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Effect of Rosemary Oil and an Emulsion of Essential Oils on Structure and Physical Properties of Chitosan Film

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The objective of this work was to study the effect of Rosemary oil (Resemary Officinalis) and an emulsion at 68 % of EOs (EA3) on *chitosan (CH)* film forming dispersion (FFD) and CH films properties. FFD were characterized in terms of rheological properties and particle size distribution. In order to study the impact of EOs into the CH matrix, microstructure (SEM), film thickness, equilibrium moisture content (EMC) and water vapour permeability (WVP) of the dried film were evaluated. Results showed that oil concentration did not affect particle size distribution whereas the type of EO have an effect on the particle size dimension. The rheological properties of the FFD were significantly affected by the presence of the EOs. While a continuous structure was observed for the CH film, the presence of EOs caused discontinuities associated with the formation of two phases in the matrix: lipid droplets embedded in a continuous polymer network. The dimension of the oil droplet were higher in the film respect to the FFD. Moreover, it was showed that the higher the EOs content, the higher the film thickness and the lower the film moisture sorption capacity. The WVTR of CH film has an average value of 2.3×10^{-3} g m^{-2} s^{-1}. The study revealed that an active chitosan film could be obtained by using the EA3 emulsion of EOs.

1. Introduction

Active packaging technologies involve interactions between the food and the packaging material to extend the shelf life of foods while maintaining their quality and safety. These materials are designed to deliberately incorporate 'active' components intended to be released into the food or to absorb substances from the food (Regulation (EC) No 1935/2004; regulation (EC) No 450/2009). Recently, given the increasing health concerns of consumers, current packaging research has focused on the use of natural compound, such as essential oil, as active agents in active edible coating materials to preserve and prolong the shelf life of food as meat, fish or minimally processed fruit (Azevedo et al., 2014; Bonilla et al., 2014; Fuenmayor et al., 2013**;** Higueras et al., 2014; Raybaudi-Massilia et al., 2008; Valencia-Chamorro et al., 2009). Among the most common oils that have been proven antimicrobial properties against spoilage microorganisms the main are Cilantro oil, Coriander oil, Oregano oil, Rosemary oil, Sage oil, Clove (bud) oil and Thyme oil (Burt, 2004). Chitosan has been studied extensively in the food industry due to its excellent film-forming, antimicrobial, physical and mechanical properties (Elsabee and Abdou, 2013) and an active antimicrobial film based on chitosan has been recently developed (Lago et al., 2014). The functional properties of edible film depend on the type of constituents and also on their interaction (Giancone et al., 2008, 2011). According to the application of EOs, it is also important to evaluate their effects on the physical and structural properties of the resulting film (Sánchez-González et al., 2010). About the role of EOs on film functional properties the results reported on literature are still contradictory, in particular regarding the effect of the oil on the water barrier properties of the film. However, it has been reported that a major role can play the type of polysaccharide or the type of oil added and their interaction on the effective structure of the film and thus on the diffusivity of the water. In fact, if by adding the oil the matrix results more open, an higher diffusion of water in the matrix can be expected and thus a negative impact on the water barrier properties expected (Perone et al., 2014). The

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composition of the film and the interaction between the constituent (polysaccharides, oil, emulsifier) began a critical aspect to design active film with desired properties (Perone et al., 2014). In this work the relation between structure and properties of chitosan film obtained with rosemary oil and an emulsion of EOs at 68 % has been investigated.

2. Material and methods

2.1. Material

Medium molecular weight Chitosan (CH MMW) with a degree of deacetylation higher than 75 – 85 %, acetic acid puriss. p.a., rosemary essential oils (Rosemary Officinalis- REO) and Tween 80 were purchased from Sigma-Aldrich (Milan, Italy). EA3 Emulsion (68 % of essential oils, 0.8 % Polisorbate Tween 80, 0.8 % Myverol 18-92, 1 % Citric Acid, 3 – 5 % $CaCl_2$) was provided from Kerry Ingredients & Flavours (Bergamo, Italy). The composition of EA3 EO were: Coriander (>10%), Clove (>10%), Caraway (10%), Cardamon (>10%), White Thyme (5-10%), Cinnamon Distilled Fraction (Nutmeg (5-10%), Black Pepper (2-5%), Anise (2-5%), Anise (<2%), Sage (<2%), Oregano (<2%), Mint (<2%).

