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Moisture and Temperature Triggered Release of a Volatile Active Agent from Soy Protein Coated Paper: Effect of Glass Transition Phenomena on Carvacrol Diffusion Coefficient

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Carvacrol release from SPI-coated papers was evaluated at different relative humidities (RH; 60, 80, and 100%) and storage temperatures (5, 20, and 30 °C). Effective carvacrol diffusivities were determined from experimental release kinetics and by using a mathematical model based on Fick's second law. Increasing storage temperature and RH lead to an increase of carvacrol diffusivity. Depending on the relative humidity, the carvacrol effective diffusivity varied from 1.71×10^{-16} to 138×10^{-16} m^2/s at 30 °C, from 0.85×10^{-16} to 8.78×10^{-16} m^2/s at 20 °C, and from 0.11×10^{-16} to 7.50×10^{-16} m^2/s at 5 °C. The combined effect of relative humidity and temperature on diffusivity was particularly marked at 30 °C and 100% RH. The temperature and relative humidity dependence of carvacrol release was related to the glass transition phenomenon and its effect on chain protein mobility and carvacrol diffusivity.

KEYWORDS: Antimicrobial packaging; carvacrol diffusivity; glass transition temperature; relative humidity; controlled release

INTRODUCTION

In most packaged solid or semisolid foods, micro-organisms primarily grow on the surfaces of the foods and significantly decrease their safety (*1*). Dipping and spraying of antimicrobial substances is done to protect food surfaces from spoilage (*2*). However, this method of antimicrobial agent addition implies the use of unnecessarily high contents of antimicrobial agents because of the migration of the active agent into the core of the product, leading to a reduction of required food surface concentration. To overcome this problem, antimicrobial packaging has been developed, in which antimicrobial agents can be slowly released from the packaging onto the food surface during storage (*3*−*5*).

Several incorporation methods of antimicrobial agent in packaging were achieved; antimicrobial agents have been impregnated into packaging materials before final extrusion (*6, 7*), dissolved into coating solvents (*8*), added in coating materials (*5, 9*), or mixed into sizing/filling materials such as paper and cardboard (*10*−*12*).

The efficacy of antimicrobial packaging is dependent on the diffusion rate of the active agent toward the food surface, the initial content in the antimicrobial agent, and the targeted food shelf life. Using a nonvolatile antimicrobial agent implies an intimate contact between the food and the packaging material, to enable a sufficient active agent diffusion, and thus concentration, on the surface of the foodstuff. Among the possibilities of antimicrobial packaging design, an interesting approach lies in introducing volatile antimicrobial agents in the packaging material. In this case, direct contact of the active packaging material with the food surface is no longer necessary because the antimicrobial agent acts via the packaging headspace atmosphere toward the food surface. The release rate of the volatile agent from the packaging system is an important parameter, highly dependent on its volatility, which relates to the chemical interactions between the volatile agent and the packaging materials (*11*).

The release of volatile compounds can be strongly dependent on environmental conditions such as temperature and relative humidity (RH) when these two parameters affect the matrix structure (*13*−*15*). The active agent release characterization is quite important to estimate the efficacy of an antimicrobial packaging and the shelf life of the packaged foodstuff.

In recent studies on the development of antimicrobial paper-based packaging, carvacrol and cinnamaldehyde, two volatile aroma compounds, were selected as antimicrobial agents and included in soy protein and modified starch coating matrix of paper (*12, 16, 17*). The ability of these matrices to retain carvacrol and cinnamaldehyde during the coating process of the paper (preparation of the coating solution, coating, and drying)

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and to limit the release during further storage in accelerated emitting conditions (30 °C and 60% RH) has been demonstrated. The antimicrobial properties of such coated papers were clearly proved by studying the growth inhibition of *Escherichia coli* or growth delay of *Botrytis cinerea*. Due to the release of the aroma compounds in high-moisture conditions, the protein-coated papers were able to rapidly create an antimicrobial atmosphere in packaging headspace. It is known that combined effect of temperature and relative humidity can affect the aroma compound release rate from biopolymer matrices in relation to glass transition change (*13*, *18*, *19*). The glass transition phenomenon is well-known to modify not only the molecular mobility of the matrix but also that of components entrapped in the matrix such as aroma compounds, for example. Changes in glass transition temperature could, thus, explain some particular behavior of release from a biopolymer matrix. Because controlling the antimicrobial agent release is of major significance for process development of antimicrobial packaging, the purpose of this study was to investigate the combined effect of temperature and relative humidity on carvacrol release from soy protein isolate (SPI)-coated paper in relation with glass transition phenomena. Different temperatures (5, 20, and 30 °C) and relative humidities (60, 80, and 100%) were tested. A mathematical model using Fick's second law was developed to determine carvacrol effective diffusivity, leading to an easier comparison of the combined effect of temperature and relative humidity. Glass transition temperatures at different relative humidities were determined using modulated differential scanning calorimetry (MDSC) and dynamic mechanical thermal analysis (DMTA) and tentatively related to the carvacrol diffusivity changes.

