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Physicochemical properties of chitosan-essential oils film-forming dispersions. Effect of homogenization treatments

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Abstract

Chitosan films enriched with basil (B) or thyme (T) essential oils (EO) were prepared by means of two homogenization treatments, without (H1) and with (H2) microfluidization. H2 treatment led to a significant decrease in the average particle size and the type of EO significantly affected d_{43} values ($p < 0.05$). ζ -potential significantly increased with the use of H2 treatment. Treatment H2 changed the rheological behaviour of the FFD. In all cases, the viscosity of the FFD was reduced by this treatment. The type of EO had a different effect on rheological behaviour depending on the homogenization treatment. CH:T were more viscous at H2 and CH:A showed higher apparent viscosity values than CH:T at H1.

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1. Introduction

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 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 than those obtained at low pressure. This is attained in the interaction chamber of the microfluidizer, where the emulsion is submitted to high shear stress. There are few studies dealing with the effect of microfluidization on the properties of the film-forming dispersions (FFD) used to obtain edible films and coatings [1]. The combination of hydrophilic constituents such as chitosan and some lipids could produce films with optimized characteristics [2]. Many lipids have been incorporated to composite films, mainly aiming to reduce the water vapour permeability of these hydrophilic materials.

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Essential oils (EO) are interesting lipids to be incorporated into the films since these plant extracts exhibit additional characteristics, such as antimicrobial and antioxidant effects [3, 4]. This would be in line with current research into the reduction of the use of chemical additives in the food industry. The aim of this work is to study the influence of homogenization treatments on particle size distribution, ζ -potential and rheological properties of chitosan-essential oils FFD as compared to pure chitosan dispersions.

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 1% w/w in 1% w/w acetic acid). Basil (B) and thyme (T) essential oils were provided by Herbes del Moli (Alicante, Spain) and $Mg(NO_3)_2$ by Panreac Quimica, S.A. (Castellar del Vallés, Barcelona, Spain).

2.2. Preparation of the film-forming dispersions

High molecular weight CH (1 wt%), dispersed in an acetic acid solution (1 % v/w), and basil (B) or thyme (T) essential oils (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. H2 treatment consisted of H1 plus high-pressure homogenization at 165 MPa in a single pass by means of a Microfluidizer® M110-P processor.

2.3 Characterization of the film-forming dispersions

2.3.1 Particle size distribution

The particle size analysis of the emulsions was carried out using a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) with ultrasound application. The samples were diluted in a sodium acetate buffer solution (175 mM) under the appropriate solvent conditions (pH = 4.8) at 2,000 rpm until an obscuration rate of 10% was obtained. Mie theory was applied considering a refractive index of 1.47 and 1.50 for T and B essential oils, respectively, and 0 absorption in both cases. Three replications per formulation were made. The volume-surface mean diameter ($d_{3,2}$) as well as the droplet size distribution were determined [5].

2.3.2 pH and ζ -potential

FFD were analyzed at 25°C in terms of pH. ζ -potential was measured using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). The electrophoretic mobility of the droplets was transformed into ζ -potential values using the Smoluchowsky mathematical model. The samples were diluted to a droplet concentration of 0.02% using a sodium acetate buffer solution (175 mM) at pH 4.8.

2.3.3 Rheological behaviour

The rheological behaviour of FFD was analysed at 25°C using a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Samples were left to rest for 5 minutes before the measurements were taken. The shear stress (σ) was measured as a function of shear rate ($\dot{\gamma}$) between 0 and 300 s⁻¹, taking 3

minutes for each (up and down) curve. 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}

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

3. Results and Discussion

3.1 Particle size distribution

The droplet size distribution of an emulsion often has a major impact on its physicochemical and sensory properties such as shelf life, appearance, flavor, and texture [6]. Figure 1 shows the size distribution in volume of emulsions of the CH-T. Table 1 shows the results of $d_{3,2}$ and the mode in size distributions of all emulsions. In all cases, the size distributions were multimodal, due to droplet flocculation taking place after the homogenization process.

H2 treatment led to significant ($p < 0.05$) reduction of the particle sizes, as compared to H1, as can be seen in figure 1. This can also be observed in table 1 through the values of the $d_{3,2}$, regardless of the type of essential oil. This is in agreement with McClements [6] who stated that emulsions that have undergone secondary homogenization (size reduction of the droplets) usually contain smaller droplets than those that have undergone primary homogenization (blending of aqueous and lipid phase to create the emulsion). The type of EO significantly affected mode values ($p < 0.05$). CH-T FFD showed the lowest particle size for both homogenization treatments. Amongst H1 emulsions, those prepared with thyme oil showed the lowest values.