2.2 Emulsion and Film making procedure

CH 1% (w/v) was prepared by dispersing chitosan powder in acetic acid solution (1%, v/v) at room temperature for 12 hours. Then, the dispersion has been centrifuged for 4 min at 5,000 rpm to eliminate the solid impurity suspended. Then, essential oils emulsion (EA3) or a mixture of REO and tween - 80 (4:1) were added to polymer solutions to reach a final concentration of 0.5 %, 1 %, and 2 % (w/v). CH-EA3 and CH-REO-tween-80 mixtures were emulsified for 4 min at 15,500 rpm at room temperature using a rotor-stator homogenizer (Ultra TurraxR, T 18, IKA, Milan, Italy) and then the solutions were de-aerated under vacuum for 15 minutes to prevent pinhole formation. 20 ml of FFD were poured onto levelled 56.7 cm^2 polystyrene Petri dishes and allowed to dry at 20° C and 50 % relative humidity (RH) for 48 h under air circulation. The dried films were peeled from the dishes and stored at 20°C and 50 % relative humidity prior to testing.

2.3. Characterization of the FFD

The particle size analysis of the FFDs was carried out by means of a laser diffractometer (Mastersizer 3000, Malvern Instruments, Worcestershire, UK). The samples were diluted in deionised water at 1,800 rpm until an obscuration rate of 10 % was obtained. Mie theory (Merkus, 2009) was applied considering a refractive index of 1.48 and absorption of 0.001 for essential oils emulsion samples (EA3) and 1.48 and 0.01 for REO samples. Three replications for formulation were made. The tenth, fiftieth and ninetieth percentile (D_{10}, D_{50}, D_{90}), has been calculated and indicated the percentage of particles with dimension inferior to the value reported.

Flow curves were obtained using of a strain controlled rheometer (RFS II, Rheometric Inc. Piscataway, NY), fitted with a coaxial cylinders. The viscosity versus shear rate was recorded at 25 °C. The shear rate ranged between 0.04-103 s^{-1}.

2.4. Characterization of the film

Microstructure of film samples was examined using an LEO EVO 40 scanning electron microscope (Zeiss, Oberkochen, Germany). Film thickness was measured using a micrometer model HO62 with a sensitivity of ±2 μm (Metrocontrol Srl, Casoria, NA, Italy). The moisture content of samples was determined by the gravimetric method. film samples were oven-dried at 105°C and accurately weighed at regular time intervals until constant weight was reached. Three measurements were performed for each sample. The moisture content was expressed as grams of water over grams of total weight (g/100g). Water vapour transmission rate (WVTR) of films was evaluated by gravimetric test according to ASTM E96 (1993) by means of a Fisher or Payne permeability Cup (Carlo Erba, Milan, Italy) at 25 °C (RH%= 0 – 85 %).

3. Results

3.1. Characterization of the FFD

Particle size of EA3 dispersion without and with polymer showed monomodal particle size distributions, and CH-EA3 dispersion showed bigger particle dimension respect to EA3 emulsion. Whereas for REO essential oil the particles dimension are reduced once they are dispersed into chitosan. Moreover, the particle size distribution changes by bimodal in absence of chitosan to monomodal in its presence. The D_{10}, D_{50}, D_{90} values are reported in Table 1.

Table 1: Particle size dimension of CH-EA3 and CH-REO

Samples (wt%)	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)
CH-EA3	*mean value and standard deviation*		
0,5	1.92 (0.27)	3.02 (0.26)	4.87 (0.15)
1	1.99 (0.24)	3.25 (0.48)	5.83 (1.59)
1,5	1.82 (0.39)	2.85 (0.53)	4.55 (0.99)
EA3	1.20 (0.01)	2.75 (0.01)	4.68 (0.01)
CH-REO			
0,5	0.120 (0.020)	0.263 (0.053)	0.566 (0.107)
1	0.128 (0.018)	0.295 (0.055)	0.672 (0.099)
1,5	0.130 (0.006)	0.283 (0.012)	0.596 (0.017)
REO	0.325 (0.022)	0.620 (0.040)	3.11 (0.711)

The typical flow curves for CH and CH-EOs FFD are shown in Figure. 1. Flow curves showed a shear-thinning behaviour for all samples. At the contrary of what expected (Vargas et al., 2011; Floury et al., 2000; Otsubo et al., 1994), as the oil content increases the viscosity decreases. The minor effect of concentrations of REO samples on viscosity can be explained by the lower size of REO particles.

