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Physical properties of chitosan-basil essential oil edible films as affected by oil content and homogenization conditions

Jeannine Bonilla, Maria Vargas *, Lorena Atarés, Amparo Chiralt

Institute of Food Engineering for Development (IUIAD) – Universidad Politécnica de Valencia, Spain

Abstract

This work studies the influence of basil essential oil, its content and the homogenization treatment on the physical properties of chitosan-based edible films. Two homogenization treatments were applied, without (H1) and with (H2) microfluidization (MF). Composite films were softer, less rigid and more stretchable than pure CH films. MF intensified these changes. H2 films showed microcracks due to the weak interactions between chitosan and oil, which affected their mechanical behaviour. In pure chitosan films, MF significantly increased water vapour permeability. Homogenization treatment greatly affected this property. Gloss was reduced by the essential oil addition, whereas MF tended to yield glossier films.

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Keywords: chitosan; essential oil; microfluidization; basil

1. Introduction

Edible films and coatings are prepared from biopolymers and are able to protect food products extending their shelf-life. The major constituents of these are polysaccharides, proteins and lipids. Chitosan (CH) is a cationic polysaccharide with excellent film-forming properties. It is obtained from chitin by deacetylation in the presence of alkali [1]. The combination of this hydrophilic constituent and some lipid could produce films with optimized characteristics [2]. Many lipids have been incorporated to composite films, mainly aiming to reduce the water vapour permeability of hydrophilic materials. Essential oils (EO) represent an interesting alternative to traditional ingredients, given the current research efforts on reducing the use of chemical additives in the food industry. Additionally, these plant extracts exhibit additional characteristics, such as antimicrobial and antioxidant effects [3]. Droplet size is a determining factor for emulsion stability, and affects other important properties of the emulsion, including its viscosity. Rotor-stator homogenizers are often used in the food industry, and are able to

* Corresponding author. Tel.: +34 96 387 7000x73642; fax: +34 963 877 956.
E-mail address: marvaco@tal.upv.es

reach particle sizes in the range of 1 μm . This can be further reduced by applying an increased pressure in the system. Microfluidization (MF) can provide emulsions with narrower particle size distributions by submitting the emulsion to high shear stress in an interaction chamber. However, there are few studies dealing with the effect of microfluidization on the properties of the film-forming dispersions used to obtain edible films and coatings [4]. The aim of this work is to study the influence of homogenization conditions and essential oil content on the physical properties (microstructure, mechanical behaviour, water vapour permeability and gloss) of CH:B edible films.

2. Materials and Methods

2.1 Materials

High molecular weight chitosan (CH) (Batch 12913CJ, Sigma-Aldrich Quimica, Madrid, Spain) was used to prepare the film-forming dispersions (FFD) (0.8 Pa s viscosity, at 2% w/w in 2% w/w glacial acetic acid). Basil (B) essential oil was provided by Herbes del Moli (Alicante, Spain), $\text{Mg}(\text{NO}_3)_2$ and P_2O_5 by Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain).

2.2. Preparation of the film-forming dispersions and casting of the films

High molecular weight CH (1 wt%), dispersed in and acetic acid solution (1 % v/w) and basil (B) (0.5 or 1 wt%) were mixed by two homogenization treatments, namely H1 and H2. FFD submitted to H1 were mixed by means of a rotor-stator homogenizer at 21500 rpm for 4 minutes. Treatment H2 consisted of H1 plus high-pressure homogenization at 165 MPa in a single pass by means of a Microfluidizer® M110-P processor. After homogenization, the formulations were degasified at room temperature with a vacuum pump. The FFD were cast at 5.6 mg solids/cm² and were poured onto a framed and leveled polytetrafluoroethylene (PTFE) plate (diameter = 15 cm) and dried at room temperature and 60 % RH. The films were peeled off from the casting plates and conditioned for one week at 5°C and 58%RH in a chamber containing an oversaturated solution of $\text{Mg}(\text{NO}_3)_2$.

2.3 Characterization of the film

2.3.1. Thickness

Film thickness was determined with a Palmer digital micrometer (Comecta, Barcelona, Spain) to the nearest 0.001 mm. Six were considered for WVP tests and four measurements were taken for the tensile tests.

2.3.2. Microstructure

Microstructure was observed by SEM in cross-sectioned cryofractured film specimens, using a JEOL JSM-5410 (Japan) electron microscope. The film samples were equilibrated in P_2O_5 to eliminate water, cryofractured by immersion in liquid nitrogen, and then mounted on copper stubs perpendicularly to their surface. After gold coating, the images were captured using an accelerating voltage of 10kV.

