Carvacrol Losses from Soy Protein Coated Papers As a Function of Drying Conditions

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ABSTRACT: The influence of drying conditions (temperature/time) on carvacrol losses was investigated for antimicrobial packaging obtained by coating paper with soy protein solutions containing 30% of carvacrol (w/w of SPI) as antimicrobial agent. The lowest carvacrol losses ranging from 25 to 30% were obtained for three drying conditions: high temperature and short time 250°C for 20 s or mild conditions 50°C for 210 s and 25°C for 3 h. In contrast, intermediate drying conditions (100°C/90 s and 150°C/45 s) led to carvacrol losses higher than 50%. The weaker losses observed for drying at 250°C compared with the intermediate temperatures could be explained by the rapid formation of a thick and protective crust that acted as a selective membrane letting to past water and retaining carvacrol. For drying at 150°C, high losses were related to water vaporization and carvacrol carrying as attested by the presence of holes observed by SEM and due to bubble formation and bursting. If drying conditions affected the carvacrol losses and its retention from coated papers during storage at 30°C and 60% relative humidity, the antimicrobial activity was found only dependent on carvacrol amount of the coated papers. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 611–620, 2007

Key words: drying; soy proteins coated paper; carvacrol; selective diffusion theory

INTRODUCTION

Since ancient times, aromatic plants such as oregano and thyme but also cloves, mint or cinnemanon are known and used for their antimicrobial activities that are often related to their major components.[1–3] Carvacrol ($C_{10}H_{14}O$) is a phenolic compound found in high concentration in thyme and oregano essential oils. As aroma compound, carvacrol is Generally Regarded as Safe (GRAS) and used as flavoring agents in baked goods, sweets, ice cream, beverages, and chewing gum.[4] Moreover, carvacrol has been found efficient against a wide range spectrum of micro-organisms both food spoilage and food-borne pathogenic micro-organisms including bacteria such as *Bacillus* and *Shigella* species, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and fungi such as *Botrytis and Penicillium* species.[5–8]

The mechanism of action of carvacrol is not completely known but its hydrophobic character allowed its accumulation in the cytoplasmic membrane, the primary site of the toxic action of hydrophobic compounds. It was suggested that carvacrol created permeability alteration with a consequent leakage of protons or a leakage of ATP or a depletion of intracellular ATP pool by not synthesising or hydrolysis of this last.[9–11] In addition, the chemical structure of carvacrol, i.e. the presence of a free hydroxyl group on aromatic cycle was essential for antimicrobial activity.[8,10]

Antimicrobial packaging is a form of active packaging that could extend the shelf-life of food product and provides microbial safety for consumer.[12] It acts to reduce, inhibit, or delay the growth of spoilage micro-organisms in packed foods.[13] Volatile and nonvolatile antimicrobial agents can be incorporated into polymers or coating solutions containing antimicrobial agent that can be applied onto polymer surfaces.[14]

Numerous agro-polymers as polysaccharides or proteins have received greatest interest for films and coating production. Besides their biodegradability, edibility in some cases, they can extend the shelf life and improve quality of the food by providing a barrier to gas transfer and carrying antimicrobial compounds in order to maintain high concentrations of preservatives on the food surface ingredients.[15–17] Among them, soy proteins have drawn attention for their film forming ability and as carrier of antimicrobial agents such as thyme oil and cinnamaldehyde for coating of pre-cooked shrimp.[18–20]

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Because of their amphiphilic character and their emulsion stabilization properties, soy proteins have successfully been used as carrier for lipophilic compound as oil or aroma compounds in encapsulation process.[21,22] Soy protein isolates were found to be the most effective for retaining orange oil by spray drying with an encapsulation efficiency of 86% compared with whey protein isolates (73%) or sodium caseinates (81.5%).[22]

Moreover, soy proteins are known to interact with aroma compounds as alcohols, aldehydes, methyl ketones, or lactones involving hydrophobic association and to some degree, hydrogen bonding.[23–25]

The present work is part of a general study on antimicrobial packaging developed by introducing carvacrol into soy protein matrix coating paper. In coated paper formation, three major processing steps occur: the coating solution preparation, the coating process, and the drying. In previous studies, the effect of different parameters such as temperature of protein dissolution and aroma concentration on structural changes of coating solution was assessed and the ability of the coated papers to retain and release carvacrol was investigated. Coating and drying were made at laboratory scale in mild conditions, i.e. at ambient temperature.[26,27]

More drastic conditions of drying are generally used in industry. The optimization of drying is complex since the drying kinetic is controlled by several phenomena and results from both operating conditions but also from physicochemical properties of the solution. It was known that drying induced changes in protein interactions with formation of new hydrophobic interactions or disulfide and hydrogen bounds.[28]

Moreover, as carvacrol is a volatile compound, drying can be a critical step that may lead to high losses about flavour retention and can be quite satisfactory if the drying conditions are properly chosen as in spray drying, the most popular technique to encapsulate and protect aroma compounds, based on entrapment of the compounds in a polymeric matrix.[29]

The objectives of this study were to investigate the effect of different times and temperatures drying combinations on structure and carvacrol losses from soy protein isolate coated papers during drying. Moreover, the carvacrol losses from the coated papers during storage in selected temperature and relative humidity (RH) were evaluated and their antimicrobial activities were assessed.

