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Effect of essential oils on properties of film forming emulsions and films based on hydroxypropylmethylcellulose and chitosan

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ABSTRACT

Film-forming dispersions (FFD) and films, prepared by incorporating different concentrations of bergamot (BO), lemon (LO) and tea tree (TTO) essential oils into hydroxypropylmethylcellulose (HPMC) and chitosan (CH) were obtained and their physico-chemical properties were characterised. Results showed that the increment of essential oil (EO) content promoted significant changes in the size and surface charge of the FFD particles. As regards the film properties, the higher the EO content, the lower the water vapour permeability and the moisture sorption capacity. In general, the addition of EO into the HPMC or CH matrix leads to a significant decrease in gloss, transparency, tensile strength and elastic modulus of the composite films. Discriminant analyses of obtained data revealed that the polymer type was the main factor which defined the FFD and composite film behaviour. For a given polymer, although both the nature and concentration of the EO influenced FFD behaviour, the concentration played a more important role. In film properties, the discriminant analyses did not reveal different groups associated to the different nature or concentration of the essential oils, although composite films with BO appeared to differ slightly from the rest.

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1. Introduction

In the next few years one of the major challenges for food technologists is the design of active food packaging. This technology appears to be a promising alternative with an increasing amount of applications due to its advantages over traditional packaging systems. The use of edible films and coatings as carriers of active substances has been suggested as an interesting option (Cuq et al., 1995; Han, 2000). Essential oil compounds, which have a well documented antimicrobial activity against spoilage microorganisms, foodborne and postharvest pathogens (Burt, 2004; Bakkali et al., 2008) are of great potential use in bioactive coatings. The mechanisms of action of essential oils (EO) have not been clearly identified but they seem to be related with the hydrophobic nature of the different terpenes (Burt, 2004; Bakkali et al., 2008).

The specific advantage of EO appears to be the synergistic effects of their compounds as evidenced in the greater activity when applied as natural EO, as compared with the sum of the effects of the individual substances (Duke and Beckstrom-Sternberg, 1992). The components of EO are important as their qualitative and quantitative composition determines the characteristics of the oils, which, in turn, could have an effect on their antimicrobial potential

(Dugo et al., 2000). The typical composition of lemon, bergamot and tea tree oil, which are the EO used in this work, is reported in Table 1. Limonene is one of the major components of citrus oils with concentrations from 88% to 95% in lemon oil, although levels in bergamot are lower with concentrations ranging from 32% to 45% (Fischer and Phillips, 2008). The composition of the essential oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO), is quite different. TTO is a complex mixture of terpenes, hydrocarbons and tertiary alcohols and its composition for determined uses is regulated by an international standard which sets levels of 14 components (Hammer et al., 2006). Its main compound is terpinen-4-ol (around 40%), which is responsible for its antimicrobial activity (Cox et al., 2001; Terzi et al., 2007).

Among the biopolymers used to obtain films/coatings, cellulose derivatives are interesting film forming compounds, as they are odourless, tasteless and biodegradable (Krochta and Mulder-Johnston, 1997). Hydroxypropylmethylcellulose (HPMC) presents a great potential for a wide range of food applications due to its biocompatibility, non-toxicity, low cost and excellent film forming capacity (Nisperos-Carriedo, 1994; Villalobos et al., 2006). HPMC films are very efficient oxygen, carbon dioxide and lipid barriers. However, they are highly permeable to water vapour, which is an important drawback that limits its application (Krochta and Mulder-Johnston, 1997), since an effective control of moisture transfer is one of the most desirable properties of the films. While the incorporation of essential oils into HPMC matrices could confer

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Table 1
Typical composition of essential oils (major volatile compounds).

Component	Composition (%)		
	Tea tree oil ^a	Bergamot oil ^b	Lemon oil ^b
Terpinen-4-ol	40.1	0.00	0.00
γ-Terpinene	23.0	–	–
α-Terpinene	10.4	0.23	0.46
1,8-Cineole	5.1	–	–
Terpinolene	3.1	–	–
ρ-Cimene	2.9	5.62	1.75
α-Pinene	2.6	1.39	0.27
α-Terpineol	2.4	0.00	1.30
Limonene	1.0	72.88	78.84
Butylacetate	–	4.97	1.47
Linalool	0.00	10.23	0.02
Valencene	–	0.00	3.34

^a Brophy et al. (1989).
^b Moufida and Marzouk (2003).

antimicrobial properties to the films, it may contribute to reduce water vapour permeability due to their hydrophobic nature.

Among the bioactive macromolecules, chitosan has a great potential for a wide range of food applications due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and film forming capacity (Li et al., 1992; Tharanathan and Kittur, 2003). Chitosan based films have been proven to present moderate oxygen barrier properties and good carbon dioxide barrier properties but high water vapour permeability, due to their hydrophilic nature (Butler et al., 1996). Combined antimicrobial effects have been described for chitosan films containing essential oils (Sánchez-González, 2010) while their water barrier properties were also improved when these hydrophobic compounds were incorporated in composite films.

Films and coatings must be designed to fulfil a number of requirements, such as to have proper mechanical properties, good appearance (adequate gloss and transparency) and adequate water and gas barrier properties. In composite films containing lipids in a biopolymer matrix, the microstructure plays a very important role (Villalobos et al., 2005; Fabra et al., 2009) in these properties, which, in turn are greatly affected by the structural properties and stability of the film-forming dispersions (FFD). The stability of FFDs is affected by their particle size and distribution, rheological behaviour, and ζ-potential of the dispersed lipid particles (McClements, 2007).

Although edible coatings with EO have been previously studied (Sánchez-González et al., 2009; Zinoviadou et al., 2009; Pranoto et al., 2005), a comparative study of the effect of different EO (of different composition) in differing polymer matrices has not been published.

The aim of this work is to evaluate how the nature of the EO (lemon, bergamot and tea tree oil) and the EO:polymer ratio affect the properties of HPMC and chitosan based films, through the characterization of the stability related parameters (particle size, rheological behaviour and ζ-potential) of the FFDs and the physical properties (barrier, mechanical and optical) of the films.

