Carbohydrate Polymers 76 (2009) 325–332

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influence of interactions on water and aroma permeabilities of ι-carrageenan–oleic acid–beeswax films used for flavour encapsulation

Maria José Fabra[a], Alicia Hambleton[b], Pau Talens[a], Fréderic Debeaufort[b,c,*], Amparo Chiralt[a], Andrée Voilley[b]

[a] Food Technology Department, Institute of Food Engineering for Development, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain
[b] ENSBANA-EMMA, Université de Bourgogne, 1 esplanade Erasme, 21000 Dijon, France
[c] IUT-Génie Biologique, Boulevard Dr. Petitjean, B.P. 17867, 21078 Dijon Cedex, France

ARTICLE INFO

Article history:
Received 10 December 2007
Received in revised form 2 October 2008
Accepted 29 October 2008
Available online 6 November 2008

Keywords:
Aroma compounds
n-Hexanal
Encapsulation
Carrageenan
Permeation
Edible films

ABSTRACT

The objective of this work is to investigate the water and aroma barrier properties of films obtained from ι-carrageenan containing glycerol and lipids mixtures of oleic acid (OA) and beeswax (BW) used for encapsulation of active compounds. Water vapor permeability (WVP) is greatly influenced by lipid composition, encapsulated aroma compound and also relative humidity. WVP decreases when films contain encapsulated aroma compound but increases when the moisture content in the films increases. When oleic acid was the main compound of lipid phase, the plasticizing effect of water revealed through water permeability is less marked. The results of ethyl acetate, ethyl butyrate, ethyl hexanoate, 2-hexanone, 1-hexanol and *cis*-3-hexenol permeabilities reveal that physicochemical interactions between aroma compounds-hydrocolloid and aroma compound-lipid induce structural changes and modify their permeability. This work gives evidence of the ability of ι-carrageenan–OA–BW films to protect encapsulated aroma compound and its influence in barrier properties.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

In the recent years, edible films and coatings have received increasing attention from researchers and industry as an interesting alternative of food packaging (Debeaufort, Quezada-Gallo, & Voilley, 1998; Tharanathan, 2003). Edible films and coatings represent a specific category of food packaging that have been defined as "a type of packaging like a film, a sheet, a thin layer or a coating which is an integral part of food and can be eaten together with" (Guilbert & Gontard, 2005). The most beneficial characteristics of edible films and coatings are their edibility and inherent biodegradability (Guilbert, 1986; Han & Gennadios, 2005; Krochta, 2002). Many biomolecules including proteins, carbohydrates and lipids have been used to prepared edible barrier films. Proteins and polysaccharides provide good mechanical properties and are effective barriers to gases and aroma compounds but they have low water barrier efficiency (Miller & Krochta, 1997; Quezada-Gallo, Debeaufort, & Voilley, 1999).

Carrageenans are sulphated polysaccharides extracted from red seaweeds (*Rhodophyceae*). The number and position of sulphate groups on the disaccharide repeating unit determine classification in three major types: κ, ι and λ. The κ-, ι- and λ-carrageenans exhibit sulphate contents of 20%, 33% and 41% (w/w), respectively. ι-Carrageenan is composed of altering α(1,3)-D-galactose-4-sulphated and β(1,4)-3,6-anhydro-D-galactose-2-sulphate. In aqueous solutions, ι-carrageenans produce thermoreversible gels on cooling below the critical temperature, where the conformation changes from a random coil of single chains to the formation of double helices of carrageenan chains, and consequently to gels (Yuguchi, Thu Thuy, Urakawa, & Kajiwara, 2002).

Carrageenan-based coatings have been applied for a long time to a variety of foods to carry antimicrobials or antioxidants, and to reduce moisture loss, oxidation or disintegration (Lacroix & Le Tien, 2005). Edible films made of ι-carrageenans display interesting advantages: good mechanical properties, stabilization of emulsions and reduction of oxygen transfers (Hambleton, Debeaufort, Beney, Karbowiak, & Voilley, 2008). However, the highly hydrophilic nature of ι-carrageenan films limits their ability to provide a significant moisture barrier. One way to improve barrier properties is to include lipidic materials in their formulation, such as fatty acids or waxes. Waxes are the more efficient substances to reduce moisture permeability because of their high hydrophobicity while unsaturated fatty acids control moisture migration less efficiently because they are more polar (Fabra, Talens, & Chiralt, 2008; Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). The addition of

---

[*] Corresponding author. Address: ENSBANA-EMMA, Université de Bourgogne, 1 esplanade Erasme, 21000 Dijon, France. Tel.: +33 (0) 3 80 39 68 43; fax: +33 (0) 3 80 39 66 11.
E-mail address: frederic.debeaufort@u-bourgogne.fr (F. Debeaufort).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.10.024

lipids to form emulsified films could be also used to encapsulate active molecules or aroma compounds (Hambleton et al., 2008; Karbowiak et al., 2008). Encapsulation of aroma compounds, vitamins and additives in hydrocolloid based films confers to them the status of active films (Reineccius, 1994). To limit aroma degradation or loss during processing and storage, it is beneficial to encapsulate volatile ingredients. Thus, encapsulation can be employed to retain aroma in a food product during storage, to protect the flavour from undesirable interactions with food, to guard against light-induced reactions and/or oxidation, to increase flavours shelf-life and/or to allow a controlled release (Reineccius, 1991; Tari & Singhal, 2002).

Edible films can be used as flavour carriers in addition to providing a barrier to aroma loss (Guilbert, 1986). The transfer of aroma compounds into the packaging creates a modification of the organoleptic properties of food during storage. The physicochemical properties of the aroma compound such as molecular weight, structure, hydrophobicity, chemical function, solubility, volatility, partial vapor pressure and polarity, have an effect on the affinity for the polymer matrix and on the transfer kinetics, also specific interactions (aroma compound-hydrocolloid and aroma compound-lipid) induce structural changes during mass transfer (Dury-Brun, Chalier, Desobry, & Voilley, 2007).