2.3.3 Mechanical properties

Mechanical properties were analysed by means of tensile tests (ASTM standard method D882, [5], to obtain stress-strain curves and mechanical parameters: elastic modulus (EM), tensile strength at break

(TS) and Hencky elongation percentage at break (%E). A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to perform the tests. Rectangular samples (25x100 mm) were cut and stored at 25°C and 58% RH in a cabinet containing Mg(NO₃)₂ saturated solution. The moisture content of the films submitted to the mechanical test ranged between 0.09 and 0.15 (wb), with a significant ($p < 0.05$) negative effect of both homogenization pressure and oil content. Equilibrated film specimens were mounted in the film-extension grips and stretched at 50 mm min⁻¹ until breakage. Six replicates of each formulation were tested.

2.3.4 Water vapour permeability (WVP)

WVP was determined gravimetrically at 5°C and 100-58% RH gradient, using a modification of the ASTM E96-95 gravimetric method [6] for hydrophilic films (Mc Hugh *et al.*, [7]). Payne permeability cups of 3.5 cm in diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium) were filled with 5 ml of distilled water (100% RH). Films were selected on the basis of a lack of defects and three round samples were cut per formulation, and thickness was measured. The films were secured and the cups were placed in pre-equilibrated cabinets fitted with a fan, at 5°C. The RH of the cabinets was held constant at 58% using oversaturated solutions of Mg(NO₃)₂. The shiny side of the films was exposed to the 58% RH atmosphere. The cups were weighed periodically after the steady state had been reached. WVP was calculated with the equations described by [3].

2.3.5 Gloss

The gloss of the films was measured with a gloss meter (Multi. Gloss 268, Minolta, Germany) on their shiny side, at an incidence angle of 60° (ASTM D523, 1999[8]). Ten replicates were made in each film. Results were expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

3. Results and Discussion

3.1 Microstructure

Characteristic SEM images of cross sections of the films are shown in figure 1 (H1 treatments) and figure 2 (H2 treatments). The microstructure was qualitatively analysed, aiming to describe the role of essential oil and homogenization procedure in the structure of the CH matrix. The films had a fragile appearance, and showed some disruption depending on the sample preparation process. Pure chitosan films were relatively homogeneous, especially those submitted to H2 treatments. In composite films, the homogenization treatment gave rise to relevant differences in the structure. In films submitted to H1, the polymer matrix showed the interruption of the continuous matrix produced by oil droplets (or their voids) (figure 1). Apparently, H1 emulsions were stable enough not to develop destabilization phenomena (flocculation, coalescence, creaming) during the drying step of the film. Films submitted to H2 showed micro-fractured matrices when essential oil are present (figure 2). In these films, oil droplets were hardly appreciable, since they were smaller and thus more intimately incorporated into the polymer matrix. The particle size reduction taking place during MF led to increased interactions between the polymer matrix and the oils, which led to a less cohesive chitosan matrix and scarcely identifiable oil droplets. Microfluidization blurs the differences between both phases and lipid particles become integrated in the polymer network. Similar results were obtained by [4].

When the oil content increases, the greater molecular contact between CH and oil compounds may weaken the polymer chain aggregation forces, making the matrix more open. In fact, for the same surface

solid density, the films were significantly thicker when they contained 1% oil ($75 \pm 2 \mu\text{m}$) than in the other cases ($52 \pm 4 \mu\text{m}$).

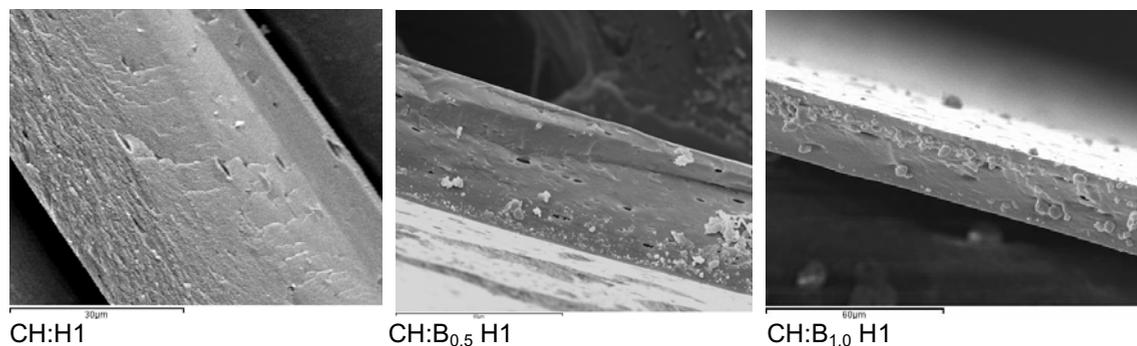


Fig. 1. Scanning electron microscopy images of transversal sections of film submitted to H1

