounds through hydrophobic bonds thanks to the grafted octenyl groups. For native starches, interactions are principally due to inclusion complex formation in the helical cavity of amylose or sorption on the surface (*39*). OSA-starches are manufactured from partially hydrolyzed starches, and there is no available information about opportunity for inclusion type interaction. Moreover, the lowest retention of cinnamaldehyde compared to carvacrol by OSA-starch-coated papers suggested the predominant hydrophobic character of the interactions.

Retention of Carvacrol and Cinnamaldehyde from SPI- and OSA-Starch-Coated Papers in Accelerated Conditions of Storage. To investigate the role of the coated matrices in antimicrobial retention over accelerated conditions of storage, the residual amount of cinnamaldehyde and carvacrol from developed papers was studied for 50 days at 30 °C and 60% RH. The conditions of temperature and relative humidity selected for this study were not drastic but sufficiently high to involve an accelerated release of the active compounds.

From the uncoated paper, the release of antimicrobial agents was total within 1 day, indicating that the paper alone was not able to retain the agents.

The carvacrol retention from SPI- and OSA-starch coated papers is depicted in **Figure 2**. When the initial concentration of carvacrol was 10% (w/w), the residual amount after 50 days was the highest, about 53% with SPI and 60% with OSA-starch as coating matrices. After 50 days of storage in accelerated conditions, both matrices were able to keep more than 50% of the initial amount of carvacrol. For an initial concentration of 30% (w/w), the carvacrol retention kinetic was close whatever the coated matrix as well as the quantity released after 50 days (about 77%). In the case of an initial concentration of 60% (w/w), the carvacrol quantity at the beginning of the storage was much more important with SPI than with OSA-starch, whereas after 50 days of storage, the residual amount of carvacrol in

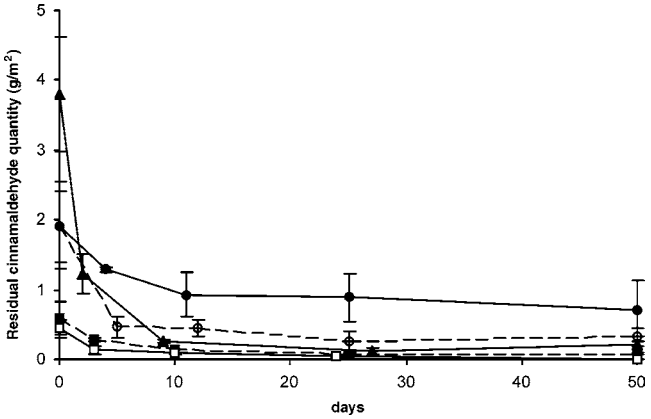

Figure 3. Kinetic cinnamaldehyde retention during storage at 30 °C and 60% relative humidity from papers coated with SPI and cinnamaldehyde (■) 10% (w/w), (●) 30% (w/w), and (▲) 60% and with OSA-starch (□) 10% (w/w), (○) 30% (w/w), and (△) 60% (w/w).

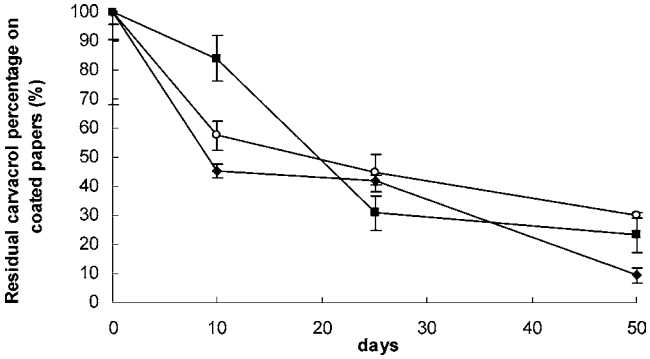

Figure 4. Kinetic carvacrol retention from coated papers containing 30% of carvacrol (w/w) during storage at 30 °C and 60% relative humidity for (■) OSA-starch-coated paper, (○) OSA-starch/SPI-coated paper, and (◆) SPI-coated paper.

the coated papers was close. However, in terms of quantity (g/m^2), the residual carvacrol amount was always higher for papers coated with SPI than with OSA-starch.