MATERIALS AND METHODS

Materials. A commercial base paper (70 g/m^2) was provided by Ahlstrom Research and Services and used as support for coating. SPIs were purchased from Seah International (SAMPROSOY 90 NB; Wimille, France). According to the supplier, the product had 8% moisture content and contained 91.8% protein. Carvacrol, the antimicrobial agent, and 2-nonanol (used as internal standard) were purchased from Sigma Aldrich (St Quentin Fallavier, France). Carvacrol is a phenolic compound found in high concentration in oregano essential oil. It is characterized by the following physicochemical properties: molecular weight, 150.22 g mol^{-1}; saturated vapor pressure at 25 °C, 6.4 Pa; and estimated hydrophobicity (log *P*), 3.52.

Preparation of Carvacrol/SPI-Coated Papers. SPI-coated papers were prepared according to the procedure previously described (*12*). SPI (10% w/v) was dissolved in distilled water heated at 50 °C, and the solution was continuously stirred for 30 min at 50 °C. After the cooling of the solution to 25 °C, carvacrol at a percentage of 30% (w/w of SPI) was added. Homogenization was carried out with an Ultra-Turrax (T-25, IKA Labotechnik, Germany) at 8000 rpm for 10 min. The coating process was performed at 25 °C: a support paper was maintained on a perforated iron plate under partial vacuum (21 cm × 30 cm), and the coating solution was applied by an adjustable micrometer thin layer chromatography applicator (Braive Instruments, Chécy, France). Then, coated papers were dried for 3 h at 23 ± 2 °C and at 50 ± 5% RH. The final material obtained is, thus, a bilayer film composed of a paper covered with a SPI coating containing the antimicrobial agent.

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

Thickness Measurement. The average thickness was determined using a hand-held caliper digital micrometer (Braive Instruments) from 10 measurements taken at random over the surface. The coating thickness was calculated from the overall thickness value minus the paper thickness. The average thickness was measured at the beginning and at the end of the release experiment. The coating thickness was found to be (30.6 ±1.6) × 10^{-6} m.

Water Sorption Kinetic. The water sorption kinetic in SPI-coated paper containing no carvacrol was determined at 20 °C (two replicas) using a Cahn microbalance (Dynamic Vapor Sorption System, SMS, London, U.K.) by using a protocol previously tested and validated (*20*). The Cahn balance allowed recording of mass product evolution as compared to time and controlled low relative humidity variations over a large range of water activity (0−0.97). Disk geometry samples (10−20 mg) with diameter of 7.5 mm were loaded and preequilibrated at 0% relative humidity (±0.4%) by a continuous flow of dry air. Preequilibration conditions were defined automatically when the change in sample mass as a function of time was lower than 0.002%/min. Samples were equilibrated at successive levels of relative humidity (from 10 to 95%). Steps in relative humidity differences were chosen to be equal to 10% from 0 to 90% of RH and equal to 5% from 90 to 95%. For each relative humidity level, the equilibrium conditions were obtained when the change in sample mass as a function of time was lower than 0.002%/min. From the equilibrium points for each relative humidity level tested, the water sorption isotherm was obtained. From the transient state of the water sorption kinetic, apparent diffusivity values of water in the SPI-coated paper were determined by using a mathematical model previously validated (*20*).

Kinetic Release of Carvacrol from SPI-Coated Papers. Pieces of papers coated or not (3 cm × 3 cm) were put on a tray of a gas flushing chamber (Bioblock, Illkirch, France) with a volume of about 370 cm^3) at the selected temperature and relative humidity. The chamber was maintained at 30, 20, or 5 °C, and for each temperature, the relative humidity (60, 80, and 100%) was adjusted using a humidified air flux (25 mL/min) and the presence of saturated salt in the incubator. At prescribed time intervals, coated papers were taken from the incubator, for analysis; carvacrol compound content of the papers was immediately determined according to the extraction method previously described (*16*, *17*). Pieces of coated papers were immersed in 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 solution was shaken for 16 h under magnetic agitation (300 min^{-1}). The organic phase containing carvacrol 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 following program of temperature was used; 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 carvacrol was performed using the internal standard method. The extraction yield was estimated by depositing a known quantity of carvacrol on the coated papers and by applying the extraction procedure described above. It was found to be about 87 ± 5% (10 replications) for SPI−carvacrol. For each condition, time, temperature, and RH, the extraction was done in triplicate from two different coated papers. The results were expressed in terms of ratio corresponding to the residual amount of carvacrol at time *t* to the initial amount after coating process and drying.

Mathematical Modeling and Apparent Diffusivity Identification. For the modeling of antimicrobial agent release from the coated paper toward atmosphere, several assumptions were made:

(1) The coated paper sample put on trays in the incubator was assumed as a plane sheet. The coating thickness is *l*.

(2) The antimicrobial agent remains mainly in the coating layer,and the migration from the coating into the paper is negligible.

(3) The antimicrobial agent transfer was supposed to be monodirectional, in the axial direction with the axis origin at the interface between the paper and coating.

(4) The apparent diffusivity (*D*) was assumed to be constant whatever the antimicrobial agent concentration.

