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Release Behavior and Stability of Encapsulated D-Limonene from Emulsion-Based Edible Films

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ABSTRACT: Edible films may act as carriers of active molecules, such as flavors. This possibility confers to them the status of active packaging. Two different film-forming biopolymers, gluten and *ι*-carrageenans, have been compared. D-Limonene was added to the two film formulations, and its release kinetics from emulsion-based edible films was assessed with HS-SPME. Results obtained for edible films were compared with D-limonene released from the fatty matrix called Grindsted Barrier System 2000 (GBS). Comparing *ι*-carrageenans with gluten-emulsified film, the latter showed more interesting encapsulating properties: in fact, D-limonene was retained by gluten film during the process needed for film preparation, and it was released gradually during analysis time. D-Limonene did not show great affinity to *ι*-carrageenans film, maybe due to high aroma compound hydrophobicity. Carvone release from the three different matrices was also measured to verify the effect of oxygen barrier performances of edible films to prevent D-limonene oxidation. Further investigations were carried out by FT-IR and liquid permeability measurements. Gluten film seemed to better protect D-limonene from oxidation. Gluten-based edible films represent an interesting opportunity as active packaging: they could retain and release aroma compounds gradually, showing different mechanical and nutritional properties from those of lipid-based ingredients.

KEYWORDS: *gluten, ι-carrageenan, emulsion-based films, D-limonene, carvone, flavor release, encapsulation*

INTRODUCTION

Edible packaging is considered a thin layer of edible material formed on a food as a coating or placed as a preformed self-standing film on or between food components.[1] It also could be defined as a film, a sheet, a thin layer, or a coating that is an integral part of a food and eaten with it.[2] Edible packaging constitutes an environmentally friendly technology that may enhance food quality, safety, stability, and the mechanical handling properties by providing a semipermeable barrier to water vapor, oxygen, and carbon dioxide between the food and the surrounding atmosphere.[3] Two categories of ingredients have been used as film-forming substances. Protein and polysaccharide are used for their mechanical, structural, and gas barrier properties, and hydrophobic substances are used for their good moisture barrier properties.[4−8] Composite films combine the advantages connected to the different components. For this study, *ι*-carrageenans (i-Cs) and wheat gluten (WG) were selected to prepare edible packaging for their capacity to create a network. Beeswax was added to obtain emulsified films. i-C, a water-soluble polymer with a linear chain mainly composed of alternated (1,2)-D-galactose-4-sulfate and (1,4)-3,6-anhydro-D-galactose-2-sulfate units, is promising as a film-forming material. In aqueous solutions, i-Cs produce thermoreversible gels when cooled below the critical temperature. Different proteins, such as WG, corn zein, and whey proteins, have been proposed as raw materials for developing edible film formulations. WG is a major functional food ingredient that is inexpensive and biodegradable and has good film-forming properties.[9] Edible films and coatings can also be used as carriers for aroma compounds, antioxidants, coloring agents, and antimicrobials that will improve food quality and safety.[10−13] In particular, encapsulation of aroma compounds with edible films hinders flavor loss, which strongly affects food quality during the processing or storage of food.[14,15] This technique allows controlled release, defined as a strategy by which one or more active agents or ingredients are made available at a desired site and time at a specific rate.[16] Edible films could be applied to food as active packaging, with the aim of gradually releasing aroma compounds with time and thus maintaining the typical flavor of food products. In this way, edible packaging represents not only an inert barrier, but it has an active role, and it interacts with the food and the surrounding media. Polysaccharides like carrageenans find limited use in flavor encapsulation, except with coacervation, because of their high viscosity, which makes difficult operations of spray-drying.[15] However, recent studies showed how i-C-based films, with or without lipids, represent a potential matrix for flavor encapsulation and flavoring,[17,18] in particular of polar aroma compounds.[19] Proteins appear to be well suited for encapsulation of volatile systems on the basis of their properties.[15] In fact, they typically provide excellent emulsification properties, show good film formation, and offer protection against oxidation. However, there is little informa-