MATERIALS AND METHODS

Materials

A commercial base paper (70 g/m^2) was provided by ''Ahlstrom Centre Research and Services'' and used as support for coating. Soy Proteins isolate (SPI) was purchased from Seah International (SAMPROSOY 90NB, Wimille, France). According to the furnisher, Samprosoy 90NB has a 8% moisture content and contains 91.8% of proteins. Carvacrol and 2-nonanol were purchased from Sigma Aldrich (St Quentin Fallavier, France). Growth media as plate count agar medium (PCA) was purchased from Biokar Diagnostic (Beauvais, France).

Strain. *E. coli* (I.P.54127) cultures were obtained from the Pasteur Institute (Paris, France) *E. coli* was cultivated on PCA media and kept at −80°C in 20% (v/v) glycerol.

Preparation of Soy protein isolate (SPI) coating solution

Soy protein isolate (10% w/v) was dissolved in distilled water heated to 50°C and the solution was continuously stirred for 30 min at 50°C. Carvacrol at a concentration 30, 60% (w/w of SPI) was added to the coating SPI solutions. 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: support papers were maintained on an iron plate (21 cm × 30 cm) and coating solutions were applied by an adjustable micrometer thin layer chromatography applicator (Braive Instrument, Chécy, France).

Drying process

Drying was carried out at 25°C during 3 hr at 50% ± 5% RH (control) or using an air dryer ''Mathis dryer'' (Zurich, Switzerland) at air velocity of 1.4 m/s. The different combinations of drying applied were: 50°C for 210 s; 100°C for 90 s; 150°C for 45 s; 250°C for 20 s. Coated papers surface's temperature was monitored with an infrared pyrometer associated with the Mathis dryer during drying, measurement increment was each 5 s.

Coated papers characterisation

Moisture content evaluation

The moisture content of coated papers was determined by drying in an oven at 105°C for 24 h using carvacrol free papers prepared in the same conditions that coated papers containing carvacrol. Indeed, carvacrol was partially eliminated during this drying and the residual content estimated by extraction method was not negligible.

Dry coated weight determination

The dry coated weight (in g/m^2) was obtained from the weight of a defined surface of coated and

uncoated paper and by subtracting from the coated weight, the moisture and carvacrol content as following:

$$\text{Dry coated weight}(g/m^2) = \text{Coated weight } (g/m^2) - \text{carvacrol content } (g/m^2) - \text{moisture content } (g/m^2)$$

Six paper pieces on different part of paper sheet were used for coated weight determination.

Scanning electronic microscopy (SEM)

Coated paper samples were preliminary frozen in liquid nitrogen, properly cut, mounted onto aluminium stubs with double-sided tape, and coated with silver. SEM observations were performed with a Hitachi S-4500 field emission scanning electron microscope (Elexience, France) operated at 8 kV.

Carvacrol kinetic retention from SPI coated papers

Pieces of coated papers (3 cm × 3 cm) were put in an oven at 30°C and at a RH of 60%. The RH was adjusted thanks to a humidified air flux through the oven (volume about 370 cm^3). Coated papers were picked out at prescribed time intervals for determining carvacrol content by extraction method as described below. Carvacrol losses were estimated from two different SPI coated papers in triplicate.

Carvacrol extraction from coated papers

The following extraction procedure was used to quantify the residual carvacrol amount of coated papers. Pieces of coated papers (3 cm × 3 cm) were immersed in water and *n*-pentane (50/50 v/v) mixture containing 100 μL of the internal standard solution (2-nonanol at 10 g/L of ethanol) and maintained for 16 h under magnetic agitation (300 min^{-1}). The organic phase (containing carvacrol and 2-nonanol) was removed, dried over anhydrous sodium sulphate, and analysed by gas chromatography. The analysis was carried out on a Varian 3800 GC equipped with a CP-Sil 5 column (Varian) (15 m × 0.32 mm, film thickness 0.25 mm) and a flame ionisation detector (FID). Hydrogen was used as a carrier gas with a flow rate of 2 mL/min. The oven temperature was programmed to rise from an initial temperature of 60°C to 150°C at 4°C/min, then to 250°C at 15°C/min and hold at 250°C for 10 min. Injector and detector temperatures were at 250°C. Injections were done in split mode with a 1 : 20 ratio.

The extraction yield was estimated by depositing a known quantity of carvacrol on a SPI coated paper, and by applying the extraction procedure described above. It was found to be about 87% ± 5% (10 replications). The quantification of carvacrol was performed thanks to the internal standard for which the response coefficient of carvacrol was determined ($K = 0.99$).

To evaluate losses after coating and drying, the theoretical carvacrol quantity was determined in relation with the dry coated weight by multiplying this latter by the percentage of added carvacrol in g per 100 g of SPI dry matter. The theoretical amount was compared with the residual amount on coated paper determined by extraction procedure and the losses were expressed in percentage.