The aim of this work is to better understand the influence of the composition of ι-carrageenan based films and interaction on water and aroma barrier properties and its capacity to protect the encapsulated active substances.

2. Materials and methods

2.1. Materials

ι-Carrageenan was supplied by Cargill (Texturizing Solutions, Baupte, France) and is the major component of the hydrophilic continuous phase for emulsion-based edible films. Beeswax (Brillocera, S.A., Valencia) and oleic acid (Panreac quimica, S.A. Castellar Del Vallés, Barcelona, Spain) were used as the hydrophobic dispersed phase, and glycerol (98% purity, Fluka Chemical, Germany) was added as plasticizer.

The aroma compound *n*-hexanal (98% purity, Sigma–Aldrich, Germany) was used as an active molecule, because of its sensitivity to oxidation, encapsulated either in ι-carrageenan matrix or in the lipid phase. It has a melting point of 128 °C, a saturated vapor pressure of 1420 Pa at 25 °C, and its solubility in water is 3.81 mg/mL.

2.1.1. Aroma compounds

The volatile compounds selected were 99.5% ethyl acetate (Aldrich Chemical Company, Inc.), 99% ethyl butyrate (Aldrich Chemical Company, Inc.), 98% ethyl hexanoate (Aldrich Chemical Company, Inc.), 98% 2-hexanone (Aldrich Chemical Company, Inc.), ⩾98% 1-hexanol (Merck-Schuchardt) and 98% *cis*-3-hexenol (Aldrich Chemical Company, Inc.). Their physicochemical characteristics are summarized in Table 1. They are chosen because they are present in many food products (Fenaroli, 1975).

The hydrophobicity is expressed by the $\log K$ (where K is the partition coefficient of the aroma compound between octanol and water). Ethyl acetate, ethyl butyrate, 2-hexanone and *cis*-3-hexenol can be considered as a hydrophilic compound ($\log K < 2$), whereas ethyl hexanoate and 1-hexanol are hydrophobic ones ($\log K > 2$).

2.2. Carrageenan-based films preparation

Two groups of films were prepared. For the first group, films containing ι-carrageenan–glycerol–lipid (1:0.3:0.4) were studied. For the second group the polysaccharide–glycerol–lipid ratio remain constant and *n*-hexanal was encapsulated. In both groups of films, lipid fraction was composed of oleic acid (OA) and beeswax (BW) in different OA:BW ratios (70:30, 50:50 and 30:70) and two of these films were prepared without lipid (control films, with and without encapsulated *n*-hexanal).

A carrageenan film-forming solution was prepared by dispersing 6 g of carrageenan powder in 200 mL of distilled water at 65 °C for 15 min under magnetic stirring. Glycerol was added at a concentration required. To prepare emulsified films, after glycerol was added to aqueous solutions of ι-carrageenan, the amount of beeswax required was melted and oleic acid was added. Once all of these components are melted under magnetic stirring, the hot solution was emulsified with a homogenizer (Ultra-Turrax model T25 IKA, Labortechnik, ODIL, France) at 24,000 rpm during 2 min. Film-forming solutions were poured into a thin layer chromatographic spreader prior to be spread at a 1000 mm thickness onto smooth polymethylmethacrylate (PMMA or Plexiglas®) plates. All of the films were dried in a ventilated chamber (KBF 20 Binder, ODIL, France) for about 8 h. Temperature and relative humidity were fixed at 30 ± 1 °C and 40 ± 2% RH, respectively.

For the second series, film-forming dispersions were prepared as for the first series, but the aroma compound was pre-solubilized (10,000 ppm) in lipid mixture before being dispersed in the film-forming solution, then the mixture was homogenized as explained previously (24,000 rpm, 2 min)

2.3. Lipid particle size analysis of emulsified films

A laser light scattering instrument *Mastersizer 2000* (Malvern Mastersizer, Malvern Instruments, Worcestershire, UK) was used to determined the volume–surface mean diameter ($D_{3,2}$) Eq. (1) of lipid particles in the film. This technique is based on measuring the scattered light intensity caused by the lipid particles. The diffraction angle is a function of the lipid particle size, its shape and the wavelength of the incident light, and is inversely proportional to particle size. Samples were prepared by dispersing 1 g of dried film in 50 mL of distilled water at room temperature with moderate magnetic stirring. To identify the incidence of lipid globule aggregation, samples were also dispersed under moderate magnetic stirring in 50 mL of a 0.1% (w/w) SDS solution at room temperature. At least three replicates of each ι-carrageenan based film formulation were tested.

An average diameter is the diameter of a hypothetical particle that represents the total number of particles in the samples. The volume–surface diameter ($D_{3,2}$) represents the average size based on the specific surface per unit volume. These mean particle diameters were defined as follows:

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \tag{1}$$

where n_i is the number of droplets in each size class and d_i is the droplet diameter.

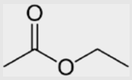
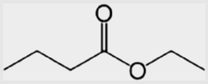
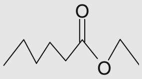
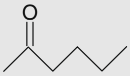
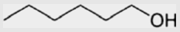
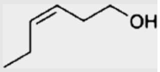
2.4. Film thickness measurements

Film thickness after drying was about 50 μm and was measured with an electronic gauge (Multichek FE SODEXIM, France) at six random positions on film samples.

2.5. Water vapor permeability measurements

The WVP of films was measured gravimetrically. The method is based on the mass variation of the permeation cells over time. This variation in weight is due to the passage of water vapor through the film put on a glass cell containing a saturated salt solution to fix the water activity. The WVP equals to:

Table 1
Physicochemical characteristics of aroma compounds.