2. Materials and methods

2.1. Materials

Hydroxypropylmethylcellulose (HPMC, E464, Methocel Food grade, Dow Chemical Company, Midland, USA), high molecular weight chitosan (CH) with a deacetylation degree of 82.7% (Batch 10305DD, Sigma-Aldrich Química, Madrid, Spain), 98% glacial acetic acid (Panreac, Barcelona, Spain) and essential oils (bergamot,

lemon, tea tree oil) supplied by Herbes del Molí (Alicante, Spain) were used to prepare the film-forming dispersions.

2.2. Preparation of the film-forming dispersions

Hydroxypropylmethylcellulose 1% w/w was dispersed in deionised water at 80 °C. Chitosan (1% w/w) was dispersed in an aqueous solution of glacial acetic acid (0.5% w/w) at 25 °C. After the dissolution of the polysaccharides, essential oils (EO) were added to polymer solutions to reach a final concentration of 0.5%, 1% and 2% (w/w). A batch without EO addition was also prepared for each type of biopolymer.

HPMC-EO and CH-EO mixtures were emulsified at room temperature using a rotor-stator homogenizer (Ultraturrax DI 25 basic-Yellowline, Janke & Kunkel, Staufen, Germany) at 13,500 rpm for 4 min. These emulsions were vacuum degasified at room temperature with a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany). Aqueous solutions of EO without polymer were prepared following the same methodology described for FFDs.

2.3. Characterization of the film-forming dispersions

The density of the FFD was measured by means of a digital densimeter DA-110 M (Mettler Toledo, Barcelona, Spain). A pH-meter C831 (Consort, Tumahout, Belgium) was used to determine the pH of the FFD at 20 °C.

2.3.1. ζ-potential measurements

In order to perform ζ-potential measurements, FFD were diluted to a droplet concentration of 0.02% EO using deionised water. ζ-potential was determined by using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). The Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into ζ-potential values.

2.3.2. Particle size measurements

Particle size analysis of the FFD was carried out by using a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The samples were diluted in deionised water at 2000 rpm until an obscuration rate of 10% was obtained. The Mie theory was applied by considering a refractive index of 1.52 and absorption of 0.1 for essential oils. Three samples of each FFD were measured in quintuplicate.

2.3.3. Rheological behaviour

The rheological behaviour of FFD was analysed in triplicate at 25 °C by means of a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti.

Rheological curves were obtained after a stabilization time of 5 min at 25 °C. The shear stress (σ) was measured as a function of shear rate (γ̇) from 0 to 512 s⁻¹, taking 5 min to reach the maximum shear rate and another 5 min to attain zero shear rate.

The power law model (Eq. (1)) was applied to determine the consistency index (K) and the flow behaviour index (n). Apparent viscosities were calculated at 100 s⁻¹.

$$\sigma = K \cdot \dot{\gamma}^n \quad (1)$$

2.4. Preparation of films

A casting method was used to obtain films. FFD were poured onto a framed and levelled polytetrafluorethylene (PTFE) plate (φ = 15 cm) and were dried in atmospheric conditions (about 60% RH and 20 °C) for 48 h. Film thickness was controlled by pouring the amount of FFD that will provide a surface density of total solids

(polymer plus EO) in the dry films of 56 g/m² in all formulations. Dry films were peeled off the casting surface and preconditioned in desiccators at 20 °C and 54.4% relative humidity (RH) prior to testing. A hand-held digital micrometer (Palmer – Comecta, Spain, ±0.001 mm) was used to measure film thickness at three different points of the same sample at least.

2.5. Characterization of the films

2.5.1. Water vapour permeability

Water vapour permeability (WVP) was measured in dry film discs ($\phi = 7$ cm), which were equilibrated at 54.4% RH and 20 °C, according to the “water method” of the ASTM E-96-95 (ASTM, 1995), using Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium). For each type of film, WVP measurements were replicated three times and WVP was calculated following the methodology described by Sánchez-González et al. (2009), at 20 °C and 54.4–100% relative humidity gradient. The equilibrium moisture content of the films at a_w 0.75 (intermediate value in the RH gradient) was determined from the weight loss when drying the equilibrated films in a vacuum oven at 70 °C.

2.5.2. Mechanical properties

Mechanical properties were measured in films equilibrated at 54.4% RH at 20 °C by using a Texture Analyser TA-XT-plus (Stable Micro Systems, Surrey, UK), with a 50 N load cell equipped with tensile grips (A/TG model) according to Sánchez-González et al. (2009). Tensile strength (TS) and percentage of elongation (%E) at break, and elastic modulus (EM) were evaluated in eight samples from each type of film.

2.5.3. Optical properties

Gloss was determined by using a gloss meter (Multi-Gloss 268, Minolta, Langenhagen, Germany) at an incidence angle of 60°, following the ASTM standard D523 (ASTM, 1999) in films previously equilibrated at 20 °C and 54.4% RH. Gloss measurements were carried out in quintuplicate over a black matte standard plate. Results were expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

The transparency of the films was determined through the surface reflectance spectra in a spectrophotometer CM-3600d (Minolta Co, Tokyo, Japan) with a 10 mm illuminated sample area. Measurements were taken from three samples in each formulation by using both a white and a black background. Film transparency was evaluated through the internal transmittance T_i (0–1, theoretical range) (Hutchings, 1999), by applying the Kubelka–Munk theory for multiple scattering to the reflection data (Sánchez-González et al., 2009). Values of T_i at 450 nm were used to compare samples.

2.6. Statistical analysis

Results were analysed by multifactor analysis of variance and discriminant analysis with 95% significance level using Statgraphics®Plus 5.1.