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tion in the literature evaluating proteins, in particular gluten, for this purpose. The interest in flavor encapsulation with gluten derives from the high barrier properties of gluten-based films toward gas and aroma compounds.[20−23] In this work, D-limonene was selected as a model aroma compound. D-Limonene is one of the aroma compounds of which lemon oil is composed. Terpen derivates such as D-limonene are important flavor components in lemon, making up over 95% of peel oil: unfortunately, they make lemon oil very susceptible to oxidation. D-Limonene can be degraded by oxidation reactions, which cause the loss of lemonlike flavor and the formation of off-flavors. D-Limonene oxidation initially results in the formation of hydroperoxides. D-Limonene hydroperoxides can undergo scission reactions that lead to the formation of products such as alcohols, epoxides, and ketones.[24] Two important D-limonene oxidation products include the limonene oxide (epoxide) and the carvone (ketone), whose release could be followed to investigate D-limonene deterioration.[25] The oxidation rate was closely related to the relative humidity: generally, the oxidation of spray-dried D-limonene increased with increasing water activity.[26] Reaction of D-limonene in the presence of Cu catalyst and air has been shown to lead to a low conversion to a mixture of limonene oxides, carvone, and carveols. This resulted in a change in odor with the development of piney, flowery notes.[27] A similar loss of D-limonene was observed using CuO, and this was prevented by the addition of either BHA or tocopherols. D-Limonene and γ-terpinene, in contrast to the other compounds of lemon oil, were both much more sensitive to UV degradation: UV irradiation caused a substantial conversion of D-limonene to the same mixture of products, resulting in a caraway-like, minty odor.[26] Because D-limonene is sensitive to oxidative degradation, several studies were focused on the possibility to encapsulate this aroma compound. Different ingredients were chosen as wall materials, such as starch, maltodextrin, and gum arabic.[25,28,29] Generally, lemon oils are added to food in the form of water-in-oil emulsions. The possibility to stabilize oil-in-water emulsions with whey proteins instead of gum arabic to inhibit D-limonene degradation was studied.[24] D-Limonene degradation and the formation of limonene oxide and carvone were less observed in the whey protein isolate (WPI)-stabilized than in gum arabic-stabilized emulsions. In agreement with that, limonene oxide, carvone, and carveol formation in microencapsulated orange oil was less in emulsions stabilized with WPI and soy protein isolate than emulsions stabilized with gum arabic.[30] Different methods were performed to quantify D-limonene and its degradation products. Several solvents can be used with the aim of extracting D-limonene from the matrix: acetone,[25] hexane,[26] and methanol.[29] Quantification of the total amount of D-limonene could be achieved using steam stripping with the aid of a Clevenger apparatus.[29] When aroma compounds are extracted from the capsule, the interactions between the aroma and the wall material could affect the recovery of the compound. Therefore, other methods are particularly adapted for the analysis of volatiles. Headspace (HS) quantification could be performed to better understand flavor release. In the last years, solid-phase microextraction (SPME) was proposed for the analysis of aroma compounds. SPME sampling through HS has been compared to classical determination methods by several authors.[31] The primary advantages of vapor-phase SPME sampling are solvent-free preparation, separation of analyte from nonvolatile and less volatile components, shorter analysis time if larger and less volatile molecules are not injected, and avoiding gas chromatographic column degradation caused by injection of large volumes of water. A method to assess D-limonene oxidation products using SPME and gas chromatography−mass spectometry (GC-MS) was set up: limonene oxides were quantified by using a PDMS fiber, which resulted in the best among several different tested SPME fibers.[32]

In this study, D-limonene was encapsulated in two different types of edible films, WG and i-C-based films. Generally, in the food industry, flavor is added to the lipid phase, for the higher affinity of aroma compounds to hydrophobic substances. Emulsion-based edible films represent a lipid phase dispersed in a continuous phase consisting of protein or hydrocolloid. In the present work, flavor was added to the lipid phase wrapped with a film of gluten or i-Cs, with the aim of better preserving flavor. i-Cs and gluten-based films have proved to have good enough gas barrier properties. Therefore, in the case of limonene encapsulation, the application of gluten and i-C as wall materials could hinder limonene oxidation because of less intake of oxygen. The aim of this work was to investigate the possibility to use edible films as flavor carriers to have a gradual release and to preserve aroma compound from oxidative degradation.

MATERIALS AND METHODS

Materials. WG, supplied by Sigma Aldrich (United States), and i-C, gently supplied by Cargill Texturizing Systems (Baupte, France), constituted the continuous matrix of films. Anhydrous glycerol (Sigma, 99.5% purity, United States) acts as a plasticizer to improve the mechanical properties of i-C and WG films. Fat used in this study, Grindsted Barrier System 2000 (GBS), supplied by Danisco (Bradbrand, Denmark), is an acetic acid ester of mono- and diglycerides made from edible, fully hydrogenated vegetable oil blended with beeswax, having a melting point of 57 °C. Glycerol monostearate (GMS) used as the emulsifier was purchased from Prolabo (99% purity, Merck eurolab, Fontenay-sous-Bois, France). The aroma compounds selected for encapsulation were D-limonene and carvone (Sigma Aldrich) with purities ≥98%. Tested were different SPME fibers varying in coating thickness and polarity of the polymer: divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), PDMS, polyethylene glycol (PEG), and polyacrylate (PA).

Methods. *Sample Preparation.* Two different film formulations were considered as follows: emulsion gluten and i-C-based films. A gluten acid film-forming dispersion was prepared following the film production method previously set up, with 10 min of ultrasound treatment.[33] An i-C film-forming solution was prepared by dispersing 6 g of i-C powder in 200 mL of distilled water at 65 °C for 15 min under magnetic stirring. Glycerol (1.8 g) was then added to the i-C solution, still under magnetic stirring. To obtain emulsion-based films, fat (GBS) was added to both film-forming solutions composed of i-C and glycerol or gluten and glycerol, at a concentration of 30%. GBS was blended with GMS (90:10 w/w). Pure D-limonene was presolubilized in melted fat before being dispersed into the film-forming solution (0.5 mL limonene/g fat). Once all of these components were melted under magnetic stirring, the heat mixture was emulsified with a homogenizer (Ultra-Turrax model T25 IKA, Labortechnik, ODIL, France) at 24000 rpm for 1 min. Dispersions were then poured onto smooth polymethylmethacrylate (Plexiglas) plates. To obtain a film, the water was removed by drying in a ventilated chamber (KBF 240 Binder, ODIL, France) for 8 h with the temperature and relative humidity fixed at 30 °C and 50% RH, respectively. Related to the fat system considered, samples were prepared by adding the aroma compounds mix to melted GBS, with the same ratio used for the edible films.

HS-SPME Procedure. To follow D-limonene and carvone release, flavored melted GBS was transferred into a 10 mL vial. Related to the

release from edible films, pieces of dried film (about 1 g weight) were overlapped into a 10 mL vial. In both cases, samples occupied the same volumes, and vials were sealed immediately with a Teflon-lined septum and screw cap. Between two measurements, vials were opened to allow aroma compound release and "oxygenation". Each measurement was carried out at the equilibrium, and a minimum time of 180 min was determined for equilibration. The samples were incubated at 25 °C and 50% RH.