Characteristic	Ethyl acetate	Ethyl butyrate	Ethyl hexanoate	2-Hexanone	1-Hexanol	*cis*-3-Hexenol
Odor[a]	Ether, pineapple	Fruit, pineapple	Fruity, banana pineapple	Like acetone but more pungent	Green grass	Green fresh grass
Chemical formula						
Molecular weight ($g\ mol^{-1}$)	88.1	116.2	144.2	100.1	102.18	100
Density at 25 °C ($g\ mL^{-1}$)	0.9	0.878	0.87	0.812	0.818	0.792
$P_{\text{saturated vapor}}$ (Pa) at 25 °C	11356	1425	120	1550[b]	10[c]	139
log K [e]	0.73[d]	1.88[d]	3.62[e]	1.38[e]	2.03[e]	1.34

[a] Fenaroli (1975).
[b] Ambrose, Ellender, Lees, Sprake, and Townsend (1975).
[c] N'Guimbi et al. (1992).
[d] Leo et al. (1971).
[e] Rekker (1977).

$$\mathrm{WVP} = \frac{\Delta m}{\Delta t \cdot \Delta p \cdot A} \cdot e$$

where $\Delta m/\Delta t$, is the weight of moisture loss per unit of time (g/s); A, the film area exposed to moisture transfer (m^2); e, the film thickness (m) and Δp, the water vapor pressure difference between the two sides of the film (Pa).

The samples were conditioned at 25 °C and 30% of relative humidity (RH) for at least 7 days prior experiments. Film samples were placed between two Teflon rings on the top of the glass cells containing the saturated salt solutions (KCl RH = 84%, NaCl RH = 75%) or distilled water (RH = 100%). Four replicates by each type of film were done.

2.6. Aroma permeability

A dynamic measurement method of aroma vapor fluxes through films was used. The apparatus has been set up by Debeaufort and Voilley (1994). The permeation cell was composed of two chambers divided by the film to be studied. The film area exposed to transfer was 15.9 cm^2. The two chambers were continuously swept by an approximately 30 mL min^{-1} nitrogen flow. The aroma concentrations in the vapor phase on the upper side of the cell were obtained by mixing two flows: one containing the volatile compound and the other dry nitrogen. Flows containing vapors were obtained from bubbling dry nitrogen through pure aroma compounds. The volatile compounds passing across the film were swept by the carrier gas (N_2). One milliliter of the carrier gas was automatically injected at periodical time in the gas chromatograph. Organic volatiles were analyzed with a flame ionizing detector (FID).

Films were equilibrated at 30% relative humidity at 25 °C before permeability determinations. Permeation measurements were carried out at 25 °C. The highest concentration of aroma in the vapor phase (saturation) was obtained by bubbling the carrier gas through pure aroma at 25 °C and atmospheric pressure. Its concentration was measured by the gas chromatograph (GC) (Chrompack CP 9000, Varian, France).

Detection threshold of aroma permeability measured by FID of the ethyl acetate, ethyl butyrate, ethyl hexanoate, 2-hexanone, 1-hexanol and *cis*-3-hexenol are 0.022, 0.07, 0.5, 0.04, 4 and 0.4 g $kPa^{-1}\ m^{-1}\ h^{-1}$, respectively.

2.7. Statistical analysis

Statistical analysis of data were performed through variance analysis (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD). Fisher's least significant difference (LSD) procedure was used.

3. Results and discussion

3.1. Lipid particle size analysis of emulsified films

Laser light scattering granulometry displays the lipid distribution in emulsified films and characterize the destabilization phenomena due to the fabrication process (spreading and drying) and also, due to the addition of the aroma compound encapsulated. Lipid particle size and distribution could influence the water and aroma transfer of the edible films. In Table 2 are given the volume–surface mean diameters ($D_{3,2}$) of films with and without SDS, which represent the average droplet size based on the specific surface per unit volume. Beeswax induces a significant increase ($p < 0.05$) of particle size as its content rise in the film matrix. Similar results were observed by Karbowiak, Debeaufort, and Voiley (2007) in ι-carrageenan-Grindsted Barrier System 2000 (GBS) films. This lipid (GBS) is an acetic acid ester of mono- and di-glycerides made from edible, fully hydrogenated vegetable oil blended with beeswax.

SDS is an anionic surfactant that charges negatively the lipid particles and prevents the particle aggregation and/or coalescence. For all OA:BW ratio, it was observed that SDS decreases the mean diameter of the lipid particles, being this effect more remarkable in film with highest beeswax content (30:70 OA:BW ratio), this means that there is a lipid particles aggregation in emulsified films due to films retraction during drying. The results show that films formulated with encapsulated aroma compound (*n*-hexanal) have significantly ($p < 0.05$) higher mean diameter for all OA:BW ratio used (with or without SDS). For the films containing more than 30% of beeswax (50:50 and 30:70 OA:BW ratio), the aroma compound disturbs the structural organization of emulsified films by the competitive affinities of lipid and aroma compound for the interface. The hexanal is less hydrophobic than the lipid mixture and behaves like an amphiphilic emulsifier. Therefore *n*-hexanal has more affinity for the interface than the emulsifier contained in the lipid mixture. The *n*-hexanal thus disturbs the emulsion stability. By contrast, when the beeswax content decreases, in 70:30 OA:BW films, the effect of both the SDS and the aroma compound is less remarkable. This can be explained because with smaller quantity of beeswax the emulsion is more homogeneous, the particle size is smaller. The emulsion is thus more stable and the aroma compound less disturbs the film structure.

Table 2
Volume–surface mean diameter ($D_{3,2}$) of lipid particles emulsified in ι-carrageenan based films with and without encapsulated aroma compound.

OA:BW ratio	*n*-Hexanal	$D_{3,2}$ water (μm)	$D_{3,2}$ SDS (μm)
70:30	Without	4.7 ± 0.03^{ab}	4.2 ± 0.14^{a}
	With	4.3 ± 0.06^{a}	4.06 ± 0.09^{a}
50:50	Without	7.3 ± 4.13^{ab}	5.8 ± 2.95^{ab}
	With	23.5 ± 0.60^{d}	18.8 ± 1.61^{d}
30:70	Without	20.27 ± 0.09^{d}	13.85 ± 0.06^{bc}
	With	19.37 ± 0.52^{d}	16.02 ± 0.32^{bc}

Means ± standard deviation.
a,b,c,d, values having the same letter are not significantly different at p level <0.05.