The internal diffusion of active agent in the coating was modeled using Fick's second law:

$$\forall x, \quad 0<x<l, \quad \forall t, \frac{\partial C}{\partial t} = D_{app}\frac{\partial^2 C}{\partial x^2} \tag{1}$$

The boundary conditions were as follows:

At the interface between the paper and the coating, the mass flux was considered to be null:

$$x=0, \quad \forall t, \quad \frac{\partial C(0,t)}{\partial x} = 0 \tag{2}$$

At the interface between the coating and the surrounding atmosphere, the active agent concentration was taken as null in the atmosphere because the air flux blew the incubator, enabling a purge of the air at each time. The corresponding active agent concentration in the film was supposed constant equal to C_∞ according to the partition phenomenon:

$$x=l, \quad t=0, \quad C(l,t)=C_\infty \tag{3}$$

The initial condition was given by

$$0<x<l, \quad t=0, \quad C(x,0)=C_0=\text{constant} \tag{4}$$

where C_0 was the initial concentration in active agent in the coating after processing.

In these conditions, and if X_t denotes the carvacrol concentration in the coated paper (g m^{-2}) at time t, X_0 is the carvacrol concentration within the coated paper (g m^{-2}) at time 0 and X_∞ is the active agent concentration in the coating at time t supposed to be infinite, the particular solution of the system of eqs 1−4 is given by Crank (*21*):

$$\frac{X_t - X_0}{X_\infty - X_0} = 1 - \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n+1)^2}\exp\left[\frac{-D_{app}(2n+1)^2\pi^2)}{4l^2}t\right] \tag{5}$$

In a first approximation and for all of the conditions, the value of X_∞ has been chosen from the maximum value of antimicrobial agent release obtained from the experiment carried out at 30 °C and 100% RH (i.e., 0.027 g/g).

Simulations of the diffusion of carvacrol in coated paper were performed using eq 5 programmed on MATLAB software (The Mathworks Inc., Natick, MA). Active agent apparent diffusivities in the coating layer for different relative humidities and temperatures were obtained from experimental data by minimizing the sum of squared error between experimentally measured and predicted values of carvacrol content using the Levenberg−Marquardt algorithm.

The root-mean-square error (RMSE) was used to estimate the quality of model fitting and was calculated as follows:

$$\text{RMSE} = \sqrt{\frac{(\hat{y}-y)^2}{(N-p)}} \tag{6}$$

where $\hat{y}$ is the vector of the predicted values, y is the vector of the experimental values, N is the number of terms in the predicted or experimental vector, and p is the number of estimated parameters. The model fitting is considered to be good if the RMSE value is close to the experimental error (average values of confidence intervals obtained for each experimental point).

Determination of Glass Transition Temperature. Modulated differential scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA) were used to determine glass transition temperatures (T_g). As the support paper properties can interfere with thermal and mechanical properties of coated layer, self-supported films were prepared. A film-forming solution of isolated soy protein (10% w/w) containing 30% (w/w) of carvacrol was made in the same conditions (temperature and stirring) as those used for coating solution preparation. The film-forming solution was spread onto a crystal PVC plate as support using a thin layer chromatography applicator. The film was dried at 25 °C for 3 h. Before DSC or DMTA analyses, the samples were equilibrated at 20 °C in different humidity-controlled containers for 2 weeks. The relative humidity was imposed by salt ($Mg(NO_3)_2$-saturated solution giving a RH of 54.4% or by water (for 100% RH).

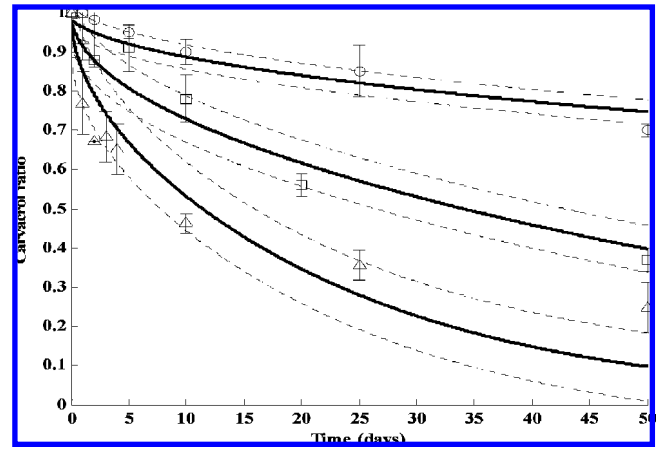

Figure 1. Comparison between experimental (symbols) and predicted (continuous curves) carvacrol retention as a function of time at 60% RH and (○) 5 °C, (□) 20 °C, or (△) 30 °C. Vertical bars and dotted lines represent, respectively, experimental and predictive errors.

MDSC. Measurements were performed on a TA instrument 2920 CE modulated DSC coupled with a refrigerant cooling system. The samples (10 mg of films) were packed down and hermetically sealed into the aluminum pans. The pans (empty and full) were weighed precisely. An empty pan was used as reference. The pans were heated at a scan rate of 2 °C /min according to the following program: equilibration at −50 °C and heating to 200 °C; amplitude of modulation at 1 °C with a period of 60 s. Reversible and nonreversible heat flow signals were separated from the total heat flow signal. The glass transition temperature was recorded from the inflection point of the changes in heat capacity and determined from the reversing heat flow signal. All samples were realized in duplicate, which were in good agreement.