After equilibration, the HS of the samples was sampled using an SPME fiber coated with PEG (60 μm), which, in set up experiments, exhibited the highest overall extraction efficiency as compared to other fibers. The fiber was manually exposed to the sample HS for 5 min at 25 °C. Finally, the fiber was withdrawn into the needle holder and immediately introduced into the GC injection port for 2 min at 230 °C for the full desorption of the fiber.

GC Conditions. The volatile flavor compounds present in the HS of film or GBS samples were analyzed with a 3800 Varian GC system equipped with a flame ionization detector (FID) and a capillar EC-wax column (length = 30m, i.d. = 0.25 mm, Alltech, United States). The oven temperature was programmed at 50 °C for 3 min, then ramped to 120 °C at 10 °C/min, and increased to 200 °C at 15 °C/min. Helium was used as the carrier gas, while hydrogen and air were used as ignition gases. The detector temperature was set at 210 °C.

Fourier Transformed Infrared (FTIR) Spectroscopy. Samples, with a 6 cm^2 surface area, were measured by means of FTI−attenuated total reflectance (FTIR-ATR) IFS Vector 22 equipment (Bruker, Germany), with a 4 cm^{-1} resolution and 3 s as the typical acquisition time. All FTIR experiments were carried out at room temperature (22 °C) done in triplicate, and the data were averaged.

Film Microstructure. Film surfaces were observed using environmental scanning electron microscopy (ESEM, model XL 30, Phillips). Small film strips (5×3 mm) were fixed on the support using double-sided adhesive tape. An accelerating voltage of 20 kV was used.

Surface Wettability: Aroma Compounds Contact Angle Measurements. Contact angles of films with D-limonene and carvone were measured with a goniometer (Kruss Gmbh, Germany) equipped with image analysis software (Drop Shape Analysis, Kruss Gmbh, Germany). In surface science, an instrument generally called a contact angle goniometer is used to measure the contact angle at which a liquid/vapor interface meets a solid surface. The relationship involving the surface tension at a point of the three-phase contact line between a smooth, rigid, solid phase S, a liquid L, and its vapor V was described as follows:

$$\gamma_{LV}\cdot\cos\vartheta = \gamma_{SV} - \gamma_{SL}$$

where γ_{LV}, γ_{SV}, and γ_{SL} are the surface tensions of the liquid−vapor, solid−vapor, and solid −liquid interfaces and ϑ is the contact angle.[34] The sessile drop method is basically an optical contact angle method to estimate wetting properties of a solid surface. A droplet of pure D-limonene or carvone was deposited on the film surface with a precision syringe. Then, the method is based on image processing and curve fitting for contact angle measurement from a theoretical meridian drop profile, measuring the contact angle between the baseline of the drop and the tangent at the drop boundary. The experimentally acquired data were as follows: contact angle (ϑ), droplet surface area exposed to air (A_S), droplet base area in contact with the film (A_B), and droplet volume (V) as a function of time t.[35] The effect of evaporation was evaluated on an impermeable reference surface: aluminum foil. All films were preconditioned in a chamber under the same environmental conditions to avoid interferences due to competing moisture exchange at the surface around the droplet. All measurements were made on both sides of the films, that is, the "support side" and the "air side".

Aroma Compound Absorption: Liquid Permeability. Liquid permeability of the films for D-limonene and carvone was estimated from drop volume variation measured with the previously described Kruss goniometer (Kruss Gmbh, Germany). The absorption flux was obtained from the drop volume kinetics taking into account the evaporation flux.[36] When an aroma compound drop is deposited onto a solid surface, two mechanisms are involved in the decrease over time of volume and contact angle: evaporation, due to the pressure difference between the drop and the surrounding atmosphere, and absorption inside the film.

$$V(t) = V(0) - V_{eva}(t) - V_{abs}(t)$$

where V_{eva} is the evaporated volume and V_{abs} is the absorbed volume. Considering absorption to be negligible in aluminum foil, it was used as a reference to estimate the evaporation flux F_{eva} as follows:

$$F_{eva} = \frac{V_{eva}(t) - V_{eva}(t + dt)}{A_S(t)\,dt} = \frac{dV_{eva}}{A_S(t)\,dt}$$

where A_S is the surface area of the water droplet on this reference material. The volume absorbed by a hydrophilic material can therefore be calculated after the subtraction of the evaporated volume. The initial volume of the droplet as well as the contact area is dependent on the measurement as they are controlled manually by the syringe. The absorbed volume at time t can be determined from the reference evaporation flux and the surface area of the droplet on the tested surface:

$$dV_{abs}(t) = V(t) - V(t + dt) - dV_{eva}(t)$$

$$dV_{abs}(t) = dV(t) - dV_{eva}(t)$$

$$dV_{abs}(t) = dV(t) - F_{eva}\cdot dt\cdot A_S(t)$$

where A_S is the surface area of the aroma compound drop on the tested surface. The absorption flux F_{abs} is thus the absorbed volume per base area unit (that corresponds to the liquid/solid interface) and per time unit:

$$F_{eva} = \frac{dV_{abs}}{A_B(t)\,dt}$$

where A_B is the base area of the aroma compound drop on the tested surface. All films were preconditioned in a chamber at the same environmental conditions to avoid interferences due to moisture exchange at the surface around the droplet. All measurements were made on the two sides of the films, the "support side" and the "air side". At least six measurements per film were carried out.