3. Results and discussion

3.1. Characterization of the film-forming dispersions

Density, pH and ζ -potential values of the different film-forming dispersions (FFD) are reported in Tables 2 and 3, for HPMC and CH FFDs. Only for the highest essential oil (EO) concentrations (2%) was a significant decrease in the density of the HPMC FFD observed. Citrus oils presented a more marked effect on density of the FFD in comparison with tea tree oil (TTO). The same tendency was observed for CH FFD density whose values were in the range of those reported by other authors at the same pH (Vargas et al., 2009). Concerning the pH of CH FFD, values were around 4.3 at room temperature and did not vary significantly ($p < 0.05$) with the incorporation of EOs, since, at the low pH value of CH dispersion, no dissociation of the weak acid EO components occurs. However, the incorporation of EO into HPMC led to a significant decrease of pH due to the acid nature and dissociation in the aqueous solution of some of the EO components, such as has been reported in a previous study (Sánchez-González et al., 2009).

Concerning the ζ -potential values of the HPMC FFD, they presented slight negative values (between -2.5 and -7 mV). These values were markedly less negative than those corresponding to the aqueous dispersion of EO (1% wt) in absence of HPMC (Table 4) (ζ -potential values of 1% TTO, BO and LO measured in water were -31 ± 2 mV, -61 ± 3 mV, and -49 ± 3 mV, respectively). Modified cellulose derivatives have been shown to present interfacial activity and can adsorb on EO droplets, thus modifying the electrophoretic mobility plane and so the ζ -potential values (Huang et al., 2001). So, results point to the fact that the HPMC chains are adsorbed on the surface of EO droplets, leading to an increase in their effective size, with a lower electrical net charge on the surface surrounding the adsorbed polymer layer. Particle sizes showed monomodal distributions whose mean size values are shown in Tables 2 and 3 for EO dispersions without and with polymer, respectively. As can be deduced from these tables, except for TTO, particles showed significantly lower mean size values when there is no polymer in the system. In general, the increment of EO did not affect the ζ -potential values of the particles, except for the HPMC-BO dispersions. In this case, the highest EO content led to more negatively charged particles.

Table 2

Properties of HPMC film-forming dispersions: pH, ζ -potential, density (ρ), Ostwald de Waale model parameters (n, k), apparent viscosity (η_{ap} at 100 s⁻¹) and particle size (d_{43} and d_{32}) values at 25 °C. Mean values and standard deviation.

FFS	pH	ζ (mV)	ρ (kg/m ³)	$0 \leq \dot{\gamma} \leq 512$ s ⁻¹				d_{43} (μ m)	d_{32} (μ m)
				n	k (Pa s) ⁿ	r^2	η_{ap} (Pa s)		
HPMC	7.87 (0.06) ^a	-3.4 (0.6) ^a	1002.5 (0.3) ^a	1.162 (0.006) ^a	0.00209 (0.00009) ^a	0.985	0.00441 (0.00006) ^a	-	-
HPMC-0.5BO	6.04 (0.02) ^b	-2.5 (0.4) ^b	1001.5 (0.9) ^a	1.028 (0.002) ^b	0.00362 (0.00008) ^b	0.970	0.00413 (0.00006) ^b	3.68 (0.02) ^a	2.721 (0.017) ^a
HPMC-1BO	5.38 (0.05) ^c	-5.1 (0.2) ^c	1002.6 (0.3) ^a	1.029 (0.002) ^b	0.00351 (0.00016) ^b	0.998	0.00415 (0.00014) ^b	4.39 (0.08) ^b	3.31 (0.04) ^b
HPMC-2BO	4.71 (0.02) ^d	-5.6 (0.2) ^c	999.10 (0.14) ^b	1.0297 (0.0008) ^b	0.0038 (0.0002) ^b	0.999	0.0044 (0.0003) ^b	4.9 (0.2) ^c	3.73 (0.15) ^c
HPMC-0.5LO	7.29 (0.05) ^e	-7.0 (0.5) ^d	1002.0 (0.8) ^a	1.0265 (0.0012) ^b	0.00367 (0.00003) ^b	0.970	0.00415 (0.00004) ^b	3.8 (0.3) ^a	2.75 (0.15) ^{a,g}
HPMC-1LO	6.39 (0.04) ^f	-6.9 (0.5) ^d	1002.5 (0.3) ^a	1.0271 (0.0012) ^b	0.00367 (0.00003) ^b	0.974	0.00416 (0.00004) ^b	4.7 (0.3) ^d	3.43 (0.13) ^d
HPMC-2LO	5.35 (0.05) ^c	-7.3 (0.4) ^d	999.16 (0.14) ^b	1.0247 (0.0016) ^b	0.0036 (0.0004) ^b	0.975	0.00431 (0.00014) ^b	5.52 (0.14) ^e	4.08 (0.05) ^e
HPMC-0.5TTO	6.97 (0.02) ^g	-2.76 (0.09) ^b	1002.4 (0.3) ^a	1.029 (0.005) ^b	0.00360 (0.00003) ^b	0.984	0.00413 (0.00009) ^b	3.30 (0.05) ^f	2.00 (0.03) ^f
HPMC-1TTO	5.74 (0.02) ^h	-2.7 (0.3) ^b	1002.3 (0.2) ^a	1.024 (0.002) ^b	0.00366 (0.00004) ^b	0.983	0.00417 (0.00014) ^b	4.13 (0.12) ^g	2.803 (0.012) ^g
HPMC-2TTO	4.70 (0.02) ^d	-2.72 (0.08) ^b	1000.74 (0.05) ^c	1.025 (0.006) ^b	0.00383 (0.00004) ^b	0.986	0.00429 (0.00009) ^b	4.6 (0.3) ^d	3.29 (0.15) ^b

a,b,c,d,e,f,g,h Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Table 3
Properties of CH film-forming dispersions: pH, ζ -potential, density (ρ), Ostwald de Waale model parameters (n , k), apparent viscosity (η_{ap} at 100 s^{-1}) and particle size (d_{43} and d_{32}) values at 25°C . Mean values and standard deviation.