3.2. Water vapor permeability

Table 3 displays the WVP of control films (without lipid) and Fig. 1 shows the mean values (and LSD intervals) of WVP of lipid containing films, with and without encapsulated *n*-hexanal, at 30/75%, 30/84%, 30/100% relative humidities conditions. As expected, water vapor permeability of ι-carrageenan based films decreases with lipid addition because of their low affinity for water and a tendency to a lower permeability can be observed as the beeswax content increases. Beeswax is more efficient to controlling moisture migration than oleic acid. This can be explained but the high hydrophobicity of its long chain fatty alcohols and long chain alkanes and its solid state (Bravin, Peressini, & Sensidoni, 2004; Fabra et al., 2008; Morillon et al., 2002; Shellhammer & Krochta, 1997). Similar behavior has been observed for sodium caseinate–OA–BW films (Fabra et al., 2008). The most significant differences were noticed for films without encapsulated aroma compound (*n*-hexanal) at 30/75% and 30/84% RH.

Usually, the effect of the relative humidity on the film WVP is significant for films with and without encapsulated aroma compound. Indeed barrier efficiency decreases when the moisture content increases in the film. The adsorbed water induces a plasticization of the carrageenan network which increases the mobility of polymer strings. As the polymer mobility increases, the diffusivity of the small molecules such as water or aroma compounds strongly increases. This phenomenon is favoured with the relative humidity increase. It may be noted that when OA ratio is higher than 30% in the lipid mixtures (70:30, 50:50 OA:BW ratio), the plasticizing effect of water revealed through water vapor permeability is less marked, then an additive plasticizing effect can be attributed to the OA.

The water barrier properties of encapsulated aroma (*n*-hexanal) of composite films depend on lipid presence. Between control films without lipid containing or not encapsulated aroma compound (*n*-hexanal), a significant increase in WVP was observed. This effect has been described in a recent work (Hambleton et al., 2008). For the lowest relative humidity gradient (30–75%), the increase of the water vapor permeability in the presence of *n*-hexanal can be attributed to a plasticization effect of the aroma compound. Indeed, at lower RH gradient, the water content in films decreases and then the plasticization caused by water is low enough that it did not hide the plasticizing effect of the *n*-hexanal. This behavior means that both water and *n*-hexanal contribute to the network plasticization. Hambleton et al. (2008) showed that *n*-hexanal was located between the ι-carrageenan lateral chains and established weak interactions that modifies the film structure and promotes water transfer. Among films containing lipid, for a determined lipid composition, the WVP values is lower for films containing encapsulated aroma compound. This effect can be related to particle size behavior. Indeed, the films containing the *n*-hexanal have less aggregated particles, i.e. a more stable emulsion because the diameters in both water and SDS are similar, as shown in Table 2.

Table 3
Water vapor permeability (WVP) of control films, with and without encapsulated aroma compound (*n*-hexanal) at 25 °C and at three RH gradients.

HR (%)	WVP (10^{-3} g kPa^{-1} m^{-1} h^{-1})	
	Without encapsulated *n*-hexanal	With encapsulated *n*-hexanal
30/75	5.58 ± 0.4^{a}	7.2 ± 2.67^{a}
30/84	8.46 ± 1.98^{b}	10.44 ± 1.86^{b}
30/100	8.352 ± 0.99^{b}	9.144 ± 0.4^{b}

Means ± standard deviation.
a,b groups homogeneous, values having the same letter are not significantly different at p level <0.05.

3.3. Aroma permeability

3.3.1. Ethyl acetate, ethyl butyrate and ethyl hexanoate permeabilities

Table 4 shows the permeability of ethyl esters (ethyl acetate, ethyl butyrate and ethyl hexanoate) through the studied films. The effect of lipid presence, OA:BW ratios and encapsulated *n*-hexanal on aroma barrier properties are evaluated by a multifactor variance analysis. The method used to discriminate the means is the Fisher's least significant difference (LSD) procedure at a confidence level of 95%. Table 4 also shows the homogeneous groups from a LSD analysis. The results show that aroma barrier efficiency is significantly influenced ($p < 0.05$) by the lipid presence for the most of the aroma compounds. Among films without encapsulated *n*-hexanal, it can be observed that permeability diminishes significantly ($p < 0.05$) for film containing lipid for both ethyl acetate and ethyl butyrate. For OA:BW ratio factor, a significant decrease ($p < 0.05$) is observed on ethyl butyrate permeability as beeswax content increases while this differences are greatly attenuated for ethyl acetate permeability. The described behavior can be attributed to structural differences in the formed matrix and to the different molecular interactions. Beeswax incorporation into composite films provides an increase of mean diameter ($D_{3,2}$) of lipid particles that could induce a more compact structure, decreasing the aroma permeability. This effect becomes more evident as beeswax content increases and for ethyl butyrate permeability. The differences can be explained by the hydrophobic character of ethyl ester compounds. As it is reported by Quezada-Gallo (1999) sorption mechanism affects the transfer of small molecules (maximum 5 carbon atoms) and reveals the polymer-volatile compound affinity while diffusion is related to their mobility within the polymeric network of the material. Diffusion plays an important role on volatile molecule with high molar volume. Ethyl acetate and ethyl butyrate transfers are mainly governed by the sorption mechanism instead of kinetic parameters (diffusivity). The higher hydrophobic character of ethyl butyrate and the lower saturated vapor pressures favours its sorption though oleic acid fraction being reduces when this compound decrease en the lipid phase, compared to ethyl acetate.