DMTA. Analyses of films were conducted with a Dynamic Mechanical Thermal Analyzer MK III (Rheometric Scientific, Piscataway, NJ), equipped with a cryogenic system fed with liquid nitrogen. Analyses were performed on rectangular samples with an approximate length of 10 mm and a width of 6 mm. The thickness of the sample was close to 1 mm, and the exact value was determined to ±1 μm using a hand-held caliper digital micrometer (Mitutoyo, Japan Braive Instrument). For each material, three samples were tested. A variable-amplitude, sinusoidal mechanical stress was applied to the sample at the frequency of 1 Hz, resulting in 0.01% strain amplitude. The compression mode of deformation was chosen for use with the sample geometry. Temperature scans (from −40 to 200 °C) were performed at a heating rate of 2 °C/min. During analysis, the stored values were the storage modulus (E'), the loss modulus (E''), and the loss tangent (tan δ). The location of the glass transition temperature (T_g) was determined from the onset of the drop of storage modulus E' ($T(E')$) or from the maximum of the tan δ peak (T(tan δ)).

RESULTS

Carvacrol Release from Coated Papers and Apparent Diffusivities. Carvacrol release from coated papers as a function of time was experimentally determined for 60, 80 and 100% RH at three temperatures, 5, 20, and 30 °C. Results are reported in **Figures 1**, **2**, and **3**. As expected, for the three RH values investigated, the release of carvacrol from the coated papers toward atmosphere was strongly enhanced by the temperature. At 60% RH, after 50 days of storage at 5 °C, the residual carvacrol amount was about 80% of the quantity initially present at the beginning of the storage, whereas at 30 °C the residual was about 30% (i.e., a decrease of the residual amount of about

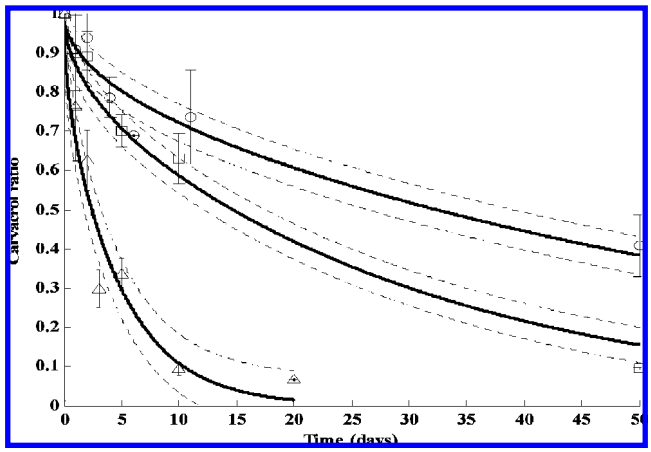

Figure 2. Comparison between experimental (symbols) and predicted (continuous curves) carvacrol retention as a function of time at 80% RH and (○) 5 °C, (□) 20 °C, and (△) 30 °C. Vertical bars and dotted lines represent, respectively, experimental and predictive errors.

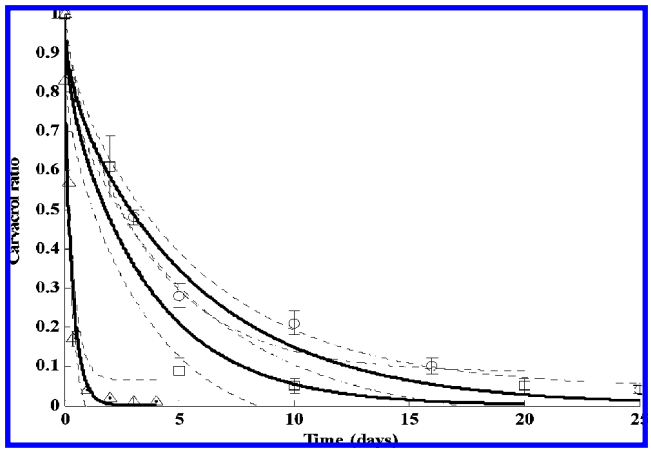

Figure 3. Comparison between experimental (symbols) and predicted (continuous curves) carvacrol retention as a function of time at 100% RH and (○) 5 °C, (□) 20 °C, and (△) 30 °C. Vertical bars and dotted lines represent, respectively, experimental and predictive errors.

3 times between 5 and 30 °C) (**Figure 1**). At 80% RH, the effect of temperature was even more pronounced, with a decrease in the residual quantity of carvacrol from 45% at 5 °C to <10% at 30 °C (i.e., a decrease of >4 times). The difference between 60 and 80% RH was less pronounced at low temperature than at high temperature. At 100% RH, the carvacrol was very quickly released (in <25 days against >50 days at 60 and 80% RH) but with differences in relation to the temperature. At 30 °C the carvacrol release was total within 1 day with a residual amount of 0.1%, whereas at 20 and 5 °C, the release was not totally achieved after 25 days. It is obvious from these results that for a given temperature, the relative humidity strongly affected the release of carvacrol, always with a higher release of the aroma compound at 100% compared to 80 and 60% RH. In agreement with these findings, Rosenberg et al. (*22*) reported that for aroma encapsulated in whey proteins release of ethyl butyrate increased with relative humidity of storage. From the results of the study, it can be concluded that the storage of coated papers at 5 °C and 60% RH could be suitable to avoid losses of carvacrol. In contrast, high temperature and relative humidity favored the fast release of carvacrol.