FFS	pH	ζ (mV)	ρ (kg/m^3)	$0 \leq \dot{\gamma} \leq 512 \text{ s}^{-1}$				d_{43} (μm)	d_{32} (μm)
				n	k (Pa s^n)	r^2	η_{ap} (Pa s)		
CH	4.28 (0.02) ^a	100 (3) ^a	1004.69 (0.13) ^a	0.785 (0.007) ^b	0.58 (0.02) ^a	0.975	0.216 (0.002) ^a	–	–
CH-0.5BO	4.31 (0.03) ^a	82 (3) ^c	1003.888 (0.005) ^b	0.777 (0.002) ^a	0.554 (0.116) ^b	0.997	0.179 (0.019) ^c	7.0 (0.4) ^a	3.9 (0.2) ^a
CH-1BO	4.25 (0.02) ^b	80.3 (1.4) ^c	1002.55 (0.05) ^d	0.7936 (0.0116) ^b	0.38 (0.04) ^d	0.996	0.150 (0.008) ^e	15.0 (0.4) ^b	6.5 (0.2) ^b
CH-2BO	4.24 (0.02) ^b	77.60 (1.02) ^d	1001.26 (0.17) ^f	0.82 (0.02) ^{bc}	0.29 (0.06) ^e	0.997	0.139 (0.015) ^e	22.1 (0.2) ^c	8.53 (0.17) ^c
CH-0.5LO	4.29 (0.02) ^a	80 (2) ^c	1004.407 (0.002) ^a	0.83 (0.02) ^c	0.36 (0.07) ^d	0.984	0.169 (0.017) ^d	4.2 (0.2) ^d	3.03 (0.04) ^d
CH-1LO	4.29 (0.02) ^a	79 (2) ^c	1003.438 (0.002) ^c	0.877 (0.013) ^d	0.25 (0.02) ^e	0.983	0.145 (0.012) ^e	9.47 (0.13) ^e	4.55 (0.07) ^e
CH-2LO	4.28 (0.03) ^a	76 (2) ^d	1002.538 (0.002) ^d	0.86 (0.02) ^d	0.25 (0.06) ^e	0.986	0.146 (0.019) ^e	18.6 (0.6) ^f	6.8 (0.2) ^f
CH-0.5TTO	4.29 (0.02) ^a	87 (3) ^b	1004.62 (0.13) ^a	0.817 (0.014) ^c	0.47 (0.03) ^c	0.970	0.202 (0.002) ^b	5.72 (0.16) ^g	2.87 (0.09) ^g
CH-1TTO	4.30 (0.02) ^a	86 (2) ^b	1003.5 (0.2) ^{bc}	0.816 (0.008) ^{bc}	0.47 (0.02) ^c	0.973	0.203 (0.002) ^b	9.87 (0.08) ^h	4.38 (0.16) ^h
CH-2TTO	4.30 (0.02) ^a	78 (3) ^d	1002.0 (0.3) ^e	0.807 (0.006) ^b	0.501 (0.005) ^b	0.975	0.201 (0.002) ^b	14.7 (0.3) ⁱ	6.2 (0.3) ⁱ

a,b,c,d,e,f,g,h,i Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Table 4
Properties of water essential oil (1% wt) dispersions: ζ -potential, density (ρ) and particle size (d_{43} and d_{32}) values at 25°C . Mean values and standard deviation.

EO	ζ (mV)	ρ (kg/m^3)	d_{43} (μm)	d_{32} (μm)
TTO	−30.6 (1.5) ^a	896.988 (0.3) ^a	4.8 (0.4) ^a	2.60 (0.08) ^a
BO	−61 (3) ^b	896.957 (0.2) ^a	3.66 (0.05) ^b	2.83 (0.04) ^b
LO	−49 (3) ^c	896.605 (0.3) ^a	3.94 (0.04) ^c	3.6 (0.4) ^c

a,b,c Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Concerning CH FFD, the same trends commented on above were observed when EOs were added to the pure CH dispersion: the mean particle size significantly ($p < 0.05$) increased and the ζ -potential of the particles decreased ($p < 0.05$), leading to bigger droplets with lower electrical net charge. In this case, the electrostatic interactions between CH and EO compounds at the pH of the FFD (4.30) will contribute to the reduction of the electrical net charge, as was described in previous studies (Vargas et al., 2009). The stability of the emulsified system was ensured by the steric stabilization promoted by the CH interfacial adsorption and the high value of ζ -potential (significantly higher than +30 mV), which implies a strong surface charge of the particles and ensures the action of the repulsive forces among these (Roland et al., 2003).

In both HPMC and CH dispersions, the increase in EO content significantly ($p < 0.05$) increased the mean particle size, although this effect was more intense in CH dispersions. Significant differences were observed as a function of the nature of the EO, depending on the type of polymer. FFD of HPMC with TTO presented the lowest particle sizes and those with LO the highest values. In CH dispersions, the particle size of FFD with LO and TTO, at a determined EO ratio, were closer and lower than those with BO.

With regard to the rheological characteristics, all FFD showed a shear thickening behaviour and no thixotropic effects were observed from the comparison of the up and down curves. So, rheological data were fitted to the Ostwald de Waale model. Tables 2 and 3 show the flow and consistency indexes, together with the apparent viscosity (η_{ap}) values at a shear rate of 100 s^{-1} for HPMC and CH FFDs. The values of the correlation coefficient were in all cases around 0.98.

Rheological parameters and apparent viscosity at 100 s^{-1} for pure HPMC dispersions agreed with those reported by Chen (2007). As expected, the addition of EO to the HPMC dispersion promoted slight but significant changes in the rheological pattern: the consistency index (k) increased whereas the flow index (n) and the apparent viscosity decreased ($p < 0.05$). So, EO incorporation made the fluid systems less viscous and less shear thinning than the pure HPMC solution. This behaviour is coherent with the adsorption of the HPMC molecules on the droplet surface, which

contributes to reduce their viscous contribution in the continuous phase while droplets are more stable and less sensitive to changes promoted by the shear forces. Neither the concentration nor the nature of the essential oil significantly affected ($p > 0.05$) the rheological parameters and the viscosity of the FFD.