In contrast, ethyl hexanoate permeability of films prepared without encapsulated aroma compound (*n*-hexanal) increases significantly ($p > 0.05$) for films containing lipid. The higher hydrophobic character of ethyl hexanoate ($\log K = 3.62$) favours its solubilization in the lipid phase. Its transfer through composite films is thus greater compared to its transfer in control films which the network is only composed of ι-carrageenan. The greater diffusion of this aroma through lipid compounds, mainly oleic acid, produces an increase of ethyl hexanoate permeability.

When comparing films with and without encapsulated *n*-hexanal, significant differences ($p < 0.05$) are observed for ethyl esters

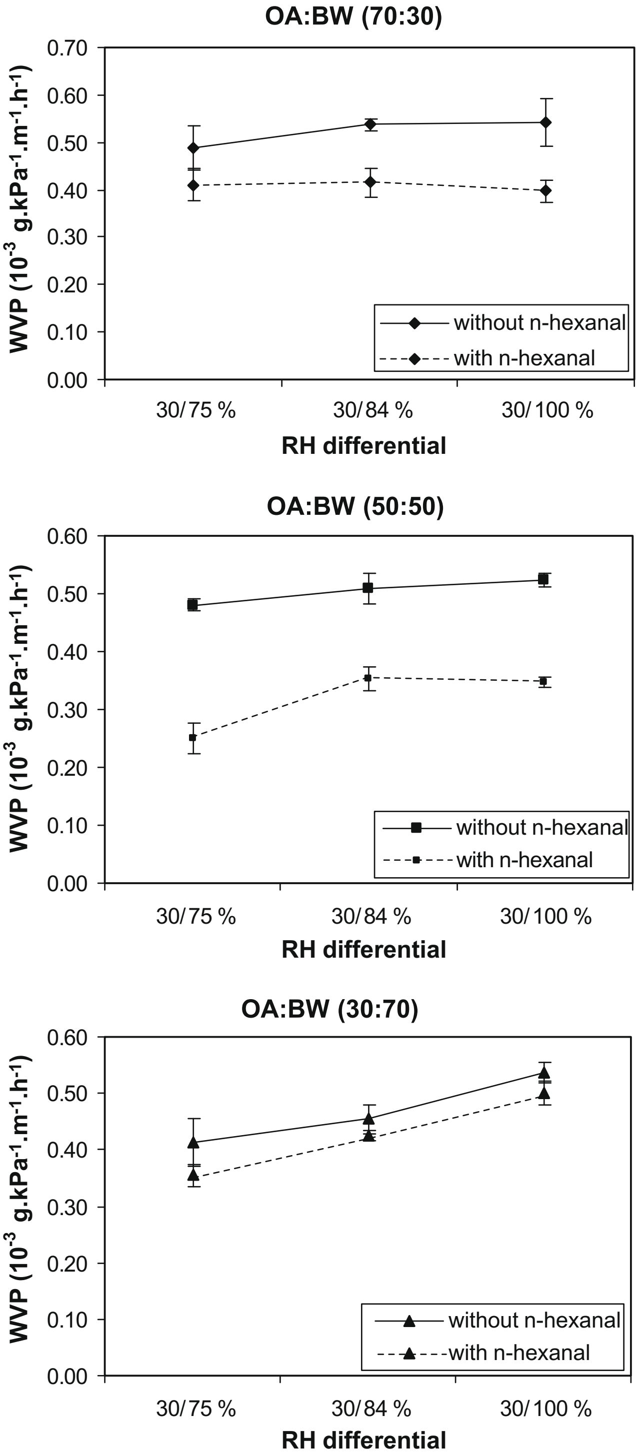


Fig. 1. Mean values (and LSD intervals) of water vapor permeability (WVP) of 2 ι-carrageenan–lipid films, with and without encapsulated aroma compound (*n*-hexanal).

permeability. Generally, encapsulated *n*-hexanal induces a decrease in aroma permeability values, except for ethyl butyrate permeability, where a significant increase is observed among lipid-based films as compared to those prepared without encapsulated *n*-hexanal. As it is shown in previous work aldehyde groups of hexanal could interact with CH$_2$OH and/or sulphate groups of ι-carrageenan in control films (without lipid) (Hambleton et al., 2008). Moreover, lipid particle size analysis gives evidence of hexanal addition inducing the formation of aggregated globules, mainly when beeswax content rises up. This could explained the differences observed between control films (without lipid, with and without encapsulated *n*-hexanal) and films prepared with different OA:BW ratios. On one hand, control films without lipid have a more compact structure when they incorporate *n*-hexanal which limits the aroma transfers. On the other hand, the encapsulated *n*-hexanal could disturb the structural organization of emulsified films by the competitive affinities of lipid mixtures and *n*-hexanal for the interface. *n*-Hexanal could have a higher affinity for the interface than the emulsifier, disturbing the emulsion stability. Moreover, the different hydrophobic character of ethyl ester plays an important role among lipid containing film. Hydrophobicity is defined as the liquid–liquid partition coefficient (*K*, a-dimensional) of the aroma compound between octanol and water expressed on a log scale (log*K*) according to Rekker (1977) and Leo, Hansch, and Elkins (1971). It indicates that the presence of encapsulated aroma compound makes the ι-carrageenan based films less homogeneous than those formulated without encapsulated *n*-hexanal and this effect is more pronounced when beeswax content increases. Thus, the aroma permeability was governed mainly by ι-carrageenan–hexanal–lipid interactions and the structure reached in the polymer network. When *n*-hexanal is added, it may interact uppermost with the lipid phase in composite films though some interactions between hexanal and CH$_2$OH and/or sulphate groups of ι-carrageenan could be also involved. Indeed, Hambleton et al. (2008) displayed through FTIR experiment that the aldehyde group of the *n*-hexanal strongly interacts with the CH$_2$OH and/or sulphate groups of the ι-carrageenan chains. Phase separation and heterogeneity are more evident in film matrix as beeswax content increases and this made the lipid action less effective. So, a part of ethyl esters could be preferentially transmitted through ι-carrageenan–hexanal zones, reaching no significantly differences ($p > 0.05$) in aroma permeability between control film (with encapsulated *n*-hexanal) and films prepared with 30:70 OA:BW ratio (see Table 4 ethyl butyrate permeability of films with encapsulated aroma compound). In addition, physicochemical properties of ethyl esters have a great effect on ethyl esters permeability. The commented difference between control and films containing lipid, with encapsulated aroma compound (*n*-hexanal), is more obvious for ethyl butyrate permeability due to lower saturated vapor pressures that makes easier their adsorption by the films surface. It may be noted that, no significant difference ($p > 0.05$) between control and lipid-based films containing the encapsulated *n*-hexanal, is observed for ethyl hexanoate permeability, probably because of the efficient barrier performance of the control films with encapsulated *n*-hexanal to ethyl hexanoate. In the case of the ethyl acetate, statistical differences between control and films containing at least 50% beeswax can be explained by the higher polarity of ethyl acetate which induces higher permeability probably in the oleic acid phase.