In contrast to carvacrol retention, the cinnamaldehyde retention pattern showed a two-step behavior with both coating matrices: a strong release was observed the first 5 days followed by a lowest release until 50 days of storage (**Figure 3**).

For SPI as coating matrix and 60% of cinnamaldehyde (w/w), the amount released was particularly high in the first days of storage; only 30% of aroma compound remained after 3 days on the coated paper. At 50 days of storage in the selected conditions, the residual amount of cinnamaldehyde in the coated paper did not exceed 5%.

Surprisingly, the cinnamaldehyde release was higher (86% after 50 days) for an initial concentration of 10% than for 30% (w/w); in the latter case, the residual amount was about 60% after 50 days. Several hypotheses can be mentioned to explain the specific release of cinnamaldehyde. It could be suggested that soy protein conformation may be modified as a function of the active agent concentration, inducing a different release behavior of cinnamaldehyde. Accordingly, changes in whey protein and sodium caseinate conformation occurring as a consequence of aldehyde addition have been reported (*40*).

To explain the low release at a concentration of 30% (w/w), the formation of a covalent bond through Schiff base, between cinnamaldehyde and proteins as reported for some aldehydes (*40*, *41*), could be suggested. However, such reactions are generally favored by more drastic conditions (temperature and pH) than those used in this study.

A plasticization effect of aroma compound at high concentration on coating matrix could be mentioned to explain the easier release of the compound. Such a phenomenon has been described for protein or polysaccharide films and was related to an increase in the diffusion of small molecules such as water, gas, or aroma compounds (*42*−*44*). This effect can also explain the faster release of carvacrol from SPI- or OSA-starch-coated paper for higher concentration of the compound.

The unexpected strong release of cinnamaldehyde for low concentration could also be explained by the effect of relative humidity on the diffusion of this compound: such phenomena may be in relation to the cinnamaldehyde water solubility. Indeed, due to its relatively polar nature, a part of cinnamaldehyde may be soluble in the water content of the matrix and may diffuse with water. However, the solubility of cinnamaldehyde in water is relatively weak, and the diffusion of cinnamaldehyde with water might be a limited phenomenon.

Cinnamaldehyde retention from OSA-starch matrixes was very weak; after 5 days of storage, for both of the coated papers, the cinnamaldehyde residual amount ranged between 25 and 30% and reached 2.7 and 17% after 50 days for initial concentrations of 10 and 30% (w/v), respectively. The relatively hydrophilic character of cinnamaldehyde and its chemical structure may explain the weak interactions with OSA-starch.

By comparison of the releases of carvacrol and cinnamaldehyde, it can be concluded that the specific nature of interactions between each active compound and each matrix strongly influenced the retention.

Carvacrol Losses and Release Time Course from OSA/SPI-Coated Papers. The industrial coating process preferentially requires solutions with dry matter ranging between 20 and 50% (w/v) (*45*) to enable a better coating weight control (*46*). Therefore, to increase dry matter, coating by combining OSA-starch and SPI was investigated. The introduction of a high SPI quantity was limited because of the high viscosity not being compatible with the coating process, and then a mixture of OSA-starch (20% w/v) and SPI (5% w/v) was assessed. Compared to cinnamaldehyde, carvacrol was the better retained compound from the tested coated matrices; it was, thus, selected to be introduced in the coating solutions at a concentration of 30% (w/w of dry matter).

The order of carvacrol introduction in the coating solution was found to be very important to avoid its exclusion from the coating matrices. Indeed, if OSA-starch and SPI were mixed together before carvacrol addition, the protein−starch interactions seemed to increase to the detriment of the carvacrol interactions, inducing its exclusion from the network. Protein and polysaccharide interactions have already been described (*47*), and it could thus be supposed that carvacrol may compete with hydrophobic OSA-starch toward proteins. Taking into account these limitations, a coating solution was prepared by adding first carvacrol to the SPI solution and subsequently OSA-starch.

The influence of carvacrol addition on OSA-starch/SPI coating solutions was studied by measurement of apparent viscosity. OSA/SPI coating solutions had a shear thinning behavior similar to that of the SPI solution, and carvacrol addition did not significantly influence the viscosity or the behavior of the OSA/SPI solution (data not shown).