For CH FFD, rheological parameters and apparent viscosity at 100 s^{-1} for pure chitosan are in the order of those found by No et al. (2006) and Vargas et al. (2009) for this polymer. The incorporation of EO promoted similar changes to those commented on above for HPMC FFD, in agreement with the polymer adsorption on the droplet surface. Nevertheless, a significant decrease in viscosity and consistency index (k) ($p < 0.05$) was observed for LO and BO when their concentration exceeded 1% in the FFD. These results could be explained by the significant reduction of the particle charge or ζ -potential, which also contributes to the system viscosity by reducing the electroviscous effects (McClements, 2005).

By considering all the determined properties of the FFDs, a discriminant analysis was carried out in order to analyse the different degrees to which the kind of polymer, the kind of EO or the EO concentration contribute to the differences in FFD behaviour. The discriminant plot obtained by considering all the properties characterised in the FFDs of both polymers, is shown in Fig. 1a in terms of functions F1 and F2 which explains 96.4% of total variance (92.2% explained by F1). Samples were clearly separated in two groups, depending on the polymer, which indicates that, more than EO (kind and amount), the kind of polymer significantly contributes to modify the characteristics of the FFD. This suggests that the properties of the aqueous continuous phase and the induced particle charge, where the polymer plays an important role, greatly influence the FFD behaviour.

Taking this result into account, similar statistical analyses were performed separately for CH and HPMC FFDs to evaluate the main factor determining the properties of FFDs: the EO nature or concentration. Discriminant plots are shown in Fig. 1b and 1c. F1 and F2 functions explain 99.5 and 97% of total variance for HPMC and CH FFDs, respectively. For both polymers, the different samples were clearly separated according to the EO type and its concentration. Function 1 separates samples with different concentration of the EOs whereas the effect of the kind of EO appeared differentiated by F2. Taking into account that F1 explains 93% or 82.5% of variance for HPMC and CH FFDs, respectively, it may be said that differences in the behaviour of FFDs of a determined hydrocolloid are mainly due to the concentration of EO. Nevertheless, as the F2 explains a low % of total variance, it could be concluded that the essential oil type impairs a difference but it does not seem very relevant, although TTO samples are clearly separated of those prepared with citrus oils, especially for CH matrix. From values of standardized coefficients of F1 (Table 5), it is possible to conclude that the differences among dispersions were mainly caused by the

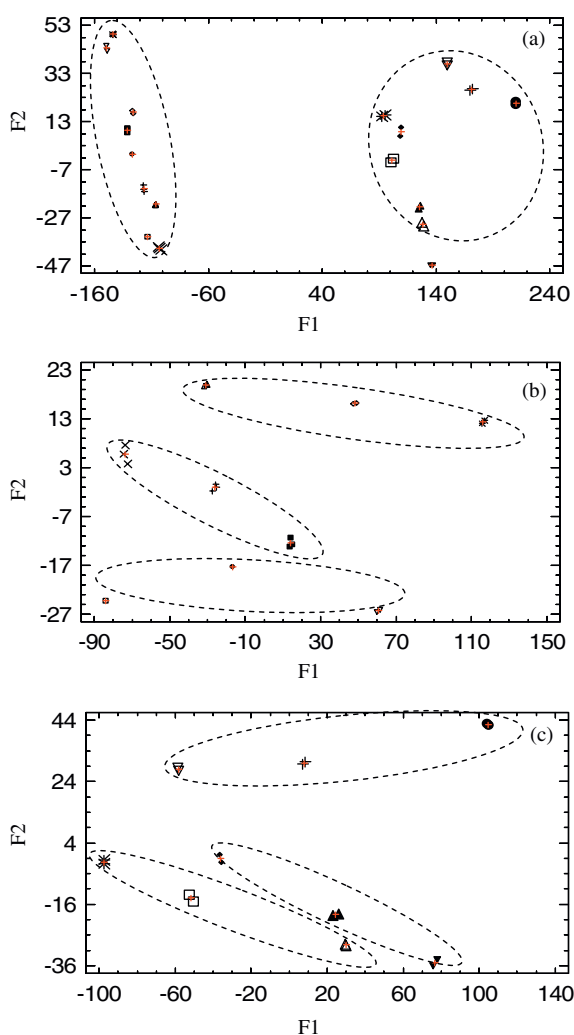


Fig. 1. Plot of discriminant functions obtained from data of both HPMC-EO, CH-EO FFD (a); HPMC-EO FFD (b) and CH-EO FFD (c). (■ - HPMC-0.5BO, + - HPMC-1BO, × - HPMC-2BO, * - HPMC-0.5LO, ◇ - HPMC-1LO, △ - HPMC-2LO, ▽ - HPMC-0.5TTO, ● - HPMC-1TTO, □ - HPMC-2TTO, ◆ - CH-0.5BO, ▲ - CH-1BO, ▼ - CH-2BO, * - CH-0.5LO, □ - CH-1LO, △ - CH-2LO, ▽ - CH-0.5TTO, + - CH-1TTO, ● - CH-2TTO).

mean particle size (d_{43} and d_{32}) and rheological properties. In terms of F2, the variables responsible for differences were the consistency index and apparent viscosity, although this aspect is less relevant since a small percentage of variance was explained by F2 (6.5% and 14.5% for HPMC and CH FFDs, respectively).

In conclusion, it is the concentration of EO more than the EO composition which induces greater differences in the behaviour of the FFD of both HPMC and CH, which are mainly explained by the particle size distribution reached under determined homogenization conditions. So, the amount of dispersed phase mainly affects affected particle size, viscosity and surface charge.