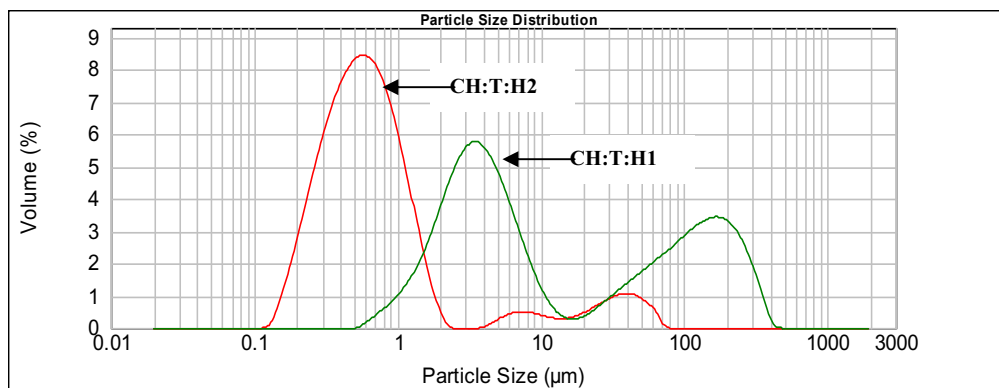


Fig. 1. Particle size distribution of CH:T FFD.

Table 1. Average diameter $d_{3,2}$, maximum peak in size distributions and ζ -potential. Average values and standard deviations, in brackets.

	Homog.	CH	CH:B	CH:T
$d_{3,2}$ (μm)	H1		9.3 (0.7) ^{x,b}	6.6 (0.6) ^{x,d}
	H2		1.30 (0.04) ^{y,b}	1.48 (0.03) ^{y,d}
Mode (μm)	H1		4.8 (0.3) ^{x,c}	3.5 (0.0) ^{x,b}
	H2		1.1 (0.0) ^{y,c}	0.5 (0.0) ^{y,b}
ζ (mV)	H1	64 (3) ^{x,a}	61 (2) ^{x,c}	55.0 (1.5) ^{x,b}
	H2	72.3 (0.6) ^{y,a}	68.0 (0.9) ^{y,c}	64 (3) ^{y,b}

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

3.2 pH and ζ -potential

The pH value of all FFD was 3.2 and no effect of essential oils and homogenization treatments was detected. According to McClements [7], if the droplet charge is sufficiently high, the emulsion may be stable against aggregation due to electrostatic repulsion. The results of ζ -potential of CH dispersions and all emulsions with 1% oil content are shown in table 1. All of them were positive due to the positive charge of the particles. The pH of the FFD was 3.2, regardless of the essential oil concentration and homogenization treatments. At this pH value, the amino groups of chitosan are positively charged ($\text{pK}_a \text{NH}_3^+ / \text{NH}_2 \approx 6.5$), which agrees with the obtained values.

Essential oils were dispersed in distilled water and the ζ -potential was measured in the absence of chitosan. The resulting average values for H1 emulsions were -27.8mV for basil and -13.4mV for thyme. For H2 emulsions, the results were -31.1mV for basil and -9.7mV for thyme. This negative charge is associated with the presence of dissociable compounds in the oils and with the adsorption of negative ions on the droplet surface. According to the obtained values, basil oil droplets showed greater surface charge than thyme oil droplets, without significant differences due to the homogenization intensity. This could be attributed to differences in the dissociation degree of ionisable compounds of the different oils.

The adsorption of chitosan on the oil droplets resulted in positively charged dispersed particles. The ζ -potential values of pure CH dispersions were the highest (64 mV and 72.3 mV, respectively for H1 and H2 homogenization conditions), and the charge was partially neutralized when adsorbed on the negatively charged oil droplets. It could be assumed that, the more positive the ζ -potential, the greater the amount of adsorbed CH at the droplet interface. All H1 emulsions could be considered as relatively stable due to their relatively high surface net charge and the steric stabilization effect of the adsorbed CH [8].

Microfluidization resulted in a ζ -potential increase ($p < 0.05$) of CH dispersions, which indicates that homogenization induced conformational changes in the polymer chain, which increased the number of charged groups in the polymer hydration surface. This is also reflected when it was adsorbed on the oil droplets, which also showed greater values of ζ - potential than when obtained by the H1 procedure. Therefore, further stabilization of the FFD was attained by H2 treatments, since additionally to the droplet size reduction, adsorption of chitosan on the oil droplet surface was favoured and the surface charge increased. The effect of the CH:EO ratio and the kind of oil was similar to that commented on for H1 samples: the greater the oil ratio, the greater the surface charge. A slightly lower charge was obtained for thyme rather than for basil oil.

3.3 Rheological behaviour

The experimental flow curves of FFD between 0 and 300s^{-1} are shown in figure 2a for treatment H1 and 2b for treatment H2, together with the curves predicted by the Ostwald de Waale model (equation 1). This equation fitted the experimental data closely. The consistency index (K) and the flow behaviour index (n) are shown in table 2, together with the apparent viscosity at 100 s^{-1} shear rate (η_{ap}). Treatment H2 changed the rheological behaviour of the FFD, which were shear thinning in H1 treatments and whose behaviour was more Newtonian after MF.