Carvacrol losses from coated papers were measured after the coating and drying process and were about 25%, whereas they

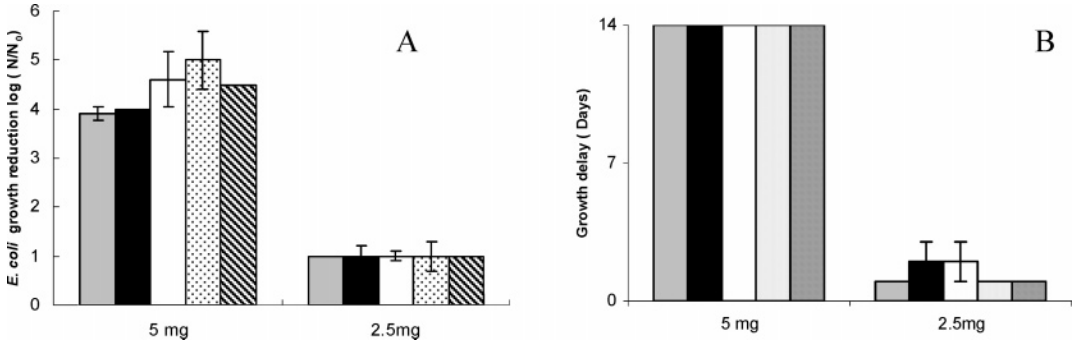

Figure 5. Antimicrobial activity of coated papers: (**A**) inhibition of *Escherichia coli* growth by the coated papers in a 1 L jar at 30 °C for 48 h; (**B**) *Botrytis cinerea* growth delay by the coated papers in a 1 L jar at 22 °C for 14 days. The bars represent, from left to right, SPI–carvacrol, OSA-starch–carvacrol, SPI–cinnamaldehyde, OSA-starch–cinnamaldehyde, and uncoated paper sprayed with carvacrol.

were about 10 and 17% from SPI and OSA-starch coated papers, respectively (**Table 1**). As mentioned previously, this result suggested the presence of specific interactions between OSA-starch and SPI, excluding therefore a part of carvacrol. The carvacrol release pattern was not obviously different from those of SPI- and OSA-starch coated papers (**Figure 4**), but the residual carvacrol quantity from OSA-starch/SPI-coated papers remaining on coated papers was the lowest (about 9% at 50 days). Mixing OSA-starch and SPI did not enhance the retention of carvacrol due, certainly, to the interaction between the two polymers. However, this mixing led to an increase of dry matter without increasing viscosity compared to the solutions alone. The use of different modified starch with a less important rate of grafting may be a possibility to avoid or to limit the interactions between polysaccharides and proteins and competition with carvacrol, responding consequently to industrial requirements.

Antimicrobial Properties of the Coated Papers. To be efficient, antimicrobial packaging had to procure two main rates in the release of active agent: low release during its storage without contact with products and fast release during its use as food packaging in close contact with products. Due to the hydrophilic nature of the coating matrices, high release of the antimicrobial agent was expected at high humidity, that is, favorable conditions for microorganism development.

The developed coated papers, (i) SPI−cinnamaldehyde, (ii) SPI−carvacrol, (iii) OSA-starch−cinnamaldehyde, and (iv) OSA-starch−carvacrol, were tested for their antimicrobial activity toward a bacterium, *Escherichia coli*, and a mold, *Botrytis cinerea*. Preliminary experiments were carried out to determine the minimal inhibition dose (MID) of carvacrol and cinnamaldehyde against *E. coli* and *B. cinerea* using uncoated papers sprayed with different aroma compound quantities in order to obtain 2.5, 5, and 10 mg of aroma compound/L of air. Microbial growth inhibition was expressed in terms of log(N/N_0) for *E. coli* (**Figure 5A**), whereas for *B. cinerea* (**Figure 5B**) growth delay was taken into account. For both aroma compounds and strains, the MID was about 5 mg/L (1 L was the volume of the experimental jar). For a concentration of 2.5 mg/L, a slight inhibition effect was observed for both microorganisms.

To test the antimicrobial efficiency of the coated papers, papers with a surface corresponding to the defined quantity (5 or 2.5 mg) were used. Depending on the ability of each matrix to retain each compound and on the concentration used [from 0.28 g/m^2 (OSA-starch and cinnamaldehyde 10%) to 7.2 g/m^2 (SPi and carvacrol 60%)], the size of the coated paper used to bring 5 mg varied between 7 and 179 cm^2. It was supposed that the major part of the amount was released in the condition of microbial testing (i.e., high humidity).