RESULTS AND DISCUSSION

Flavor Release Kinetics from Films and Fat Support. In this study, the possibility to use edible films as flavor carriers was investigated. Two different matrices were compared. As in the food industry, aroma compounds are added into lipid ingredients, and a sample constituted only by fat (GBS) was used as the reference matrix. Two different preparations of emulsified edible films, WG and i-C-based, were realized with the addition of GBS. In the three matrices (GBS and edible films), D-limonene was solubilized into the lipid ingredient. Related to the possibility of using edible films as flavor carriers, the D-limonene release kinetics from the different matrices was measured by dosing the quantity of aroma compound in the HS.

A preliminary setup phase was carried out to select analysis conditions. About the HS-SPME extraction, the fibers coated with PEG resulted in the most suitable for the detection of limonene and carvone. Then, different exposure times of the fiber to the vapor over the sample were tested to optimize aroma compound extraction. After 5 min of exposure time, the quantity of limonene extracted was the highest. The equilibrium temperature equal to 25 °C was selected as the exposure temperature. Because the samples were opened to allow limonene release and closed only the time needed to reach thermodynamic equilibrium, essays were performed to determine the time necessary to achieve equilibrium: even if the maximum quantity of D-limonene was detected after 40 min,

the HS-SPME analysis was performed after an equilibration time of 180 min.

In Figure 1, the D-limonene release from the three different matrices is represented. The results are given as $A_{limonene}t_i/A_{limonene}t_0$, as a function of time. For the three systems, the D-limonene percentage in HS rapidly decreased in the first period of time. As observed, in the case of GBS, the D-limonene percentage in the HS decreased for the first 50 h, and it settled at 50%, remaining constant for the next sampling. D-Limonene added to the i-C film was rapidly released in the first 20 h, until its amount in HS reached a quantity too low to be detected. As observed, the D-limonene release rate was higher in the i-C film as compared to the other matrices. D-Limonene is a compound with hydrophobic characteristics, as it is demonstrated by its log *P* (4.38), whereas carrageenans are supposed to form a hydrophilic network.[37] These results were in accordance to those obtained for permeability measurements.[38] Indeed, D-limonene permeability to i-C films resulted in values higher than values obtained for other aroma compounds, that is, ethyl esters. Considering its hydrophobic nature, D-limonene seemed not to show high affinity to the hydrocolloids layer. The initial very low amount of D-limonene after film drying seemed to confirm that this aroma compound is rapidly released from the matrix, even before the equilibration in vial: indeed, at time 0, the D-limonene area counts detected for GBS, WG, and i-C were 1 047 470, 329 438, and 32 855, respectively. The behavior of D-limonene encapsulated in i-C was very different as compared to the release of the other aroma compounds considered. Indeed, the esters and methyl ketones used in the previous study exhibited a weaker release rate.[19] This could be explained considering that D-limonene is characterized by a higher hydrophobicity (high Log *P*) than all of the other aroma compounds previously analyzed.

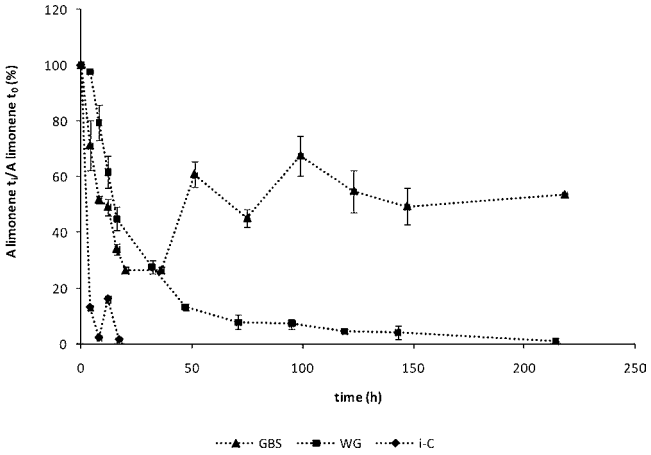

Figure 1. D-Limonene release from fat (GBS), i-Cs film, and WG film as a function of time.

D-Limonene release from GBS and WG followed the same trend (Figure 1). The D-limonene amount in the HS decreased rapidly in the first period to settle at a quite constant value in the second part of the kinetics, with a different area ratio as a function of the matrix. In both cases, the quantity of aroma in the HS remained quite constant after 50 h. This behavior could suggest an unsteady diffusion phase, during which the aroma compound reaches the matrix−air interface. After this first phase, the aroma compound evaporated once the surface is reached. Moreover, because of its higher affinity for more hydrophobic matrices, D-limonene interacts more strongly and then remains linked to the matrix.

The aroma compound release from the two samples seemed to follow a second order reaction. Indeed, the D-limonene ratio decreased in an exponential way, which could indicate a diffusion limited release.

Comparing i-Cs with gluten-emulsified films, the latter showed more interesting encapsulating properties: D-limonene was retained in fact by the gluten film during the process needed for film preparation, and it was released gradually during analysis time. D-Limonene did not show great affinity to i-Cs films, maybe due to high aroma compound hydrophobicity.

Moreover, concerning the structure of WG films, cross-sections of WG films and i-C films were compared (Figure 2). Observing the images obtained with SEM, the WG films showed a structure rather different from carrageenans films. In both cases, fat was not present as globules but as numerous layers. In the case of WG, those seemed to be more indented. Therefore, gluten or i-Cs networks did not wrap fat particles forming a continuous layer: fat was probably distributed between different layers. The WG films structure could represent a higher resistance to D-limonene mass transfer, contributing to explain the lower release rate.