Antimicrobial activity

For this fast antimicrobial activity test, a coated paper with a known area (8 cm^2) was placed on the surface of a solid medium, PCA, which has previously been inoculated with an overnight bacterial suspension of *E. coli*. After 24 h of incubation at 30°C, bacterial growth in the vicinity of the paper was observed.

RESULTS AND DISCUSSION

Influence of drying process on carvacrol losses from SPI coating papers

The effect of drying condition on papers coated with SPI solutions containing an initial concentration of 30% of carvacrol (w/w of SPI) was studied on carvacrol losses and on the coating layer structure.

Preliminary tests were carried out to determine for each selected drying temperature, the applied time to obtain a final moisture content for all the coated papers close to 6–7% as usually done in industrial paper drying. The selected drying temperature/time couples were: 50°C/210 s; 100°C/90 s; 150°C/45 s; 250°C/20 s. The moisture content of the different coated papers exhibiting close-coated weight, range from 6.1 to 6.9% w/w of dry matter (Table I). A quite linear relation was observed between the logarithm of drying time as a function of drying temperature (Fig. 1). As expected the time required to reach the defined moisture content is drastically reduced

TABLE I
Drying Parameters (Time and Temperature Couples) Applied, Coated Weight and Moisture Content Determination of SPI-Carvacrol Coated Papers (Containing Carvacrol at 30% w/w of SPI)

Drying temperature	Drying time	Dry coated weight (g/m^2)	Moisture content in coated papers (%)
25°C	3 h	12.4 ± 1.8	6.9 ± 0.9
50°C	210 s	13.2 ± 1.6	6.6 ± 0.9
100°C	90 s	12.7 ± 2.1	6.1 ± 0.9
150°C	45 s	13.4 ± 1.5	6.2 ± 1.0
250°C	20 s	11.9 ± 1.1	6.9 ± 0.7

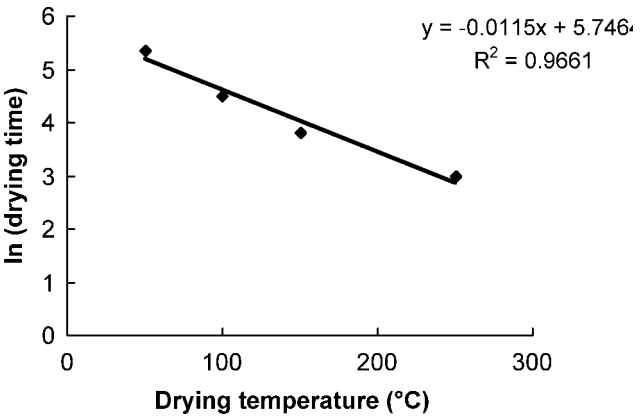

Figure 1 Relation between the drying time and the drying temperature of the SPI-caravcrol coated papers.

by increasing drying temperature. The effect of these drying temperature and time conditions on carvacrol losses were compared with smooth drying carried out at 25°C for 3 h at 50% RH.

The temperature of the coated papers surface was monitored by an infrared pyrometer during drying (Fig. 2). For the inlet drying temperature of 50°C, evolution of the coated paper surface temperature during the drying process displayed a first constant temperature period corresponding to the constant rate drying period for 40 s and then the temperature increased asymptotically to reach the inlet drying temperature. This second period corresponded to the falling rate period. During drying at 100°C, the temperature of the coated papers surface increased rapidly, the falling period reached quickly inducing a none detection of the first period. The same phenomenon occurred for papers dried at 150 and 250°C with a period of constant rate drying not detectable in the condition of data monitoring. However, the second period began at temperatures higher (110 and 150°C against 45°C) and the temperature increase of coated paper surface was faster than for a drying at 100°C.

It can be noted that the 238°C carvacrol boiling temperature was never reached during the drying.[4] However, it is known that high–boiling compounds are particularly volatile in dilute aqueous system because their weak vapour pressure are compensated by a relative high activity coefficient.[30]

After the drying process, the carvacrol losses from the SPI coated papers were calculated by subtracting the initial calculated carvacrol deposited on coated papers (g/m^2) from the extracted carvacrol quantity (g/m^2) remaining on dried coated papers. Carvacrol losses appeared to be highly dependant on time/temperature couples applied during drying and varied from 25 to 55% (Fig. 3). The lower losses ranging