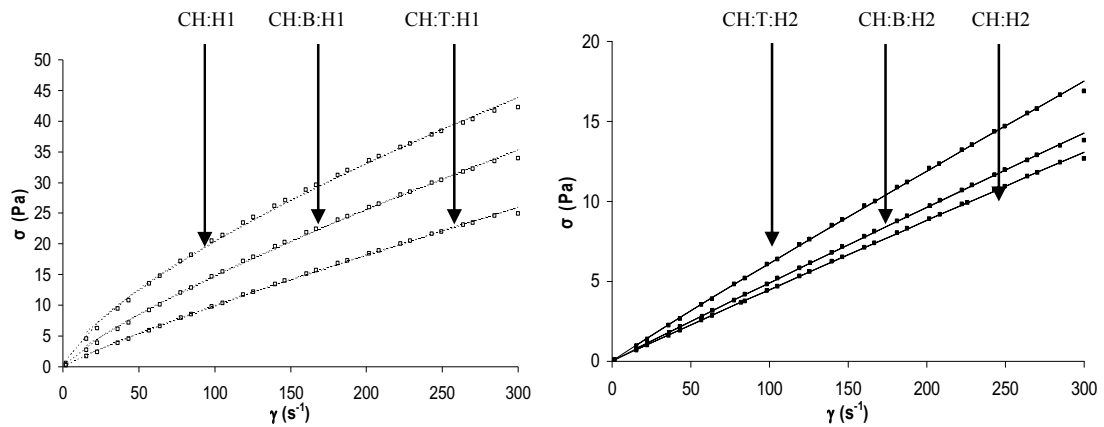


Fig 2. Typical Flow curves of FFD for treatment H1 (a) or H2 (b) at 25°C

In the case of pure CH dispersions, an important viscosity decrease was observed (78.5% reduction) when microfluidized, which could be due to the molecular weight reduction caused by MF and the conformational change of the chains [9]. Similar results were obtained by Vargas *et al.*[1].

FFD submitted to H1 and containing 1% EO were less viscous than pure chitosan dispersions. This behaviour can be explained by taking into account two opposite effects: the presence or the increase in the dispersed phase (oil) content that tends to increase the viscosity of the system and the adsorption of the polymer on the droplet surface, which provokes a decrease in the effective thickening concentration in the aqueous phase. Thus, viscosity of the FFD was reduced after MF, in accordance with both the observed reduction in droplet size and the increase in the amount of chitosan adsorbed on the oil droplet surface, which in turn is in agreement with the growth of the interfacial area in the system.

The oil type had a significant impact on η_{ap} at 100s^{-1} for H1 dispersions ($p < 0.05$), although for H2 samples, no notable differences among viscosity of basil and thyme emulsions were detected. In the case of emulsions submitted to H1, those with thyme oil were the least viscous, which may be due to the fact that they have smaller droplets at the same oil content.

Table 2. Flow behaviour index (n), consistency index (K), and apparent viscosity at 100s^{-1} (η_{ap}). Average values and standard deviation, in brackets.

	Homog.	CH	CH:B	CH:T
n	H1	0.70 (0.00) ^{x,a}	0.791 (0.002) ^{x,c}	0.871 (0.002) ^{x,e}
	H2	0.98 (0.00) ^{y,a}	0.967 (0.005) ^{y,c}	0.96(0.00) ^{y,e}
k (Pa.s)ⁿ	H1	0.838 (0.013) ^{x,a}	0.389 (0.007) ^{x,c}	0.182 (0.004) ^{x,e}
	H2	0.05 (0.00) ^{y,a}	0.060 (0.006) ^{y,c}	0.076 (0.002) ^{y,e}
η_{ap} (100 s⁻¹) (Pa.s)	H1	0.200 (0.002) ^{x,a}	0.144 (0.002) ^{x,c}	0.097 (0.002) ^{x,e}
	H2	0.043 (0.00) ^{y,a}	0.050 (0.004) ^{y,c}	0.06 (0.00) ^{y,e}

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

4. Conclusions

The analyses of the FFD properties allow us to conclude that CH adsorbs on the thyme and basil droplet surface. The greater the particle size reduction, the greater the CH adsorption, which promoted emulsion stability, especially when the samples were submitted to microfluidization. High pressure homogenization treatment promoted a significant reduction in particle size and viscosity of the FFD. The type of essential oil used to prepare the FFD did not promoted great differences in the properties of the FFD after microfluidization. However, H1 treatments gave rise to less positively charged, smaller particles and less viscous dispersions in chitosan-thyme essential oil FFD. The differences could be explained by the different affinity among CH and the droplet surface charge of both types of oils. The later affected the polymer interfacial adsorption, which, in turn, had an influence on the effective droplet size.

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