Lipid ingredients are used in food formulations as flavor carriers because of the hydrophobic nature of the great majority of the aroma compounds. However, both flavors and lipid ingredients are affected by oxidative degradation that could result in off flavors formation. D-Limonene is a very susceptible

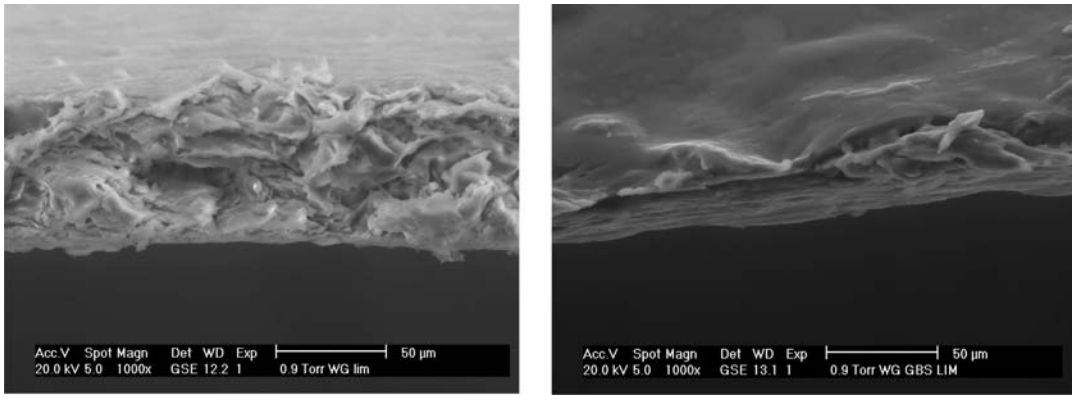

Figure 2. Cross-section of WG-emulsified film (left) and i-C-emulsified film (right), obtained with E-SEM.

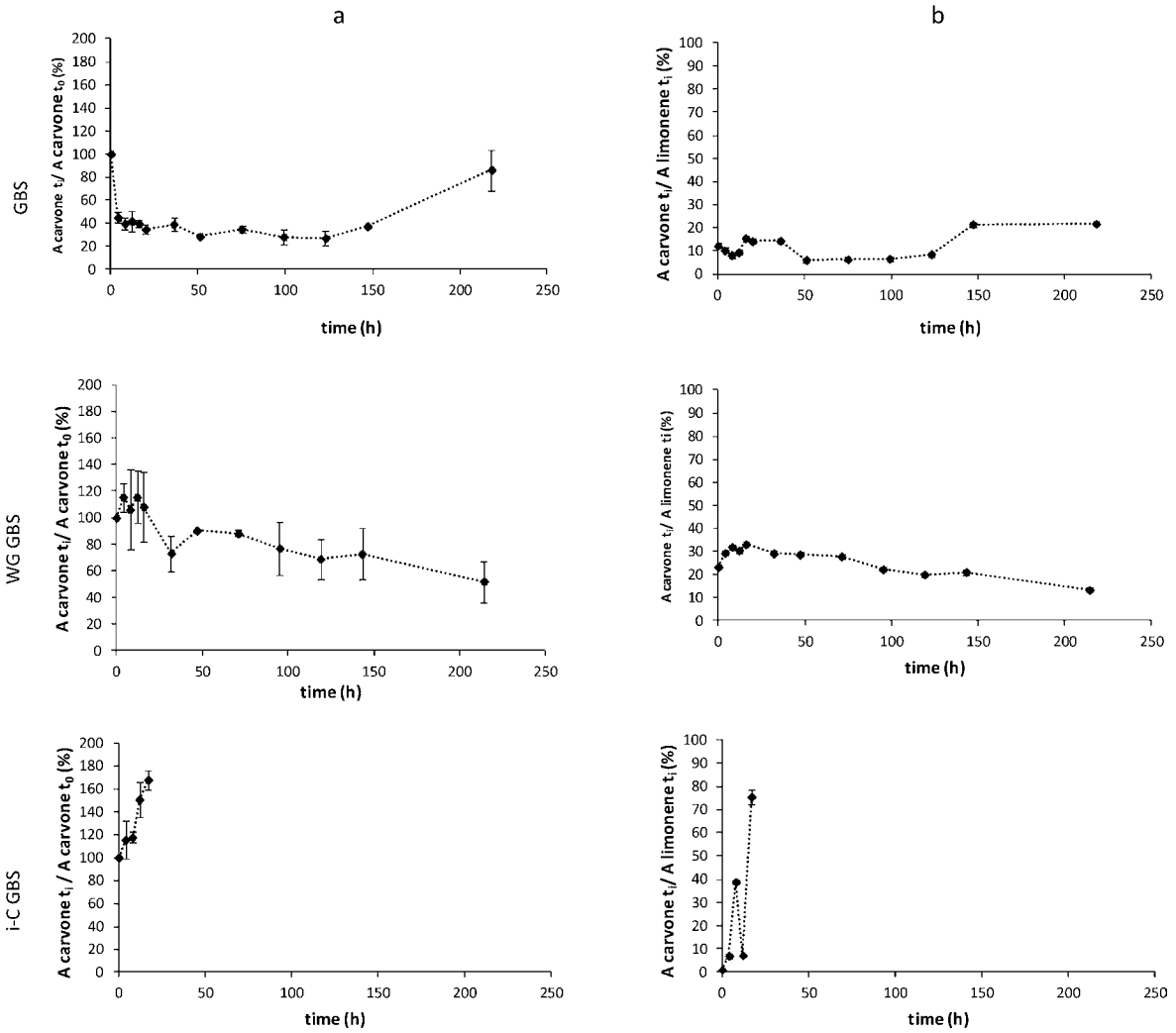

Figure 3. Carvone release from three different matrices as a function of time.