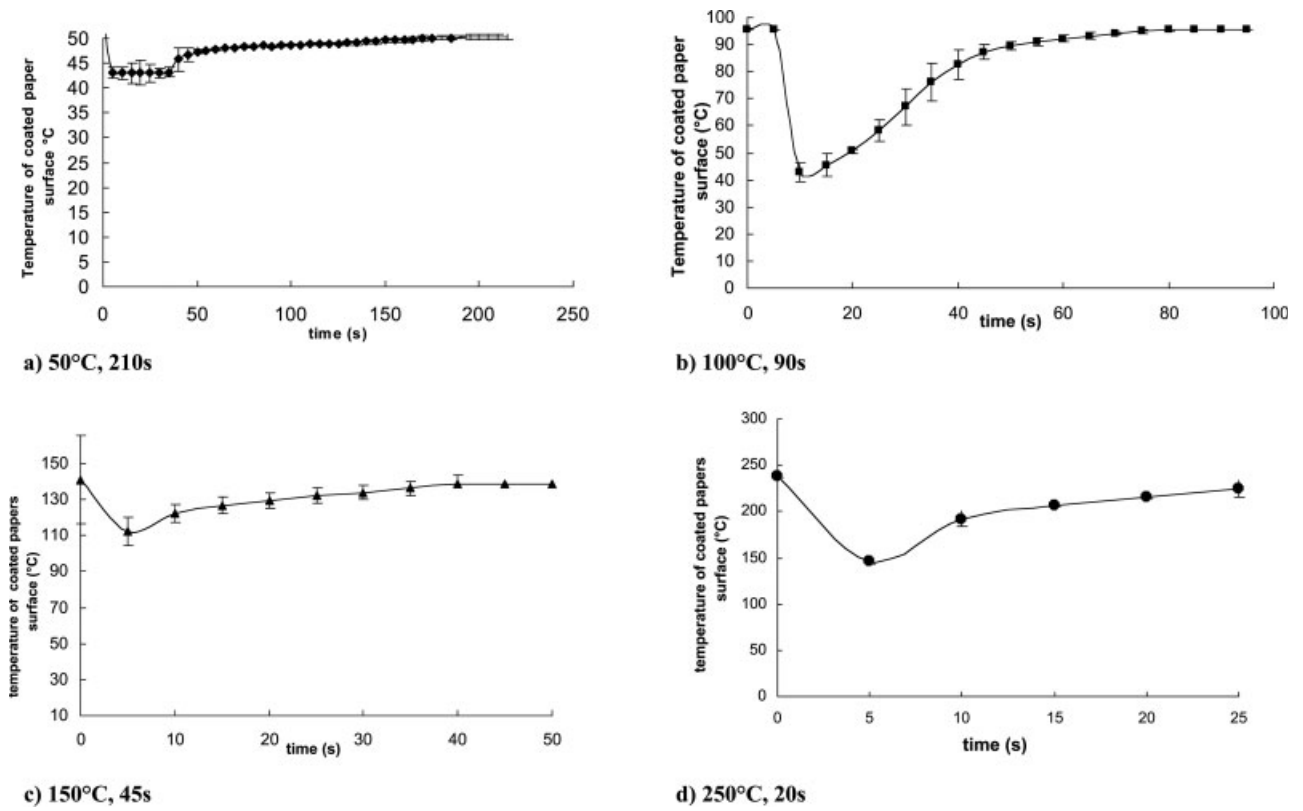

Figure 2 Temperature of coated papers surface monitored during the drying process.

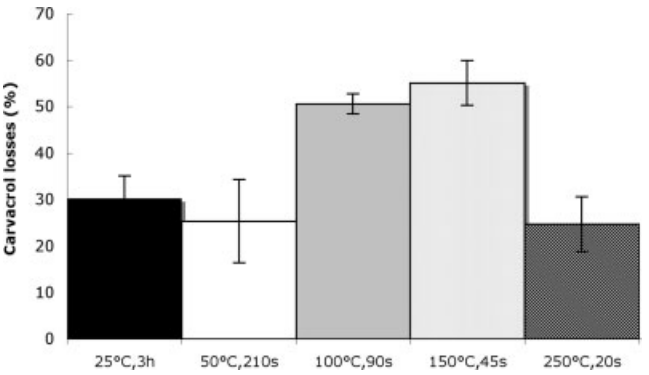

Figure 3 Carvacrol losses from Soy protein coated papers after coating and drying process as a function of drying treatment: (■) ambient conditions (25°C) for 3 h, (□) 50°C for 3 min 30, (▩) 100°C for 90 s, (▩) 150°C for 45 s, (▩) 250°C for 20 s.

from 25 to 30% were obtained for high drying temperature and short time (250°C/20 s) and for mild condition of temperature (25°C/3 h) and 50°C/210 s. In contrast, intermediate drying conditions (100°C/90 s and 150°C/45 s) led to carvacrol losses higher than 50%. A good retention of aroma compounds is generally reported for rapid drying rate such as applied in atomization process for aroma compounds encapsulation. This high retention is related to a crust formation at the surface of the product and to the selective diffusion theory.[29–33] The crust, a layer of high solid contain material, is formed in early stage of drying at high temperature and is reported to behave as a selective membrane: most of the water passes through this crust while a large part of the bigger volatile compounds are retained within the matrix. It was reported that the formation of this membrane was dependent on a critical water concentration at the surface; as soon as the interface water concentration felt bellow the critical moisture content, the selective membrane was formed.[30,34] Drying conditions, leading to a high surface rate of water evaporation, permitted to achieve high aroma retention.[34] Our results suggested that the drying at 250°C authorized the rapid formation of the selective membrane avoiding high losses of carvacrol while at 150°C and below this temperature the gradient of concentration was not sufficiently steep to decrease rapidly the water content and to achieve the crust's formation. The high losses observed for temperature of 100 and 150°C compared with 25 and 50°C could be also related to the increase of water and carvacrol diffusion with temperature.[30]