To quantify the effect of relative humidity and temperature on the release of carvacrol from SPI matrix, apparent diffusivities of carvacrol were identified for each RH and temperature

Table 1. Apparent Diffusivity of Carvacrol in SPI Coating and RMSE Obtained for the Different Temperatures and Relative Humidities[a]

temp (°C)	RH (%)	apparent diffusivity (10^{-16} m^2/s)	RMSE	exptl error
5	60	0.11 (0.06−0.16) A	0.03	0.05
	80	0.66 (0.43−0.89) B	0.05	0.08
	100	7.50 (5.79−9.21) C	0.04	0.03
20	60	0.85 (0.39−1.31) B	0.06	0.05
	80	1.46 (0.96−1.96) BD	0.04	0.06
	100	8.78 (3.76−13.8) C	0.08	0.04
30	60	1.71 (0.94−2.48) D	0.09	0.04
	80	8.93 (5.65−12.21) C	0.07	0.05
	100	138 (101−175) E	0.07	0.03

[a] Values within parentheses are 95% confidence intervals on the identifed pararameter given by the model. Values in the same column with different letters are significantly different by multiple comparisons using MATLAB software.

investigated using eq (5), the experimental data, and the optimization procedure. By using eq 5 several assumptions have been made, which now have to be gone into more detail and justified. First, eq 5 was applied to the coating layer only, the carvacrol diffusion through the paper being neglected. Indeed, the diffusivity of carvacrol in the paper is widely higher than that observed in the SPI layer (590×10^{-16} in the paper against 1.7×10^{-16} m^2/s in the SPI layer at 30 °C and 60% RH). Consequently, even if some carvacrol migrates into the paper layer, the rate-limiting compartment for the carvacrol diffusion was the SPI layer (diffusivity in the paper was 345-fold higher than that in the SPI layer) and not the paper. Consequently, the paper acted as a support and the carvacrol diffusion in the paper layer could be neglected. The second hypothesis was that the carvacrol diffusion takes place in a homogeneous media as previously observed by SEM analysis (*17*). The third hypothesis made by using eq 5 is that the flux of water from the external atmosphere to the SPI layer was much faster than the carvacrol release and that, consequently, the SPI layer could be assumed at a constant water content during the entire experiment. To justify this assumption, the water sorption kinetic in the SPI-coated paper was carried out. Using the transient state data, a diffusivity value in the SPI-coated paper of 1.2×10^{-11} m^2/s for the humidity range of 60−100% was identified. By using this value in mathematical simulations, it was found that the time required to reach the moisture equilibrium would be 30 min at 60% RH, 50 min at 80% RH, and 100 min at 100% RH. As the release kinetics of carvacrol was measured on 50 days, the impact of SPI local water concentration was supposed to not interfere with the carvacrol diffusion. The carvacrol diffusivity identified for each migration experiment was obtained, in fact, for a constant moisture content value of coated SPI paper. In agreement with sorption isotherm, this content was equal to 0.0857 g/g dry weight for 60% RH, 0.1349 g/g dry weight for 80% RH, and 0.2678 for 99% RH. Finally, the last hypothesis made is that the thickness of the SPI layer remains constant during the migration experiment. This was justified because the variations in thickness of the SPI-coated paper were negligible between the beginning and the end of the migration experiment.

Experimental and simulated data are compared in **Figures 1−3**. The model fitted well the experimental results for all of the tested temperatures and relative humidities. The RMSE values, which represent the quality of the model fitting, were always in the same range or lower than the experimental errors (**Table 1**). This quite satisfying fitting justified the assumptions and allowed us to suppose no impact of local carvacrol concentration on the diffusivity values. Depending on the relative humidity and temperature, the apparent diffusivities

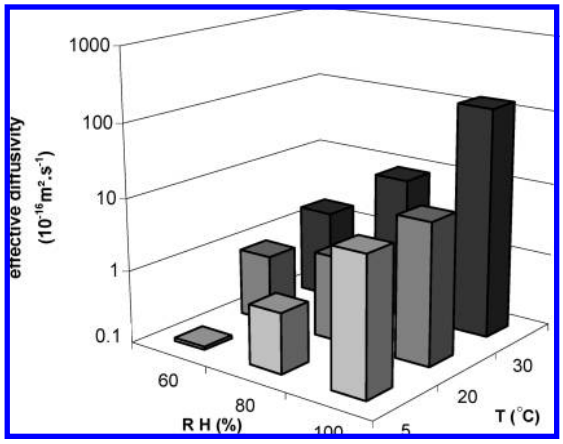

Figure 4. Apparent diffusivity of carvacrol (m^2/s) in the SPI coating layer as a function of relative humidity and temperature.

Table 2. MDSC Data Obtained from Reversible and Nonreversible Heat Flow for SPI Films Equilibrated at Two Relative Humidities

nonreversible heat flow		reversible heat flow			
T °C endo		T °C exo		T_g °C	
54.4% RH	100% RH	54.4% RH	100% RH	54.4% RH	100% RH
125	70	110	60	59	1