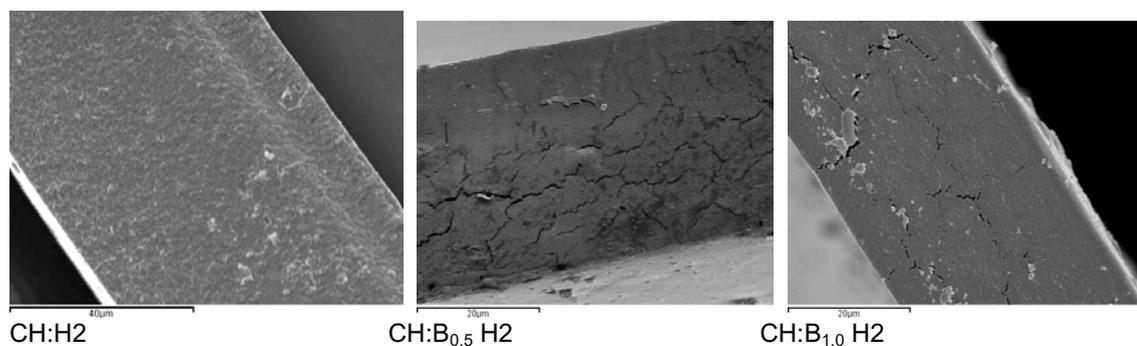


Fig. 2. Scanning electron microscopy images of transversal sections of film submitted to H1

3.2 Mechanical properties

In pure chitosan films, the MF process produced some reduction in rigidity (lower EM, $p < 0.05$). This was previously observed by Vargas *et al.* [4] in pure chitosan films submitted to different pressure homogenization treatments (62, 83, 124 and 165 MPa) and has been attributed to the reduction in the molecular weight of the polymer during microfluidization. The correlation between the molecular weight of chitosan and the mechanical resistance of chitosan films has been previously reported [9]. Kasaai *et al.* [10] investigated the chain scission of chitosan as affected by microfluidization pressure. The obtained results suggest that, at 165 MPa pressure, the molecular weight of the chitosan is sufficiently reduced to have a significant impact on the Elastic Modulus. However, the rest of the mechanical properties of pure polymer films were not significantly affected by homogenization pressure.

Regardless of the homogenization technique, the addition of B led to a significant decrease in EM and TS, and a marked increase in the stretchability, as a function of the oil content (Table 1). The greater the oil content, the greater the effect. This could be attributed to the discontinuities induced in the chitosan matrix by oil droplets (as previously observed in the micrographs, figures 1 and 2), which caused a loss of

the film cohesion and mechanical resistance. However, the interruption of the CH chain aggregations makes the chain displacement during stretching easier, which gives the film a greater ability to be deformed without breaking. This has also been found in previous studies [11, 12].

Microfluidization at high pressure intensified the effects described for composite films, giving rise to the least rigid, the softest and most stretchable films ($p < 0.05$). Moreover, the σ vs. ϵ_H curves (Figure 3) reveal the micro-fractures taking place during the first tensile step (curves with small peaks), which are caused by the film fragility associated to the high degree of contact of the oil-CH molecules, which makes the CH chain aggregation forces weaker. In this case, the EM values reported correspond to an apparent value since microstructural failures occurs, as commented in the microstructural analysis.

Table 1. Mechanical properties of the films: a) EM b) TS c) %E. Mean values and 95% LSD intervals.

FFD	EM (MPa)		TS (MPa)		E (%)	
	H1	H2	H1	H2	H1	H2
Ch	2448 (252) ^{a,x}	2078 (323) ^{a,y}	74 (11) ^{a,x}	75 (10) ^{a,y}	4.6 (0.8) ^{a,x}	6 (2) ^{a,y}
Ch:B _{0.5}	1932 (341) ^{b,x}	428 (68) ^{b,y}	68 (18) ^{b,x}	32 (13) ^{b,y}	19 (9) ^{c,x}	38 (8) ^{c,y}
Ch:B _{1.0}	1199 (138) ^{c,x}	415 (33) ^{c,y}	43 (6) ^{c,x}	32 (5) ^{c,y}	18 (10) ^{b,x}	22 (6) ^{b,y}