The presence of the crust was not observable by scanning electron microscopy (SEM) (Figs. 4 and 5). Whatever the drying, the coated papers exhibited a smooth surface [Fig. 4(b–e)]. The coating matrix filled the support paper leading to a continuous and dense layer clearly observed. However, in the case of drying at 150°C/45 s or at 250°C/20 s, SEM revealed the presence of holes or craters [Fig. 4(d,f,g)] on the surface. Their presence was strongly linked to the boiling water temperature. Indeed, these structures were due to the formation of bubbles bursting through the surface when vaporization of water occurred. The surface temperature of coated papers dried at 150 and 250°C were clearly above the boiling water temperature (Fig. 2). Steam water acted as carrier of carvacrol favouring losses.

Such holes were also reported for encapsulation matrix after spray drying when the applied conditions were not optimum. This phenomenon is called ''ballooning'' and occurs when sufficiently high inlet air temperature is used. Steam is then formed in the interior of the matrix causing the droplet to puff-up (or balloon) thereby producing a thin-walled hollow particle and several holes observed on capsule surface by SEM.[29]

The coated papers dried at high temperature presented a similar aspect to coated papers dried at 25°C/3 h and 100°C/90 s [Fig. 5(a–d)]. However, comparing the pictures at the same magnification (×2500), it clearly appeared that in spite of close coating weight, the thickness of the coating layer was different (6, 8.5, 8.5, and 12 μm for coated papers dried at 25, 100, 150, 250°C, respectively). It seems that at low drying temperature, the required long drying time may favour impregnation of the fibre network leading to reduce the thickness of the continuous surface coating layer. Moreover, the penetration of the coating solutions has been reported to decrease with an increasing protein concentration and consequently viscosity increase, a high penetration being favoured by a low viscosity. For instance, wheat gluten or corn gluten meal induced an impregnation percentage of paper which varied from 4.8 to 63.3% depending on the nature and concentration of the coating agent.[34] SPI coating solutions are known to form gel under heat when the denaturation temperatures of the major proteins were reached.[35] Therefore coating solutions could thick rapidly in the case of drying at high temperature preventing the fibre impregnation by decreasing molecules mobility in the network. Moreover, aggregation of protein might contribute to the formation of the crust. The denaturation temperature of the two major proteins β-conglycinin and glycinin were found in solution near 76°C and 94°C respectively but varied with RH.[36] Heat-induced denaturation and aggregation occur more difficultly at lower RH because the temperature of denaturation are higher than at strong humidity.

It could be suggested that for a drying at 100°C, in agreement with the weak thickness of coating, the soy proteins were not completely denaturated