aroma compound, whose oxidation causes the loss of lemonlike odors and the formation of mintylike odor compounds. Many compounds derived from D-limonene oxidation: among off-flavors compounds, carvone was selected as a marker of D-limonene oxidation, as it is one of the earliest D-limonene oxidation products.[26] The aim of this second part of the research project was to investigate whether edible films could protect D-limonene from oxidative degradation: indeed, emulsified edible films represent a lipid phase with a second layer constituted by gluten or carrageenans that are known to be good barriers against gases.[2,14,17] Carvone was detected in the HS by using SPME fiber for the three matrices: GBS, WG, and i-C films. Very few works concerned the study of D-limonene oxidative degradation in solid systems: in the majority of cases, a solvent extraction was performed with the aim of analyzing oxidation products.[27] More recently, the HS-SPME method was set up to dose D-limonene oxides.[32] Carvone release from the three different matrices as a function of time is shown in Figure 3. Results are presented as $A_{carvone}t_i/A_{carvone}t_0$ (%, column a) and $A_{carvone}t_i/A_{limonene}t_i$ (%, column b). Carvone release followed different mechanisms as a function of the matrix. In the case of GBS and WG, carvone release was quite constant. For the first sample, there was an increase in carvone concentration after 150 h, while for the latter carvone, concentration weakly decreased in the second part of the kinetic. In the case of i-Cs film, carvone HS quantity increased since the beginning as a function of time; carvone and limonene release from i-C had an opposite behavior, indicating that limonene initially present in the matrix was completely released or oxidized.

As displayed, carvone was present in all matrices at time 0. The presence of carvone since the beginning of the kinetic suggested that oxidation started not only during samples conservation but also during sample preparation. This could be related to process conditions too intense for the lipid ingredient and for the flavor. Indeed, this degradation was especially favored by the drying step in the film-making process.

In the systems analyzed, it was not possible to separate carvone formed by oxidation in the matrix from carvone formed by oxidation in the HS, once D-limonene was released. Therefore, even if edible films used in this work are supposed to be a good barrier to oxygen, their capacity to protect flavor from oxidation could not be evaluated only by HS-SPME analysis. Comparing carvone release from GBS and WG films, the area ratio is greater in the case of WG film. This behavior is the opposite of that obtained for D-limonene release. In addition, carvone is characterized by physicochemical properties different from D-limonene: log *P* measured for carvone is 3.07, which indicates a lower affinity to the lipidic system.

Table 1. Contact Angle, Measured at Time 0 and after 10 s, and Absorption Flux for GBS and WG Film Samples

matrix	aroma compound	side	ϑ_0	ϑ_{10}	F_{abs} (g m^{-2} s^{-1})
WG	D-limonene	air	24.20 ± 3.08	13.70 ± 1.93	3.31 ± 0.83
WG	carvone	air	27.33 ± 3.73	6.57 ± 0.90	11.33 ± 1.42
WG	D-limonene	support	19.79 ± 3.06	11.83 ± 1.27	0.81 ± 0.09
WG	carvone	support	27.13 ± 3.77	14.95 ± 1.51	0.87 ± 0.15
GBS fat	D-limonene		23.93 ± 2.42	21.53 ± 2.63	1.32 ± 0.19
GBS fat	carvone		30.45 ± 3.34	14.80 ± 0.99	3.18 ± 0.19