varied with a factor superior to 1000, from 0.11×10^{-16} to 138×10^{-16} m^2/s. The highest value of carvacrol apparent diffusivity was clearly obtained at 30 °C for 100% RH (**Figure 4**). Within the same temperature of storage and according to the confidence intervals, carvacrol apparent diffusivity values were significantly different related to the relative humidity, except at 20 °C for 60 and 80% RH, at which the two diffusivity values identified were not significantly different. Carvacrol apparent diffusivity increased with the relative humidity with always the highest value at 100% RH, but its effect was more obvious at 5 and 30 °C than at 20 °C. For a given relative humidity, the effect of temperature increase on apparent diffusivity values was less pronounced than that of relative humidity. Indeed, for a given RH, if all of the diffusivity values increased with the temperature, according to the confidence interval obtained on the identified diffusivity value, this effect was significant only for 60% RH between 5 and 20 °C and for 80 and 100% RH between 20 and 30 °C. Close diffusivity values were observed for different conditions of storage such as 60% RH at 20 °C and 80% RH at 5 °C or 60% RH at 30 °C and 80% RH at 20 °C or 80% RH at 30 °C and 100% RH at 5 and 20 °C, showing the combined effect of temperature and relative humidity. This effect of temperature and RH could be related to structural changes in the SPI matrix caused by these two parameters. From this consideration, the glass transition temperature of the SPI matrix was measured as a function of relative humidity.

Effect of Relative Humidity on Glass Transition Temperature. The effect of relative humidity on T_g was measured only at two extremes of relative humidity at 54.4% (corresponding to equilibration with salt-saturated solution of ($Mg(NO_3)_2$)) and at 100% RH by equilibration with water.

The thermal characteristics of the film obtained from total, reversing, and nonreversing heat flow signals from MDSC data for the two RH conditions are summarized in **Table 2**. As expected, in the studied interval of temperature, the flow signals of the sample maintained at the highest RH were different from the sample maintained at the lowest RH. The major endothermic peak measured from the nonreversible flow decreased with the increase of RH. From reversible flow heat signal, an exothermic peak and the glass transition temperature were observed; a shift of the values of the onset temperature of exothermic peak (from 110 to 60 °C) and of glass transition temperature (from 59 to 1 °C) was clearly found with the increase of relative humidity. In the literature (*23*), for native SPI stored at 50% RH, an endothermic peak was found at 103 °C, the onset temperature of exothermic peak around 73 °C, and the T_g around 50 °C. These authors have also studied the effect of relative humidity, and they have observed a decrease of exothermic peak onset temperature (from 85 to 70 °C) with relative humidity (from 23 to 85% RH). These results were in agreement with the changes observed for the antimicrobial films; the difference in values could be explained by the use of pure component (SPI) against films containing carvacrol in this study. Indeed, the presence of carvacrol could modify the thermal properties of the SPI films. The study of hexyl acetate on the thermodynamic behavior of 11S globulin, a legumin protein, had shown that aroma compound could affect the thermal properties (*24*). These authors had shown that hexyl acetate concentration depends on thermodynamic properties. Below the level of saturation for the protein according to the hexyl acetate binding isotherm, the interaction with hexyl acetate caused an apparent rise in the conformational stability of the native protein, whereas an excess of hexyl acetate concentration led to some decrease in the values of thermodynamic parameters of the heat denaturation of the protein.

To complete the glass transition temperature study, the thermomechanical behavior of the carvacrol SPI films preequilibrated at the two relative humidities was studied. Representative DMTA curves (storage modulus E' and tan δ vs temperature) obtained with film samples maintained at 54.4 and 100% RH are shown in **Figure 5**. The α relaxation process defined by a maximum of tan δ associated with a drop-in storage modulus corresponding to drastic changes in the sample physical state was observed. This event corresponds to the transition zone from glass-like to rubber-like consistency of the sample (*25*). Above this zone, noisy values of tan δ were obtained as E' and E'' dropped by $>10^3$ Pa from the glassy to the rubbery state. This can be due to sample partial collapse and led to invalid tan δ and modulus values, which were not reported in **Figure 5**. A similar phenomenon has been observed with wheat gluten thermoplastic materials (*26*). Independently, the glass transition temperatures (T_g) were identified to the temperature of the maximum of the tan δ. Increasing the RH moved the relaxation phenomenon toward lower temperatures and caused the T_g to shift from 33 to −5 °C. A second peak was observed at 48 °C as multiple events occurred. However, these values were slightly different from those obtained by MDSC analysis. It is usual that the T_g from DMTA differed from those measured from MDSC due to the dynamic nature of DMTA, but the plasticizing effect of water was manifested in the two cases. Moreover, DMTA analyses were realized at ambient relative humidity (not in controlled relative humidity), and it could be assumed that the samples' water content changed in relation to their initial content. These changes could modify the accuracy of T_g determination. T_g of SPI films plasticized with different concentrations of glycerol have been determined by DMA (*27*), and multiple T_g values associated with glycerol and protein-rich phases were observed. However, T_g decreased with the increase of plasticizer (glycerol). For the lowest concentration of glycerol, a T_g of 112 °C associated with soy protein was found. This high value compared to the values found in our