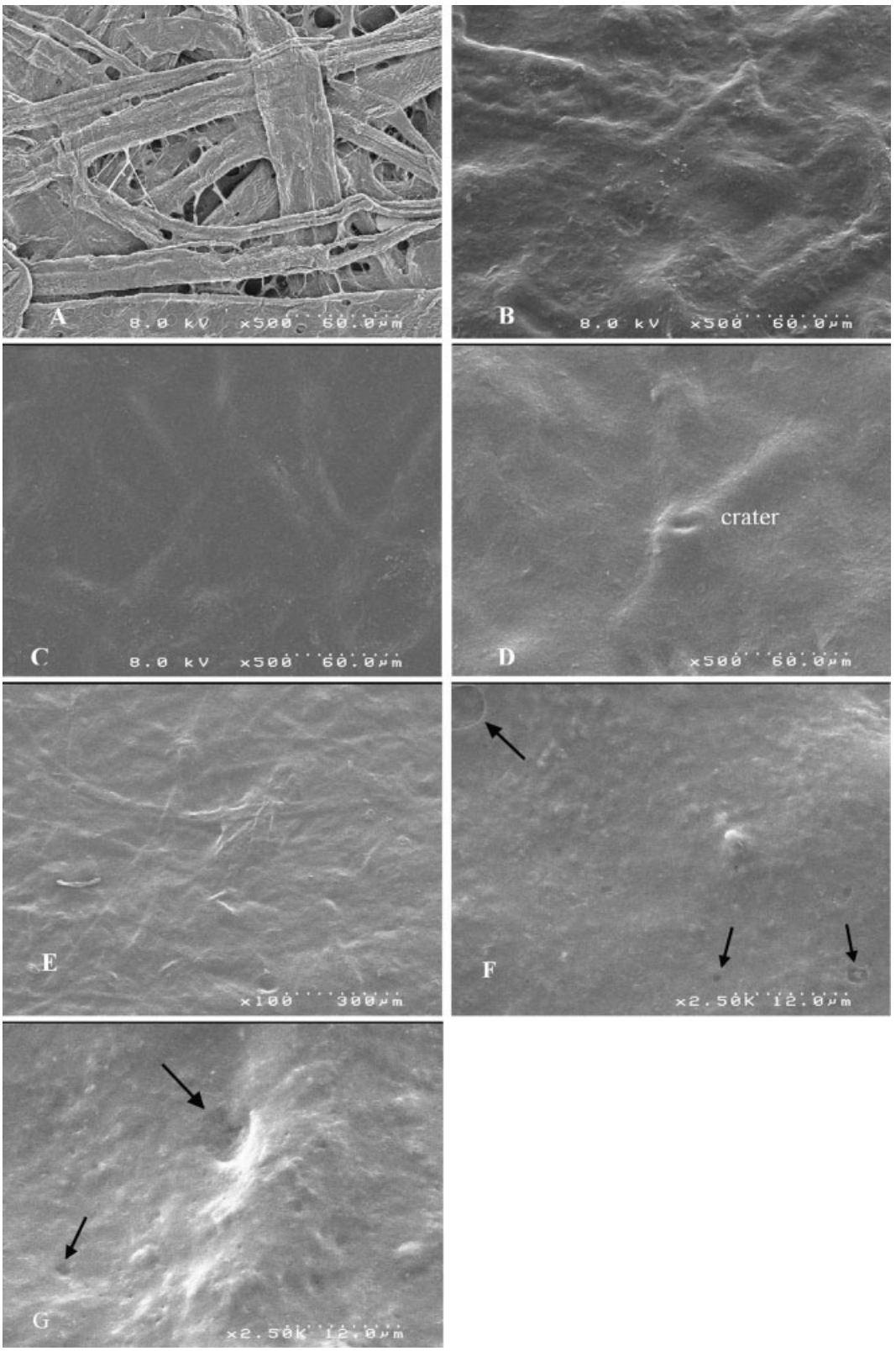

Figure 4 SEM (magnification ×500) of surfaces: (a) uncoated paper, (b) SPI-carvacrol coated paper dried at 25°C for 3 h, (c) SPI-carvacrol coated paper dried at 100°C for 60 s, (d) SPI-carvacrol coated paper dried at 150°C for 45 s, (e), carvacrol-soy proteins coated paper dried at 250°C for 20 s. Image (f,g) corresponds to (d,e) magnified to 2500.

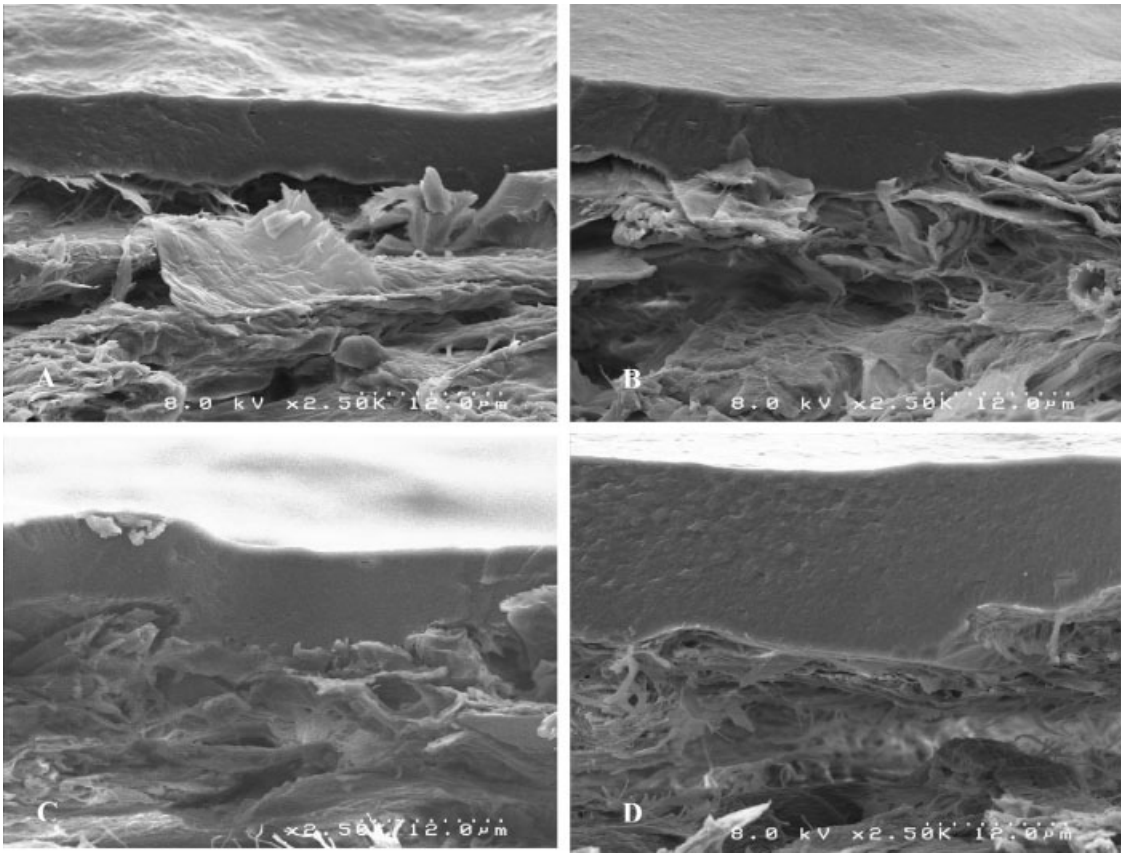

Figure 5 SEM (magnification ×2500) of cross sections of: (a) SPI-carvacrol coated paper dried at 25°C for 3 h, (b) SPI-carvacrol coated paper dried at 100°C for 60 s, (c) SPI-carvacrol coated paper dried at 150°C for 45 s, (d) SPI-carvacrol coated paper dried at 250°C for 20 s.

because the surface temperature and then the bulk temperature stayed under 95°C. Moreover, crust formation was not favoured and as expected the losses of carvacrol were high in relation to the high diffusion of compound at this temperature.