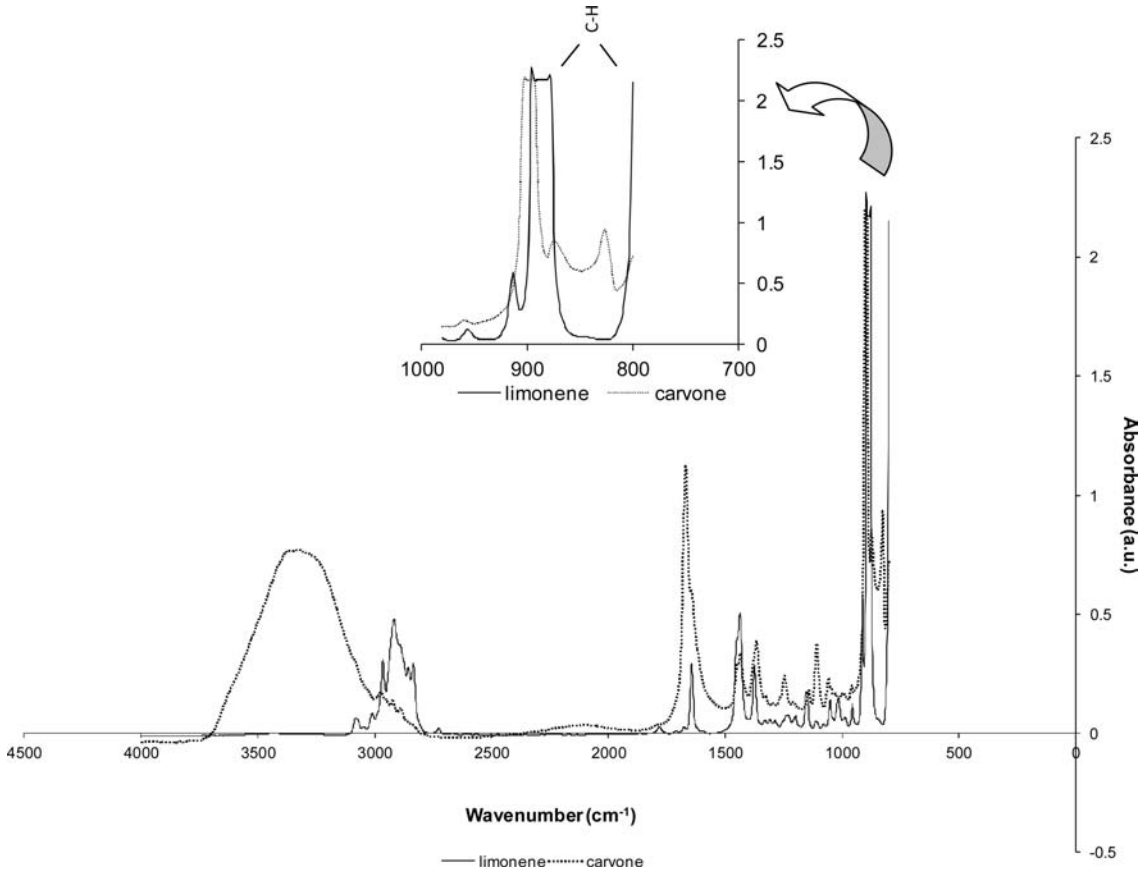

Figure 4. FTIR spectra obtained for pure D-limonene (continuous line) and pure carvone (dotted line).

Film Surface Wettability and Aroma Compounds Permeability. To better understand the previous results obtained for aroma compound release, the absorption and permeation processes of D-limonene and carvone have been studied. Therefore, the aroma contact angle and absorption rate measurements have been performed. The contact angles obtained immediately after deposition (ϑ_0) and after 10 s (ϑ_{10}) as well as the aroma compound absorption flux (F_{abs}) are given in Table 1. The absorption flux was calculated from the droplet volume kinetics.[36] GBS and WG film, prepared with and without fat addition, were characterized. Concerning film samples, the two sides were analyzed: support side and air side (i.e., side exposed to air during film drying). In the case of gluten films without fat, D-limonene was fastly absorbed on the surface of the solid, and it was not possible to determine accurately its contact angle. The phenomenon occurred on both sides of the film. The rate of absorption would be theoretically considered as infinite. For WG films (emulsified films), there were no significant differences between the contact angle measured on each side (support side and air side), either at time 0 or after 10 s. This is in contrast with results obtained for carrageenans films, where the water contact angle was found higher on the support side than on the air side.[37] The absorption flux can be related to an apparent liquid permeability and compared with the aroma vapor permeability expressed as an aroma transfer rate or flux:[38] comparing D-limonene F_{abs} in GBS and WG support side, it is possible to observe that the absorption flux was higher for GBS. This could suggest a slower D-limonene permeability to WG film. Comparing D-limonene F_{abs} with carvone F_{abs} in WG, results obtained were different. Carvone F_{abs} was greater than D-limonene F_{abs}. This could indicate a higher liquid permeability to carvone of WG films. As it is possible to observe, F_{abs} were different as a function of film side considered. F_{abs} values of the air side were higher than F_{abs} values of the support side. This difference could be explained observing that the two surfaces of gluten films were not identical: after the drying step, two different surfaces characterized the WG film. In the case of the side in contact with the support during drying, it resulted as more homogeneous and smooth as compared to the side in contact with air during drying. The air side could be considered