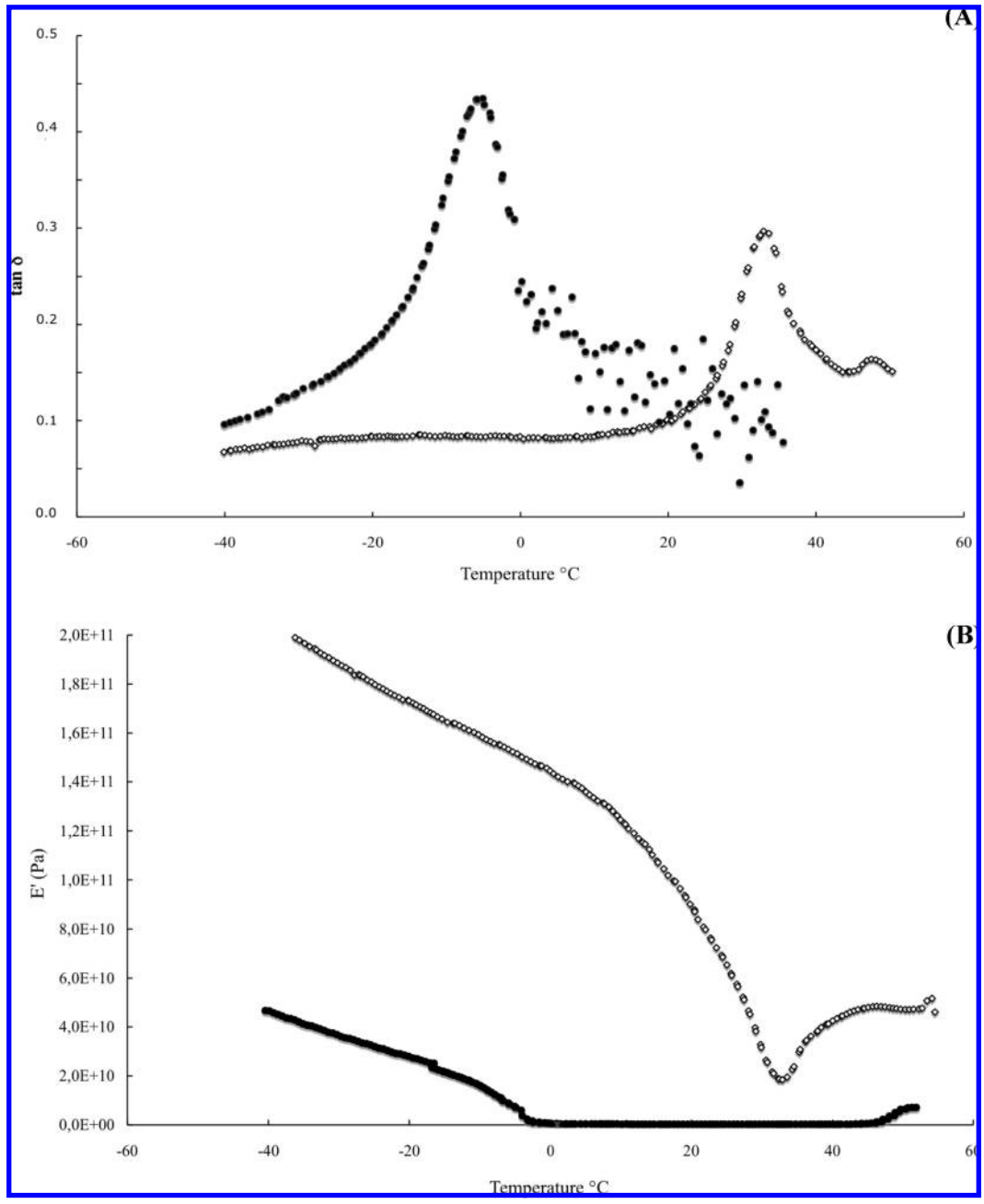

Figure 5. Changes in tan δ (**A**) and in storage modulus (**B**) with temperature for SPI films containing carvacol at 54.4 and 100% RH.

study could be due to difference in RH of film (data not given) and as suggested below by the presence of carvacrol. The T_g value of a polymer is also governed by chemical composition and structural features, which can depend on physical conditions of film processing.

DISCUSSION

The influence of temperature and relative humidity of storage on carvacrol release from SPI-coated paper might be explained by structural changes occurring in the soy protein matrix as a function of external conditions (RH and temperature) as mentioned before for aroma compounds encapsulated in biopolymer matrices (*15, 21, 22*). The carvacrol apparent diffusivity increase with RH and temperature can clearly be correlated to the glass transition changes of protein matrix. Below the T_g, the material is rigid in a "glassy state" and above the T_g, it becomes viscoelastic in a "rubbery state". The change of state induces an increase of specific chain mobility of the polymer and an increase of small molecules diffusivity. As observed, the T_g of SPI films was significantly affected by the presence of moisture, and it can be supposed that papers coated with SPI were affected in the same way. According to T_g determination at 54.4% RH, the SPI matrix was in a glassy state. Confirming this, the carvacrol apparent diffusivity was particularly low but affected by the temperature increase. As the RH increases and, thus, the water concentration, the transition occurs, leading to a high release of the aroma compound. At 100% RH for the temperatures, the SPI matrix was clearly in a rubbery state (**Table 2**) or in the glass transition zone for the temperatures very close to 5 °C. The glass transition temperature at 80% RH has not been measured, but it can be deduced from diffusivity results (similar diffusivity values for 30 °C/80% RH and 20 °C/100% RH) that the transition occurred near or above 20 °C.