3.2. Characteristics of the films

Tables 6 and 7 show the physical properties characterized in the CH and HPMC films. Mechanical properties (in samples equilibrated at 54.4% and 20 °C) were measured in terms of the percentage of elongation at break (E%), tensile strength (TS) and elastic modulus (EM). TS represents the film's resistance to elongation or its stretching capacity and EM is a measure of the stiffness of the film. The values of mechanical properties obtained for pure HPMC and CH films agreed with those found by other authors

(De Moura et al., 2009; Sánchez-González et al., 2009; Zivanovic et al., 2005). As can be deduced from Tables 6 and 7, pure CH films were mechanically more resistant to fracture and more stretchable (greater TS, EM and E% values) than pure HPMC films. The mechanical response of the films from both polymers presented similar trends when the EO was incorporated into the matrix, although the changes were more pronounced when using CH matrix. Within the concentration range under consideration, the addition of EO led to a decrease not only in the elastic modulus, but also in the tensile strength and deformation at break. In CH films, these changes in the mechanical parameters provoked by the EO concentration were in general only significant at the highest EO concentration levels, whereas in the case of HPMC, only the TS parameter varied significantly when the EO concentration increased. These effects coincide with the results reported by other authors when adding essential oil to a chitosan matrix (Pranoto et al., 2005; Zivanovic et al., 2005). The presence of structural discontinuities in the polymer network, provoked by the incorporation of the lipid dispersed phase, explains the smaller degree of film stretchability (lower E% values) and resistance to break (lower TS values). This response was usually observed in many composite films. In general, the nature of the EO did not significantly affect the mechanical behaviour of HPMC films. However, in CH films and at a determined level of EO ratio, BO generally induces a greater decrease of the film elastic modulus, stretchability and resistance to break than LO and TTO, which is more accused at low concentrations.

The thicknesses of pure HPMC, CH and composite films are reported in Tables 6 and 7. Composite films were not as thick as pure films ($p < 0.05$) and the thickness was even more reduced when the EO concentration increased. This result suggests that possible losses of oil could occur during film drying which reduce the total amount of solids contributing to the film thickness, since the amount of FFD poured to obtain the film was determined taking into account the total solid (polymer plus EO) concentration of the dispersion (56 g/m²).

The water vapour permeabilities (WVP) of the films at 100/54.4 RH gradient and 20 °C are also reported in Tables 6 and 7. WVP values were in the range of those reported by other authors for films based on HPMC (Sebti et al., 2002; Villalobos et al., 2006; Sánchez-González et al., 2009) and CH (Park and Zhao, 2004; Vargas et al., 2009), respectively. Regardless of the type of polymer, WVP values showed a decrease in line with the increase in the EO concentration, this being more significant ($p < 0.05$) when the EO ratio increased. This behaviour is expected, as an increase in the hydrophobic compound fraction usually leads to an improvement in the water barrier properties of films. Concerning composite films, at low levels of essential oil (up to HPMC-EO ratio of 1:1), the nature of the oil did not significantly affect WVP. However, when incorporating higher EO concentrations (2%), citrus oils (BO and LO) showed better water vapour barrier properties than TTO. The incorporation of 2% BO or LO caused a reduction of about 50% in the WVP values, whereas only 20% reduction was obtained with the same TTO concentration. The greater hydrophobic nature of the main component of both citrus oils (limonene) as compared with the major component of TTO (α -terpineol) (Jordan, 1999) could explain the observed differences. On the contrary, the WVP reduction in CH composite films was less affected by the nature of the EO (around 30-40% in all cases), probably due to the greater hygroscopic nature of the chitosan matrix, reflected in the higher equilibrium moisture contents as compared with HPMC films (Tables 6 and 7). The greater moisture content and its impact on the molecular mobility may mask the different effect of each EO on the WVP of CH films.

The gloss and transparency of the films are relevant properties since they have a direct impact on the appearance of the coated product. Film transparency was evaluated through the internal

Table 5
Standardized coefficients and statistics of discriminant functions of FFDs and films.

Properties	CH/HPMC		CH		HPMC	
	Function 1	Function 2	Function 1	Function 2	Function 1	Function 2
d_{32}	0.750	-0.382	2.745	0.041	2.854	2.490
d_{43}	0.184	-0.115	-1.181	-0.592	-3.493	-1.437
ρ	-0.065	0.105	-1.743	-0.556	0.097	-0.177
k	-1.787	-0.186	4.685	0.044	-1.788	-4.523
n	-0.069	-0.056	4.316	0.673	-1.185	-2.678
pH	-0.207	0.721	1.467	-0.048	2.128	0.518
ζ -Potential	0.130	-0.186	0.525	-0.013	-0.677	-0.427
η_{ap}	1.992	0.620	1.556	1.475	0.930	3.248
<i>Statistics</i>						
Variance (%)	92.19	4.21	82.48	14.39	92.92	6.62
Auto-value	33075.0	1509.44	7838.52	1367.29	6187.11	440.90
α	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
<i>Films</i>						
Gloss (60°)	-0.122	0.305	0.329	-0.643	0.344	-0.831
E	-0.822	-0.041	0.175	-1.069	0.212	-0.932
EM	-0.776	-0.270	-1.061	-0.184	0.120	-1.236
TS	0.305	-0.629	-2.101	1.941	0.230	0.102
Ti	1.077	0.214	-1.371	0.510	0.597	0.020
WVP	0.339	1.302	2.668	-1.167	0.717	0.915
<i>Statistics</i>						
Variance (%)	64.14	16.16	63.72	22.11	59.78	22.54
Auto-value	56.46	14.22	54.25	18.82	20.42	7.70
α	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 6
Properties of HPMC and HPMC-EO composite films: Elongation (E), tensile strength (TS), elastic modulus (EM), thickness, water vapour permeability (WVP), equilibrium moisture content at $a_w = 0.75$ (We), internal transmittance (Ti) and gloss values. Mean values and standard deviation.