For a drying at 150 or 250°C, it could be supposed that the proteins might be completely denaturated and that therefore the crust formation favoured. However, high losses were found at 150°C and the thickness of the coating layer was clearly inferior to the layer of paper dried at 250°C. During drying at 150°C, even if the surface temperature exceeds 110°C, the conditions didn't let the gel to form. Moreover, the important vaporization of water as attested by the presence of holes in SEM could contribute to carry the carvacrol out of the matrix and explained the strong losses.

The vaporization of water also occurred for a drying at 250°C but the impact of this phenomenon on carvacrol losses was limited by the crust formation.

These results pointed out the different mechanisms involved in carvacrol losses during drying and also the importance of drying time/temperature conditions as key parameters for controlling these losses.

Influence of carvacrol concentration and drying parameters on carvacrol losses from SPI coated papers

In this part, the influence of carvacrol concentration was studied. Indeed, aroma compounds concentration is an important parameter since aroma compounds are known to alter or to modify proteins conformation.[37,38] In addition to the concentration of 30% (w/w) of carvacrol, coated papers were prepared with a concentration of 60% (w/w) and two drying conditions (25°C/3h and 250°C/20 s) providing the best carvacrol retention were applied After drying at 25°C/3 h, carvacrol losses were close, about 30%, for both coated papers (Table II). After drying at 250°C/20 s, the carvacrol losses were 2.5 times higher for papers containing 60% (w/w) than for paper containing 30% (w/w).

A too high concentration of carvacrol such as 60% (w/w of SPI) may affect the soy proteins retention ability leading to weaker interactions between carvacrol and SPI. Subsequently, a high drying temperature could destabilize soy proteins-carvacrol interactions. Carvacrol was found to have an influence on soy protein solutions behaviour: an increase of aroma compounds from 10 to 30% induced a viscos-

TABLE II
Influence of Temperature/Time Drying and Carvacrol Initial Concentrations on Carvacrol Losses From Coated Papers

Initial carvacrol concentration (w/w of SPI)	Drying parameters	Dry coated weight (g/m^2)	Carvacrol losses (%)
30%	25°C, 3 h	12.4 ± 1.8	30.6 ± 4.7
	250°C, 20 s	11.9 ± 1.1	24.7 ± 3.8
60%	25°C, 3 h	11.2 ± 1.5	31.9 ± 0.4
	250°C, 20 s	12.8 ± 1.1	73.9 ± 5.9

ity decrease followed by a plateau from 30 to 60% (w/w of SPI).[26] The viscosity was also modified in different manner with the presence of carvacrol and the temperature of the coating solution. Such results suggested that modification of soy proteins-carvacrol network might take place under the effect of concentration and drying conditions.

Moreover, high aroma compounds concentrations are reported to result in lower retention from the matrices.[22,39] High aroma compounds concentrations implied greater proportions of volatiles close to the drying surface, thereby shortening the diffusion path length to the air/particle interface and inducing greater losses. Indeed, water of coating solutions tends to force the hydrophobic compounds such as carvacrol to the air/water interface and out of the solution. Consequently, the compounds are more easily removed. This phenomenon is more pronounced at high aroma compounds concentration, when their homogenisation in the coating solution is more difficult to obtain. To limit such losses during encapsulation process, volatile compounds are generally introduced at a ratio of aroma compound to carrier solid of 1–5 or 1–4 (20–25% w/w of dry matter).[22,29] In the present study, the ratio of 30% w/w was correct since the losses after a drying 250°C/20 s were relatively weak. In contrast, when the carvacrol was used at a ratio of 60% (w/w of SPI), the higher carvacrol amount found near the surface of coating layer could be easily vaporized in drastic conditions of drying inducing high losses.

Influence of drying process on carvacrol retention from SPI coated papers during storage