The effect of volatile compound on aroma permeability was evaluated by the multifactor variance analysis. For aroma transfer of a homologous series of ethyl esters (ethyl acetate, ethyl butyrate and ethyl hexanoate) through ι-carrageenan based films, statistical significant differences ($p < 0.05$) are observed. These differences can be explained by the mechanism (sorption and diffusion) implied in aroma transfers and also by the different hydrophobicity between aroma compounds. As commented previously, ethyl acetate and ethyl butyrate transfers are mainly governed by the sorption mechanism. In contrast, the differences between these aroma compounds and ethyl hexanoate could be better understood by the higher hydrophobic character (log*K*) and the lower saturated vapor pressure of the last one which allows its transfer though ι-carrageenan based films mainly when oleic acid is the main compound in lipid fraction.

Table 4
Ethyl acetate, ethyl butyrate and ethyl hexanoate permeability of ι-carrageenan–OA–BW films without encapsulated aroma compound.

Encapsulated *n*-hexanal	OA:BW ratio	Ethyl acetate permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})	Ethyl butyrate permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})	Ethyl hexanoate permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})
Without	Control	0.015 ± 0.002^{a}	0.35 ± 0.02^{a}	$<0.0005^{a}$
	70:30	0.0035 ± 0.0001^{b}	0.047 ± 0.002^{b}	3.0 ± 0.2^{b}
	50:50	0.0023 ± 0.0001^{b}	0.036 ± 0.001^{c}	1.2 ± 0.2^{bc}
	30:70	0.0030 ± 0.0001^{b}	0.027 ± 0.001^{d}	0.70 ± 0.07^{c}
With	Control	0.0061 ± 0.0006^{c}	0.209 ± 0.005^{e}	$<0.0005^{a}$
	70:30	0.0039 ± 0.0001^{bc}	0.105 ± 0.004^{f}	$<0.0005^{a}$
	50:50	0.00270 ± 0.00003^{b}	0.144 ± 0.009^{g}	$<0.0005^{a}$
	30:70	0.00271 ± 0.00004^{b}	0.248 ± 0.0008^{e}	$<0.0005^{a}$

Means ± standard deviation.
a–g, groups homogeneous for each aroma permeability, values having the same letter are not significantly different at p level <0.05.

3.3.2. 2-Hexanone, 1-hexanol and cis-3-hexenol permeabilities

In order to evaluate the influence of the polarity and chemical group of aroma compound, permeability of 2-hexanone, 1-hexanol and *cis*-3-hexenol of ι-carrageenan, with and without encapsulated *n*-hexanal, films were investigated and compared with ethyl hexanoate permeability. Table 5 shows the 2-hexanone permeability and homogeneous groups from a LSD analysis of studied films. The incorporation of the oleic acid: beeswax mixtures induces a statistically decrease ($p < 0.05$) of the aroma permeability of ι-carrageenan films which does not contained *n*-hexanal whereas this effect is less significant for films having the encapsulated aroma compound (*n*-hexanal). These are in accordance with the results observed for the previous aroma permeability values (see Table 4). For films prepared without encapsulated *n*-hexanal, beeswax presence induces an increase of the mean diameter of the lipid particles and a more compact structure is obtained. In contrast, the presence of encapsulated aroma compound (*n*-hexanal) disturbs the structural organization of emulsified film due to the competitive affinities of lipid mixtures and encapsulated aroma compound for the interface. As consequence, a decrease in barrier efficiency could be observed due to the more heterogeneous matrix obtained. It is note that for films formulated with encapsulated aroma compound, no significant differences ($p > 0.05$) are observed between control and films containing lipid, though it is observed a tendency to increase the mean permeability value as beeswax content increases in the lipid phase. This behavior can be compared to those obtained for ethyl butyrate permeability.

Table 5 also displays 1-hexanol permeability values and homogenous groups from a LSD analysis of studied films. Contrarily to what has been observed for the previous aroma compound permeability, for films without encapsulated aroma compound, lipid presence induces a significant increase ($p < 0.05$) of 1-hexanol permeability values but this is reduced as beeswax content increases in lipid mixtures. These results suggest that the hydrophobic character of 1-hexanol ($\log K = 2$) limits its transfers through hydrophilic control films while its diffusion through lipid phase is easier, mainly when oleic acid is the main compound. In addition, the transfer of 1-hexanol through sodium caseinate–OA–BW films may induce interactions between polar groups (such as hydrogen bonds) leading to a more plasticized structure, specially at high content of oleic acid. On the contrary, as the wax content increases, the aroma permeability decreases as also shown by Quezada-Gallo, Debeaufort, Callegarin, and Voilley (2000). This can be explained by a lower diffusivity through beeswax in oleic acid. Indeed, diffusion is reduced by the solid fat content and crystalline ratio. Moreover, a more compact structure is obtained with the beeswax content.