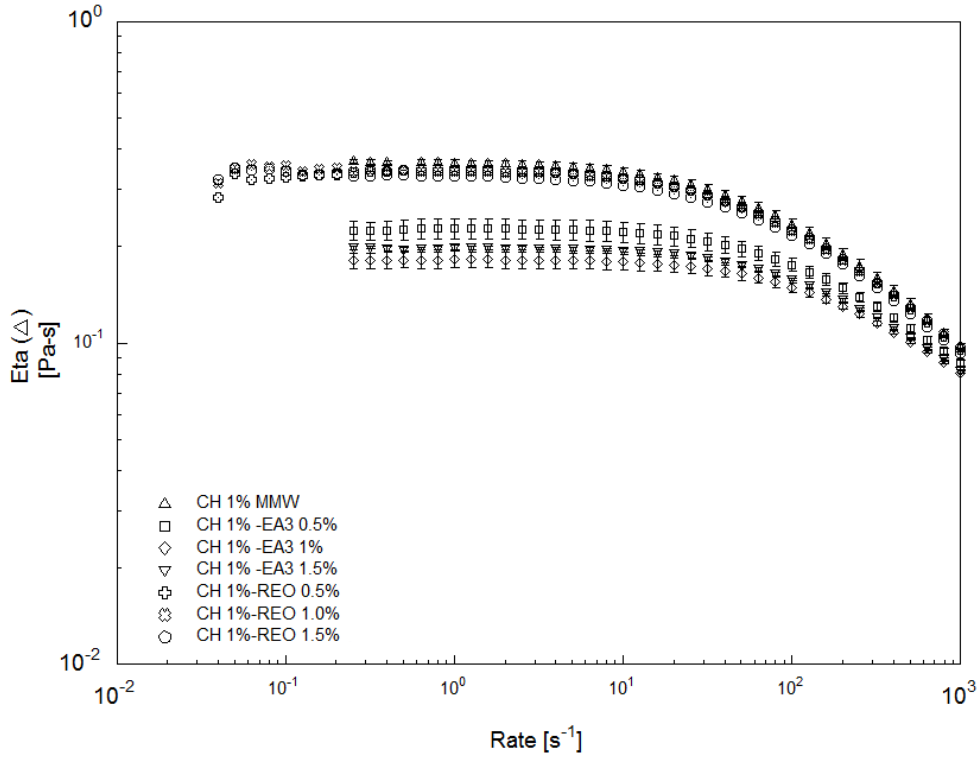

Figure 1: Viscosity (Pa s) of chitosan film added with EOs as function of shear rate (s^{-1})

3.2. Characterization of the film

Characteristic SEM images of cross section of the films are shown in Figure 2. The oil droplets were uniformly distributed along the thickness but its sizes are not homogenous and it are bigger than in the FFD.