Whatever the matrix or the antimicrobial agent, a coated paper containing a quantity of 5 mg of carvacrol or cinnamalddehyde induced *E. coli* growth inhibition from 4 to 5 log and a growth delay up to 21 days for *B. cinerea*. A lower tested quantity of 2.5 mg inhibited by 1 log *E. coli* growth and increased *B. cinerea* growth delay by 1 day.

According to these results, no difference of antimicrobial activity between the different coated papers and the uncoated paper were noted. In the experimental conditions, high relative humidity, the coated matrices were able to release high amounts of antimicrobial agent (more than 90%), leading to a sufficient antimicrobial activity. This last assumption was confirmed by extraction of the residual amount of the agent on the papers after antimicrobial testing: only 10% of the agent was always present in the coated paper. It can be suggested that the high relative humidity in the jar due to the presence of agar favored the fast release of carvacrol and cinnamaldehyde, which generated an antimicrobial atmosphere.

Then, the sole determinant factor of antimicrobial activity was the amount of antimicrobial agent in the coated paper, which was related to the size of coated papers and to the ability of the matrix to retain the agent during the coating and drying process.

Therefore, it could be concluded that SPI- and OSA-starch-coated papers were able to retain in storage conditions carvacrol and cinnamaldehyde used as antimicrobial agents and to release them in favored conditions of microorganism development. Although some difference in retention and release (in a moderate condition of humidity) occurred depending on the matrices and the compound nature, both coated papers were able to create an antimicrobial modified atmosphere due to the fast active agent release by the matrices in favored conditions.

Selected active compounds could be thus immobilized in the SPI- and OSA-starch-coated papers and subsequently released, thereby inhibiting target microorganisms. However, a matrix based on soy proteins appeared to be a more suitable carrier, especially with carvacrol, than a matrix based on OSA-starch. Cinnamaldehyde, the more polar compound, was retained less than carvacrol, suggesting predominant hydrophobic interactions.

SPI−carvacrol-coated papers could act as a reservoir, able to gradually or totally release the antimicrobial agent and to maintain a constant microbial inhibitory effect. The ideal rate of antimicrobial release that provides the most effective inhibitory effect on microbial growth of a food product may depend on the environmental conditions such as temperature and relative

humidity. Further study has to be performed to investigate the effect of these parameters on the antimicrobial agents release time course.

For food product application, because the selected active agents are flavor compounds, their impact on organoleptic properties of the product has to be taken into account. It should be noted that this impact could be reduced by using a mixture of volatile organic compounds exhibiting an antimicrobial synergistic effect (*48*).

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LITERATURE CITED

(1) Torres, J. A.; Motoki, M.; Takano, M. Microbial stabilisation of intermediate moisture food surface: I. Control of surface preservative concentration. *J. Food Process. Preserv.* **1995**, *9*, 75−92.

(2) Chervin, C.; Westercamp, P.; El-Kereamy, A.; Rache, P.; Tournaire, A.; Roger, B.; Goubran, F.; Salib, S.; Holmes, R. Ethanol vapours to complement or suppress sulfite fumigation of table grapes. *Acta Hortic.* **2003**, *628*, 779−784.

(3) Padgett, T.; Han, I. Y.; Dawson, P. L. Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *J. Food Prot.* **1998**, *61*, 1330−1335.

(4) Han, J. H. Antimicrobial food packaging. *Food Technol.* **2000**, *54* (6), 56−65.

(5) Appendini, P.; Hotchkiss, J. H. Review of antimicrobial food packaging. *J. Appl. Polym. Sci.* **2002**, *3*, 113−126.

(6) Quintavalla, S.; Vicini, L. Antimicrobial food packaging in meat industry. *Meat Sci.* **2002**, *62*, 373−380.

(7) Suppakul, P.; Miltz, J.; Sonneveld, K.; Bigger, S. W. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *J. Food Sci.* **2003**, *68*, 408−420.

(8) Ouattara, B.; Simard, R. E.; Piette, G.; Begin, A.; Holley, R. A. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int. J. Food Microbiol.* **2000**, *62*, 139−148.

(9) Gennadios, A.; Weller, C. L. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* **1991**, *36*, 1004−1009.