For films prepared with encapsulated aroma compound (*n*-hexanal), no significant differences ($p > 0.05$) are observed between control and lipid containing films, obtaining permeability values for *cis*-3-hexenol under the equipment threshold. As explained previously, for films with encapsulated *n*-hexanal, aroma transfers are governed mainly by ι-carrageenan–hexanal–lipid interactions and the structure reached in the polymer network. Size particle analysis suggests that phase separation could occur and a part of aroma compounds could preferentially be transmitted through ι-carrageenan–hexanal zones. In this sense, it could be expected that permeability's values increases with beeswax content reaching no significant differences in aroma permeability between control film and films prepared with higher beeswax content, as it was shown for ethyl butyrate permeability (Table 4). However, similarly to ethyl hexanoate permeability, no significant difference is observed between control and films containing lipid (with encapsulated aroma compound) and this can be explained to the efficient barrier performance to 1-hexanol of control films (formulated with encapsulated *n*-hexanal).

1-Hexanol, 2-hexanone and ethyl hexanoate permeability values are statistically different ($p < 0.05$) for in ι-carrageenan based films. Concerning films prepared without encapsulated aroma compound, different behaviors are also observed between control and lipid containing films. For control films (without lipid), 1-hex-

Table 5
2-Hexanone, 1-hexanol and *cis*-3-hexenol permeability of ι-carrageenan–OA–BW films without encapsulated aroma compound.

Encapsulated *n*-hexanal	OA:BW ratio	2-Hexanone permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})	1-Hexanol permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})	*cis*-3-Hexenol permeability (10^{-3} g kPa^{-1} m^{-1} h^{-1})
Without	Control	0.081 ± 0.004^{a}	$<0.004^{a}$	$<0.0004^{a}$
	70:30	$<0.00004^{b}$	83.0 ± 2.0^{b}	$<0.0004^{a}$
	50:50	$<0.00004^{b}$	19.8 ± 0.13^{c}	$<0.0004^{a}$
	30:70	$<0.00004^{b}$	$<0.004^{a}$	$<0.0004^{a}$
With	Control	0.034 ± 0.001^{c}	$<0.004^{a}$	$<0.0004^{a}$
	70:30	0.018 ± 0.001^{d}	$<0.004^{a}$	$<0.0004^{a}$
	50:50	0.036 ± 0.001^{c}	$<0.004^{a}$	$<0.0004^{a}$
	30:70	0.048 ± 0.006^{e}	$<0.004^{a}$	$<0.0004^{a}$

Means ± standard deviation
a–e, groups homogeneous for each aroma permeability, values having the same letter are not significantly different at p level <0.05.

anol and ethyl hexanoate exhibited the lowest permeability values probably due to its higher hydrophobicity (log$K$ = 2.03 and 3.62, respectively) which limits their affinity though control films where the hydrophilic character is more pronounced. In contrast, lipid presence induces an increase in aroma permeability of ethyl hexanoate and 1-hexanol as compared to 2-hexanone. This can be attributed to the great sorption and diffusion of hydrophobic aroma compound (1-hexanol and ethyl hexanoate) through lipid phase (the highest oleic acid the highest diffusion). It may be noted that this increase in aroma permeability is more pronounced for 1-hexanol as compared to ethyl hexanoate permeability and these differences become more important with the oleic acid content. Polar groups of 1-hexanol and oleic acid probably interacts with ι-carrageenan film network, inducing a plasticization which favours their transfer.

As regards to films prepared with encapsulated *n*-hexanal, a sharp reduction in 1-hexanol and ethyl hexanoate permeability values is observed compared to 2-hexanone. As explained previously, these differences could be attributed to structural changes and the higher hydrophobicity of 1-hexanol and ethyl hexanoate which provides better aroma barrier properties for hydrophilic films.

As shown in Table 5, *cis*-3-hexenol values permeability of studied films are lower than equipment threshold, showing significantly reduced permeability. These differences can be attributed to the insaturation presence that implies higher molecular volume and diameter of *cis*-3-hexenol. Thus, intrinsic molecular mobility of this aroma compound is reduced compared to linear aroma compounds with the same chain length and thus its ability to diffuse is strongly diminished. Linear molecules such as 1-hexanol could be sorbed and diffuser easily in the film matrix.

4. Conclusion

The influence of OA:BW ratio and encapsulated *n*-hexanal presence on water and aroma barrier properties of ι-carrageenan based films was evaluated. Water vapor permeability decreases as relative humidity increases but this difference becomes less significant with oleic acid content. Water barrier properties also revealed the plasticizing effect of both *n*-hexanal and oleic acid. Physicochemical properties of aroma compound and encapsulated *n*-hexanal play an important role in aroma permeability. It was observed a plasticizing effect of ethyl acetate and ethyl butyrate into control films without encapsulated *n*-hexanal. The addition of *n*-hexanal to control films reduces the aroma permeability due to the better organization of the gel network and the more compact structure obtained. In films containing lipid, the aroma permeability depends on OA:BW ratio and *n*-hexanal presence. For films prepared without encapsulated *n*-hexanal, the permeability is governed mainly by the greater diffusion through oleic acid. Consequently, the increase of beeswax content produces a decrease in the aroma permeability values. On the contrary, *n*-hexanal encapsulation induces a more heterogeneous matrix. Then, the aroma transfers are probably mainly governed by the ι-carrageenan–hexanal interactions. This work reveals that ι-carrageenan can be a very good support for the encapsulation because of low aroma permeability as long as no plasticization by encapsulated aroma occurs.

Acknowledgments

The authors acknowledge the financial support from Spanish Ministry of Culture and Education throughout the project AGL2007-65503 and from the Conselleria de Educacion de la Comunidad Valenciana Through the Project GVPRE/2008/352. Author M.J. Fabra thanks Spanish Ministry of Culture & Education for a FPU grant (AP2005-3562). Author A. Hambleton thanks CONACyT of Mexico for their support. Authors also thank M. Desprairies from Cargill for its advice and council in using hydrocolloids, Dr. C. Brun-Dury from ENSBANA, for her assistance in the use of gas chromatography (GC) and Prof. J.P Gay for the manuscript improvement.