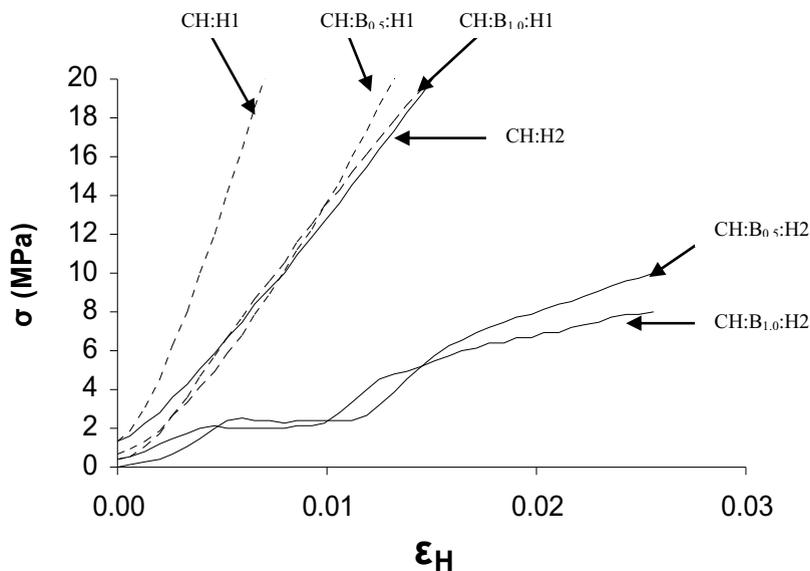


Fig. 3. Detail of the typical true stress (σ) vs. Hencky strain (ϵ_H) curves obtained in tensile tests carried out on some composite films submitted to H1 (dotted lines) and H2 (solid lines) homogenization processes

3.3 Water vapour Permeability

Values of water vapour permeability are shown in table 2. In pure chitosan films, H2 produced a significant increase in WVP ($p < 0.05$), which could be explained by the reduction in the polymer chain length and the subsequent increase in its hydrophilic nature, as reported by Gocho *et al* [13]. These

authors studied the effect of molecular weight on the water sorption isotherms of chitosan, and they found that the lower the molecular weight of chitosan, the higher the water affinity.

Barrier properties of films submitted to H1 were not significantly affected by oil addition when CH oil ratio was 1:0.5 ($p > 0.05$), although a WVP increase was found when the ratio was 1:1 ($p < 0.05$). This could be due to the relatively hydrophilic nature of the main components of these essential oil and their negative effect on the cohesion forces of the CH matrix, as commented on above, which enhance transport phenomena through the film. Nevertheless, the application of MF led to a significant decrease in WVP when the CH:B was higher than 1:1. Probably, at low essential oil concentration, the effect of the overall increase in the hydrophobic nature of the matrix predominated over the effect of the loss of matrix cohesion and, then, water vapour transport was limited.

3.4 Gloss

The gloss of the films was studied because it has a direct influence on the appearance of the coated product. Table 2 shows the values obtained at 60° incidence angle. Norm ASTM D523 [8] recommends this geometry when results are comprised between 10 and 70, which was the case for most the obtained results. All values were lower than 30, meaning that all the studied films were only slightly glossy.

The gloss of the films is linked to the morphology of their surface [1] and generally, the smoother the surface, the glossier the film. Hence, composite films were less glossy than pure chitosan films because of the occurrence of discontinuities in the chitosan matrix. These discontinuities increased the roughness of the film surface and decreased the specular reflectance. Thus, gloss was reduced by the addition of B for both homogenization treatments, showing very small differences among samples.

Nevertheless, H2 process tended to yield glossier films, particularly for pure CH films, which can be attributed to the particle size reduction and the subsequent surface roughness decrease. Similar results were found by Vargas *et al.* [4] in chitosan-oleic acid films submitted to MF.

Table 2. Water vapour permeability (WVP) and Gloss (60°) of the films at 58-100%RH gradient and 5°C. Average values and standard deviations, in brackets.

Parameter	Homog.	CH	CH:B _{0.5}	CH:B _{1.0}
WVP (g s ⁻¹ m ⁻¹ Pa ⁻¹) x 10 ¹¹	H1	61 (6) ^{x,a}	61 (7) ^{x,a}	73 (6) ^{x,b}
	H2	83 (7) ^{y,a}	42.6 (0.6) ^{y,b}	80 (9) ^{x,a}
Gloss (60°)	H1	14 (4) ^{x,c}	7 (2) ^{x,a}	9 (3) ^{x,b}
	H2	20 (5) ^{y,c}	11 (3) ^{x,a}	9 (2) ^{x,b}

The same superscript (^{abc} to be compared horizontally the same homogenization conditions and ^{xy} vertically the same formulation) in LSD test.

4. Conclusion

Homogenization conditions greatly influenced the final film microstructure and thus the mechanical behaviour of chitosan-basil essential oil films. The induced close contact between the polymer and the oil compounds gave rise to more fragile films of increased stretchability. This can be explained by the weakening effect on the CH matrix where chain interaction forces were reduced. This effect was also observed in films obtained from mild homogenized dispersions, but it was less marked. Water barrier properties of the films were dependent on the chitosan:essential oil ratio and the homogenization technique. Microfluidization increased the WVP of pure CH films while the addition of basil oil was only effective in improving water barrier properties when incorporated at the lowest ratio in film-forming

dispersions submitted to microfluidization. Gloss of chitosan films was reduced by the addition of basil essential oil. However, the application of microfluidization tended to yield glossier films.

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