Film	E (%)	TS (MPa)	EM (MPa)	Thickness (μm)	WVP ($\text{g Pa}^{-1} \text{s}^{-1} \text{m}^{-1}$) $\times 10^{11}$	We ($\text{g H}_2\text{O/g d m}^{-1}$) [*]	Ti ($\lambda = 450 \text{ nm}$)	Gloss (60°)
HPMC	7.9 (0.6) ^a	56 (7) ^a	643 (74) ^a	61.6 (0.6) ^a	71 (7) ^a	0.073	0.851 (0.006) ^b	50 (5) ^a
HPMC-0.5BO	5.1 (0.8) ^b	54 (5) ^a	473 (180) ^a	46.5 (0.7) ^b	65 (5) ^{ab}	0.089	0.820 (0.007) ^d	11 (2) ^b
HPMC-1BO	4.7 (0.5) ^b	47 (5) ^a	420 (107) ^a	35.3 (0.8) ^c	52 (6) ^b	0.057	0.826 (0.014) ^d	11 (2) ^b
HPMC-2BO	2.9 (0.7) ^b	39 (3) ^b	444 (75) ^a	31.1 (1.7) ^d	f31 (4) ^c	0.062	0.789 (0.035) ^e	11 (2) ^b
HPMC-0.5LO	6.0 (0.6) ^b	54 (3) ^a	515 (230) ^a	40.6 (0.4) ^e	68 (5) ^a	0.072	0.849 (0.005) ^b	11.9 (0.3) ^b
HPMC-1LO	4.5 (0.9) ^b	50 (1) ^a	459 (13) ^a	32.8 (1.5) ^d	65 (7) ^a	0.058	0.857 (0.002) ^a	11.8 (0.8) ^b
HPMC-2LO	3.9 (0.4) ^b	40 (4) ^b	397 (139) ^a	25.6 (2.8) ^f	41 (6) ^c	0.053	0.842 (0.005) ^c	8.2 (1.2) ^b
HPMC-0.5TTO	7.6 (0.4) ^b	42 (6) ^b	696 (104) ^a	32.8 (1.2) ^d	70 (5) ^a	0.061	0.863 (0.003) ^a	24 (3) ^c
HPMC-1TTO	4.2 (0.4) ^b	35 (2) ^c	542 (160) ^a	27.5 (1.6) ^f	66 (4) ^{ab}	0.057	0.859 (0.002) ^a	20 (2) ^d
HPMC-2TTO	4.2 (0.2) ^b	34 (5) ^c	365 (124) ^a	22.3 (1.2) ^g	57.3 (1.5) ^b	0.056	0.83 (0.02) ^d	16 (3) ^e

^{a,b,c,d,e,f,g} Different letters in the same column indicate significant differences among formulations ($p < 0.05$).

^{*} Values obtained from the sorption isotherm curves (Sánchez-González et al., 2010).

Table 7
Properties of CH and CH-EO composite films: Elongation (E), tensile strength (TS), elastic modulus (EM), thickness, water vapour permeability (WVP), equilibrium moisture content at $a_w = 0.75$ (We), internal transmittance (Ti) and gloss values. Mean values and standard deviation.

Film	E (%)	TS (MPa)	EM (MPa)	Thickness (μm)	WVP ($\text{g Pa}^{-1} \text{s}^{-1} \text{m}^{-1}$) $\times 10^{11}$	We ($\text{g H}_2\text{O/g d m}^{-1}$) [*]	Ti ($\lambda = 450 \text{ nm}$)	Gloss (60°)
CH	22 (5) ^a	113 (20) ^a	2182 (277) ^a	52.0 (1.7) ^a	129 (10) ^a	0.235	0.801 (0.013) ^a	32 (5) ^a
CH-0.5BO	7 (4) ^b	65 (10) ^{de}	766 (205) ^{cd}	55 (2) ^a	130 (1) ^a	0.189	0.761 (0.012) ^c	9 (3) ^c
CH-1BO	5.5 (0.7) ^b	63 (21) ^{de}	799 (163) ^{cd}	41 (2) ^b	108 (15) ^b	0.174	0.746 (0.014) ^{de}	4.9 (1.2) ^b
CH-2BO	6 (2) ^b	50 (8) ^b	747 (225) ^b	36 (3) ^b	92 (9) ^b	0.137	0.744 (0.012) ^e	8.8 (1.5) ^c
CH-0.5LO	18.1 (0.8) ^c	94 (9) ^f	1534 (185) ^e	41 (2) ^b	101.9 (1.5) ^b	0.163	0.764 (0.015) ^c	17 (2) ^{de}
CH-1LO	14.6 (0.4) ^c	57 (7) ^d	1466 (160) ^e	36.0 (1.4) ^b	88 (4) ^b	0.140	0.765 (0.009) ^c	15 (2) ^d
CH-2LO	6.4 (0.2) ^b	37 (3) ^c	954 (113) ^d	36 (3) ^b	77 (3) ^c	0.110	0.782 (0.009) ^b	9.9 (1.8) ^c
CH-0.5TTO	20 (8) ^c	74 (15) ^e	1447 (308) ^e	39 (3) ^b	99 (4) ^b	0.209	0.757 (0.018) ^d	28 (5) ^a
CH-1TTO	17 (6) ^c	72 (12) ^e	1419 (322) ^e	38 (3) ^b	100.1 (1.3) ^b	0.159	0.764 (0.009) ^c	19 (2) ^e
CH-2TTO	8 (2) ^b	54 (5) ^d	652 (157) ^c	24.3 (1.2) ^c	74.7 (1.8) ^c	0.134	0.789 (0.013) ^b	5.7 (1.2) ^b

^{a,b,c,d,e,f} Different letters in the same column indicate significant differences among formulations ($p < 0.05$).

^{*} Values obtained from the sorption isotherm curves (Sánchez-González et al., 2010).

transmittance, Ti (0–1, theoretical range). An increase in Ti can be assumed as an increase in transparency (Hutchings, 1999). Ti values at $\lambda = 450 \text{ nm}$ of the HPMC, CH and HPMC-EO, CH-EO composite films were reported in Tables 6 and 7. In general, significant

differences were observed associated with the nature and amount of the essential oil. Ti values were significantly lower in films incorporating the highest amounts of EO. The composite films were more opaque than pure CH and HPMC films. These results coincide

with those found in previous studies (Sánchez-González et al., 2009; Vargas et al., 2009). This phenomenon is related with the light scattering provoked by lipid droplets (with a different refractive index) distributed throughout the film network. Regarding the nature of the oil, BO films were the least transparent (lower Ti values) probably due to a selective absorption of some components of this EO (at 668 nm), leading to lower transmittance values.