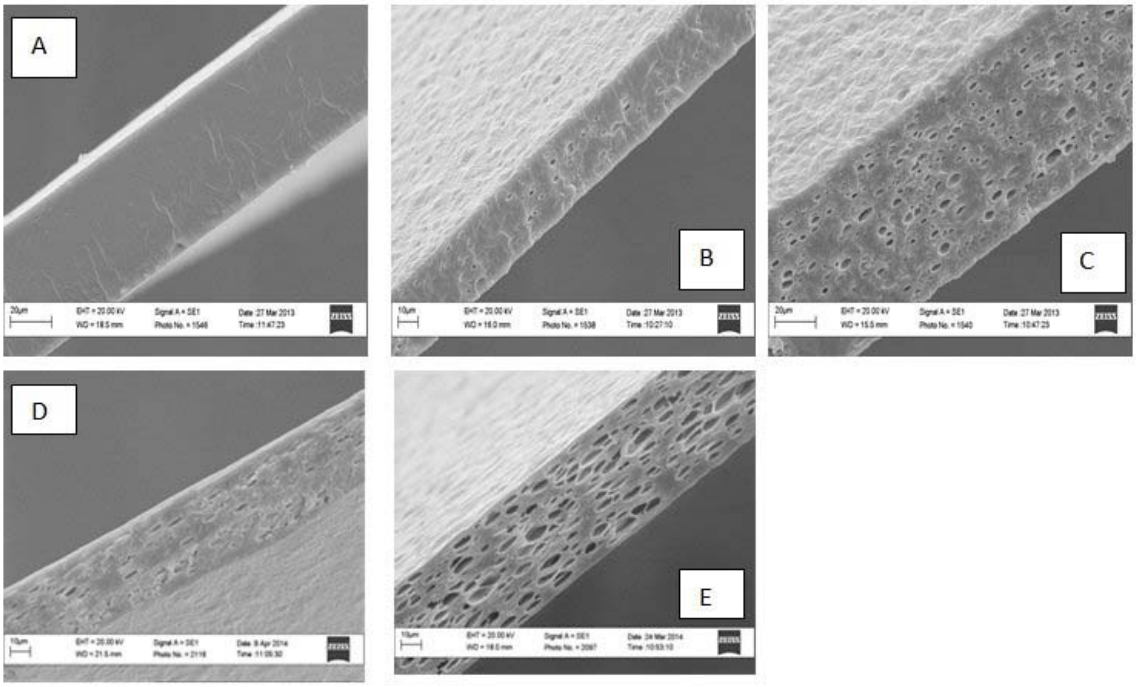

Figure 2: SEM micrographs of 1 % Chitosan film at 0% EA3 (A), 0.5% oil (B), 1.5 % oil (C), and of 1 % Chitosan film at 0.5 % REO (D), 1.5 % REO oil (E).

As reported in Table 2, the addition of EOs led to an increase of films thickness. The films made from CH-REO, which showed a smaller particle sizes, were thinner than the others.
In general, equilibrium moisture content (EMC) at a_w equal to 0.5 decreased as EO was incorporated into chitosan-based film (Table 2). Chitosan-based films containing REO essential oil had less equilibrium moisture content than those containing EA3 essential oils when the same level of essential oil was present (0.5, 1 %) in each film. Lipid fraction caused to formation of covalent bonds between the functional groups of chitosan chains, leading to a decrease in the availability of hydroxyl groups and limiting polysaccharide–water interactions by hydrogen bonding and resulting in a decrease of moisture content value of edible films.
Chitosan film had a WVTR value of 2.3×10^{-3} g m^{-2} s^{-1} (Table 2). EA3 did not have a significant effect on the WVTR of chitosan film. Different results have been obtained when REO has been added to chitosan film. The WVTR of chitosan film increased to almost 50 % when REO has been added. The higher particle size of REO in CH film by disrupting the compact structure of the polymer can enhance moisture passing through the films and thereby increase the water vapour transmission rate values of the films. Moreover, as suggested by Perdones et al. (2014), possible interactions of the EO compounds with CH, as also deduced from water sorption data and SEM results, make the matrix more open to the transport of water molecules, despite the theoretical increase of the hydrophobic nature of the matrix due to the presence of lipids. Anyway, the differences between CH film with EA3 and REO EOs can be attributed to the different dimension of the oil particle in the film. Thus, the good stability of the emulsion during casting is very important for the WVTR of the film.

4. Conclusions

EOs concentration did not showed any effect on the particle size dimension in chitosan FFD. All the dispersion CH-EA3 showed bigger particle dimension respect to EA3 emulsion, whereas for REO essential oil the particles dimension are reduced once they are dispersed into chitosan. The oil had a significant effect of viscosity of FFD that decreased as oil concentration increased. The structure of the CH film has been well observed by SEM images: A continuous structure was observed for the CH film while the presence of EOs caused discontinuities associated with the formation of two phases in the matrix: lipid droplets embedded in a continuum polymer network. The oil droplets were uniformly distributed along the thickness but its sizes were not homogenous and it were bigger than in the FFD. The addition of EOs led to an increase of films thickness and a decreased of moisture content as the oil concentration increased. EA3 did not have a significant effect

on the WVTR of chitosan film, but the WVTR of chitosan film increase of almost 50 % when REO has been added. Chitosan is a promising biopolymer for active food packaging and chitosan-EA3 film showed better properties that chitosan-REO film.