In the conception of antimicrobial packaging, it was important to control the losses of antimicrobial agent during the process but also the losses during storage because the phenomena involved can be different. It was verified that in mild conditions of storage (temperature 20°C and 50% RH) the carvacrol losses were insignificant. Accelerated conditions of storage 30°C and 60% RH were applied to papers containing 30% (w/w of SPI) and dried at 25, 150, and 250°C (Fig. 6). These papers were selected since these drying conditions affected in a different manner the coated papers microstructure. From the kinetic at 30°C and 60% RH, two retention phases could be noticed: a rapid and a slow drying step similar for all the papers. The most drastic drying treatment induced the most important release in the first step. The drying at 150°C did not lead to higher carvacrol release from coated paper than those from papers dried at 250°C. Soy proteins have a particularly plastic behaviour and can rearrange in a different manner during drying depending on the time and temperature conditions. These modifications in particular at high temperature could involve less strong interaction between soy proteins and carvacrol that favoured its release. In a previous study, it was shown that during the first 3 days, carvacrol release was the most rapid when coated papers were prepared from SPI solutions heated at 90°C compared with solution prepared at 25 or 50°C.[26] High temperatures are known to affect the affinity between aroma compounds and proteins: heating can cause a significant increase in the number of binding sites in the protein molecule, but can induce a decrease in the binding affinity.[38] Numerous binding sites with weak affinity to carvacrol may be present in soy proteins when coated papers were dried at elevated temperature. Moreover, the conditions of the kinetic study (30°C, 60% RH) may promote the water uptake and induce conformational changes depending on the fixed network structure after drying. The transfer rate of a volatile compound from a matrix in the glassy state such as soy protein coating is primarily via Fickian diffusion. This rate is directly proportional to the molecular diffusion of the flavour component which is strongly dependent upon water concentration.[40] It was found that diffusion coefficients in foods between 0.7 and 44% RH may vary by a factor 10^3 due to the resulting physical and physicochemical changes which occurs in the food

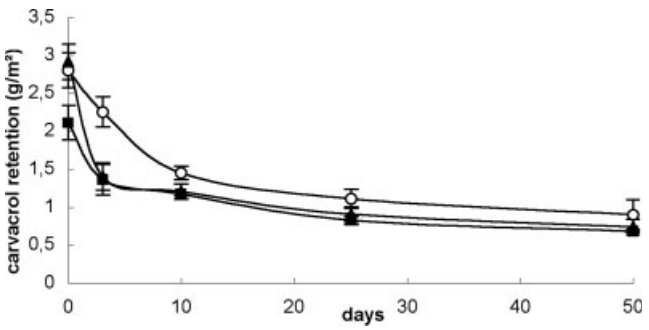

Figure 6 Kinetic carvacrol retention from SPI-carvacrol (30% w/w of SPI) dried at different conditions: (○) ambient temperature for 3 h (■) drying at 150°C for 45 s, (▲) drying at 250°C for 20 s. The retention was followed in selected conditions: at 30°C, and 60% RH through an humidified air flow of 25 mL/min.

TABLE III
Antimicrobial Activity of Coated Papers With the Same Area (8 cm^2) but Dried in Different Conditions Against *E. coli*

Initial carvacrol concentration (w/w of SPI)	Drying parameters	Residual carvacrol (g/m^2)	*E.coli* growth
0	25°C, 3 h	0	+
30	25°C, 3 h	2.6	−
	150°C, 45s	2.1	+
	250°C, 20s	2.7	−
60	25°C, 3 h	4.6	−
	250°C, 20s	2.0	+

+ signified growth and − no growth.

upon exposure to water.[29] The slow release step could be explained by strong interactions between carvacrol and soy proteins that are not affected by drying conditions and by the conditions of RH and temperature of kinetic release. Furthermore, after 50 days, for the three coated papers, the released carvacrol amount was similar and ranged between 72 and 75%.

Antimicrobial activity of coated papers

To test the antimicrobial activity of the different coated papers, agar diffusion tests were realized with a fixed area piece of coated paper and a model strain, *E. coli*, which is used as an indicator of faecal contamination in several food products.

A bacterial growth was observed with a paper containing 30% of carvacrol and dried at 150°C/45 s or a paper containing 60% of carvacrol and dried at 250°C/20 s whereas no growth was observed for the other papers (Table III). The quantity of carvacrol brought by the fixed area (8 cm^2) varied between 1.6 and 1.8 mg for ineffective papers while for the antimicrobial papers, carvacrol amount brought was always equal or superior to 2 mg accordingly to carvacrol content after drying while. This quantity corresponded to the minimal inhibition concentration in these experimental conditions. It could be concluded that the efficiency of the coated paper was related to their residual carvacrol amount.

CONCLUSION

The temperature/time drying parameters but also carvacrol concentration influenced carvacrol retention from SPI coated papers. It is interesting to note that the lower losses were observed for a ratio of carvacrol of 30% (w/w of SPI) and mild drying conditions (25°C/3 h, 50°C/210 s) or a high drying temperature and short drying time (250°C/20 s). It was suggested that for this combination, a crust or selective membrane had rapidly been formed leading to past water but retaining carvacrol. Because of the fast drying and the good repartition of carvacrol on the matrix, its losses by vaporization of water, bubbles formation and bursting were weak while the same phenomena induced high losses at 150°C and a time of 45 s. The drying process was found to affect the kinetic retention of carvacrol from the coated papers stored at 30°C and 60% RH but not their anti-microbial properties.

To avoid much important losses of antimicrobial agent during drying, the following parameters; time/temperature, nature and concentration of aroma compounds, nature of the matrix should be taken account. The impact of air drying combined by infrared drying (i.e. drying at industrial scale of coated papers) should be investigated to overcome the process feasibility of the SPI-carvacrol coated papers as antimicrobial packaging.

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