(10) Gontard, N.; Guilbert, S.; Cuq, J. L. Edible wheat gluten films—influence of the main process variables on film properties using response-surface methodology. *J. Food Sci.* **1991**, *57*, 190−195.

(11) Guilbert, S.; Cuq, B.; Gontard, N. Recent innovations in edible and/or biodegradable packaging materials. *Cereal Foods World* **1997**, *14*, 741−751.

(12) Carlin, F.; Gontard, N.; Reich, M.; Nguyen-The C. Utilization of zein coating and sorbic acid to reduce *Listeria monocytogenes* growth on cooked sweet corn. *J. Food Sci.* **2001**, *66*.

(13) Cha, D. S.; Choi, J. H.; Chinnan, M. S.; Park, H. J. Antimicrobial films based on Na-alginate and kappa-carrageenan. *Lebensm. Wiss.-Technol.* **2002**, *8*, 715−719.

(14) Dole, P.; Joly, C.; Espuche, E.; Alric, I.; Gontard, N. Gas transport properties of starch based films. *Carbohydr. Polym.* **2004**, *58*, 335−343.

(15) Oussalah, M.; Caillet, S.; Salmieri, S.; Saucier, L.; Lacroix, M. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *J. Agric. Food Chem.* **2004**, *52*, 5598−5605.

(16) Pranoto, Y.; Rakshit, S. K.; Salokhe, V. M. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *Lebensm. Wiss.-Technol.* **2005**, *38*, 859−865.

(17) Reineccius, G. A. The spray drying of food flavors. *Drying Technol.* **2004**, *22*, 1289−1324.

(18) Kim, Y. D.; Morr, C. V. Microencapsulation properties of gum arabic and several food proteins: spray-dried orange oil emulsion particles. *J. Agric. Food Chem.* **1996**, *44*, 1314−1320.

(19) Qi, Z. H.; Xu, A. Starch-based ingredients for flavor encapsulation starch. *Cereal Foods World* **1999**, *44*, 460−465.

(20) Coma, V.; Sebti, I.; Pardon, P.; Deschamps, A.; Pichavant, F. H. Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. *J. Food Prot.* **2001**, *64*, 470−475.

(21) Hoffmann, K. L.; Han, I. Y.; Dawson, P. L. Antimicrobial effects of corn zein films impregnated with nisin, lauric acid and EDTA. *J. Food Prot.* **2001**, *64*, 885−889.

(22) Zivanovic, S.; Chi, S.; Draughon, A. F. Antimicrobial activity of chitosan films enriched with essential oils. *J. Food Sci.* **2005**, *70*, M45−M51.

(23) Helander, I. M.; Alakomi, H. L.; Latva-Kala, K.; Mattila-Sandholm, T.; Pol, I.; Smid, E. J.; Gorris, L. G. M.; von Wright, A. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.* **1998**, *46*, 3590−3595.

(24) Ultee, A.; Slump, A.; Stecging, G.; Smid, E. J. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. *J. Food Prot.* **2000**, *63*, 620−624.

(25) Roller, S.; Sheedhar, P. Carvacrol and cinnamic acid inhibit microbial growth in fresh cut melon and kiwifruit at 4 and 8 °C. *Lett. Appl. Microbiol.* **2002**, *35*, 390−394.

(26) Kim, H. O.; Park, S. W.; Park, H. D. Inactivation of *Escherichia coli* O157:H7 by cinnamic aldehyde purified from *Cinnamomum cassia* shoot. *Food Microbiol.* **2004**, *21*, 105−110.

(27) Kwon, J. A.; Yu, C. B.; Park, H. D. Bactericidal effects and inhibition of cell separation of cinnamic aldehyde on *Bacillus cereus*. *Lett. Appl. Microbiol.* **2003**, *37*, 61−65.

(28) Ben Arfa, A.; Combes, S.; Presiozi-Belloy, L.; Gontard, N.; Chalier, P. Antimicrobial activity of carvacrol related to its chemical structure. *Lett. Appl. Microbiol.* **2006**, *43*, 149−154.

(29) El-Sadi, H.; Carreau, P.; Esmail, N. Changes in the rheological and colloidal properties of paper coating liquids with paper-yellowing inhibition additives. *J. Colloid Interface Sci.***2004**, *271*, 496−503.