Table 2: EMC (0.5 aw), WVTR, WVP and thickness of Chitosan film with different concentration of EO

Samples (EOs wt %)	EMC (g_w/g_{dm})	Thickness (μm)	WVTR x 10^{-3} (g m^{-2} s^{-1})
CH-EA3			
0	22 (2)	46 (9)	2.6 (0.4)
0,5	15 (2)	63 (5)	2.19 (0.03)
1	13 (1)	86 (4)	2.04 (0.05)
1,5	10 (1)	97 (9)	2.28 (0.07)
CH-REO			
0	22 (2)	46 (9)	2.6 (0.4)
0,5	12 (1)	53 (4)	5.6 (0.1)
1	10 (1)	65 (5)	5.2 (0.6)
1,5	9 (1)	69 (4)	4.3 (0.9)

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References

Azevedo, A.N., Buarque, P.R., Oliveira Cruz, E.M., Fitzgerald Blank, A., Barreto Alves, P., Nunes, M.L., Lins de Aquino Santana, L. C. (2014). Response surface methodology for optimization of edible chitosan coating formulations incorporating essential oil against several foodborne pathogenic bacteria. Food Control, (43), 1-9.

Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology, 94, 223-253.

Floury, J., Desrumaux, A., Lardières, J. (2000). Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Innovative Food Science & Emerging Technologies, 1, 127-134.

Giancone T., Torrieri E., Di Pierro P., Mariniello L., Moresi M., Porta R., Masi P. (2008). Role of constituents on the network formation of hydrocolloid edible films. Journal of Food Engineering, 89 (2), 195-203.

Giancone T., Torrieri E., Di Pierro P., Cavella S., Giosafatto C.V.L., Masi P. (2011). Effect of surface density on the engineering properties of high methoxyl pectin-based edible films. Food and Bioprocess Technology, 4, 1228-1236.

Elsabee, M.Z., Abdou, E.S. (2013). Chitosan based edible films and coatings: A review. Materials Science and Engineering, (C33), 1819-1841.

Fuenmayor, C.A., Mascheroni, E., Cosio, M.S., Piergiovanni, L., Benedetti, S., Ortenzi, M., Schiraldi, A., Mannino, S. (2013). Encapsulation of R-(+)-Limonene in Edible Electrospun Nanofibers, Chemical Engineering Transaction, 32, 1771-1776.

Higuera, L., Lopez-Carballo, G., Hernandez-Munoz, P., Català, R., Gavara, R. (2014). Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. International Journal of Food Microbiology, (188), 53-59.

Lago M.A., Sendon R., de Quiros A.R.B., Sanches-Silva A., Costa H.S., Sanchez-Machado D.I., Valdez H.S., Angulo L., Aurrekoetxea, G.P., Torrieri E., Lopez-Cervantes J., Paseiro P. (2014). Preparation and Characterization of Antimicrobial Films Based on Chitosan for Active Food Packaging Applications. Food and Bioprocess Technology, 1 (10), 2932-2941.

Merkus, H. G. 2009. Particle Size Measurements. Fundamentals, Practice, Quality. Springer, pp. 259-285.

Otsubo Y., Prud'homme R. K. (1994). Rheology of oil-in-water emulsions. Rheologica Acta, 33, 29-37.

Perdones A., Vargas M., Atares L., Chiralt A. 2014. Physical, antioxidant and antimicrobial properties of chitosan-cinnamon leaf oil films as affected by oleic acid. Food Hydrocolloids, 36, 256-264.

Perone N., Torrieri E., Cavella S., Masi P. (2014). Effect of rosemary oil on structure and functional properties of HPMC film at several concentrations. Food and Bioprocess Technology, 7 (2), 605-609.

Valencia-Chamorro S.A., Pérez-Gago M.B., del Río M., Palou L. (2009). Effect of antifungal hydroxyl propyl methylcellulose (HPMC)–lipid edible composite coatings on postharvest decay development and quality attributes of cold-stored 'Valencia' oranges. Postharvest Biology and Technology, 54, 72-79.

Vargas M., Perdones Á., Chiralt A., Cháfer M., González-Martínez C. (2011). Effect of homogenization conditions on physicochemical properties of chitosan-based film-forming dispersions and films. Food Hydrocolloids, 25, 1158-1164.