For a given RH, the strong modification of diffusivity with temperature could be also explained by the variation of carvacrol

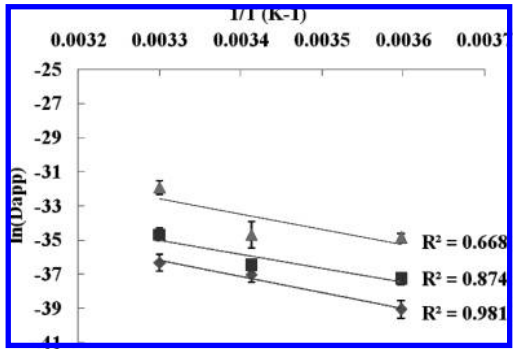

Figure 6. Arrhenius plot of apparent diffusivity of carvacrol in the SPI coating (m^2/s) as a function of $1/T$ (▲, 100% RH; ■, 80% RH; and ◆, 60% RH).

volatility. The vapor pressure of an aroma compound and consequently its volatility are exponentially affected by the temperature. Between 5 and 30 °C, the vapor pressure of carvacrol increases 16-fold. The variations of diffusivity between these two temperatures at 60, 80, and 100% RH were about 15.5-, 13.5-, and 18-fold, respectively, showing a relatively good correlation with vapor pressure variation, particularly at the smallest relative humidity. The effect of temperature was tentatively described by the Arrhenius equation (eq 7) adapted to effective diffusivity:

$$D = D_0 \exp^{-E_a/RT} \quad (7)$$

At 60% RH, the Arrhenius law was validated with sufficient accuracy ($R^2 > 0.95$), whereas for the highest relative humidity the Arrhenius law was not validated ($R^2 = 0.87$ and 0.67 for 80 and 100% RH, respectively) (**Figure 6**). These results confirm that the SPI matrix was in a glassy state over the entire range of temperature investigated as stated by the MDSC analysis. On the contrary, the nonlinearity of log D_{app} as a function of $1/T$ at 80 and 100% RH suggests a modification of the matrix structure, probably due to the glass transition phenomenon. As a consequence E_a would be not constant in the temperature range tested for 80 and 100% RH. This effect of glass transition on E_a values was previously noted on wheat gluten films (*28*). Glass transition is often characterized by a temperature change, but changes such as the higher mobility of small molecules can be observed for a zone largely inferior or superior to the T_g. This could explain the nonvalidation of Arrhenuis law when the SPI matrix was supposed to be in a rubbery state at 100% RH whatever the temperature between 5 and 30 °C.

The carvacrol diffusivity values (10^{-14}–10^{-17} m^2/s) in SPI-coated papers were relatively low compared to values found for other antimicrobial agents such as sorbic acid (7.6×10^{-12} m^2/s) in gluten film or potassium sorbate in whey protein films, for which the values varied between 5.4×10^{-11} and 9.8×10^{-11} m^2/s depending on the film composition (*29*, *30*). In soy protein matrix drug delivery systems, the diffusivity of theophylline was found to be $(2.54–37.5) \times 10^{-9}$ m^2/s depending on formulation and pH (*31*). Diffusivity can be affected not only by the size, the steric hindrance, the concentration of the molecule, the matrix nature, and its characteristic but also by environmental factors (*32*). It can be also supposed that the vapor state of the antimicrobial agent affected the diffusion. The diffusivity of gas such as O_2 or CO_2 in gluten films varied between 10^{-13} and 10^{-12} m^2/s (*33*).

The weak diffusivity of carvacrol in SPI-coated paper, specifically at low temperature and RH, is an advantage to store

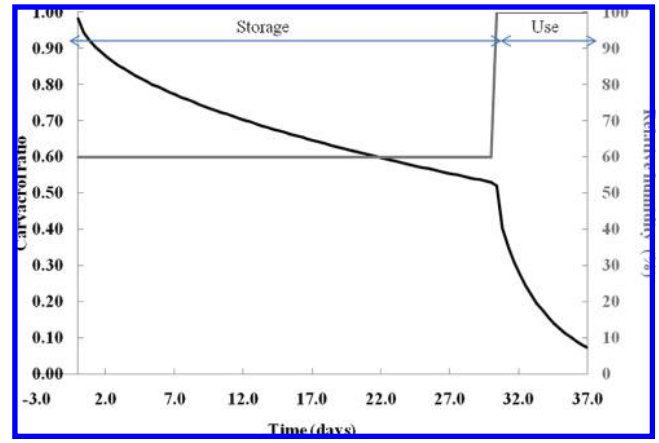

Figure 7. Simulated effect of temperature abuse during storage of a biological product (100% RH) packed in a SPI-coated paper containing carvacrol as antimicrobial compound (in gray, control stored at 20 °C; in black, storage at 20 °C with temperature abuse of 1 day at 30 °C after 2 days of storage at 20 °C).

the antimicrobial packaging without losses of the antimicrobial agent. The modification of the protein network structure by water uptake leading to the glass transition temperature shift to lower temperatures resulted in a higher mobility of the agent in conditions of microorganism growth. As reported in **Figure 7**, it was possible to predict the carvacrol losses from a coated paper during controlled storage conditions and the consecutive release of carvacrol when conditions of use were applied (i.e., in contact with a high relative humidity food).

Glass transition temperature determination in relation with effective diffusivity analysis is of great importance for in-depth understanding of the mechanisms involved in the controlled release of volatiles from protein-based matrix toward atmosphere. This improved knowledge allows optimization of the use of antimicrobial SPI coated papers, for example, their storage conditions and their use in activation conditions in the presence of a food product.

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