Gloss values of the films measured at incidence angle values of 60° were reported in Tables 6 and 7. The addition of EO to the HPMC and CH matrix led to a decrease of the gloss, especially for citrus oils, regardless of EO concentration. For TTO, a smaller gloss reduction was observed, which, in this case, was dependent on the EO concentration. Gloss reduction in composite films containing lipids was also observed by different authors (Trezza and Krochta, 2000; Villalobos et al., 2005; Sánchez-González et al., 2009). The gloss of the films is related with the surface morphology reached during film drying. In general, the smoother the surface, the higher the gloss (Ward and Nussinovitch, 1996). In this sense, the decrease in gloss with the incorporation of EO could be explained by an increase of the surface roughness of the composite films. This

roughness appears as a consequence of the migration of droplets or aggregates to the top of the film during film drying, which leads to surface irregularities. Flocculation and creaming of oil droplets occurred during film drying and this effect on gloss seems more intense in citrus oils (BO and LO) than in TTO. This could be related with the greater stability of the TTO emulsions reflected in the smaller droplets and narrower particle size distribution deduced from the smaller difference between d_{43} and d_{32} values.

By considering all the properties measured in the films (WVP, optical and mechanical properties), a discriminant analysis was performed in order to analyse how much the kind of polymer, the kind of EO or the EO concentration contribute to the different behaviour of the films. The discriminant plot is shown in Fig. 2a in terms of functions F1 and F2 which explains 80% of total variance (64% being explained by F1). As for the FFDs, different samples were clearly separated as a function of the polymer type. So, similar statistical analyses were performed separately for CH-EO and HPMC-EO composite films to evaluate what the main factor causing differences was: the EO nature or its concentration. Discriminant plots are shown in Fig. 2b and c. Functions F1 explain 64% and 60% of total variance for CH and HPMC films, respectively. Regardless of the type of polymer, the separate analysis of films reveals differences by the type of essential oil (BO), which is discriminated by the F1. From values of standardized coefficients of F1 and F2 (Table 5), it is possible to conclude that the variables which caused the greatest differences among CH films were tensile strength at break, water vapour permeability and internal transmittance (Ti), whereas in HPMC films these were WVP and Ti. So, differences in film behaviour must be mainly attributed to the polymer that forms the continuous matrix, whereas the incorporation of different EO or ratios did not impart clearly differentiated behaviour, except when BO is used as samples appear as an independent group.

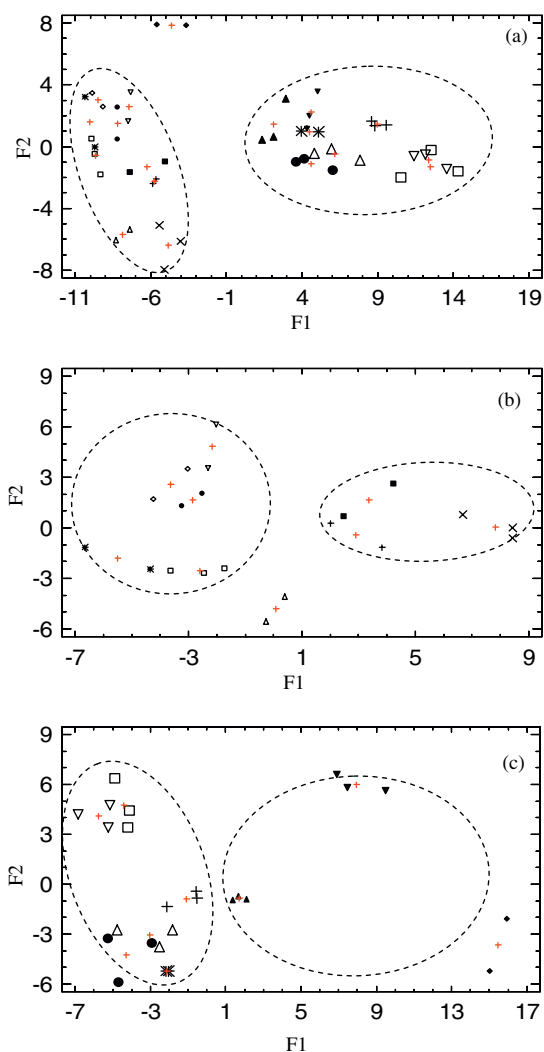


Fig. 2. Plot of discriminant functions obtained from data of both CH-EO, HPMC-EO composite films (a); HPMC-EO films (b) and CH-EO films (c). (■ - HPMC-0.5BO, + - HPMC-1BO, × - HPMC-2BO, * - HPMC-0.5LO, ◇ - HPMC-1LO, △ - HPMC-2LO, ▽ - HPMC-0.5TTO, ● - HPMC-1TTO, □ - HPMC-2TTO, ◆ - CH-0.5BO, ▲ - CH-1BO, ▼ - CH-2BO, * - CH-0.5LO, □ - CH-1LO, △ - CH-2LO, ▽ - CH-0.5TTO, + - CH-1TTO, ● - CH-2TTO).

4. Conclusion

HPMC and CH are good polymer matrices for entrapping essential oils (EO). The incorporation of EO led to significant changes in the properties of film-forming dispersions and films. Different EO concentration promotes changes in particle size and size distribution and in ζ -potential. HPMC and CH contribute to the emulsion stability by adsorption on the oil droplet surface, although the film gloss data reflected that flocculation and creaming of oil droplets occurred during film drying. The higher the EO content, the lower the water vapour permeability. In both HPMC and CH matrices, the addition of EO led to a significant decrease in gloss, transparency and tensile strength and elongation at break of the composite films. Discriminant analyses revealed that the type of polymer is the main factor inducing differences in both FFD and film behaviour. For films of both polymers, both concentration and type of EO contribute in the differentiation of groups of film-forming dispersions associated with their behaviour, the type of the oil playing a more important role. As regards film properties, discriminant analyses did not reveal clearly different groups associated with the nature or concentration of essential oil, although composite films with BO were differentiated from the rest.

Uncited reference

Srinivasa et al. (2007).

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