(30) Ben Arfa, A.; Chakrabandhu, Y.; Presiozi-Belloy, L.; Chalier, P.; Gontard, N. Coating papers with soy protein isolates as inclusion matrix of carvacrol. *Food Res. Int.* **2007**, *40*, 22−32.

(31) Williams, P. A.; Phillips, G. O. Introduction to food hydrocolloids. In *Handbook of Hydrocolloids*; Williams, P. A., Phillips, G. O., Eds.; Woodhead Publishing: Cambridge, U.K., 2000; pp 1−19.

(32) Damodaran, S.; Kinsella, J. E. Interaction of carbonyls with soy protein—thermodynamic effects. *J. Agric. Food Chem.* **1981**, *29*, 1249−1253.

(33) Risch, S. J.; Reineccius, G. A. Spray-dried orange oil: effect of emulsion size on flavor retention and shelf stability. In *Flavour Encapsulation*; Risch, S. J., Reineccius, G. A., Eds.; ACS Symposium Series 370; American Chemical Society: Washington, DC, 1988; pp 67−77.

(34) Mongenot, N.; Charrier, S.; Chalier, P. Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. *J. Agric. Food Chem.* **2000**, *48*, 861−867.

(35) Sorgentini, D. A.; Wagner, J. R. Comparative study of foaming properties of whey and isolate soybean proteins. *Food Res. Int.* **2002**, *35*, 721−729.

(36) Molina Ortiz, S. E.; Wagner, J. R. Hydrolysates of native and modified soy protein isolates: structural characteristics, solubility and foaming properties. *Food Res. Int.* **2002**, *35*, 511−518.

(37) Semenova, M. G.; Antipova, A. S.; Wasserman, L. A.; Misharina, T. A.; Golovnya, R. V. Binding of aroma compounds with legumin. II. Effect of hexyl acetate on thermodynamic properties of 11S globulin in aqueous medium. *Food Hydrocolloids* **2002**, *16*, 565−571.

(38) Guichard, E. Flavour retention and release from protein solutions. *Biotechnol. Adv.* **2006**, *24*, 226−229.

(39) Conde-Petit, B.; Escher, F.; Nuessli, J. Structural features of starch−flavor complexation in food model systems. *Trends Food Sci. Technol.* **2006,** *17*, 227−235.
(40) Meynier, A.; Rampon, V.; Dalgalarrondo, M.; Genot, C. Hexanal and t-2-hexenal form covalent bonds with whey proteins and sodium caseinates in aqueous solution. *Int. Dairy J.* **2004**, *14*, 681−690.
(41) Gremli, H. A. Interaction of flavor compounds with soy protein. *J. Am. Oil Chem. Soc.* **1974**, *51*, A95−A97.
(42) Quezada-Gallo, J. A.; Debeaufort, F.; Voilley, A. Interactions between aroma and edible films. 1 Permeability of methyl cellulose and low density polyethylene films to methyl ketones. *J. Agric. Food Chem.* **1999**, *47*, 108−113.
(43) Han, J. H.; Krochta, J. M. Wetting properties and water vapor permeability of whey-protein-coated paper. *Trans. ASAE* **1999**, *42*, 1375−1382.
(44) Pommet, M.; Redl, A.; Guilbert, S.; Morel, M. H. Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials *J. Cereal Sci.* **2005**, *42*, 81−91.
(45) Laudone, G. M.; Matthews, G. P.; Gane, P. A. C. Observation of shrinkage during evaporative drying of water-based paper coatings. *Ind. Eng. Chem. Res.* **2004**, *43*, 712−719.
(46) Vartiainen, J.; Skytta, E.; Enqvist, J.; Ahvenainen, R. Properties of antimicrobial plastics containing traditional food preservatives. *Packaging Technol. Sci.* **2003**, *16*, 223−229.
(47) De Kruif, C. G.; Tuinier, R. Polysaccharide protein interactions. *Food Hydrocolloids* **2001**, *15*, 555−563.
(48) Tunc, S.; Chollet, E.; Chalier, P.; Preziosi-Belloy, L.; Gontard,N. Combined effect of volatile antimicrobial agents on the growth of *Penicillium notatum*. *Int. J. Food Microbiol.* **2007**, *113*, 263−270.

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