References

Ambrose, D., Ellender, J. H., Lees, E. B., Sprake, C. H. S., & Townsend, R. (1975). Thermodynamic properties of organic oxygen compounds XXXVIII. Vapour pressures of some aliphatic ketones. *Journal of Chemical Thermodynamics, 7*, 453–472.

Bravin, B., Peressini, D., & Sensidoni, A. (2004). Influence of emulsifier type and content on functional properties of polysaccharide lipid-based edible films. *Journal of Agricultural Food Chemistry, 52*(21), 6448–6455.

Debeaufort, F., & Voilley, A. (1994). Aroma compound and water vapour permeability of edible films and polymer packagings. *Journal of Agricultural Food Chemistry, 42*, 2871–2875.

Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coating: Tomorrow's packagings: A review. *Critical Reviews in Food Science, 38*(4), 299–313.

Dury-Brun, C., Chalier, P., Desobry, S., & Voilley, A. (2007). Multiple mass transfers of small volatile molecules through flexible food packaging. *Food Reviews International, 23*, 199–255.

Fabra, M. J., Talens, P., & Chiralt, A. (2008). Tensile properties and water vapour permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *Journal of Food Engineering, 85*, 393–400.

Fenaroli, G. (1975). In T. E. Furia & N. Bellanca (Eds.). *Handbook of flavor ingredients* (Vol. 2, pp. 3–566). Cleveland, OH: CRC Press.

Guilbert, S. (1986). Technology and application of edible films. In M. Mathlouthi (Ed.), *Food packaging and preservation* (pp. 371–394). New York: Elsevier Applied Science Publishers.

Guilbert, S., & Gontard, N. (2005). Agro-polymers for edible and biodegradable films: Review of agricultural polymeric materials, physical and mechanical characteristics. *Innovations in Food Packaging*, 263–276.

Hambleton, A., Debeaufort, F., Beney, L., Karbowiak, T., & Voilley, A. (2008). Protection of active aroma compound against moisture and oxygen by encapsulation in biopolymeric emulsion-based edible films. *Biomacromolecules, 9*(3), 1058–1063.

Han, J. H., & Gennadios, A. (2005). Edible films and coatings: A review. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 239–262). London: Elsevier Academic Press.

Karbowiak, T., Gougeon, R. D., Rigolet, S., Delmotte, L., Debeaufort, F., & Voilley, A. (2008). Diffusion of small molecules in edible films: Effect of water and interactions between diffusant and biopolymer. *Food Chemistry, 106*(4), 1340–1349.

Karbowiak, T., Debeaufort, F., & Voilley, A. (2007). Influence of thermal process on the structure and functional properties of emulsion-based edible films. *Food Hydrocolloids, 21*(5–6), 879–888.

Krochta, J. M. (2002). Proteins as raw materials for films and coatings: Definitions, current status, and opportunities. In A. Gennadios (Ed.), *Protein-based films and coatings* (pp. 1–32). Boca Raton, London, New York, Washington, DC: CRC Press.

Lacroix, M., & Le Tien, C. (2005). Edible films and coatings from non-starch polysaccharides. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 338–361). London: Elsevier Academic Press.

Leo, A., Hansch, C., & Elkins, D. (1971). Partition coefficients and their uses. *Chemical Reviews Washington, 71*(6), 525–616.

Miller, K. S., & Krochta, J. M. (1997). Oxygen and aroma barrier properties of edible films: A review. *Trends in Food Science and Technology, 8*, 228–237.

Morillon, V., Debeaufort, F., Blond, G., Capelle, M., & Voilley, A. (2002). Factors affecting the moisture permeability of lipid-based edible films: A review. *Critical Review in Food Science and Nutrition, 42*(1), 67–89.

N'Guimbi, J., Kasehgari, H., Mokbel, I., & Jose, J. (1992). Tensions de vapeur d'alcools primaires dans le domaine 0 3 Pa a 1.5 kPa. *Thermochimica Acta, 196*, 367–377.

Quezada-Gallo, J. A. (1999). Influence de la structure et de la composition de réseaux macromoléculaire pour les transferts de volatiles (eau et arômes). Applications aux emballages comestibles et plastiques. Thèse de doctorat, ENSBANA, Université de Bourgogne, Dijon, pp. 168.

Quezada-Gallo, J. A., Debeaufort, F., & Voilley, A. (1999). Interactions between aroma and edible films. 1. Permeability of methylcellulose and low-density polyethylene films to methyl ketones. *Journal of Agricultural Food Chemistry, 47*, 108–113.

Quezada-Gallo, J. A., Debeaufort, F., Callegarin, F., & Voilley, A. (2000). Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based edible films. *Journal of Membrane Science, 4678*, 1–10.

Reineccius, G. A. (1991). Carbohydrates for flavor encapsulation. *Food Technology, 45*, 144–147.

Reineccius, G. (1994). Flavor encapsulation. In J. M. Krochta, E. A. Baldwin, & M. O. Nisperos-Carriedo (Eds.), *Edible films to improve food quality* (pp. 105–120). Basel: Technomic Publishing Co. Inc..

Rekker, R. F. (1977). *The hydrophobic fragmental constant*. New York: Elsevier.

Shellhammer, T. H., & Krochta, J. M. (1997). Whey protein emulsion film performance as affected by lipid type and amount. *Journal of Food Science, 62*(2), 390–394.

Tari, T. A., & Singhal, R. S. (2002). Starch based spherical aggregates: Reconfirmation of the role of amylose on the stability of a model flavouring compound, vanillin. *Carbohydrates Polymers, 50*, 279–282.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology, 14*(3), 71–78.

Yuguchi, Y., Thu Thuy, T. T., Urakawa, H., & Kajiwara, K. (2002). Structural characteristics of carrageenan gels: Temperature and concentration dependence. *Food Hydrocolloids, 16*, 515–522.