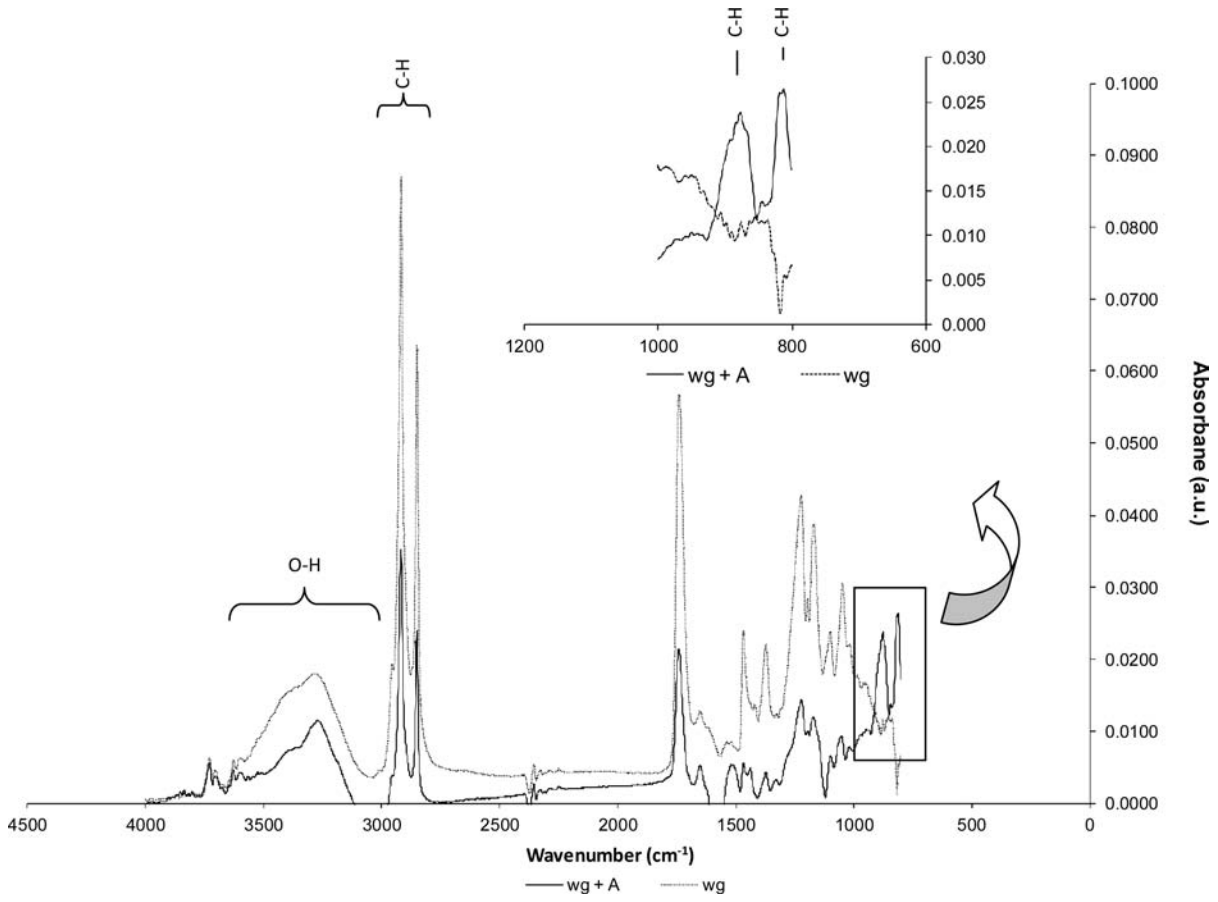

Figure 5. FTIR spectra of WG-emulsified films with (continuous line) or without D-limonene (dotted line).

more porous than the other side, probably allowing a fast permeation of the aroma in the film.

These results could confirm release data: probably carvone presence in the HS of WG samples could be due to D-limonene oxidation within the matrix but also to the higher diffusion of carvone through the matrix to the surface. A more accurate analysis of oxidation and interactions between aroma compounds and matrices have been characterized by FTIR.

D-Limonene Degradation/Interactions within Films Displayed by FTIR. To better understand if the carvone dosed in the HS was the result of D-limonene oxidation within the matrix or in the HS after D-limonene release, FTIR spectroscopy analysis of i-Cs and gluten films was carried out. Because of the complexity of the matrices considered, we compared spectra obtained for the film without aroma compounds with those obtained for film with encapsulated aroma compound. The analysis was affected by the low quantity of aroma compound in the matrices. The spectra obtained for D-limonene and carvone are compared in Figure 4. D-Limonene showed typical bands at a wavelength of 885 cm^{-1}, corresponding to CH deformation. This band was selected for this study. This band has previously been selected by other authors to monitor changes in D-limonene concentration, because no asymmetries or alterations in the positions were observed.[39] FTIR measurements were carried out on three different film preparations and triplicated. Reproducibility of the experiments was found to be high (>95%). Three different samples were analyzed with the aim of investigating the effectiveness as encapsulating matrix: i-Cs emulsified films, WG films without fat, and WG emulsified films. The FTIR spectrum of each sample was compared to the spectrum obtained for the same matrix containing initially D-limonene.

The second purpose of this experimental part was to investigate the protective effect of i-Cs or gluten against oxidation, and, therefore the presence of carvone as an indicator of limonene oxidation. Even if all of the samples mentioned were analyzed with FTIR, only in the case of WG films (emulsified) with and without limonene differences were observed. For this reason, only spectra of aroma compounds, limonene and carvone, and WG emulsified films with and without limonene are shown in Figures 4 and 5. The other spectra (i-C, gluten film without GBS) were only commented. In the case of i-C, the FTIR spectra obtained were overlapped in the region of CH deformation: this suggested the absence of aroma compounds within the matrix. The absence of band shifts in this region suggested that there was no visible interaction between i-Cs and D-limonene. The absence of typical bands of D-limonene or carvone in the whole spectra suggested that no aroma compound was in the film or that the quantity was too low to be detected. This is in agreement with results obtained in the study of release: i-Cs films seemed to lose the most quantity of aroma compound in the first hours after preparation. To understand the influence of gluten on D-limonene encapsulation, four different samples of gluten films were prepared: gluten film without GBS, gluten with and without D-limonene, and gluten films with GBS, with and without D-limonene. By this way, it was possible to check if gluten could encapsulate flavor even if there was no lipid phase. As observed for i-C samples, the typical bands associated with

the presence of limonene were no detected for gluten films without fat.

Spectra obtained for WG films with fat and with or without aroma compound are shown in Figure 5. Observing the spectra related to films with D-limonene, it is possible to observe the presence of typical bands of D-limonene. At 885 cm^{-1}, there was a band, present also in D-limonene spectra and related to the CH deformation. This indicated that D-limonene is encapsulated in the film after the drying step, confirming the better capacity of gluten to retain flavors. The spectra of WG film with D-limonene did not reveal the presence of carvone. Accordingly, the band at 900 cm^{-1}, related to its presence, was undetected. These results suggested that D-limonene oxidation did not occur in the WG matrix but in the HS or that the carvone concentration was too low to be detected.

This study considered investigating the possibility of using edible films as active packaging. In particular, the chance to use edible films as flavor carriers was studied. Comparing i-Cs with gluten-emulsified film, the latter showed more interesting encapsulating properties: in fact, D-limonene was retained by the gluten film during the process needed for film preparation, and it was released gradually during analysis time. D-Limonene did not show great affinity to i-Cs film, maybe due to high aroma compound hydrophobicity. Another aspect investigated was the possibility to protect D-limonene from oxidation: both gluten and i-Cs films are supposed to have good barrier properties to gases, in particular oxygen. This aspect deserves further investigation, because HS-SPME and FTIR analysis did not allow clarifying if oxidation affected encapsulated aroma compound. Preliminary results, however, seemed to show that WG film protected D-limonene from degradative reactions: an increase in carvone release was probably due to oxidation in the HS once D-limonene was released and not within the matrix. Gluten films could represent an interesting opportunity as active packaging: they could retain and release aroma compounds gradually, showing mechanical and nutritional properties different from those typical of lipidic ingredients.

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Notes

The authors declare no competing financial interest.

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